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Floral polymorphism and the fitness implications of attracting pollinating and florivorous insects

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• *Background and Aims* Floral polymorphism is frequently attributed to pollinator-mediated selection. Multiple studies, however, have revealed the importance of non-pollinating visitors in floral evolution. Using the polymorphic annual daisy *Ursinia calenduliflora*, this study investigated the importance of different insect visitors, and their effects on fitness, in the maintenance of floral polymorphism.

• *Methods* The spatial structure of a discrete floral polymorphism was characterized based on the presence/absence of anthocyanin floret spots in *U. calenduliflora*. A 3-year observational study was then conducted in polymorphic populations to investigate differences in visitation rates of dominant visitors to floral morphs. Experiments were performed to explore the floral preference of male and female *Megapalpus capensis* (the dominant insect visitor) and their effectiveness as pollinators. Next, floral damage by antagonistic florivores and the reproductive success of the two floral morphs were surveyed in multiple populations and years.

• *Key Results* Floral polymorphism in *U. calenduliflora* was structured spatially, as were insect visitation patterns. *Megapalpus capensis* males were the dominant visitors and exhibited strong preference for the spotted morph in natural and experimental observations. While this may indicate potential fitness benefits for the spotted morph, female fitness did not differ between floral morphs. However, as *M. capensis* males are very efficient at exporting *U. calenduliflora* pollen, their preference may likely increase the reproductive fitness of the spotted morph through male fitness components. The spotted morph, however, also suffered significantly greater costs due to ovule predation by florivores than the spotless morph.

• *Conclusions* The results suggest that pollinators and florivores may potentially exert antagonistic selection that could contribute to the maintenance of floral polymorphism across the range of *U. calenduliflora*. The relative strength of selection imposed by each agent is potentially determined by insect community composition and abundance at each site, highlighting the importance of community context in the evolution of floral phenotypes.

Key words: Antagonistic selection, bee flies, community context, floral polymorphism, florivory, monkey beetles, ovule predation, pollen export, pollinator-mediated selection, *Ursinia calenduliflora*, *Megapalpus capensis*.

INTRODUCTION

Floral polymorphism is a common phenomenon in angiosperm species (Galen, 1999; Ellis and Anderson, 2012). It includes variation in floral traits such as tube length (Anderson *et al.*, 2010), size (Schlumpberger *et al.*, 2009), scent (Ayasse *et al.*, 2000; Peter and Johnson, 2014) and, most commonly, colour (Schemske and Bierzychudek, 2001; Warren and McKenzie 2001; De Jager *et al.*, 2011; Sun *et al.*, 2014). Although intraspecific variation in floral colour has been associated with variation in plant size (Rausher and Fry, 1993), flower production (Levin and Brack, 1995) and survivorship (Coberly and Rausher 2008), its most researched role is in pollinator attraction (Waser and Price, 1981; Stanton, 1987; Jones and Reithel, 2001). Since pollinators may exhibit differential preference for floral colour morphs that can affect plant fitness (Johnson, 1997; Boyd, 2004), they are often considered the drivers of floral polymorphism. Such pollinator-mediated selection is viewed as ubiquitous and has given rise to the floral syndrome

concept (Fenster *et al.*, 2004), which has been used to predict pollinator type based on floral phenotype alone (Pauw, 2006; Armbruster *et al.*, 2011; but see De Merxem *et al.*, 2009).

Floral visits by non-pollinating species, however, can also have a strong influence on the evolution of floral form (reviewed in Strauss and Whittall, 2006). For example, both floral size (Galen, 1999) and colour (Irwin *et al.*, 2003) have been shown to respond to selection exerted by florivores, which may be even stronger than selection imposed by pollinators on some floral traits (Cariveau *et al.*, 2004; Parachnowitsch and Caruso 2008). Pollinator-mediated selection may thus only achieve its full potential in the absence of florivores (Herrera, 2000). What is more, when pollinators and florivores exhibit preference for the same floral traits, there is potential for antagonistic selection (Strauss and Whittall 2006). In polymorphic wild radish, pollinators and a subset of florivore species prefer floral morphs without anthocyanin pigments and both groups are likely to exert selection on this trait (Stanton, 1987; Irwin *et al.*, 2003). Variation in the presence of anthocyanin is one of the most common

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forms of floral polymorphism (Levin and Brack, 1995; Warren and McKenzie, 2001; Strauss and Whittall, 2006), suggesting that antagonistic selection via pollinators and florivores may potentially be widespread.

Species that exhibit anthocyanin-based floral polymorphism in sympatry may provide an ideal system to explore potential antagonistic selection, since spatial variation in the dominance of a given floral morph across its range will likely be controlled by the interplay between pollinator and florivore-mediated selection. Using the polymorphic annual daisy *Ursinia calenduliflora* as a model, we investigated the importance of pollinators and florivores for floral polymorphism. Specifically, we asked whether (1) there is spatial variation in the distribution of floral morphs across the landscape; (2) the morphs are interfertile and self-compatible; and (3) there are any differences in insect visitation patterns, florivory damage and reproductive success between the two morphs in polymorphic populations.

MATERIALS AND METHODS

Study system

Ursinia calenduliflora grows in the Succulent Karoo winter rainfall biome in Namaqualand, South Africa. It bears solitary inflorescences on the end of long peduncles and exhibits two floral morphs: a spotted anthocyanin-containing morph characterized by a red ring with black spots at the base of all ray petals; and a spotless plain morph without markings on its orange ray petals (Fig. 1A, B). Although different insect species visit *U. calenduliflora*, the bee fly, *Megapalpus capensis*, is a very common visitor in all populations (Fig. 1C). In this biome these flies pollinate another annual, *Gorteria diffusa*, which exhibits elaborate fly-mimicking spots on its ray florets (Ellis and Johnson, 2009). *Megapalpus capensis* flies are attracted to these dark spots (Johnson and Midgley, 1997), especially the males, which exhibit mate-searching behaviour on them (Ellis and Johnson, 2010a; De Jager and Ellis, 2012), suggesting the possibility that male *M. capensis* may also prefer the spotted morph of *U. calenduliflora*.

Spatial variation in the distribution of floral morphs

To explore variation in the distribution of different inflorescence morphs across the landscape we surveyed *U. calenduliflora* populations in Namaqualand during 2010–2012. We estimated the percentage of plain inflorescences by walking random transects through sites, scoring about 500 inflorescences per site as either spotted or plain in a 1 m section along the transect. We sampled one population per site and treated any populations containing both morphs as polymorphic. We used a generalized linear model (GLM) with an underlying negative binomial distribution and log link function to investigate the influences of latitude, longitude and altitude on the percentage of plain inflorescences at a site. Since there was no significant difference in percentages at a site over multiple years, we used the average percentage of plain inflorescences for sites sampled in more than one year.

Insect visitation patterns

Field observations. To determine whether the two morphs were attracting different insect visitors, we conducted observations in two large polymorphic populations over 3 years (Nourivier, 2010–2012, 34 h; Bovlei, 2012, 18 h). We observed patches roughly 1 m² in size for 30 min, recording the number of spotted and plain inflorescences in each patch as well as the identity of all insect visitors to each morph. From this dataset, we calculated the mean number of visits per inflorescence per morph for each observational patch by the dominant visitors (*M. capensis* flies – Diptera: Bombyliidae; monkey beetles – Coleoptera: Hopliini; blister beetles – Coleoptera: Meloidea). We separated *M. capensis* flies into male and female visitors. Males can be distinguished from females by sex-specific mate searching behaviour (De Jager and Ellis, 2012). We used a generalized linear mixed model (GLMM) with a gamma distribution and log link function to analyse these data and investigate the visitation rates at the Nourivier site, for which we had 3 years of data. We investigated the effects of morph (fixed factor), year and patch nested within year (random factors). Individual analyses were run for all dominant visitor groups and we obtained F statistics for the fixed factor (morph) and Wald Z statistics for the random factors.

To explore variation in visitation patterns over larger spatial scales, we also investigated the effects of morph and site on the visitation rates of dominant visitors during 2012 between the Bovlei (18 h of observation) and Nourivier (14 h of observation) sites. For this analysis we employed a GLM with a gamma distribution and a log link function. We obtained Wald χ^2 statistics as well as the estimated means and standard errors of the visitation rates for all dominant visiting groups to the two morphs at each site. We also analysed differences in the overall number of inflorescences that male and female individuals of *M. capensis* visited with Mann–Whitney U-tests to estimate their potential efficiency as pollinators.

Preferences of male and female M. capensis. Since *M. capensis* males are strongly attracted to dark petal spots in other daisies (Ellis and Johnson, 2010a; De Jager and Ellis, 2012) we experimentally explored the preference of male and female flies for the two floral morphs of *U. calenduliflora*. We caught flies in a large polymorphic population of *U. calenduliflora* (Bovlei) in 2009 and transported them to the Succulent Karoo Knowledge Centre in Kamieskroon for experiments. We confirmed the genders of the flies visually before releasing them into a 1 m³ mesh cage containing an array composed of ten fresh inflorescences of each of the spotted and plain morphs in an alternating pattern in four rows. We observed each fly for 10 min and recorded the number of landings it made on each floral morph. To model the influence of gender on fly preferences we employed generalized estimating equations (GEEs) using fly identity as our repeated subject variable. We coded all fly choices as binary responses and used a binomial distribution with a logit link function. We selected an exchangeable correlation construct, which assumed that choices are equally correlated within each fly. From this analysis, we obtained the estimated marginal means and 95 % Wald confidence intervals of each gender's preference.

Megapalpus capensis *males as effective pollinators of the different morphs.* To confirm that *M. capensis* were effective pollinators

Fig. 1. (A) Plain and (B) spotted floral morphs of *Ursinia calenduliflora*. (C) The common bee fly visitor *Megapalpus capensis* drinking nectar from a plain inflorescence. (D) A monkey beetle resting on the ray florets. These beetles often cause damage to ray florets, as seen in (E) and (F), where a monkey beetle is foraging on *U. calenduliflora* ray petals. Photographs: Marinus de Jager.

and to compare the effectiveness of male flies between the two floral morphs, we conducted pollen export and deposition experiments using fluorescent powder (Dayglo Color, Cleveland, OH, USA) as a pollen analogue in 2010. We applied powder to all exerted pollen presenters on two random inflorescences in an array containing 24 fresh inflorescences of either the spotted or the plain morph before releasing individual male flies ($n = 9$) into cages containing one of the arrays. We left males for 20 min before catching them and releasing them on an array of the other morph. We always used different colour powders on the two floral arrays and randomized colours and arrays before experiments. We confirmed the export of fluorescent powder

with UV light and replaced all inflorescences that received powder before starting a new experiment. We used *t*-tests for dependent samples to analyse these data as we used the same flies on both arrays.

Florivory damage

Damage to ray florets in natural populations. During transect surveys of the spatial structure of floral polymorphism in *U. calenduliflora*, we also estimated the incidence of damage to ray florets by florivores for all scored inflorescences in polymorphic populations. We coded all inflorescences as damaged or undamaged based on evidence of missing floral tissue due to the foraging activities of florivorous insects (Fig. 1E, F). We analysed these data with a GLMM using a binomial distribution with a logit link function and year and site as random factors and morph as a fixed factor.

Damage to ovules inside maturing infructescences. To explore the extent of ovule predation, a potentially severe cost of florivory, we collected approximately 15 mature infructescences from each of five populations at the end of the flowering season during 2010–2012. We identified mature infructescences by their dried ray florets and nodding habit, which occurs after flowering but before their seeds are dispersed by wind. We dissected each infructescence under a dissection microscope by making a cross-section through the involucre (and thus all of the disk florets) and inspected it for evidence of ovule predation (i.e. the presence of larvae or pupae inside dissected infructescences and the remains of ovules consumed by florivorous insects). We also measured the diameter of the infructescences to control for variation in inflorescence size. We analysed the incidence of ovule predation by florivores with a GLMM using an underlying binomial distribution and logit link function. We treated year, site and diameter as random factors and morph as a fixed factor.

Reproductive success of floral morphs

Number of fertilized ovules across sites and years. To investigate the reproductive success of the two floral morphs we counted the total number of ovules in each dissected infructescence. We then recorded the number of fertilized ovules that we identified by their larger size and considerable swelling of the ovary walls, which were dark green compared with those of unfertilized ovules. We excluded any infructescences that exhibited evidence of ovule predation from this analysis in order to avoid underestimation of reproductive fitness. We analysed these data with a GLMM with a Poisson distribution and a log link function, treating year, site and diameter as random factors and morph as a fixed factor. For all GLMMs we investigated interactions terms and excluded them from the final analysis if they were not significant.

Breeding system of U. calenduliflora. We collected seeds from natural populations in Namaqualand during 2010 and grew them in a greenhouse under ambient conditions and a set water cycle at Stellenbosch University during 2011. We sowed seeds in pots containing a mixture of sand and compost (1:1). At 84 days after planting, we measured seedling heights (from the Bovlei population) and thinned seedlings to the tallest individual per pot. For the remainder of the experiment we added additional water and nutrients (Nitrosol, Fleuron, South Africa) as required.

Focal plants (see Fig. 5 for sample sizes) received some or all of four treatments: (1) outcross pollen from another individual of the same morph; (2) outcross pollen from another individual of the other morph; (3) self pollination; (4) no pollination (inflorescences bagged before opening). We applied pollen with artist brushes that we dipped in alcohol between treatments to remove pollen grains. Kruskal–Wallis ANOVA was used to analyse differences in seed production between the various pollination treatments. Statistical analyses were carried out in the SPSS 19 package (SPSS, Chicago, IL, USA).

RESULTS

Spatial variation in the distribution of floral morphs

During 3 years of sampling we found 21 *U. calenduliflora* populations (Fig. 2). Only eight of these were polymorphic, one of which contained extremely few plain inflorescences. The rest of the populations were monomorphic for the spotted morph. We found no monomorphic plain morph populations. Results from our GLM analyses revealed that altitude and latitude had significant effects on the percentage of plain morphs at a site (Table 1).

Insect visitation patterns

Field observations. In our analyses of visitation rates at the Nourivier site nearly all dominant flower-visiting groups showed significant variation between floral patches (Table 2), indicating substantial variation in insect visitation over small spatial scales. *M. capensis* males, which were the most common visitors to both morphs (Fig. 3), were the only visitors to exhibit overall morph preference, although monkey beetles exhibited nearly significant morph preferences. As year was not a significant factor for any of the visiting groups, we excluded it from the final model. Results from our analyses of visitation rates between the Bovlei and Nourivier sites during 2012 also revealed significant spatial variation for all visiting groups (Table 3). Morph was a significant factor, with both *M. capensis* males and monkey beetles (only at the Bovlei site) preferring to visit the spotted morph (Fig. 3). Overall, *M. capensis* males also visited significantly more inflorescences during a visiting bout than females (Mann–Whitney *U*-test: $U = 2841$, d.f. $= 259$, $P < 0.0001$).

Preferences of male and female M. capensis. Male individuals of *M. capensis* exhibited a significant preference for the spotted morph of *U. calenduliflora* during controlled cage experiments, while females showed no preference (Fig. 4). This pattern mirrors that of our field observations. If *M. capensis* males act as effective pollinators, their preference for the spotted morph could potentially result in increased fitness.

Megapalpus capensis *males as effective pollinators of the different morphs.* Our experiments investigating the effectiveness of *M. capensis* males as pollinators revealed that they successfully collected and deposited pollen on both floral morphs of *U. calenduliflora*. There were no significant differences (*t*-test for dependent samples: $t = 0.42$, d.f. $= 8$, $P = 0.68$) between pollen analogues exported on floral arrays of the plain (mean inflorescences receiving pollen analogue $= 5.22$) and
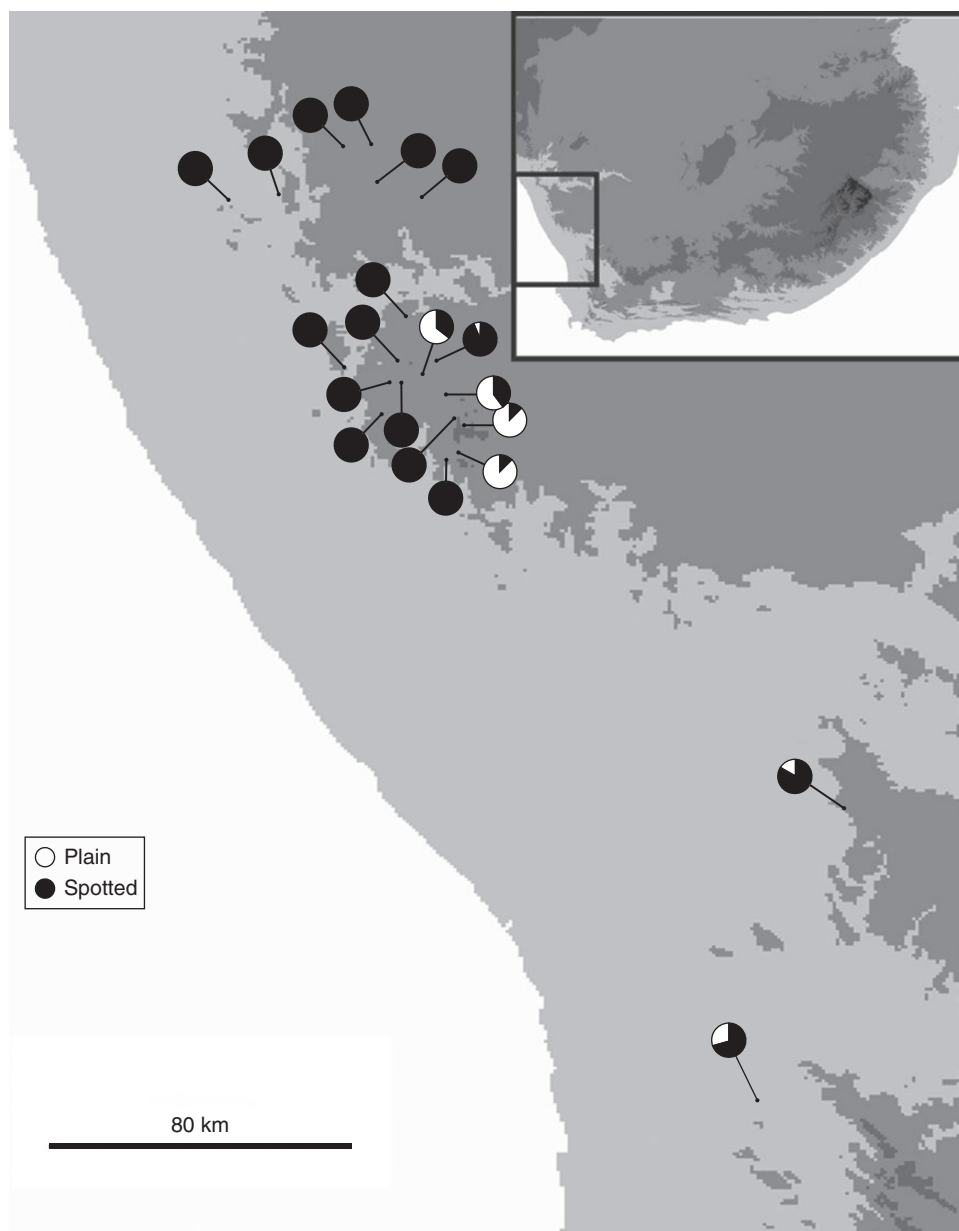
FIG. 2. Spatial pattern of floral polymorphism in *U. calenduliflora* within Namaqualand in South Africa. Pie charts show the proportion of plain and spotted inflorescences within populations (as indicated in the key). Average proportions were used for polymorphic sites sampled in more than one year.

TABLE 1. *GLM analyses investigating the effects of latitude, longitude and altitude on the percentage of plain inflorescences throughout* U. calenduliflora's *range*

Source	B	Wald χ^2	P
Latitude	−4·847	9·707	**0·002**
Longitude	−2·010	0·510	0·475
Altitude	0·011	27·781	**<0·001**

Significant *P*-values are highlighted in bold.

the spotted morph (mean inflorescences receiving pollen analogue = 4·78), indicating they can effectively pollinate both morphs.

Florivory damage

Damage to ray florets in natural populations. Results from GLMM analyses of transect data across all populations revealed that there was no variation in the incidence of damage to ray florets by florivores between morphs or years, although there was a nearly significant effect of site (Table 4). During transect surveys and field observations we often observed monkey beetles and blister beetles foraging on the ray florets of both morphs of *U. calenduliflora*. In 2012 we detected significant effects of site (GLM binomial distribution with logit link: Wald $\chi^2 = 4·969$, $P<0·05$) and morph (Wald $\chi^2 = 5·000$, $P<0·05$) on ray floret damage between the two insect observation sites (Bovlei and Nourivier). The spotted morph had

TABLE 2. *GLMM analyses investigating the effects of morph and patch on the visitation rates of the dominant insect visitors to natural patches of* U. calenduliflora *in the Nourivier population*

Source	Test	M. capensis males		M. capensis females		Monkey beetles		Blister beetles	
		Value	P	Value	P	Value	P	Value	P
Morph	F	58·907	**<0·001**	0·264	0·621	3·252	0·076	0·018	0·895
Patch	Z	3·845	**<0·001**	2·283	**0·022**	2·297	**0·003**	0·198	0·843

Significant *P*-values are highlighted in bold.

greater damage at both sites and the Bovlei site had greater damage than the Nourivier site, presumably due to the greater number of monkey beetles and blister beetles at this site (Fig. 3). There was no difference in other pollination/florivory measures (damage to ovules and number of fertilized ovules) between these two sites.

Damage to ovules inside maturing infructescences. During dissections we found multiple unidentified larvae and pupae inside the infructescences of *U. calenduliflora*. The presence of larger larvae and pupae generally resulted in more ovule damage. Typically, less than 30 % of all ovules were damaged, although this was difficult to quantify. The proportion of predated inflorescences per morph per site ranged from 0 to 40 %. Results of GLMM analyses showed that the only significant factor affecting ovule damage by florivores was morph, the spotted morph suffering much greater damage than the plain morph (Table 5; mean percentage of predated inflorescences per site was 16 % for the spotted and 4 % for the plain morph).

Reproductive success of floral morphs

Number of fertilized ovules across sites and years. We detected no significant differences in the number of fertilized ovules between morphs, sites or years (Table 5). There was, however, a significant effect of diameter, the larger infructescences containing more fertilized ovules. Diameter was strongly correlated with the total number of available ovules per infructescence (Spearman rank correlation: $r = 0.805$, $P < 0.001$; $n = 369$) and thus a good predictor of reproductive potential. Infructescence diameter did not differ between the two morphs (GLMM with normal distribution: $F = 0.153$, $P = 0.696$).

Breeding system of U. calenduliflora. This species is largely self-incompatible as most inflorescences in the self-pollination treatment produced very few seeds (spotted, mean $= 1.53 \pm 5.56$, $n = 10$; plain, mean $= 1.44 \pm 1.97$, $n = 10$) and those in the unmanipulated bagged treatment produced no seeds at all ($n = 13$). There were no significant differences between outcross treatments as analysed by Kruskal–Wallis ANOVA (Fig. 5), except for the plain × plain cross, which produced significantly fewer seeds than the spotted × spotted cross ($Z = 2.97$; $P = 0.018$). The two morphs are thus interfertile and can freely outcross in natural populations. There was no difference in seedling height between the two morphs as measured at day 84 (*t*-test for independent groups: $t = -1.62$, d.f. $= 42$, $P = 0.11$).

DISCUSSION

Our results reveal potential antagonistic selection on the same floral trait by different classes of insect visitors. Male *M. capensis* flies, the dominant visitors and effective pollinators of *U. calenduliflora*, always exhibit preference for the spotted morph. This is probably a result of preferences of male flies for dark spots in a mate-searching context, a behavioural modality that is known to be exploited for pollination by other daisies in the region (Ellis and Johnson, 2010a; De Jager and Ellis, 2012). Florivory damage due to insect larvae feeding on ovules and developing fruits was also significantly more prevalent in the spotted morph. These two opposing forces of selection may thus play an important and interactive role in the maintenance of floral polymorphism throughout the range of *U. calenduliflora*.

Selection exerted by florivores via ovule predation will directly affect seed set and reduce female reproductive success for the spotted morph. In contrast, the preference of male *M. capensis* pollinators for this morph did not result in increased female fitness. This pattern may suggest that *U. calenduliflora* populations are not pollen-limited and that pollinator-mediated selection via female fitness is not an important pathway of selection. However, male pollinator preference for the spotted morph could influence reproductive success via increased pollen export. Our experiments revealed that these males are effective exporters of *U. calenduliflora* pollen and that they visit significantly more inflorescences than female flies. Male flies in the field also visited the spotted morph nearly five times more often than the plain morph, suggesting considerably greater likelihoods of spotted morphs siring seeds within natural populations. Previous studies on the spotted daisy *Gorteria diffusa* have revealed that enhanced pollen export is the main advantage of attracting *M. capensis* males (Ellis and Johnson, 2010a).

Monkey beetles also exhibited preference for the spotted morph in the Bovlei population, but not the Nourivier population. A recent experimental study in the Namaqualand region demonstrated preference of monkey beetles of the tribe Hopliini for spotted flowers, although variation in preference between experimental sites was also evident (Van Kleunen *et al.*, 2007). Monkey beetles in the Hopliini tribe are known to comprise many different species with high turnover rates between sites in this region (Colville *et al.*, 2002). If species vary in preference, this may explain why we only found a preference of monkey beetles for spotted *U. calenduliflora* at some sites. Flowering plants can potentially benefit by attracting monkey beetles if they act as effective pollinators (Steiner, 1996; Van Kleunen *et al.*, 2007). In *U. calenduliflora*, this may be unlikely because of their destructive foraging habits on its
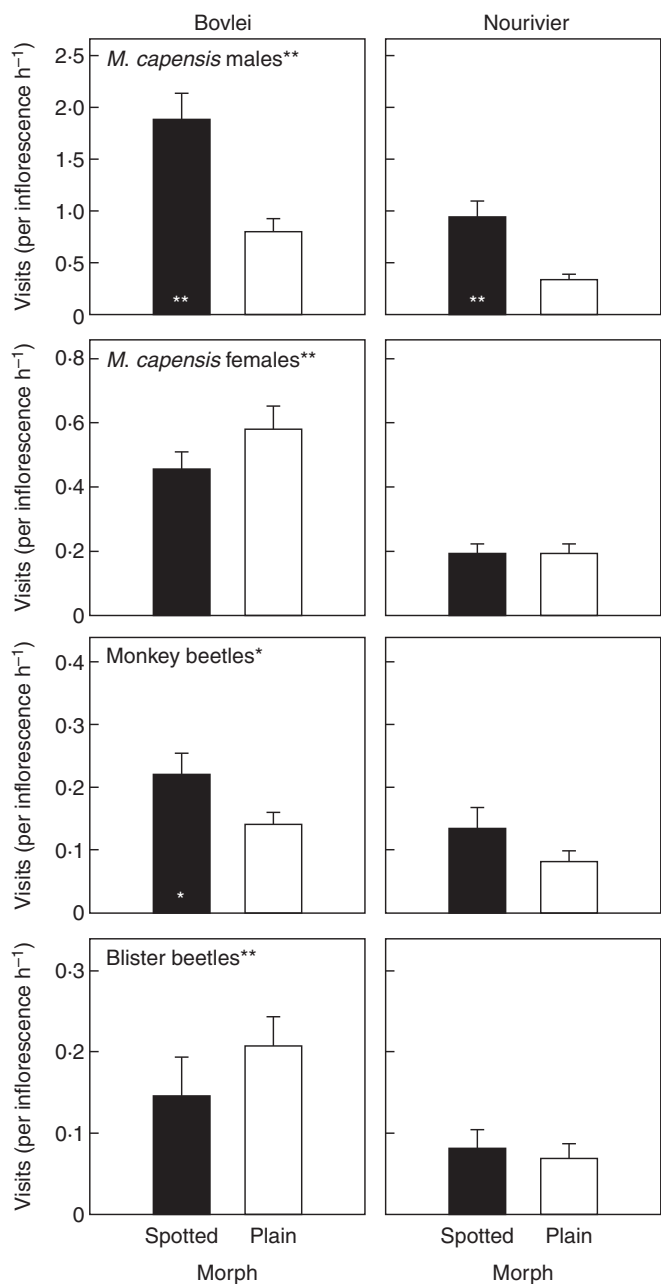
Fig. 3. Means (+ s.e.) for the number of visits per inflorescence per hour to spotted and plain floral inflorescences of *U. calenduliflora* made by (A) *M. capensis* males, (B) *M. capensis* females, (C) various species of monkey beetle and (D) various species of blister beetle at two sites in 2012. Significant differences between visits to plain and spotted floral morphs are indicated with white asterisks within the black bars, and differences between the two sites are indicated with black asterisks at the end of the heading in each graph. Number of floral patches observed at each site: Bovlei = 38; Nourivier = 28. *$P<0.05$; **$P<0.005$.

ray florets and their relative immobility compared with *M. capensis* flies (M. de Jager, pers. obs.), although we have no data on their role in the pollination of *U. calenduliflora*. Conversely, the preference of monkey beetles for the spotted morph may negatively affect plant fitness as damage to floral tissue by florivores can decrease pollinator attraction (reviewed

in McCall and Irwin, 2006). Although we observed monkey beetles actively foraging on the ray florets of *U. calenduliflora*, their larvae presumably are not responsible for the ovule damage inside infructescences as monkey beetle larvae are typically soil dwellers that feed on plant roots (Colville *et al.*, 2002).

The greater frequency of florivorous larvae and pupae observed in spotted infructescences could be a result of preference in egg-laying females or differential protection from herbivory in the two floral morphs. The latter may be more likely as the anthocyanin pigments responsible for the spotted morph's ray floret spots are the end-products of the same biochemical pathway that produces anti-herbivore compounds, such as flavones, flavonols and tannins (Fineblum and Rausher, 1997). An increase in anthocyanin production may thus be associated with a decrease in anti-herbivory compounds. If, for instance, mutations block the production of floral anthocyanin in one morph, that morph may accumulate more defensive compounds than inflorescences still producing anthocyanins, which can reduce observed florivory rates. However, this will only occur if the mutations blocking anthocyanin production do not also affect the production of intermediate, defensive compounds (i.e. only end-products are affected; Fineblum and Rausher, 1997). Since this biosynthetic pathway can link pollinator attraction to herbivory defence, it provides a potential mechanism whereby both pollinators and florivores can exert selection on the same floral trait.

An experimental study of herbivore performance on floral morphs of wild radish found that lepidopteran larvae performed better on morphs that contained anthocyanin (Irwin *et al.*, 2003), which may offer some support for this mechanism. Other herbivore species in that study, however, performed better on the morphs without anthocyanin and some herbivore species preferred the morphs without anthocyanin when given a choice (Irwin *et al.*, 2003). A study of *Claytonia virginica* also found both pollinator and herbivore preference for increased floral redness (anthocyanins), resulting in antagonistic selection on this trait (Frey, 2004). Other floral traits that have been documented to be under antagonistic selection from pollinators and herbivores/florivores include flower shape (Galen and Butchart, 2003), size (Ashman *et al.*, 2004) and number (Cunningham, 1995), as well as calyx length (Cariveau *et al.*, 2004).

Such antagonistic selection suggests that variation in the composition and abundance of pollinators versus herbivores/florivores across the range of a species may generate spatial structure in the floral trait on which they exert selection. In our study, the distribution of floral morphs in *U. calenduliflora* showed significant spatial structure. We also observed significant spatial structure in the visitation rates of dominant visitors over both small and large spatial scales. These visitors included pollinators as well as florivores. If, for instance, a site has many pollinators but few florivores, the spotted morph may be favoured. However, we did not find spatial structure in ovule predation that could produce variation in florivore-mediated selection between sites. Damage to ray florets exhibited a nearly significant site effect across populations. During our insect observations at two sites in 2012, ray floret damage also differed significantly between sites. The spotted morph suffered greater ray floret damage at both of these sites, potentially due to preference by monkey beetles. Floral tissue damage has been reported to strongly decrease pollinator attractiveness (Krupnick *et al.*,

TABLE 3. *GLM analyses investigating the effects of morph and site on visitation rates of the dominant insect visitors to two populations of* U. calenduliflora

		M. capensis males		M. capensis females		Monkey beetles		Blister beetles	
Source	Test	Value	P	Value	P	Value	P	Value	P
Morph	χ^2	36·853	**<0·001**	0·787	0·375	5·642	**0·018**	0·104	0·747
Site	χ^2	25·714	**<0·001**	45·641	**<0·001**	6·895	**0·009**	10·412	**0·001**

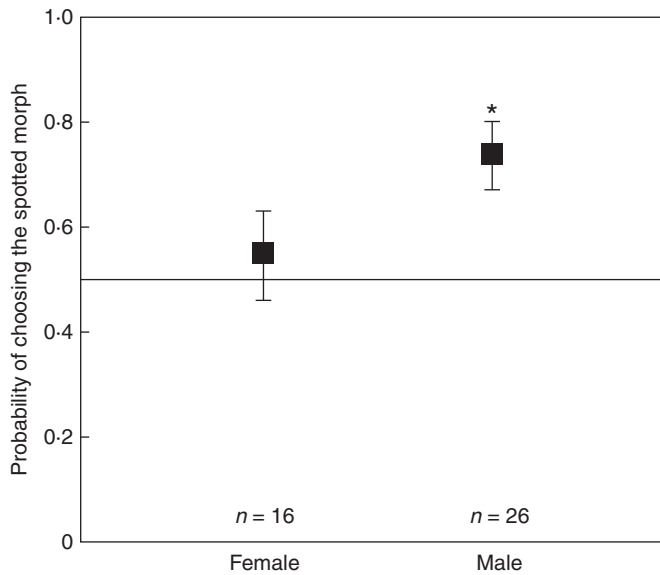Significant *P*-values are highlighted in bold.

FIG. 4. Estimated marginal means with their 95 % Wald confidence intervals for male and female *M. capensis* flies are shown for their probability of choosing to land on the spotted morph of *U. calenduliflora* during cage experiments with mixed arrays of both floral morphs. *$P < 0.05$.

TABLE 4. *GLMM analyses investigating the effects of morph, year and site on the incidence of ray floret damage due to florivores in* U. calenduliflora

Source	Test	Value	P
Morph	F	0·129	0·719
Year	Z	0·985	0·324
Site	Z	1·658	0·097

TABLE 5. *GLMM analyses investigating the effects of morph, diameter, year and site on the number of fertilized ovules, and the incidence of ovule predation in* U. calenduliflora

		Fertilized ovules		Ovule predation	
Source	Test	Value	P	Value	P
Morph	F	2·548	0·111	13·738	**<0·001**
Diameter	Z	2·694	**0·007**	0·439	0·661
Year	Z	0·978	0·328	0·722	0·470
Site	Z	1·245	0·213	0·671	0·502

Significant *P*-values are highlighted in bold.

1999), indicating that ray floret damage in *U. calenduliflora* may affect fitness.

Other studies than our own have also reported on the variable nature of insect visitation rates to flowers. In a 7-year field study, Price *et al.* (2005) documented significant variation in the communities of insects visiting *Ipomopsis aggregata* between years, as well as variation in visitation rates between sites, for both pollinators and florivores. A study of the generalist Mediterranean herb *Paeonia broteroi*, which exhibits geographical floral variation, also revealed significant differences in insect visitation patterns between sites (Sánchez-Lafuente, 2002). Thompson (2001) also reported great variation in insect visitors to flowers of *Jasminum fruticans* between sites and years in France and Spain, as well as variation in the responses of different visiting species to floral display size. These results reveal the potential for variable selection to be exerted by different floral visitors across the landscape. While this may potentially contribute to the maintenance of floral polymorphism across the range of a species, other factors that we did not investigate are likely responsible for the maintenance of floral polymorphism within each population.

Apart from biotic interactions, abiotic factors such as the presence of drought conditions may also be important in the maintenance of floral polymorphism across a species' range. Floral morphs containing anthocyanin often exhibit higher reproductive fitness during drought than floral morphs without anthocyanin (Schemske and Bierzychudek, 2001; Warren and McKenzie, 2001). This is probably due to the role that anthocyanin plays in maintaining metabolic activity under stressed conditions (Tholakalabavi *et al.*, 1997). In contrast, morphs that do not contain anthocyanin often produce more seeds than anthocyanin-containing morphs under well-watered conditions (Warren and McKenzie, 2001). Although we detected an altitude effect on the percentage of plain inflorescences across the landscape, which may potentially indicate increased water availability at high-altitude sites, we also found plain inflorescences at low altitudes, as well as monospecific spotted populations at high altitudes. Our greenhouse experiments also offer no support for a plain morph advantage under well-watered conditions as crosses between plain morphs produced significantly fewer seeds than crosses between spotted morphs and there were no
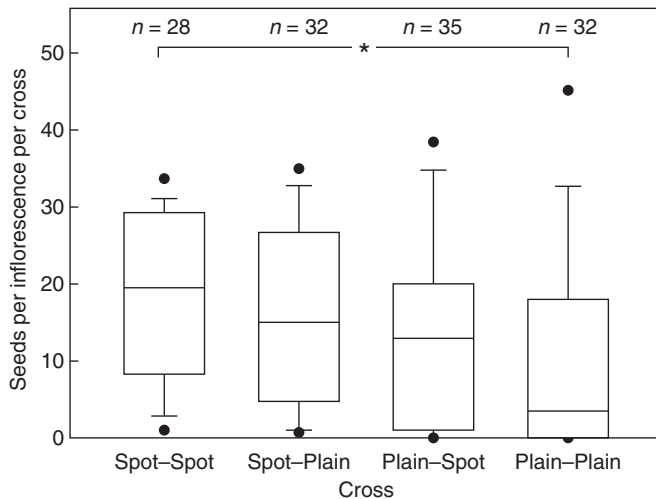
F I G . 5 . Median and 95th and 5th percentiles of the number seeds produced per inflorescence per cross between the morphs of *U. calenduliflora*. The number of plants receiving each pollination treatment is indicated on the *x*-axis. The only significant difference was between the plain × plain and the spotted × spotted cross. *$*P<0.05$.

differences between the seedling heights of plain and spotted morphs under well-watered conditions.

We thus suggest that geographical variation in the presence and abundance of insect visitors across the landscape may be more important than abiotic factors for the maintenance of floral polymorphism. Such spatial variation in selective regimes may offer some insight as to why many studies fail to detect pollinator-mediated selection (reviewed in Harder and Johnson, 2009). Many studies also report substantial spatial variation in the strength of selection exerted by pollinators between sites (Ellis and Johnson, 2010*b*; Lay *et al.*, 2011). This may be a result of geographic variation of antagonistic interactions, as pollinator-mediated selection is often only significant in the absence of herbivores/florivores (Herrera, 2000; Herrera *et al.*, 2002). These patterns highlight the many players in the selective arena that affects floral phenotype and illustrates the value of a more inclusive approach to the study of floral evolution. Future studies may help elucidate the possible importance of antagonistic selection by exploring its strength and structure across the landscape, especially within widespread plant species with a high diversity of floral visitors.

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