

Gender-specific pollinator preference for floral traits

Marinus L. de Jager and Allan G. Ellis*

Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland, 7602 South Africa

Summary

1. Shifts between alternative pollinator types are regarded as the main source of divergent selection underlying angiosperm floral diversification. However, pollinating species can exhibit substantial intraspecific variation, particularly between genders, in key morphological and behavioural traits determining their interactions with flowers. This potential mechanism of floral diversification remains largely unexplored.

2. The bee fly, *Megapalpus capensis*, is the predominant pollinator of the remarkable array of floral forms of the sexually deceptive daisy *Gorteria diffusa*. Flies exhibit strong gender-specific interactions with the variable insect-like spots which characterize *G. diffusa* inflorescences.

3. In order to explore variation in the preferences of male and female pollinators for the visual, tactile and olfactory components of these spots, and its implications for floral diversification, we used a sequence of binary choice tests where we manipulated individual spot components.

4. Male and female flies exhibited contrasting preferences for spot components with females preferring simplistic spots and avoiding UV highlights, whilst males prefer any additional visual and tactile phenotypic complexity.

5. Floral odour alone elicited significant preference in females only, indicating that, in contrast to orchids, sexual deception in *G. diffusa* is achieved largely through visual mimicry of female pollinators.

6. Our results clearly show that elaboration of the insect-like spots has evolved in response to male preferences and suggest that a trade-off exists between the attraction of male and female flies, which may have contributed to the divergence in floral phenotype between morphotypes of *G. diffusa*.

7. Pollinators exhibit gender differences in floral preferences and behaviour which is another potential source of divergent selection contributing to angiosperm floral diversification.

Key-words: behaviour, divergent selection, floral diversification, mating signals, pollen export, sexual deception

Introduction

The use of different pollinator species, which can vary substantially in their floral preferences (Bradshaw & Schemske 2003) and sensory systems (Chittka 1992; Troje 1993), is regarded as the main source of divergent selection underlying the remarkable diversification of angiosperm flowers (Johnson, Linder & Steiner 1998; Whittall & Hodges 2007). However, pollinators also exhibit considerable intra-specific variation, particularly between genders, in sensory, morphological and behavioural traits relevant to their interaction with flowers that could impose divergent selection on floral traits (reviewed in Ellis & Anderson 2012).

Previous studies have reported differences between genders in colour preference and behaviour within Lepidopteran (Rusterholz & Erhardt 2000; Alarcón *et al.* 2010), Hymenopteran (Ne'eman *et al.* 2006) and Dipteran (Ellis & Johnson 2010) pollinators, respectively. If there is spatial variation in the importance of different genders as pollinating agents, such gender-specific preferences and behaviour could contribute to floral diversification between isolated populations. In one of the few studies investigating the role of gender-specific selection in floral diversification, Temeles & Kress (2003) revealed that differences in foraging preferences of male and female hummingbirds drive divergence in floral shape and nectar production within the *Heliconia* species they pollinate.

Sexually deceptive orchids that achieve pollination through the attraction and elicitation of copulation

*Correspondence author. E-mail: agellis@sun.ac.za

attempts from male pollinators only (Schiestl *et al.* 2003; Mant, Peakall & Schiestl 2005) are well-known examples of flowers shaped by gender-specific pollinator-mediated selection. Diversification of sexually deceptive orchid lineages, however, does not result from gender-based differences in their pollinators, as only male pollinators are involved. In contrast, *Gorteria diffusa* Thund., a sexually deceptive African daisy (Ellis & Johnson 2010), employs both male and female pollinators and therefore offers a unique opportunity to investigate the effect of gender-specific pollinator behaviour on floral divergence. *Gorteria diffusa* exhibits geographically structured floral variation and comprises distinct floral morphotypes, despite being pollinated by a single species of bee fly (Ellis & Johnson 2009). Both male and female flies visit all the *G. diffusa* morphotypes, but only males exhibit mate searching and copulation behaviour on a subset of these morphotypes (Ellis & Johnson 2010).

Male sexual responses are elicited by black spots on the ray florets of *G. diffusa*, which are required for the attraction of these flies (Johnson & Midgley 1997). These spots vary in complexity and show substantial differentiation between floral morphotypes. In this study, we investigate male and female responses to visual, olfactory and tactile components of the spot phenotype. First, we ask which components of spot phenotype influence pollinator preference and behaviour, and secondly whether male and female pollinators exhibit differences in their preference for these components. If gender-specific preferences have contributed to floral diversification within this system, we might expect that males and females will show different, or even opposing, preferences for some spot components. If spot complexity is associated with the mimicry of females, we may also expect to find male, but not female, preference for increasing complexity within the spots.

Materials and methods

STUDY SYSTEM

Gorteria diffusa is a self-incompatible spring flowering annual daisy from the arid winter-rainfall areas of South Africa. It has a prostrate growth form with branches up to 0.5 m long that carry a profusion of inflorescences (10–60 per plant) on individual peduncles. Inflorescences vary in colour from orange through pale yellow and are characterized by black spots at the base of some, or all, of the ray florets. These spots vary in complexity from simple black pigment patches to three-dimensional structures containing specialized epidermal cells (Thomas *et al.* 2009). Allopatric populations vary substantially in spot phenotype as well as ray floret shape, number and colour, which has led to the description of 14 distinct floral morphotypes, all pollinated predominantly by the bee fly *Megapalpus capensis* Wiedemann (Ellis & Johnson 2009). Although all floral morphotypes induce feeding behaviour in *M. capensis* males and females, five morphotypes induce inspection/mate-searching behaviour predominantly in males whilst three sexually deceptive morphotypes elicit copulation attempts exclusively from males (Ellis & Johnson 2010). These gender-specific differences

in behaviour on *G. diffusa* morphotypes suggest that *M. capensis* males and females exhibit differential responses to the variation in floral traits observed across the range of *G. diffusa* (see Fig. 1).

Megapalpus capensis, like many bombyliids, is a common flower visitor and is regularly seen visiting a range of spring flowering species in Namaqualand. Mating takes place on the open inflorescences of daisies such as *G. diffusa*. Females typically sit within inflorescences and feed whilst males exhibit mate-searching behaviour by flitting between inflorescences, landing on the petal spots and other flies when present. They often exhibit mating attempts on the spots of sexually deceptive *G. diffusa* identical to those exhibited on females (Ellis & Johnson 2010). Mating attempts with female flies, however, seldom result in copulation; perhaps indicating that females only mate once during their lifetime or are only receptive for a limited period. Males, however, will repeatedly land on and exhibit mating behaviour towards females along their flight path, as well as in captivity (M. de Jager, personal observation), indicating that they are likely polygynous and that the first males to mate could experience paternity advantage.

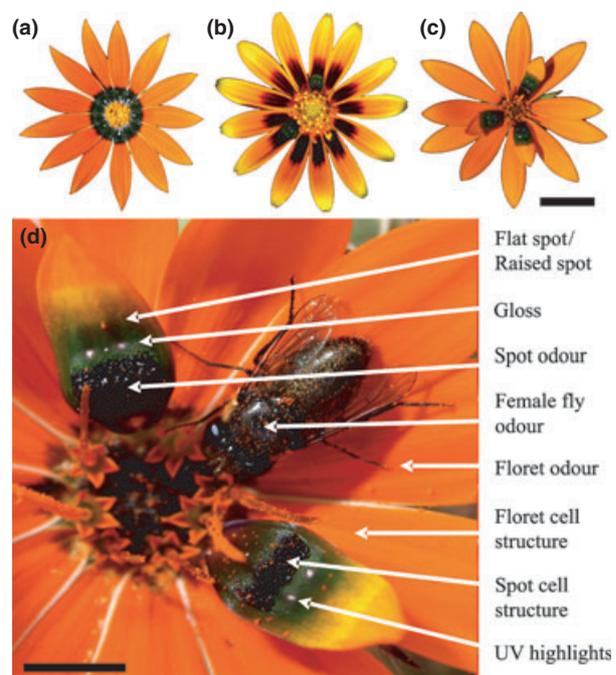


Fig. 1. Photos *a* – *c* depict floral morphotypes representing the three functional categories found within *Gorteria diffusa*: (*a*) is a feeding morphotype (Garies) that induces only feeding behaviour in *Megapalpus capensis* males and females; (*b*) represents an inspection morphotype (Okiep) which induces feeding behaviour in both genders, as well as mate searching behaviour from *M. capensis* males and (*c*) a sexually deceptive morphotype (Spring) which induces feeding behaviour in both genders, but elicits copulation behaviour exclusively from *M. capensis* males. Note that all morphotypes exhibit dark spots at the base of some or all ray florets and that these spots increase in complexity from left to right. (*d*) Shows a close up of the sexually deceptive Spring morphotype in (*c*) with its pollinator, the bee fly *M. capensis*. Arrows indicate the main ray floret spot components that we investigated in this study and include visual, olfactory and potential tactile components. Scale bar in (*c*) = 1 cm and (*d*) = 0.5 cm. Photos by Allan Ellis.

EXPERIMENTAL SET-UP

In order to determine the influence of various components of spot phenotype on gender-specific pollinator preference, we designed a sequential series of binary choice experiments, starting with simplistic spot models and gradually increasing complexity by adding visual, tactile and olfactory components. Spot models were attached with re-useable adhesive to model inflorescences (3 cm diameter orange paper discs) that had a similar reflectance spectrum to the spotted ray florets of the Spring morphotype of *G. diffusa* (see Fig. S1, Supporting Information). Each model inflorescence contained a single spot model and was presented 5 cm above ground level. Model inflorescences used in binary choice pairs were placed 3 cm apart and differed only in the specific spot component under investigation. The Spring morphotype of *G. diffusa* was selected as the basis for the complex spot models in our experiments as it has been shown to frequently elicit mating behaviour from *M. capensis* males (Ellis & Johnson 2010). *Megapalpus capensis* individuals naïve to the floral spots under investigation were caught on daisy inflorescences at a site where no *G. diffusa* occurs (S 30, 12, 33.3; E 18, 2, 58.4). Gender was visually determined before releasing the flies individually into a 1 m³ gauze cage that contained a binary choice of model inflorescences. Each fly was observed for 10 min and its preference for spot models in each choice experiment, as well as its behaviour on them, was recorded with a digital voice recorder. We used flies on the same day we caught them and exposed them to the various binary choice experiments in a random order, generally using each fly for a given experiment only once in order to exclude potential learn-

ing. Our experiments were conducted between August and September in 2006, 2009, 2010 and 2011 on warm sunny days between 11 am and 4 pm when *M. capensis* are most active. Sample sizes for all experiments are reported in Table 1.

Visual signals

Black spots on a coloured ray floret have previously been suggested as pollinator attractants in *G. diffusa* (Johnson & Midgley 1997). To confirm this we offered flies a choice between a plain orange model inflorescence and one containing a centrally placed 1 cm diameter matt black paper spot (Experiment 1). Because *G. diffusa* spots are often raised above the ray floret surface, we also offered flies a choice between a plain orange model inflorescence and one containing a centrally placed 5 mm diameter odourless black plastic bead (Experiment 3). Flies potentially perceive two visual components of the raised spot which may be important for pollinator attraction, the reflective highlight associated with a convex surface and the three-dimensionality itself. In order to tease apart these components, we first offered flies a choice between black paper spots (1 cm diameter) painted with either gloss or matt transparent acrylic paint (Experiment 2) and then between a flat gloss spot and a raised odourless black plastic bead (Experiment 4). The gloss-matt choice tested the importance of reflectance off the black surface whereas the gloss-raised choice tested the importance of three-dimensionality, although the nature of reflectance from these two surfaces likely also differed.

Table 1. Details of the choice experiments to determine the preference of male and female flies for the various spot components present within *Gorteria diffusa*

Experiment	Spot	Gloss	Raised spot	Spot odour	Female odour	Spot cell structure	UV highlights	Sensory system investigated	<i>N</i> (flies) ♂/♀	<i>N</i> (visits) ♂/♀
1. Matt spot No spot	X 0							Visual	11/11	26/54
2. Gloss spot Matt spot	X X	X 0						Visual	43/37	194/153
3. Raised spot No spot	X 0	X 0	X 0					Visual	17/15	40/30
4. Raised spot Gloss spot	X X	X X	X 0					Visual	29/5	104/28
5. Spot odour No odour	X X	X X	X X	X 0				Olfactory	33/26	308/146
6. Female odour No odour	X X	X X	X X		X 0			Olfactory	24/12	74/48
7. Spot cell structure Floret cell structure	X X	X X	X X			X 0		Tactile/visual	21/33	162/152
8. UV highlights No highlights	X X	X X	X X			X X	X 0	Tactile/visual	30/25	170/191
9. UV highlights +Spot odour UV highlights	X X	X X	X X	X 0		X X	X X	Tactile/ visual/ olfactory	12/11	78/96

Experiment numbers are listed with the binary choices flies were exposed to in each, increasing from top to bottom in spot complexity. Along each row the presence (X) or absence (0) of these spot components are indicated for each choice in every experiment. The relevant sensory system investigated in each experiment is also indicated, as well as the sample size of each fly gender we used and the total number of visits they made in each experiment

Olfactory signals

Floral odour has often been shown to attract pollinators (reviewed in Raguso 2008), especially within sexually deceptive orchids which employ surface hydrocarbons to attract mate seeking visitors (Schiestl *et al.* 2003; Mant, Peakall & Schiestl 2005). In order to test its importance within *G. diffusa*, we extracted cuticular compounds from the sexually deceptive Spring morphotype of *G. diffusa* by individually submerging spotted ray florets for 4 min in 8 mL glass vials containing 200 µL hexane (C₆H₁₄). Using the odourless black plastic beads from experiment 4, we then offered flies a choice between model inflorescences with a black plastic bead covered in either spotted ray floret extract or pure hexane (50 µL each – Experiment 5). To determine if the spots produce any unique compounds that males might be responding to we also offered males a choice between model inflorescences with a black plastic bead covered in either spotted ray floret extract or non-spotted ray floret extract (50 µL each). As a control to test whether our extraction method was effective in capturing the putative hydrocarbon compounds that might affect pollinator preference, we caught two females of *M. capensis* in copula in the field and extracted them separately in 400 µL hexane for 2 min (Mant, Peakall & Schiestl 2005). We then offered flies a choice between model inflorescences with a black plastic bead covered in receptive female extract or pure hexane (100 µL each – Experiment 6). All extracts were applied with a 500 µL SGE LC glass syringe (Supelco, St. Louis, MO, USA).

Tactile signals

The surface cell structure of ray floret spots within sexually deceptive *G. diffusa* morphotypes is complex and contain specialized multicellular papillae (Thomas *et al.* 2009) which may have a tactile effect on pollinators. To explore the importance of these potential tactile signals we used the protocol of Whitney *et al.* (2009) to create odourless epoxy casts of spotted and non-spotted ray florets of the sexually deceptive Spring morphotype. These casts captured all the minute details present on the surface of the ray florets at a µm scale, but were odourless and did not include visual differences as we used black pigment to colour all epoxy casts uniformly black. Using these casts we offered flies a choice between a black epoxy cast of a spotted ray floret and a black epoxy cast of a non-spotted ray floret (Experiment 7).

Combination of signals

Previous studies demonstrated that pollinators can use multiple sensory modalities to respond to flowers and that multiple floral traits can differentially impact pollinator choice and behaviour, compared to a single trait (Kunze & Gumbert 2001; Raguso & Willis 2002). We therefore tested the effect of different combinations of spot components on fly preference and behaviour, using the same protocols described above. As the ray floret spots of many *G. diffusa* morphotypes contain white UV reflective highlights, we used Titanium oxide pigment to strategically add white UV reflective highlights to the black epoxy casts of spotted ray florets from experiment 7. We then offered flies a choice between spotted ray floret epoxy casts with or without UV reflective highlights (Experiment 8). Finally, in order to test the combined effect of visual, olfactory and tactile spot components, we used the black epoxy casts described in experiment 8 and added the floret odour extracts from experiment 5. Flies were thus offered a choice between a spotted ray floret epoxy cast with UV highlights and either spotted ray floret extract or pure hexane (50 µL each – Experiment 9).

STATISTICAL ANALYSES

We ran separate Generalized Estimating Equations (GEE) analyses for each binary choice experiment in order to test for significant differences between male and female preference for the more complex model. We chose to use GEE's, which control for fly identity, as our data are correlated responses. The influence of gender on preference for the more complex spot model was modeled using an exchangeable correlation structure, which assumes that observations within a subject are equally correlated. We used a binomial distribution with a logit link function to obtain the estimated marginal means and their 95% confidence intervals (CIs) which we back-transformed before plotting. Departure of preference from random choice was confirmed for each gender when the 95% CIs did not overlap with the random expectation of 50% preference for the more complex model. We also ran separate Generalized Linear Model (GLM) analyses for each experiment using only the first choice of each fly as dependant variable, but as these results were qualitatively similar to our GEE analyses we do not report results here. Next we ran GEE's with the same parameters to model the influence of the model type used on the behavioural responses of each gender during our binary choice experiments. We used preference for landing on the spot model attached to the orange model inflorescence, as opposed to landing on the model inflorescence itself, as our dependant variable and ran a single analysis for each gender, grouping all data by the model type used. Analyses were carried out in the spss 19 statistical package (SPSS Inc., Chicago, IL, USA).

Results

VISUAL SIGNALS

Although female flies significantly preferred model inflorescences with a simple black spot over those without (Fig. 2, Experiment 1), they exhibited no preference for visual elaboration of the black spot, whether this involved three-dimensionality or glossiness. In contrast, males showed significant preference for these spot elaborations when contrasted with spotless models or those with a simple matt spot. Results suggest that both reflectance from the shiny black surface (gloss – Experiment 2) and three-dimensionality (raised spot – Experiment 3) contribute to male preference, although preference was stronger for three-dimensionality (Experiment 4). Despite these differences, the preferences of males and females were only significantly different for the choice between a raised spot and no spot at all.

OLFACTORY SIGNALS

In contrast to the visual components, males did not exhibit preference for the floral odour of spotted *G. diffusa* ray florets, whereas females did (Experiment 5). Male flies ($n = 14$) also did not discriminate between spotted and non-spotted ray floret extracts (Goodness of fit Test; $G = 0.05$, d.f. = 13, $P = 0.82$) suggesting that spotted ray florets do not produce any unique compounds relevant to the attraction of male flies. Interestingly, the control for our extraction method revealed significant differences between genders with males, but not females, exhibiting

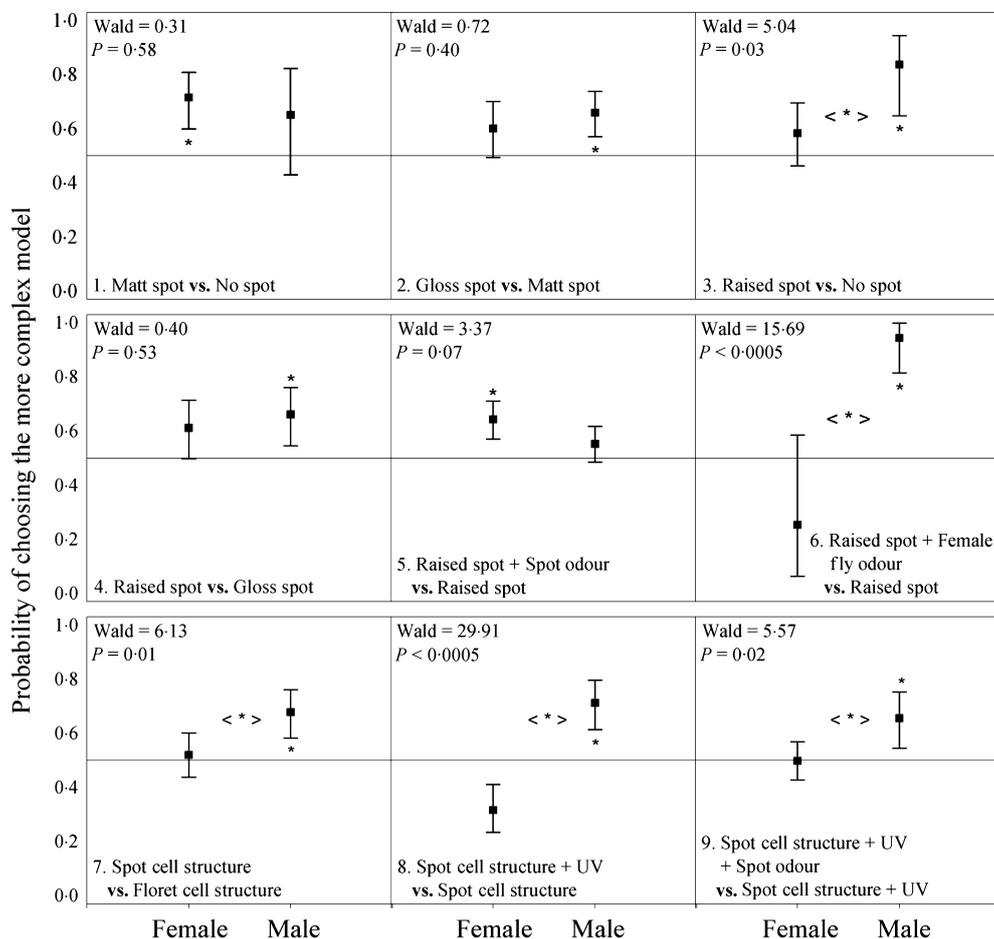


Fig. 2. Probability that *Megalopus capensis* females and males will choose the more complex spot model (first description in the explanation within each panel) in each choice experiment (experiments 1–9, details in methods). Backtransformed marginal means from Generalized Estimating Equations models with 95% Wald confidence intervals (CI) are shown. *Indicates significant ($P < 0.05$) preference for one model over the other in male and female flies (i. e., CI do not overlap with the 0.5 expectation under random visitation). Wald Chi square and P values are reported for the differences between male and female preference and significance is indicated with '*’.

strong preference for the odour extracts of receptive female flies (Experiment 6). This result confirms that our extraction protocol captured compounds involved in male sexual responses.

TACTILE SIGNALS

When investigating further structural elaboration of the spots with regards to the specialized papillae present within the spot epidermis of some *G. diffusa* morphotypes we again found male, but not female, preference for the more complex models (Experiment 7). Male and female preferences also differed significantly.

COMBINATION OF SIGNALS

When we combined visual and tactile components by adding UV reflective highlights to the black epoxy casts of spotted ray florets bearing epidermal papillae, we again found significant preference exhibited by males only (Experiment 8). In contrast to the random choices exhibited by females in previous experiments, females

significantly avoided models containing UV highlights, indicating a potential trade-off for *G. diffusa* when producing this floral trait. When we combined visual and tactile, as well as olfactory traits, only males showed significant preference for the more complex model bearing all three stimuli (Experiment 9). Again, there were significant differences between male and female preferences.

Although copulation behaviour was not observed on our spot models, male flies exhibited significantly different behaviour from females by landing on various spot models significantly more often than on the orange model inflorescences to which they were attached (Fig. 3). Exceptions were the most simplistic of spot models (black matt and gloss spot), epoxy models of the non-spotted ray florets which do not bear papillae (non-spotted floret cell structure) and the most complex spot models (spot cell structure with UV and either hexane or spotted ray floret odour extract). In contrast, females always significantly avoided landing on the spot models, except for the raised spot containing female fly odour on which they exhibited no landing preference.

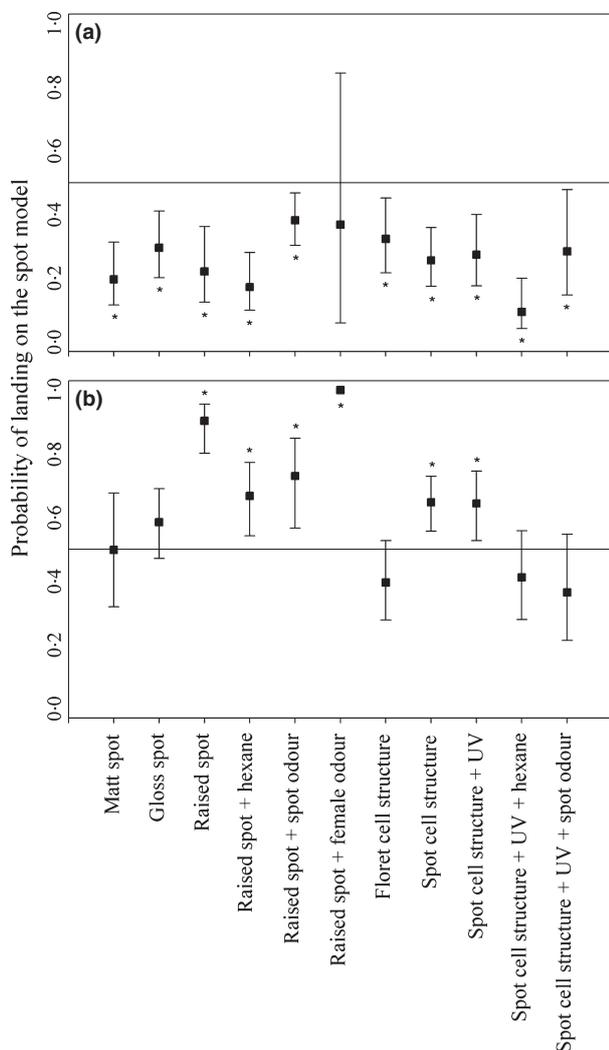


Fig. 3. Different behavioural responses of (a) female and (b) male flies to the model inflorescences they visited during our choice experiments. The probability of landing on the spot model attached to model inflorescences, as opposed to landing on the model inflorescence itself, is indicated. Female flies significantly avoided landing on the spots in most cases whereas males tended to land on the spots. Backtransformed estimated marginal means are plotted with the upper and lower 95% Wald confidence intervals for each model type used. *Indicate significant ($P < 0.05$) deviation from the random expectation of equal expression of both behaviours.

Discussion

Our results show that male and female bee flies exhibit contrasting preferences for most of the spot components of *G. diffusa*. Such divergent selection (or opposing selection in the case of UV reflective highlights) imposed by different fly genders can generate floral diversification within this system without the need for pollinator shifts. Male flies select for any visual elaboration of the spots (including glossiness, raised three dimensionality and UV reflective highlights), whilst females only exhibit significant preference for the most simplistic visual spot models. This result strongly suggests that visual elaboration of spot

complexity has evolved under selection exerted by male flies. The fact that most of *G. diffusa*'s morphotypes bear some reflective UV highlights (Ellis & Johnson 2009) suggests that selection through male flies in this system is widespread. Johnson & Midgley (1997) suggested that the UV highlights within *G. diffusa* spots mimic the reflective highlights on the convex thorax of *M. capensis* flies, implying that the attraction of male flies to these UV highlights may be related to mate searching. The presence of UV highlights in sexually deceptive orchids has also been attributed to the visual mimicry of females in order to attract males searching for mates (Gaskett & Herberstein 2010).

In contrast to studies on sexually deceptive orchids where olfaction is the key stimulus used to attract male Hymenopteran pollinators (Schiestl *et al.* 2003; Mant, Peakall & Schiestl 2005) we found female, but not male, preference for the spotted ray floret odour extracts from sexually deceptive *G. diffusa* when used in isolation. Applying floral odour extract in our study also did not elicit copulation behaviour from male flies, suggesting that it does not contain gender-specific pheromonal signals, as is the case in sexually deceptive orchids (Schiestl *et al.* 2003). We did however find male, but not female, preference for the odour extract of sexually receptive females. This result indicates that there are indeed gender-specific compounds produced by receptive *M. capensis* females which males are responding to, but which *G. diffusa* has not employed in its mimicry of female flies. It should be noted that males did not exhibit mating behaviour in response to the receptive female extracts either. This suggests that male bee flies use signals besides olfaction to detect and react to receptive females, although it is possible that our extraction method did not capture all the relevant compounds dictating male mating behaviour. A previous study on Mediterranean bee flies, however, found male mating behaviour in response to simple black ink spots (Johnson & Dafni 1998) which strongly suggests that odour signals are less important in governing mating behaviour than in Hymenoptera.

Our experiments investigating the importance of the specialized epidermal papillae structures present within sexually deceptive *G. diffusa* spots also revealed male, but not female preference, indicating that these potential tactile components also evolved under selection from male flies. Studies on sexually deceptive orchids bearing papillae have suggested that these features might help mimic the body textures of females (Blanco & Barboza 2005). Although males did not exhibit mating behaviour on our spot models bearing papillae, they did significantly prefer them over models without papillae and therefore must have been able to discriminate them prior to landing. It has been observed that the raised papillae in *G. diffusa* spots possess light reflecting tips that appear similar to the dorsal surface of *M. capensis* flies (Johnson & Midgley 1997), making it possible that this spot component is also involved in visual mimicry of *M. capensis* females.

Interestingly, our experiment combining visual, tactile and olfactory spot components contrasts with our experiment testing olfaction in isolation by finding male preference for the spotted ray floret odour extract of *G. diffusa*. This could be due to a hierarchal system used by flies in decision making, much like Hymenopteran pollinators confronted with the floral traits of sexually deceptive orchids. Streinzer, Paulus & Spaethe (2009) found that bees are firstly attracted to the flowers of sexually deceptive orchids in the genus *Ophrys* through olfactory signals, but once they are close enough they also use visual signals in decision making. Similarly, *G. diffusa* may also be using multimodal signals to attract and elicit responses from mate seeking *M. capensis* males. Multimodal signals can improve signal detection and performance by simultaneously stimulating different sensory systems within the receiver (Candolin 2003). This may be important in *M. capensis* because females appear unresponsive to most mating attempts and may only mate once, thus placing males under strong selection to detect potential mates quickly.

POTENTIAL CAUSE AND EFFECTS OF GENDER-SPECIFIC VARIATION IN POLLINATOR PREFERENCE

As the UV reflective highlights and the specialized papillate structures within raised *G. diffusa* spots are proposed bee fly mimics (Johnson & Midgley 1997), the preponderance of male preference for these elaborate traits might be linked to mate searching behaviour. Male insects are known to devote more time to mating behaviour than females, which spend more time collecting nutritional rewards related to brood care (Alcock *et al.* 1978). *Gorteria diffusa* spots might therefore be exploiting various search images that males use to locate potential mates in its mimicry of female bee flies. This is further supported by the fact that males behaved significantly differently from females by consistently landing on elaborate spot models during our binary choice experiments. One benefit of exploiting mate seeking behaviour in males is that they move more between plants than females, which can increase pollen export (Ellis & Johnson 2010). Increased outcrossing is one of the most compelling hypotheses invoked to explain the evolution of sexual deception in orchids (Scopece *et al.* 2010) as it can increase the quality of the seeds produced (Peakall & Beattie 1996). Within *G. diffusa*, however, only sexually deceptive morphotypes gain the benefit of increased pollen export from mate seeking male flies (Ellis & Johnson 2010), which begs the question why non-sexually deceptive morphotypes also bear UV highlights. One explanation might be that most of the *G. diffusa* morphotypes experience selection by mate seeking male flies, which move more between plants than females (Ellis & Johnson 2010), and may therefore relatively increase outcrossing rates even without the elicitation of copulation attempts. The observed female avoidance of black spot models bearing UV highlights

may stem from an attempt to reduce competition, either for food or for potential mates. This result also suggests that females do not aggregate together to form leks, which is supported by the observation that solitary females are typically found within inflorescences.

Female pollinators have also been found to spend more time per flower and move less between plants than males (Ne'eman *et al.* 2006; Ellis & Johnson 2010). This behaviour is probably related to differences in energy requirements. Within Hymenoptera it has been noted that females spend more time feeding than males (Alcock *et al.* 1978) and that they collect more pollen in order to provide for their young (Michener 2000). Female butterflies have also been found to move less between flower patches than males and to target nectar sources high in amino acids that are probably linked to egg development (Rusterholz & Erhardt 2000). Whereas attraction of male insects can increase pollen export as a consequence of mate searching behaviour, attraction of females may contribute more to pollen import, relative to males, if spending more time on flowers results in greater loads of conspecific pollen. This has been found to be the case with female hawkmoths (Alarcón *et al.* 2010), as well as female bee pollinators (Ne'eman *et al.* 2006).

Variation in the effectiveness of male and female pollinators can produce allopatric divergence in floral phenotype when the relative importance of males and females as pollinators varies geographically, but is constant within populations (Ellis & Anderson 2012). One geographically variable factor that can produce this is pollen limitation. As the importance of selection acting through pollen import has been shown to be the highest when pollinators are limited (Ashman & Morgan 2004), selection through pollen export is likely to become disproportionately important under pollinator abundance. Muchhala *et al.* (2010) found similar results in a modelling study, but also showed that under high visitation rates specialization tends to evolve due to competition acted out through pollen export. Sexually deceptive *G. diffusa* are unlikely to experience pollen limitation, as the removal of ray floret spots does not result in decreased fruit set within a sexually deceptive morphotype (Johnson & Midgley 1997). Under such conditions, selection exerted by mate seeking male flies will be strong as they can increase relative fitness through increased pollen export (Ellis & Johnson 2010). The evolution of sexual deception will therefore be favoured in these populations, resulting in complex spots specialized on the attraction and elicitation of mating behaviour from male flies. Geographic variation in pollen limitation might therefore affect the strength of selection exerted by male and female pollinators in different populations.

Another mechanism that could generate asymmetry in the importance of male and female pollinators is variation in the sex ratio of flies between populations. If ratios within a population remain stable over generations, selection exerted by the dominant gender may influence and determine floral phenotype. The presence and importance

of alternative pollinators within each population may also have an impact on the effectiveness of a given *M. capensis* gender and the strength of selection it exerts. Some of the *G. diffusa* morphotypes are visited by insects other than *M. capensis* (Ellis & Johnson 2009), although there is no obvious relationship between their presence and floral phenotype. Lastly, the strength of selection exerted by food seeking female pollinators will likely be affected by the availability and attractiveness of alternative rewarding plant species in the community and thus the degree of reliance of *M. capensis* on *G. diffusa* as a food source. These are some mechanisms by which male and female pollinators can exert differential selective pressures in different populations and highlight the importance of ecological context in the evolution of specialization. As specializing on different pollinating genders will differentially affect plant fitness, floral diversification within plants pollinated by a single species is also likely to occur and may hitherto have been an overlooked mechanism of floral divergence.

Acknowledgements

We would like to thank W. Augustyn and C. Conradie for help in the field, the Succulent Karoo Knowledge Centre for providing a base during fieldwork and Alison Brody and two anonymous reviewers for helpful comments. Funding was provided by the South African National Research Foundation (A.G.E.) and Stellenbosch University (A.G.E. and M.D.J.). Permits were obtained from the Northern Cape Conservation Board (1488/2009, 1487/2009, 1418/2010, 1417/2010, 1198/2011, 1268/2011). All experiments conducted conform to the legal requirements of South Africa.

References

- Alarcón, R., Riffell, J.A., Davidowitz, G., Hildebrand, J.G. & Bronstein, J. L. (2010) Sex-dependent variation in the floral preferences of the hawkmoth *Manduca sexta*. *Animal Behaviour*, **80**, 289–296.
- Alcock, J., Barrows, E.M., Gordh, G., Hubbard, L.J., Kirkendall, L., Pyle, D.W., Ponder, T.L. & Zalom, F.G. (1978) The ecology and evolution of male reproductive behaviour in the bees and wasps. *Zoological Journal of the Linnean Society*, **64**, 293–326.
- Ashman, T. & Morgan, M.T. (2004) Explaining phenotypic selection on plant attractive characters: male function, gender balance or ecological context? *Proceedings of the Royal Society B: Biological Sciences*, **271**, 553–559.
- Blanco, M.A. & Barboza, G. (2005) Pseudocopulatory pollination in *Lepanthes* (Orchidaceae: Pleurothallidinae) by fungus gnats. *Annals of Botany*, **95**, 763–772.
- Bradshaw Jr, H.D. & Schemske, D.W. (2003) Allele substitution at a flower colour locus produces a pollinator shift in monkey flowers. *Nature*, **426**, 176–178.
- Candolin, U. (2003) The use of multiple cues in mate choice. *Biological Reviews*, **78**, 575–595.
- Chittka, L. (1992) The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. *Journal of Comparative Physiology A*, **170**, 533–543.
- Ellis, A.G. & Anderson, B.C. (2012) Pollinator mediated floral divergence in the absence of pollinator shifts. *Evolution of Plant–Pollinator Relationships* (ed. S. Patiny), pp. 237–262. Cambridge University Press, Cambridge.
- Ellis, A.G. & Johnson, S.D. (2009) The evolution of floral variation without pollinator shifts in *Gorteria diffusa* (Asteraceae). *American Journal of Botany*, **96**, 793–801.
- Ellis, A.G. & Johnson, S.D. (2010) Floral mimicry enhances pollen export: the evolution of pollination by sexual deceit outside of the Orchidaceae. *The American Naturalist*, **176**, E143–E151.
- Gaskett, A.C. & Herberstein, M.E. (2010) Colour mimicry and sexual deception by tongue orchids (*Cryptostylis*). *Naturwissenschaften*, **97**, 97–102.
- Johnson, S.D. & Dafni, A. (1998) Response of bee-flies to the shape and pattern of model flowers: implications for floral evolution in a Mediterranean herb. *Functional Ecology*, **12**, 289–297.
- Johnson, S.D., Linder, H.P. & Steiner, K.E. (1998) Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). *American Journal of Botany*, **85**, 402–411.
- Johnson, S.D. & Midgley, J.J. (1997) Fly pollination of *Gorteria diffusa* (Asteraceae), and a possible mimetic function for dark spots on the capitulum. *American Journal of Botany*, **84**, 429–436.
- Kunze, J. & Gumbert, A. (2001) The combined effect of color and odor on flower choice behavior of bumble bees in flower mimicry systems. *Behavioral Ecology*, **12**, 447–456.
- Mant, J., Peakall, R. & Schiestl, F.P. (2005) Does selection on floral odor promote differentiation among populations and species of the sexually deceptive orchid genus *Ophrys*? *Evolution*, **59**, 1449–1463.
- Michener, C.D. (2000) *The Bees of the World*. Johns Hopkins University Press, Baltimore, MD.
- Muchhala, N., Brown, Z., Armbruster, W.S. & Potts, M.D. (2010) Competition drives specialization in pollination systems through costs to male fitness. *The American Naturalist*, **176**, 732–743.
- Ne'eman, G., Shavit, O., Shaltiel, L. & Shmida, A. (2006) Foraging by male and female solitary bees with implications for pollination. *Journal of Insect Behavior*, **19**, 383–401.
- Peakall, R. & Beattie, A.J. (1996) Ecological and genetic consequences of pollination by sexual deception in the orchid *Caladenia tentaculata*. *Evolution*, **50**, 2207–2220.
- Raguso, R.A. (2008) Wake up and smell the roses: the ecology and evolution of floral scent. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 549–569.
- Raguso, R.A. & Willis, M.A. (2002) Synergy between visual and olfactory cues in nectar feeding by naïve hawkmoths, *Manduca sexta*. *Animal Behaviour*, **64**, 685–695.
- Rusterholz, H. & Erhardt, A. (2000) Can nectar properties explain sex-specific flower preferences in the Adonis blue butterfly *Lysandra bellargus*? *Ecological Entomology*, **25**, 81–90.
- Schiestl, F.P., Peakall, R., Mant, J.G., Ibarra, F., Schulz, C., Franke, S. & Francke, W. (2003) The chemistry of sexual deception in an orchid-wasp pollination system. *Science*, **302**, 437–438.
- Scopece, G., Cozzolino, S., Johnson, S.D. & Schiestl, F.P. (2010) Pollination efficiency and the evolution of specialized deceptive pollination systems. *The American Naturalist*, **175**, 98–105.
- Streinzer, M., Paulus, H.F. & Spaethe, J. (2009) Floral colour signal increases short-range detectability of a sexually deceptive orchid to its bee pollinator. *Journal of Experimental Biology*, **212**, 1365–1370.
- Temeles, E.J. & Kress, W.J. (2003) Adaptation in a plant-hummingbird association. *Science*, **300**, 630–633.
- Thomas, M.M., Rudall, P.J., Ellis, A.G., Savolainen, V. & Glover, B.J. (2009) Development of a complex floral trait: the pollinator-attracting petal spots of the beetle daisy, *Gorteria diffusa* (Asteraceae). *American Journal of Botany*, **96**, 2184–2196.
- Troje, N. (1993) Spectral categories in the learning behaviour of blowflies. *Zeitschrift für Naturforschung*, **48**, 96–104.
- Whitney, H.M., Kalle, M., Andrew, P., Chittka, L., Steiner, U. & Glover, B.J. (2009) Floral iridescence, produced by diffractive optics, acts as a cue for animal pollinators. *Science*, **323**, 130–133.
- Whittall, J.B. & Hodges, S.A. (2007) Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature*, **447**, 706–709.

Received 23 February 2012; accepted 28 May 2012

Handling Editor: Alison Brody

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Spectral reflectance of *G. diffusa*'s Spring morphotype and the various models used in this study.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.