

The impact of floral spot and ring markings on pollinator foraging dynamics

Marinus L. de Jager¹ · Edward Willis-Jones² · Samuel Critchley² · Beverley J. Glover²

Received: 1 March 2016 / Accepted: 13 July 2016 / Published online: 11 August 2016
© Springer International Publishing Switzerland 2016

Abstract Linear areas of contrasting floral colour, or “nectar guides”, are recognized to enhance pollinator attraction. Few studies, however, have investigated the role of other types of floral marking in pollinator behaviour. In this study, we explore the impact of petal spots and petal rings on bumblebee foraging dynamics. Using model flowers with discrete spot or bullseye ring patterns we investigate the responses of *Bombus terrestris* foragers towards rewarding and unrewarding flowers. We find that the presence of petal spots and rings reduce the search time of pollinators to locate rewarding model flowers. Only the rewarding petal ring phenotype, however, is readily learned and significantly increases the foraging efficiency of experienced bees over four foraging bouts. Although the rewarding spot phenotype induces random foraging over this time frame, employing differential conditioning with a strong aversive stimulus over ten foraging bouts reveal that bees have the capacity to correctly identify rewarding spot phenotypes with more training. Once a strong association between petal marking and reward is formed, bees continue to respond to marked phenotypes even when unrewarding, suggesting rewardless plants that exhibit petal marking could potentially exploit their pollinators. We conclude that petal marking, whether organised discretely in a spot or in a continuous ring around the centre of a flower, have a significant and complex influence on pollinator foraging dynamics.

Keywords *Bombus* · Deception · Insect behaviour · Learning · Nectar guide · Plant–pollinator interaction

Electronic supplementary material The online version of this article (doi:[10.1007/s10682-016-9852-5](https://doi.org/10.1007/s10682-016-9852-5)) contains supplementary material, which is available to authorized users.

✉ Marinus L. de Jager
mdj@sun.ac.za

¹ Department of Botany and Zoology, Stellenbosch University, Matieland, Stellenbosch 7602, South Africa

² Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK

Introduction

The floral traits of most angiosperms are shaped by their interactions with animals. Selection on flower morphology, for instance, is often mediated by pollinators (Harder and Johnson 2009). Floral colour has emerged as a crucial factor governing pollinator attraction in multiple experimental studies investigating monochromatic colour (Spaethe et al. 2001; Bradshaw and Schemske 2003; Campbell et al. 2010). Most flowers, however, are dichromatic and typically comprise areas of contrasting colour, often in close proximity to the placement of rewards (pollen or nectar). The role of these “nectar guides” was first considered by Sprengel over two centuries ago (Sprengel 1793), who argued that they help pollinators locate the flower’s nectary.

This hypothesis has been confirmed in fly (Johnson and Dafni 1998; Dinkel and Lunau 2001) and bee (Lunau et al. 2006; Goodale et al. 2014) pollinators that locate the nectary or the central area typically holding rewards more often on actinomorphic model flowers that bear contrasting guide marks. Naïve bumblebees also consistently choose model flowers with contrasting colours over monochromatic models (Simonds and Plowright 2004), suggesting floral patterning may be innately attractive and easier to detect. Nectar guides thus also function to make flowers more conspicuous, as evident by a reduction in pollinator search time when contrasting nectar guides are added to plain model flowers (Leonard and Papaj 2011). Studies with *Delphinium nelsonii* found that enhancing the inferior nectar guides of albino flowers reduced bumblebee and hummingbird search time to similar levels as normal blue flowers that bear contrasting nectar guides (Waser and Price 1985).

The majority of studies investigating the effect of contrasting floral colours have focussed on linear nectar guides. Many plant species, however, produce a different type of floral patterning referred to as petal spots. Like linear nectar guides, these round to oval spots develop through localised accumulation of pigments and are widespread across angiosperms, including many families in both the eudicots and the monocots (Thomas et al. 2009). Darwin was the first to examine the contrasting dark floret in the centre of white inflorescences of wild carrot, *Daucus carota*, although he concluded it plays no role in pollination and may simply be an ancestral trait (Darwin 1888). Eisikowitch (1980) argued that its function is to attract flies and experimentally revealed that naïve flies clearly preferred “spotted” inflorescences, as well as model flowers containing black spots or dead conspecific flies. Dark petal spots may thus be attractive to pollinators through a deceptive pathway, that of exploiting their aggregative response, which has been termed the “fly-catcher effect” (Wickler 1968). Evidence for this effect in natural populations of *D. carota*, however, has been ambiguous (Lamborn and Ollerton 2000).

Much clearer evidence for the role of petal spots in pollinator attraction comes from the South African daisy, *Gorteria diffusa*. This species exhibits an astounding range of floral morphotypes varying in spot complexity (Ellis and Johnson 2009). Its bee-fly pollinators are strongly attracted to inflorescences bearing spots (Johnson and Midgley 1997), with male pollinators preferring complex spots while females prefer simplistic spots (de Jager and Ellis 2012). The most intricate spots have been shown to be sexually deceptive, as male flies exhibit copulation behaviour on them that enhances plant fitness through increased pollen export (Ellis and Johnson 2010). Apart from flies, beetle pollinators have also been found to be strongly attracted to dark spots on model flowers (Dafni et al. 1990; Van Kleunen et al. 2007). The effect of dark petal spots on bee pollinators is still unclear, since studies on *Clarkia* species polymorphic for petal spots report either slight preference

for spot phenotypes (Jones 1996) or frequency dependant preference (Eckhart et al. 2006). Petal spots on model flowers, however, have been found to elicit antennal reactions from naïve bumblebees (Lunau et al. 2006) that may aid feeding if spots are placed close to the location of rewards.

A variant of the petal spot patterning is sometimes found in actinomorphic flowers, especially in the Asteraceae family, that exhibit a full ring of connected black petal spots (Midgley 1993) forming a bullseye pattern around the rewarding central area. Very little research has been done on these petal rings (Dinkel and Lunau 2001), and its contribution to pollinator behaviour relative to petal spots requires investigation. In fact, the influence of petal rings and petal spots on pollinator search time, whether they improve foraging success and whether they could promote plant exploitation of pollinators is unknown. In this study, we explore the behavioural responses of the bumblebee, *B. terrestris*, to investigate the impact of petal rings and spots on pollinator foraging dynamics, and ask four specific questions. (1) Does petal marking reduce the search time of pollinators, and does this differ between rings and spots? (2) How readily do pollinators learn to distinguish rewarding rings and spots, and how does this influence the rewards obtained? (3) Can pollinators be trained to form a strong association between petal marking and reward? (4) Could petal marking facilitate pollinator exploitation once a strong association with reward has been formed?

Materials and methods

Experimental setup

All experiments were conducted with colonies of flower-naïve *B. terrestris* workers (Koppert Ltd., UK). The bumblebees were supplied with polyfloral pollen (Naturally Green, UK) ad libitum and housed in a plastic nest box connected to a 110 × 100 × 70 cm flight arena via a transparent plastic tube with manually operated gates, allowing controlled entry into the arena. Illumination was provided by six Sylvania Activa 172 Professional 36 W fluorescent tubes, modified with Philips high frequency ballasts with a flicker frequency greater than 1200 Hz on a 12 h light/dark cycle. Model flowers comprised epoxy discs with the lid of a 1 ml Eppendorf tube fixed in the center. Epoxy discs were made by combining 1.5 g epoxide resin; 1.2 g hardener and 0.2 g orange



Fig. 1 The three model phenotypes investigated in this study

powder dye (Cornelissen Pigments, UK) before casting into a dental wax impression of a *Lilium* sp. tepal to provide a petal-like surface texture and sheen visually similar to natural flowers.

To create the ring phenotype we drew a single 2.5 mm thick ring with a black marker pen on the epoxy discs (40 mm diameter) around the central lid (Fig. 1). For the spot phenotype we drew three 4 mm diameter spots on the epoxy discs at equal distance around the central lid. Before and between experiments we allowed bees to feed from six uniformly grey epoxy discs (40 mm diameter) containing 30 % v/v sucrose solution (used as reward throughout experiments) in the central lid to train them to feed from artificial flowers. We marked individual foragers with water-soluble paint for identification purposes. Bees were typically tested within a single day, in the few instances where a bee did not complete all responses during 1 day we scored remaining responses the following morning. No bee was used in more than one experiment or exposed to more than one floral model.

Does petal marking reduce the search time of pollinators?

To investigate the effect of petal marking on pollinator search time we set out either three orange plain model flowers, three orange ring models or three orange spot models on the corners of a 30 cm equilateral triangular layout in the middle of the flight arena, following the protocol of Spaethe et al. (2001). The central lids of model flowers were filled with 30 μ l sucrose solution as reward prior to the start of each experiment. A single bee was then allowed to enter the arena and the time taken from departing the 1st model to landing on the 2nd model was recorded as its search time. To ensure that bees were actively searching for food we did not incorporate the time taken to locate the 1st model flower as bees sometimes flew around inside the arena before settling down to feed.

Similarly, we excluded the time taken to locate the 3rd model flower because bees often returned to the 1st model, thereby potentially confounding search time with the effects of memory. Once a bee returned to the nest box (constituting one foraging bout) model flowers were cleaned with 30 % ethanol before being randomized to avoid scent marking and spatial learning (done across all experiments). Each bee performed five foraging bouts. Bees were filmed for this experiment to allow accurate quantification of the time taken. Model phenotype (plain, ring or spot), foraging bout and their interaction were used as factors in generalized estimating equation (GEE) analyses (see “[Statistical analysis](#)” section for details).

How readily do pollinators learn to distinguish rewarding rings and spots, and how does this influence foraging success?

To explore pollinator foraging success in relation to petal rings and spots we placed six model flowers comprising two phenotypes 4 cm apart in a rectangular layout in the arena. Treatments included either three plain models and three ring models, or three plain models and three spot models. Ring and spot models were filled with 30 μ l sucrose solution and scored as rewarding, while plain models were filled with 30 μ l water and scored as unrewarding. Single bees were allowed to enter the arena and the total number of visits made, whether they landed on the rewarding model and if so, whether they consumed sucrose were recorded. When sucrose was consumed, bees typically drained the lid in the centre of the model flower. We refilled the emptied lid as soon as the bee landed on the next model flower. We thus also calculated the foraging success for each bee during each

bout as the amount of sucrose consumed per visit by dividing the number of lids drained by the number of visits made during that foraging bout. Each bee performed four foraging bouts. The rewarding phenotype (ring or spot), foraging bout and their interaction were used as factors in the GEE analyses.

Can pollinators be trained to form a strong association between petal marking and reward?

Since bees increased their probability of choosing the rewarding ring phenotype over four bouts in the prior differential conditioning experiment, but not the rewarding spot phenotype (see “Results” section; Fig. 3), we investigated whether they possess the capacity to accurately and consistently differentiate spot models from plain models by employing quinine as a more aversive stimulus (Dyer and Chittka 2004). Quinine is bitter-tasting and acts as reinforcing punishment, but cannot be detected by sight or scent (Whitney et al. 2008), making the presence or absence of spots the only cue available to bees for identifying quinine. Five spot models and five plain models were randomly placed in the flight arena, with spot models containing 20 μ l sucrose solution (rewarding) and plain models containing 20 μ l 0.12 % w/v (saturated) quinine hemisulphate salt solution (punishing).

Single bees were allowed to make 100 responses, which typically required ten foraging bouts. For this experiment, responses were scored as correct when a bee landed on a rewarding model or rejected a punishing model (by approaching within 2 cm of the model and hovering in front of it before flying away). Responses were scored as incorrect when a bee landed on a punishing model or rejected a rewarding model. These measures were used in order to facilitate 100 responses during a single day. Bee choices were analysed in sequential bouts of ten visits each, which served as the only factor in GEE analysis.

Could petal marking facilitate pollinator exploitation once a strong association with reward has been formed?

To determine whether the use of petal rings and spots as reward signals may deceive pollinators when no rewards are present, we used absolute conditioning to train bees on either the ring, or the spot phenotype. Six model flowers of a given type were placed randomly in the arena and filled with 30 μ l sucrose solution. Each bee was allowed 15 successive foraging bouts with sucrose refilled between every bout. When the last foraging bout ended, three plain models and three ring models, or three plain models and three spot models (depending on phenotype bees were trained on) without any rewards were immediately placed in a rectangular pattern. Once the bee entered the arena the gate was shut and each visit scored as landing on the training model or the plain model until 20 visits had been made. The model phenotype (ring or spot) that bees were trained on was analysed as the only factor in GEE analysis.

Statistical analysis

We used GEE’s for all analyses as multiple responses for each bee were recorded. Bee identity was used as the repeated subject variable employing an exchangeable correlation matrix to account for correlated responses within individuals. All sample sizes are displayed in figures. Data for search time was analyzed with a Gamma distribution and a log link function, while the sucrose consumed per visit data conformed to normality and was

analyzed with a Gaussian distribution. The binomial distribution and a logit link function were used for all other analyses. Estimated marginal means and 95 % confidence intervals (CI's) were obtained and back transformed from the logit scale before plotting. All statistical analyses were carried out in SPSS 23 (SPSS, Chicago, USA).

Results

Petal marking reduces the search time of pollinators

Bee search time was much faster on marked model flowers than on plain models (Wald $\chi^2 = 26.704$, $df = 2$, $p < 0.001$). Differences in search time between spot and plain models were highly significant, while the estimated 95 % CI's of search time on ring and plain models overlapped by <0.06 s (Fig. 2). This overlap is meaningless in the biological context of locating flowers when considering the variation in search time found within a given treatment. There was no difference between search times on ring and spot models. Foraging bout (Wald $\chi^2 = 3.873$, $df = 4$, $p = 0.423$) and its interaction with model phenotype (Wald $\chi^2 = 2.034$, $df = 8$, $p = 0.980$) were not important in predicting search times, suggesting that bees innately find marked phenotypes more quickly. When analysing only the first foraging bout of each bee (i.e. naïve bees), although the same general trend was observed with search times being slightly faster on marked phenotypes (Fig. S1), this effect was much weaker (GLM, Wald $\chi^2 = 4.501$, $df = 2$, $p = 0.105$), suggesting that some improvement in search time may in fact be gained with experience.

Pollinators readily learn to distinguish rewarding rings and increase their foraging success with experience

Repeated exposure to rewarding ring and spot model flowers revealed that bees had higher foraging success on the ring phenotype (Fig. 3). Bees were also more successful during later foraging bouts. However, this pattern was mostly driven by greater foraging success on the ring phenotype during later bouts, as evident from the highly significant interaction

Fig. 2 Search times in seconds on plain, ring and spot model flowers. Estimated means and 95 % CI are shown. Letters indicate significant differences between treatments

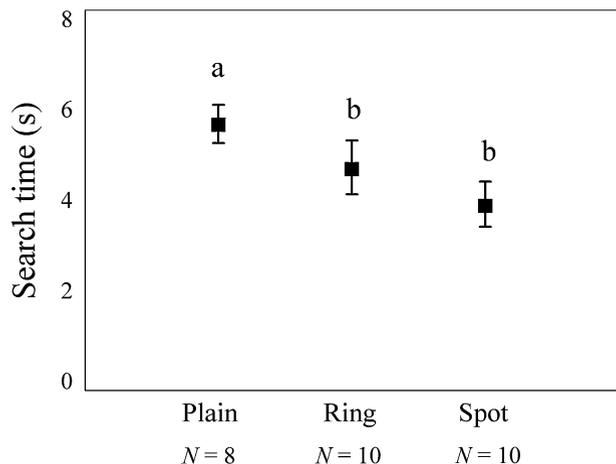
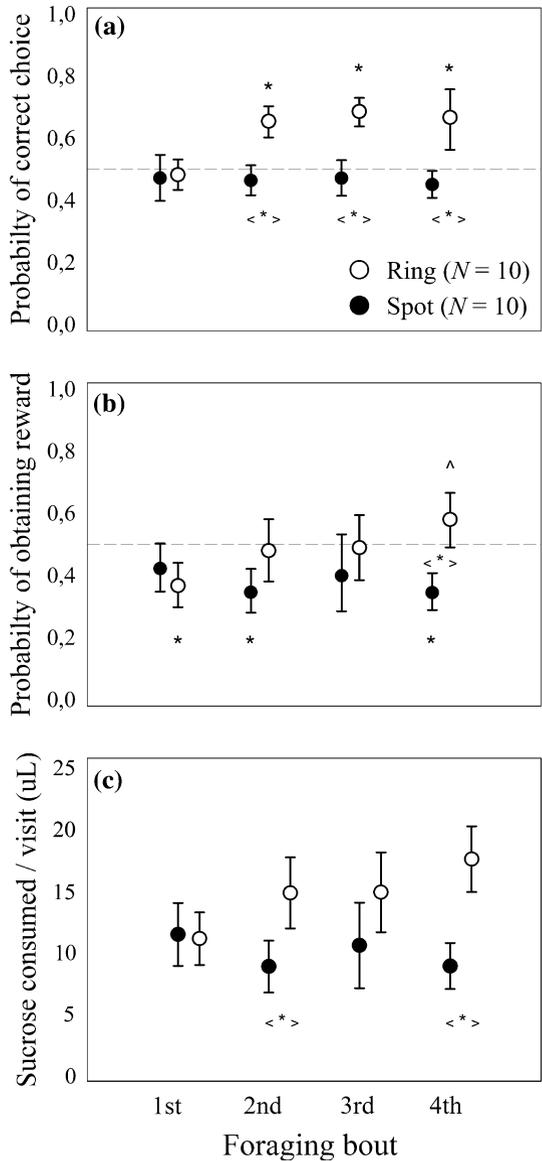


Fig. 3 Bees had greater foraging success on rings than on spots in terms of **a** the probability of choosing the rewarding model, **b** the probability of obtaining the reward and **c** the amount of sucrose consumed per visit. Estimated means and 95 % CI are shown. *Asterisk* indicates significant deviation from random foraging, while *asterisk between less than and greater than* indicates differences between spot and ring models within a foraging bout. *Hat symbol* indicates near significance



between model type and foraging bout (Table 1). While foraging success on the ring phenotype increased with foraging bout and indicates a learned component, foraging success remained constant across bouts on the spot phenotype (Fig. 3). When analysing only the first foraging bout of naïve bees, there were no differences between model types for the probability of choosing the rewarding model (GLM, Wald $\chi^2 = 0.819$, $df = 1$, $p = 0.365$), the probability of obtaining a reward (Wald $\chi^2 = 0.201$, $df = 1$, $p = 0.654$) or the amount of sucrose consumed per visit (Wald $\chi^2 = 0.013$, $df = 1$, $p = 0.909$), indicating that only experienced bees exhibit increased foraging success on marked flowers.

Table 1 GEE results of the effects of repeated exposure to rewarding marked phenotypes on foraging success

Source	Choose rewarding			Reward obtained			Sucrose consumed <i>p</i> /visit		
	Wald χ^2	<i>df</i>	<i>p</i>	Wald χ^2	<i>df</i>	<i>p</i>	Wald χ^2	<i>df</i>	<i>p</i>
Model type	33.928	1	<0.001	7.381	1	<0.010	8.831	1	<0.010
Foraging bout	25.479	3	<0.001	11.415	3	<0.050	12.342	3	<0.010
Model type \times foraging bout	24.427	3	<0.001	30.770	3	<0.001	39.927	3	<0.001

Pollinators can be trained to form a strong association between petal marking and reward

Although bees in our previous experiment did not increase their probability of choosing the rewarding spot model over four foraging bouts, the average number of choices made per bee was only 32.9 ± 7.07 (mean \pm SD). This experiment revealed that by increasing their exposure to 100 visits per bee and punishing wrong choices with bitter-tasting quinine, bees greatly increased their probability of choosing the rewarding spot models over ten foraging bouts (Wald $\chi^2 = 263.506$, $df = 9$, $p < 0.001$; Fig. 4).

Petal marking can facilitate pollinator exploitation under a strong association with reward

Bees trained on rewarding ring or spot phenotypes preferred to land on these models over plain models, even when they were no longer rewarding (Fig. 5), indicating that the use of petal markings for identifying rewards could be used to facilitate deceptive interactions

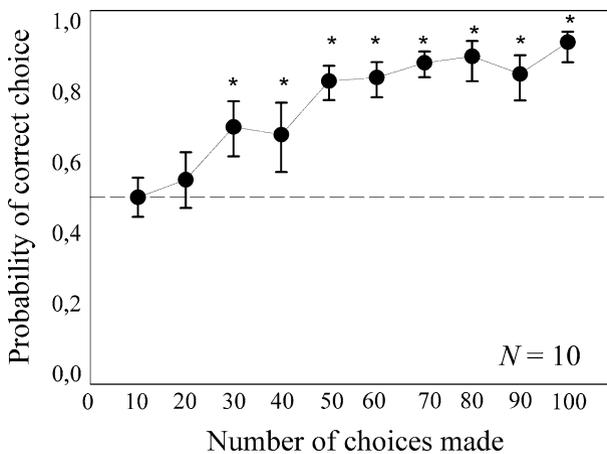


Fig. 4 The probability of correctly identifying the rewarding spot model during differential conditioning with bitter-tasting quinine. Estimated means and 95 % CI are shown. Asterisk indicates significant deviation from random foraging

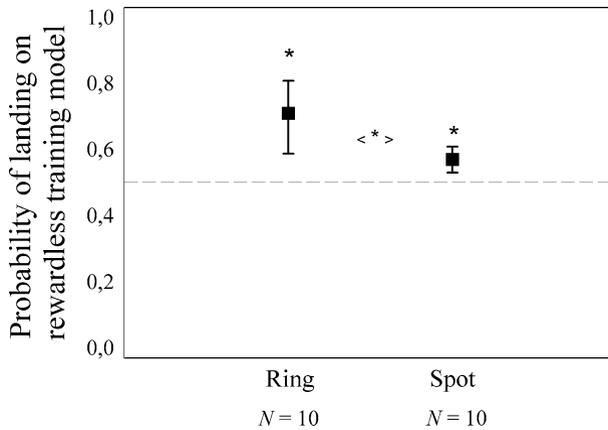


Fig. 5 The probability of landing on a rewardless training phenotype after absolute conditioning. Asterisk indicates significant deviation from random, while asterisk between less than and greater than indicates significant differences between bees trained on spot or ring models

with pollinators. This tendency was stronger for bees trained on the ring phenotype than the spot phenotype (Wald $\chi^2 = 4.522$, $df = 1$, $p = 0.033$), in line with our previous results that indicate a stronger learned response on the ring phenotype.

Discussion

Our results reveal various consequences of petal marking for plants and pollinators. Bees locate marked floral phenotypes faster, which could result in increased visitation rates for plants bearing flowers with spots and rings. Faster search times on marked phenotypes will also increase the foraging efficiency of pollinators on rewarding flowers, as predicted under optimal foraging theory (MacArthur and Pianka 1966). Petal marking may thus lead to reciprocal benefits for plants and pollinators, as documented in the iris *Lapeirousia oreogena* (Hansen et al. 2012). However, we observed increased pollinator foraging success only on the ring phenotype, driven by the bees' greater probability of visiting this rewarding phenotype over four foraging bouts. Although bees on the spot phenotype did not increase their foraging success during this time frame and appeared to be foraging randomly, extending their exposure and punishing them for choosing non-spot phenotypes greatly increased their probability of visiting the rewarding spot phenotype.

Why bees in our study exhibited slower learning curves on the spot phenotype is uncertain. Although slower *B. terrestris* learning rates on yellow spots than dark spots may be a result of yellow spots mimicking rewarding anthers (Pohl et al. 2008), both spots and rings in our study were similarly dark and thus unaffected by such colour properties. Our observations, however, may potentially be related to the limitations of bee vision. Temporal constraints on visual processing result in reduced flight speed and height when model flowers are small, in order to maintain a given detection area (Spaethe et al. 2001). While the small dark spots on our model flowers thus likely required longer search time for detection, bees exhibited the fastest search time on the spot phenotype, making it uncertain whether they clearly resolved the spots. The detectability of a spot may also be influenced by the angle of approach, whereas a dark ring should be visible from all angles, which will

likely assist the learning process. These learning constraints on the spot phenotype were easily overcome by punishing wrong choices with quinine and extending bees exposure. This procedure probably produced the observed increase in accuracy at the expense of slower search times (although not measured in this experiment), as documented in previous learning experiments with *B. terrestris* (Chittka et al. 2003).

When analysing only the first responses of naïve bees a similar, but non-significant trend was observed of faster search times on the spot phenotype. This suggests that petal marking mostly benefits experienced pollinators in terms of increased foraging success. A study on the bumblebee, *Bombus impatiens*, reported a significant benefit of linear nectar guides to naïve bees in terms of reduced search time, with this advantage becoming stronger over time (Leonard and Papaj 2011). Learning thus seems to modify the interaction between petal marking and pollinator behaviour, with experienced pollinators enjoying greater foraging success. This interaction may not always favour both players and could be transformed into a deceptive one. Bees in our absolute conditioning experiment continued to exhibit preference for previously rewarding marked phenotypes even when they became rewardless, confirming previous findings in the congener *B. impatiens* (Leonard and Papaj 2011). While these results may suggest plants can easily exploit pollinators that associate contrasting petal markings with reward, bee preference for petal markings can also be reversed with multiple exposures to non-rewarding marked phenotypes (Simonds and Plowright 2004).

Bee preference for marked flowers may thus vary with each pollinator's experience of rewarding and unrewarding phenotypes, making it difficult to generalize to a population level. Indeed, in a recent study on the bee pollinated non-rewarding orchid, *Anacamptis morio*, which bears petals with highly contrasting patches containing dark spots, no evidence of pollinator-mediated selection was found on the proportion of petal area with dark petal markings (Sletvold et al. 2016). Strong pollinator selection, however, was documented on the amount of floral contrast, with plants exhibiting increased petal colour contrast having higher fitness (Sletvold et al. 2016). High contrast between spots and rings and their background floral colour has experimentally been shown to elicit the greatest attraction in both bees (Lunau et al. 1996) and flies (Dinkel and Lunau 2001), and may thus also be relatively important for pollinator deception.

Most morphotypes of the fly-pollinated *G. diffusa* also exhibit highly contrasting dark markings on yellow to orange inflorescences (Ellis and Johnson 2009). It is curious to note that all deceptive morphotypes within this system invariably exhibit a spot phenotype, whereas non-deceptive morphotypes typically possess a ring phenotype (Ellis and Johnson 2010). While this could suggest that slow pollinator learning rates on spot phenotypes (if fly responses are similar to bees) promoted deceptive interactions in *G. diffusa*, studies investigating pollinator learning on a deceptive *G. diffusa* spot morphotype report considerable pollinator learning behaviour to avoid repeated deception (de Jager and Ellis 2013, 2014a). This learned behaviour may potentially be even faster on the ring phenotype, which could help explain why no *G. diffusa* morphotypes bearing petal rings (as well as other daisies that likely share its bee-fly pollinator—*Dimorphotheca pinnata*, *Ursinia calenduliflora*) are deceptive, but experimental evidence is required to address this possibility.

While our bumblebee experiments suggest that petal rings may instead be more conducive to pollinator exploitation than petal spots, it is important to note that this pattern matches, and is likely driven by, the greater tendency of bees to visit rings than spots. Flies, however, display a strong innate attraction to dark petal spots (Eisikowitch 1980; de Jager and Ellis 2013) related to their aggregative and sexual behaviour, that may make them

more likely to be exploited by petal spot-bearing plants than bees. Petal spots have recently also been shown to play a large role in the deception of fly pollinators in the mushroom-mimicking orchid, *Dracula lafleurii* (Policha et al. 2016), indicating that petal spots are likely involved in pollinator deception across various types of mimicry.

Besides their strong impact on plant–pollinator interactions, petal spots and rings also influence plant interaction with herbivores. In Southern Africa, some beetles forage directly on Asteraceae flowers bearing petal markings. These include the families Scarabaeidae that feed on flower ovules, florets and pollen (Picker and Midgley 1996; de Jager and Ellis 2014b) and Meloidae that feed on the ray florets of daisies (de Jager and Ellis 2014b). In a study investigating floral markings in the polymorphic daisy, *U. calenduliflora*, it was shown that while the morphotype exhibiting dark ray floret markings experienced increase visitation rates from its bee-fly pollinators, it also had the highest incidence of floral predation (de Jager and Ellis 2014b), suggesting the production of petal markings likely results in fitness trade-offs in this, and possibly other species bearing petal markings. The net effect of petal markings on plants and the animals they interact with is thus far from clear and although we show evidence in favour of a predominantly positive effect, further study across multiple pollinator groups is required in order to fully comprehend this interesting plant–animal interaction.

Acknowledgments We thank Matthew Dorling for excellent plant care and for maintaining bee colonies, and Rachel Walker, Corneile Minnaar and Willem Augustyn for helpful discussions. This project was supported by a Royal Society Joint International Project Grant to BJG.

References

- Bradshaw HD, Schemske DW (2003) Allele substitution at a flower colour locus produces a pollinator shift in monkey flowers. *Nature* 426:176–178
- Campbell DR, Bischoff MB, Lord JM, Robertson AW (2010) Flower color influences insect visitation in alpine New Zealand. *Ecology* 91:2638–2649
- Chittka L, Dyer AG, Bock F, Dornhaus A (2003) Bees trade off foraging speed for accuracy. *Nature* 424:388
- Dafni A, Bernhardt P, Shmida A et al (1990) Red bowl-shaped flowers: convergence for beetle pollination in the Mediterranean region. *Isr J Bot* 39:81–92
- Darwin C (1888) *The different forms of flowers on plants of the same species*, 3rd edn. John Murray, London
- de Jager ML, Ellis AG (2012) Gender-specific pollinator preference for floral traits. *Funct Ecol* 26:1197–1204. doi:10.1111/j.1365-2435.2012.02028.x
- de Jager ML, Ellis AG (2013) The influence of pollinator phylogeography and mate preference on floral divergence in a sexually deceptive daisy. *Evolution* 67:1706–1714. doi:10.1111/evo.12070
- de Jager ML, Ellis AG (2014a) Costs of deception and learned resistance in deceptive interactions. *Proc R Soc B* 281(2013):2861
- de Jager ML, Ellis AG (2014b) Floral polymorphism and the fitness implications of attracting pollinating and florivorous insects. *Ann Bot* 113:213–222. doi:10.1093/aob/mct189
- Dinkel T, Lunau K (2001) How drone flies (*Eristalis tenax* L., Syrphidae, Diptera) use floral guides to locate food sources. *J Insect Physiol* 47:1111–1118. doi:10.1016/S0022-1910(01)00080-4
- Dyer AG, Chittka L (2004) Fine colour discrimination requires differential conditioning in bumblebees. *Naturwissenschaften* 91:224–227. doi:10.1007/s00114-004-0508-x
- Eckhart VM, Rushing NS, Hart GM, Hansen JD (2006) Frequency dependent pollinator foraging in polymorphic *Clarkia xantiana* ssp. *xantiana* populations: implications for flower colour evolution and pollinator interactions. *Oikos* 112:412–421
- Eisikowitch D (1980) The role of dark flowers in the pollination of certain Umbelliferae. *J Nat Hist* 14:737–742. doi:10.1080/00222938000770611
- Ellis AG, Johnson SD (2009) The evolution of floral variation without pollinator shifts in *Gorteria diffusa* (Asteraceae). *Am J Bot* 96:793–801. doi:10.3732/ajb.0800222

- Ellis AG, Johnson SD (2010) Floral mimicry enhances pollen export: the evolution of pollination by sexual deceit outside of the Orchidaceae. *Am Nat* 176:E143–E151. doi:[10.1086/656487](https://doi.org/10.1086/656487)
- Goodale E, Kim E, Nabors A et al (2014) The innate responses of bumble bees to flower patterns: separating the nectar guide from the nectary changes bee movements and search time. *Naturwissenschaften* 101:523–526. doi:[10.1007/s00114-014-1188-9](https://doi.org/10.1007/s00114-014-1188-9)
- Hansen DM, Van der Niet T, Johnson SD (2012) Floral signposts: testing the significance of visual “nectar guides” for pollinator behaviour and plant fitness. *Proc Biol Sci* 279:634–639. doi:[10.1098/rspb.2011.1349](https://doi.org/10.1098/rspb.2011.1349)
- Harder LD, Johnson SD (2009) Darwins’ beautiful contrivances: evolutionary and functional evidence for floral adaptation. *New Phytol* 183:530–545
- Johnson SD, Dafni A (1998) Response of bee-flies to the shape and pattern of model flowers: implications for floral evolution in a Mediterranean herb. *Funct Ecol* 12:289–297
- Johnson SD, Midgley JJ (1997) Fly pollination of *Gorteria diffusa* (Asteraceae), and a possible mimetic function for dark spots on the capitulum. *Am J Bot* 84:429–436
- Jones KN (1996) Behavior and postpollination reproductive in alternative success floral phenotypes of *Clarkia gracilis*. *Int J Plant Sci* 157:733–738
- Lamborn E, Ollerton J (2000) Experimental assessment of the functional morphology of inflorescences of *Daucus carota* (Apiaceae): testing the “fly catcher effect”. *Funct Ecol* 14:445–454
- Leonard AS, Papaj DR (2011) “X” marks the spot: the possible benefits of nectar guides to bees and plants. *Funct Ecol* 25:1293–1301. doi:[10.1111/j.1365-2435.2011.01885.x](https://doi.org/10.1111/j.1365-2435.2011.01885.x)
- Lunau K, Wacht S, Chittka L (1996) Colour choices of naive bumble bees and their implications for colour perception. *J Comp Physiol A* 178:477–489
- Lunau K, Fieselmann G, Heuschen B, Van De Loo A (2006) Visual targeting of components of floral colour patterns in flower-naive bumblebees (*Bombus terrestris*; Apidae). *Naturwissenschaften* 93:325–328. doi:[10.1007/s00114-006-0105-2](https://doi.org/10.1007/s00114-006-0105-2)
- MacArthur RH, Pianka ER (1966) On optimal use of a patchy environment. *Am Nat* 100:603. doi:[10.1086/282454](https://doi.org/10.1086/282454)
- Midgley JJ (1993) An evaluation of Hutchinson’s beetle-daisy hypothesis. *Bothalia* 23:70–72
- Picker MD, Midgley JJ (1996) Pollination by monkey beetles (Coleoptera: Scarabaeidae: Hopliini): flower and colour preferences. *Afr Entomol* 4:7–14
- Pohl M, Watolla T, Lunau K (2008) Anther-mimicking floral guides exploit a conflict between innate preference and learning in bumblebees (*Bombus terrestris*). *Behav Ecol Sociobiol* 63:295–302. doi:[10.1007/s00265-008-0661-x](https://doi.org/10.1007/s00265-008-0661-x)
- Policha T, Davis A, Barnadas M et al (2016) Disentangling visual and olfactory signals in mushroom-mimicking *Dracula* orchids using 3D printed flowers. *New Phytol*. doi:[10.1093/biostatistics/manuscript-acf-v5](https://doi.org/10.1093/biostatistics/manuscript-acf-v5)
- Simonds V, Plowright CMS (2004) How do bumblebees first find flowers? Unlearned approach responses and habituation. *Anim Behav* 67:379–386. doi:[10.1016/j.anbehav.2003.03.020](https://doi.org/10.1016/j.anbehav.2003.03.020)
- Sletvold N, Trunschke J, Smit M et al (2016) Strong pollinator-mediated selection for increased flower brightness and contrast in a deceptive orchid. *Evolution* 70:716–724. doi:[10.1111/evo.12881](https://doi.org/10.1111/evo.12881)
- Spaethe J, Tautz J, Chittka L (2001) Visual constraints in foraging bumblebees: flower size and color affect search time and flight behavior. *Proc Natl Acad Sci* 98:3898–3903. doi:[10.1073/pnas.071053098](https://doi.org/10.1073/pnas.071053098)
- Sprengel CK (1793) *Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen*. Friedrich Vieweg, Berlin
- Thomas MM, Rudall PJ, Ellis AG et al (2009) Development of a complex floral trait: the pollinator-attracting petal spots of the beetle daisy, *Gorteria diffusa* (Asteraceae). *Am J Bot* 96:2184–2196. doi:[10.3732/ajb.0900079](https://doi.org/10.3732/ajb.0900079)
- Van Kleunen M, Nänni I, Donaldson JS, Manning JC (2007) The role of beetle marks and flower colour on visitation by monkey beetles (Hopliini) in the Greater cape floral region, South Africa. *Ann Bot* 100:1483–1489. doi:[10.1093/aob/mcm256](https://doi.org/10.1093/aob/mcm256)
- Waser NM, Price MV (1985) The effect of nectar guides on pollinator preference: experimental studies with a montane herb. *Oecologia* 67:121–126
- Whitney HM, Dyer A, Chittka L et al (2008) The interaction of temperature and sucrose concentration on foraging preferences in bumblebees. *Naturwissenschaften* 95:845–850. doi:[10.1007/s00114-008-0393-9](https://doi.org/10.1007/s00114-008-0393-9)
- Wickler W (1968) *Mimicry*. Weidenfeld and Nicholson, London