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MIMICS AND MAGNETS: THE IMPORTANCE OF COLOR AND ECOLOGICAL FACILITATION IN FLORAL DECEPTION

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Abstract. Plants that lack floral rewards can attract pollinators if they share attractive floral signals with rewarding plants. These deceptive plants should benefit from flowering in close proximity to such rewarding plants, because pollinators are locally conditioned on floral signals of the rewarding plants (mimic effect) and because pollinators are more abundant close to rewarding plants (magnet effect). We tested these ideas using the non-rewarding South African plant *Eulophia zeyheriana* (Orchidaceae) as a study system. Field observations revealed that *E. zeyheriana* is pollinated solely by solitary bees belonging to a single species of *Lipotriches* (Halictidae) that appears to be closely associated with the flowers of *Wahlenbergia cuspidata* (Campanulaceae), a rewarding plant with which the orchid is often sympatric. The pale blue color of the flowers of *E. zeyheriana* differs strongly from flowers of its congeners, but is very similar to that of flowers of *W. cuspidata*. Analysis of spectral reflectance patterns using a bee vision model showed that bees are unlikely to be able to distinguish the two species in terms of flower color. A UV-absorbing sunscreen was applied to the flowers of the orchid in order to alter their color, and this resulted in a significant decline in pollinator visits, thus indicating the importance of flower color for attraction of *Lipotriches* bees. Pollination success in the orchid was strongly affected by proximity to patches of *W. cuspidata*. This was evident from one of two surveys of natural populations of the orchid, as well as experiments in which we translocated inflorescences of the orchid either into patches of *W. cuspidata* or 40 m outside such patches. Flower color and location of *E. zeyheriana* plants relative to rewarding magnet patches are therefore key components of the exploitation by this orchid of the relationship between *W. cuspidata* and *Lipotriches* bee pollinators.

Key words: Batesian mimicry; bee vision; Eulophia zeyheriana; *facilitation;* Lipotriches; *magnet species; Orchidaceae; pollination success; UV;* Wahlenbergia cuspidata.

Introduction

Deceptive pollination systems are known from at least 32 angiosperm families (Renner 2006). However, the vast majority of deceptive species belong to the Orchidaceae, a very large family in which ~30% of species lack floral rewards (van der Pijl and Dodson 1966). The most common deceptive systems in orchids involve the exploitation of food-seeking animals, and these have been considered to operate either through mimicry of specific rewarding flowers (Batesian floral mimicry) or nonspecific resemblance to rewarding flowers (generalized food deception; Jersáková et al. 2006).

Batesian floral mimics tend to exploit relatively specialized mutualisms between rewarding plants and their pollinators (Bronstein 2001, Anderson et al. 2005). This can require rather precise mimicry of the size (Galizia et al. 2005), shape (Johnson et al. 2003*a*), and color (e.g., Nilsson 1983, Johnson 1994, 2000, Anderson et al. 2005) of the floral display of rewarding plants. Roy and Raguso (1997) suggest that in a floral mimicry system, visual signals such as inflorescence color, size, and shape may be more important than scent, at least for bee pollinators. Recently, Galizia et al. (2005) examined the importance of scent and color in the Batesian mimicry system involving *Bellevalia flexuosa* (model) and *Orchis israelitica* (mimic). They found no evidence for scent mimicry in this system and point to visual similarity as being the key to the successful deception by *O. israelitica*.

Close matching of reflectance spectra between the flowers of Batesian mimics and their models has been demonstrated in several studies (Nilsson 1983, Johnson 1994, 2000, Johnson et al. 2003*a*, Anderson et al. 2005). It is often claimed that this similarity is adaptive, yet most studies fail to show that a mimic is more similar in color to a model than are conspecifics of the mimic. In one study, indirect evidence for adaptation was obtained from evidence that intraspecific variation in flower color of a Batesian mimic was correlated with the color of model flowers (Johnson 1994). Johnson et al. (2003*a*) furthermore argued that the cream color of a putative orchid mimic is likely to be adaptive because this trait is evolutionarily derived in its phylogenetic context.

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Galizia et al. (2005) showed that the flowers of a putative Batesian mimic are more similar in color to those of the model than are flowers of this orchid's nearest relatives.

Mimics may require not only overall similarity in flower color to their models, but also specific color patterning. Mimics that exploit mutualisms in which bees collect both nectar and pollen from flowers may need to imitate pollen rewards of their models. Nilsson (1983), for example, suggested that cream patches on the flowers of *Cephalanthera rubra* imitate pollen on the anthers of *Campanula* flowers. Heuschen et al. (2005) show that yellow UV-absorbing patches on flowers of many plants are attractive to pollen-collecting bees. They suggest that this is because these patches have a generalized resemblance to pollen.

There is increasing recognition that facilitation is an important ecological process in plant pollination systems (Feinsinger 1987, Callaway 1995, Feldman et al. 2004). The two main mechanisms of facilitation suggested by Moeller (2004), "joint attraction of pollinators" and "joint maintenance of pollinators," are bidirectional in the sense that facilitation benefits all of the interacting plants and apply mainly to situations in which all interacting species produce floral rewards. Unidirectional facilitation in pollination systems, on the other hand, is exemplified by the "magnet species effect" and "Batesian floral mimicry." In the magnet species effect (Thomson 1978), a rewarding "magnet" plant species increases local pollinator abundance in an area to the benefit of other plant species that have inferior rewards or lack rewards altogether (cf. Laverty 1992, Johnson et al. 2003*b*). In Batesian mimicry systems, deceptive mimics benefit from rewarding model species because these models condition pollinators to visit flowers of the mimic (cf. Nilsson 1983, Johnson 1994, 2000, Anderson et al. 2005). Some pollination systems apparently contain elements of both the magnet effect and Batesian floral mimicry. For example, pollination of the deceptive orchid *Anacamptis* (*Orchis*) *morio* is enhanced both by proximity to rewarding plants and the similarity of flower color to that of potential magnet plants (Johnson et al. 2003*b*). Gumbert and Kunze (2001) similarly showed that pollinators were more likely to approach flowers of the deceptive orchid *Anacamptis* (*Orchis*) *boryi* when foraging on rewarding flowers with similar colors to those of the orchid, suggesting a case of generalized Batesian floral mimicry. This is consistent with evidence that pollinators are more likely to switch foraging between similarly colored species (Chittka et al. 1997, Chittka and Raine 2006).

Johnson et al. (2003*b*) proposed that there may be a subtle continuum between generalized food deception and floral Batesian mimicry. This continuum may provide an evolutionary pathway, whereby natural selection might favor floral phenotypes of the deceptive species that resemble a co-occurring rewarding species, particularly if that rewarding species also facilitates pollination through a magnet effect. This could lead to a fine-tuned mimicry of the rewarding species in terms of morphology and color and perhaps also scent. However, there are still few studies of ecological facilitation in the pollination of deceptive plants (Dafni and Ivri 1979, Johnson 1994, Alexandersson and Ågren 1996, Gumbert and Kunze 2001, Johnson et al. 2003*b*), and we are aware of only one previous study in which the floral phenotype of a putative floral mimic has been manipulated in order to test its importance for pollination (Johnson et al. 2003*a*).

While searching for pollinators of the non-rewarding terrestrial plant *Eulophia zeyheriana* (Orchidaceae), we noticed that many of the bees sleeping in the pale blue flowers of a sympatric rewarding species, *Wahlenbergia cuspidata* (Campanulaceae), carried pollinaria of the orchid. The similarity in color of *E. zeyheriana* and *W. cuspidata* flowers and the fact that bees apparently visit both species led us to hypothesize that *E. zeyheriana* is to some degree a Batesian mimic of the flowers of *W. cuspidata*.

To test the idea that *E. zeyheriana* is a specialized exploiter of the relationship between *W. cuspidata* and its bee pollinators, we first asked whether the pollination success of *E. zeyheriana* was positively influenced by the local presence of *W. cuspidata* plants. We then investigated whether color of *E. zeyheriana* is more similar to that of its putative model in terms of the bee visual system than is the case for congeners of the orchid and whether manipulation of the flower color would influence visitation by bees. We also examined whether *E. zeyheriana* and *W. cuspidata* have similar distribution ranges, habitats, and phenology.

Material and Methods

The study species

Eulophia zeyheriana (Orchidaceae; Fig. 1A–C), the putative mimic, is restricted to the Drakensberg Mountains of the summer rainfall regions of South Africa (Appendix A: Fig. A1). The small, pale-blue flowers are borne on sparse, slender inflorescences reaching 20 cm in height. The pollinaria of this genus consists of two hard, solid pollinia and a stipe that undergoes rapid reconfiguration following removal of the pollinaria by pollinators. This correctly orients the pollinia to be deposited on the stigmas of flowers on subsequent visits and likely protects against geitonogamous pollen transfer (Peter and Johnson 2006*b*). Like all *Eulophia* species, this species is deceptive, and no nectar was discovered in any of the many flowers inspected.

Wahlenbergia cuspidata (Campanulaceae), the putative model, grows in clumps (Fig. 1D) at higher altitudes in the eastern parts of South Africa (Appendix A: Fig. A1). The pale-blue flowers are strongly protandrous. The anthers deposit pollen along the style that functions as a pollen presenter (Fig. 1E). After a period of about two days and following the removal of the majority of pollen

FIG. 1. (A) *Eulophia zeyheriana* (Orchidaceae) forms scattered groups in short grassland. (B) The small, pale blue flowers are arranged in (C) lax inflorescences. (D) *Wahlenbergia cuspidata* (Campanulaceae) grows in scattered clumps. (E) Flowers are at first male, with pollen exposed on the outside of the style that forms a pollen presenter. (F) After a period of 2–3 days the pollen has been removed and the tips of the pollen presenter flare open to expose the lobes of the stigma. (G) It appears that *Lipotriches* bees frequently visit the deceptive *Eulophia zeyheriana* flowers and most male *Lipotriches* bees bear pollinaria between their antennae. (H) The bees alight on the flowers and position themselves on the pollen presenter/style to probe the nectaries at the base of the petals. Scale bars: (A) 10 mm; (B, C, E–H) 5 mm; (D) 40 mm.

by pollinators, the stigma unfolds, becomes receptive, and the flower enters a female phase (Fig. 1F). Preliminary observations of single male- and female-phase flowers showed that small amounts of nectar are produced in concealed nectaries at the base of the pollen presenter/gynoecium (nectar volume [male phase, 0.46 μL; female phase, 1.76 μL], concentration [male phase, 25.70%; female phase, 27.33%], sucrose : hexose : fructose ratio [male phase, 44:56:0; female phase, 50:50:0]). These measurements were made from unbagged flowers at 09:00 and represent the standing crop of nectar available to bees. The flowers of this species rapidly close and nod over on the approach of inclement weather or in the evening, apparently in response to changing light conditions. This mechanism may serve to protect the pollen from water damage.

Study sites

Work was conducted primarily on the summit of Mount Gilboa at the extreme southwest of the Karkloof mountain range in central KwaZulu-Natal in eastern South Africa (Appendix A: Fig. A1). The Karkloof range is separated from the escarpment of Drakensberg Mountains by ~80 km. The area is burnt during the austral winter (primarily June–August) in most years. The vegetation of the summit of Mount Gilboa is an exceptionally diverse grassland community (~400 plant species). Following winter fires, the vegetation during the rainy summer months is characterized by short grass and numerous plants blooming en masse. At this site, *E. zeyheriana* plants are widely scattered over the summit and intermingled with more discrete clumps of *W. cuspidata* plants. The second study site was at Cobham, along the banks of the Pholela river in the foothills of the Drakensberg (Appendix A: Fig. A1). Here, the vegetation was dominated by relatively mature grasses and scattered plants of *E. zeyheriana*, while *W. cuspidata* plants were mainly found growing along footpaths. Few other species were in flower. At a large spatial scale at both sites, the orchids outnumbered *W. cuspidata* clumps by ~2:1, although at smaller spatial scales in the vicinity of *W. cuspidata* clumps, individual flowers of the orchid were usually outnumbered by those of *W. cuspidata*. Fieldwork was conducted between December 2001 and January 2002 as well as January and February of 2004.

Distribution and flowering phenology

Distribution and flowering phenology data were collected over the course of this study and supplemented with data from specimens housed in the Natal Herbarium (NH), University of KwaZulu-Natal Herbarium (NU), Schonland Herbarium, Grahamstown (GRA), and the Pretoria Herbarium (PRE). Flowering dates of herbarium specimens and our observations were renumbered, with 1 July being the first day of the season and 30 June the last day to span the austral summer. The distributions of renumbered flowering dates for *E. zeyheriana* and *W. cuspidata* were compared using the Kolmogorov-Smirnov test.

Pollinators

Bees were collected from flowers of *W. cuspidata* at Mount Gilboa and Cobham. Bees were either found sleeping in closed flowers in the late afternoon or during cloudy weather or foraging during sunny weather. Collected bees were identified by a specialist entomologist (C. Eardley, Department of Agriculture, South Africa), and voucher specimens were deposited in the Albany Museum, Grahamstown. Granular pollen loads on the bees were examined using Beattie's (1971) technique. Pollinaria of *E. zeyheriana* are easily distinguished by their size from those of co-occuring congeners.

We conducted 30 h of observations at Cobham, 35 h at the Mount Gilboa site, and 14 h at other sites in the Drakensberg Mountains. These observations span from mid-morning to late afternoon. Observation focused on patches of *W. cuspidata* and involve catching bees or observing their activity.

Survey of pollination success and translocation experiment

To determine the pollination success of *E. zeyheriana* and its relationship to the proximity of *W. cuspidata* plants, we recorded the proportion of flowers on each inflorescence that had pollinaria removed and those with pollinia deposited on their stigmas, as well as the distance to the nearest clump of *W. cuspidata*. We also determined the overall proportion of flowers on an inflorescence showing evidence of visitation (pollinarium removal and/or pollinia deposition). At Gilboa this included scoring flowers with only their anther caps removed, representing a failed visit. The first survey was conducted at Cobham during February 2002, and we scored the visitation rates and distance to the nearest *W. cuspidata* plant for all orchids encountered. A second survey was conducted on Mount Gilboa in February 2004. Due to the larger number of *W. cuspidata* and *E. zeyheriana* plants on Mount Gilboa, six transects were laid out, each at a random bearing from a different focal clump of *W. cuspidata*. Orchids within 1 m of the transect line were scored for visitation and distance from the *W. cuspidata* clump. Proportional visitation data were arcsine square-root transformed and related to the natural log of the distance to *W. cuspidata* patches using simple linear regression.

To complement these analyses based on natural distributions, we performed a translocation experiment at Mount Gilboa. *Eulophia zeyheriana* inflorescences were collected and visited flowers were removed before being placed in water containing glass pill vials taped to stakes and positioned in pairs either within patches of *W. cuspidata* or 40 m outside such patches. These were left for 9 d before being reexamined for signs of visitation. Proportion data were arcsine square-root transformed and compared using a two-tailed paired *t* test.

Reflectance spectra

The reflectance spectra of various floral parts of the two species were measured using an Ocean Optics S2000 spectrophotometer (Ocean Optics, Dunedin, Florida, USA), coupled to an Ocean Optics Mini–D2T light source as described in Johnson et al. (2003*a*). We measured spectra of adaxial petal surfaces for both *E. zeyheriana* and *W. cuspidata* flowers, as well as the prominent white papillose area of the labellum of *E. zeyheriana* and the pollen-covered pollen presenter of male-phase *W. cuspidata* flowers. We also measured the reflectance spectra of 19 other species of *Eulophia* including all the likely sister taxa of *E. zeyheriana* (Hall 1965). Where more than one individual of each of these other species were measured, the average locus for the species was calculated in the color space.

Measured spectra were analyzed using the Chittka model to derive color loci in the bee color space (Chittka 1992, Chittka and Kevan 2005). This model uses the spectral sensitivity of honey bee receptors to calculate color loci. The sensitivities of Hymenoptera are phylogenetically conservative (Briscoe and Chittka 2001), and it is likely that the *Lipotriches* (Halictidae) bees involved in this pollination system have similar receptor sensitivities to honey bees. Perceptual color distances between average color loci were calculated using the equations of Chittka (1992). It seems likely that bumble bees (Dyer and Chittka 2004*a*, *b*) and honey bees (Giurfa 2004) can distinguish color loci with distances down to ~0.06 units apart, depending upon the color involved.

To determine whether flowers of the two species possess patterning in the UV region of the spectrum, flowers were photographed with a B + W 403 black filter (Jos. Schneider Optische Werke, Bad Kreuznach, Germany) that removes all wavelengths of light above 400 nm. Konica 400 ISO black and white film (Konica-Minolta, Tokyo, Japan) was used as it is sensitive to near-UV (~350–400 nm), but required exposures of ~90 s. The gray scale of Kevan et al. (1973) was used to judge the exposures.

Color modifications

To investigate the importance of color for pollinator attraction in this mimicry system, the reflectance of the adaxial and abaxial surfaces of the lateral petals of *E. zeyheriana* flowers were changed by painting them with a UV-absorbent mixture. This consisted of Parsol 1789 and Parsol MCX (Roche, Basel, Switzerland) dissolved in duck preen gland fat developed for modifying the reflective properties of birds feathers (Andersson and Amundsen 1997) and used by Johnson and Andersson (2002) to modify the color of *Hypoxis* flowers. Control flowers included flowers painted only with the preen fat and those with no treatment. Control flowers had a spot of the UV absorbing mixture applied behind the flower bract as a precaution to control for any potential odor of the active compounds, even though the active compounds are not volatile (Andersson and Amundsen 1997). These treatments were applied to plants growing in small groups at the Mount Gilboa site. After four days the flowers were scored for visitation. Transformed data were analyzed using an ANOVA. The effects of these manipulations on flower color were assessed using UV photography and analysis of reflectance spectra using the model of Chittka (1992).

Breeding systems

Inflorescences were bagged and virgin flowers were either self-pollinated, outcrossed with pollinia from other individuals growing more than 10 m away, or left untreated to test for autogamy. In most cases, one of each of these three treatments was applied to each inflorescence.

RESULTS

Distributions and phenology

The distributions of *E. zeyheriana* and *W. cuspidata* overlap broadly and both are confined to grasslands at higher altitudes in the eastern parts of South Africa (Appendix A: Fig. A1). At six separate sites we have visited, the two species co-occur. Therefore, the apparent absence of *Wahlenbergia* from some sites where the orchid has been collected (Appendix A: Fig. A1) likely reflects under-collecting of herbarium specimens rather than true incongruence. Flowering of *E. zeyheriana* overlaps broadly with *W. cuspidata* (Appendix B: Fig. B1). While flowering of *E. zeyheriana* peaks ~10 days earlier than *W. cuspidata*, at both sites there is no significant difference in the distribution of flowering times of the two species (Kolmogorov-Smirnov test, $P > 0.10$ for Gilboa, $P > 0.05$ for Cobham).

Pollinators

In total, we observed more than 70 individual bees bearing pollinaria of *E. zeyheriana* and caught 46 of these bees. All belonged to a single *Lipotriches* species (Halictidae), the identity of which remains unresolved due to the uncertain taxonomy of this genus (C. Eardley, *personal communication*). During the 2001–2002 flowering season, we caught 11 *Lipotriches* bees bearing *E. zeyheriana* pollinaria or viscidia between their antennae (Fig. 1G) on Mount Gilboa and nine at Cobham. All bees were either roosting in *W. cuspidata* flowers or foraging from the concealed nectaries at the base of the flowers (Fig. 1H). This bee was the only insect species observed to visit the flowers of *W. cuspidata*. In January and February 2004, ~50 *Lipotriches* bees bearing pollinaria were observed in *W. cuspidata* flowers on Mount Gilboa and 26 of these bees were captured. At the Cobham site, 79% of the bees collected bore at least one pollinarium or viscidium, while 59% of bees collected or observed at Gilboa carried pollinaria or viscidia. A number of bees carried more than one

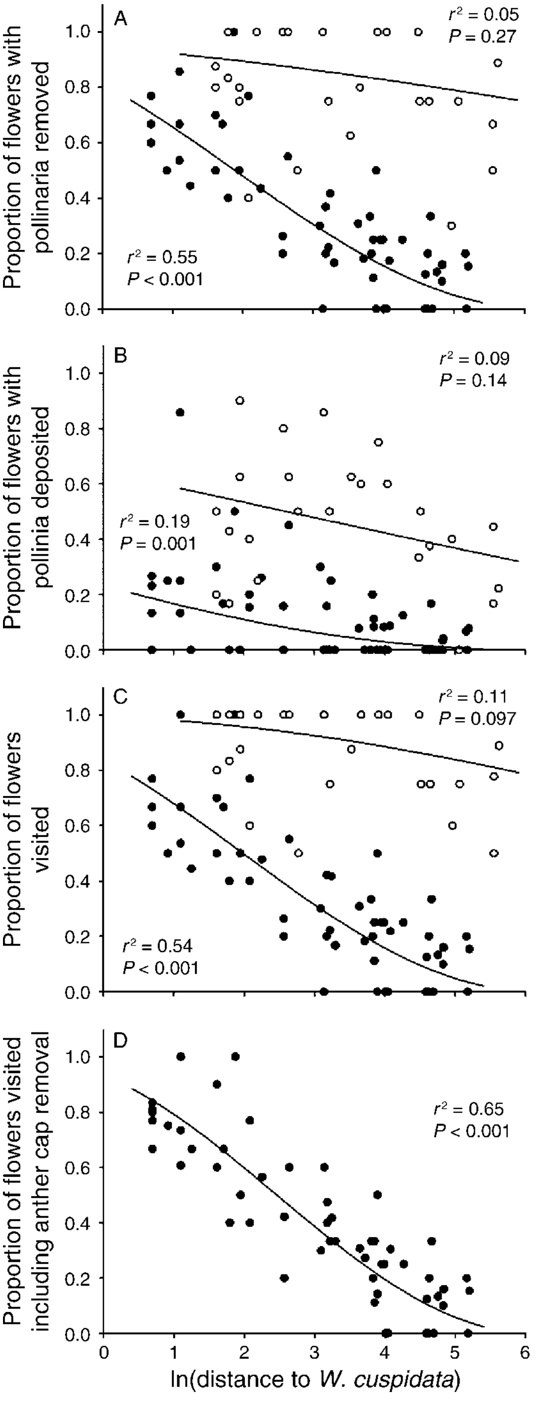

FIG. 2. Pollination success of *Eulophia zeyheriana* at increasing distances from patches of *Wahlenbergia cuspidata* plants at Cobham (open circles) and Mount Gilboa (solid circles). This includes the proportion of flowers on an inflorescence (A) with pollinaria removed, (B) with pollinia deposited, (C) with signs of either pollen removal or deposition, and (D) showing any sign of visitation, including those flowers with their anther caps dislodged. Proportion data were arcsine square-root transformed, and distance data were natural log-transformed for regression analysis. Distances were measured in meters. The back-transformed proportion data and regression lines are presented.

pollinarium, one individual bearing four viscidia as well as a complete pollinarium. Of the 218 *E. zeyheriana* flowers found to have pollinia deposited on their stigmas, 58% contained a single pollinium, 40% contained two pollinia, three stigmas had three pollinia deposited, and a single flower had four pollinia deposited on its stigma. We observed two direct visits to flowers of *E. zeyheriana* in 2004. Each visit lasted 20–25 s and entailed the bee alighting on the flower, positioning itself on the labellum, and probing around base of the labellum and column, apparently inspecting the spur for nectar. In one case, a bee deposited pollinia it was carrying and removed a second pollinarium.

All of the 46 *Lipotriches* bees collected over two flowering seasons at both sites were male. This includes bees sleeping in *W. cuspidata* flowers, bees foraging on the *W. cuspidata* flowers, and the two bees collected following their visits to *E. zeyheriana* flowers.

Although granular pollen of other taxa was represented on all bees, only *W. cuspidata* pollen was found on every bee inspected (Appendix C: Table C1). Bees from Mount Gilboa carried significantly more pollen grains of *W. cuspidata* than bees from Cobham ($t_{29} = 2.3$, $P = 0.0160$). They also had significantly more pollen grains from other taxa ($t_{29} = 3.8$, $P = 0.0003$), and significantly more taxa are represented in these pollen loads at Mount Gilboa ($t_{29} = 9.5$, $P < 0.0001$; Appendix C: Table C1). Other taxa well represented in the pollen loads of the bees inspected include species of Asteraceae and Fabaceae (Appendix C: Table C1).

Population survey and translocation experiments

Pollination success and overall rates of visitation in *E. zeyheriana* declined with increasing distance from focal *Wahlenbergia cuspidata* plants at both sites (Fig. 2), but these univariate relationships were significant only for the Mount Gilboa site (Fig. 2). Overall visitation success of *E. zeyheriana* was significantly greater at the Cobham site (ANCOVA, $F_{1,78} = 4.99$, $P = 0.028$; Fig. 2C).

A similar trend of decreased pollination success with increased distance from *W. cuspidata* plants was revealed by the translocation experiment. Measures of pollinaria removal and overall visitation were both significantly lower in inflorescences that were translocated to a position 40 m from the nearest *W. cuspidata* plant compared to those translocated within a clump of *W. cuspidata* (Fig. 3). Pollinia deposition was also lower in plants translocated outside of *W. cuspidata* clumps, but not significantly so.

Reflectance spectra

Flowers of both *E. zeyheriana* and *W. cuspidata* appear pale blue to humans with pale blue to white pollen presenters or labellae (Figs. 1 and 4A, C). However, in the near-UV region, the "white" papilose area of the rolled labellum of *E. zeyheriana* and the pollen presenter of *W. cuspidata* appear strongly UV absorbent (Fig. 4B, D).

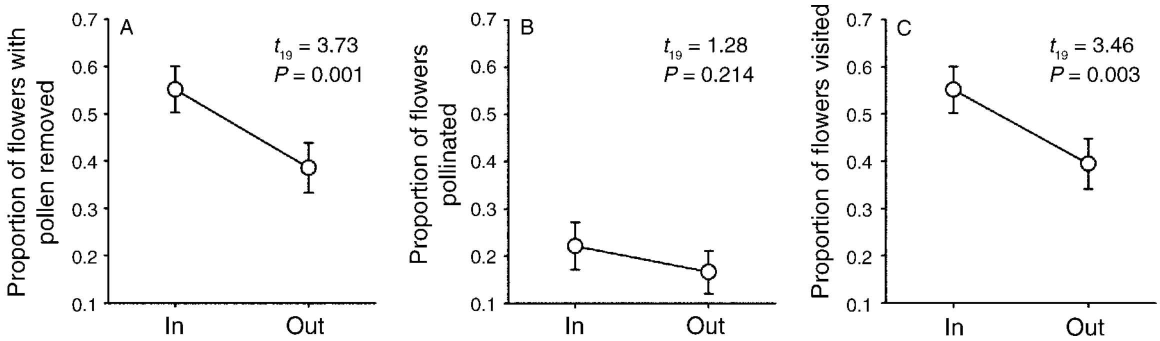

FIG. 3. Pollination success (mean ± SE) of *Eulophia zeyheriana* inflorescences translocated into patches of *Wahlenbergia cuspidata* plants (in) and inflorescences translocated 40 m from patches of *W. cuspidata* (out). This includes the proportion of flowers on an inflorescence (A) with pollinaria removed, (B) with pollinia deposited, and (C) with signs of either pollen removal or deposition. Proportion data were arcsine square-root transformed. Two-tailed P values were determined using a paired t test ($n = 20$ pairs of inflorescences).

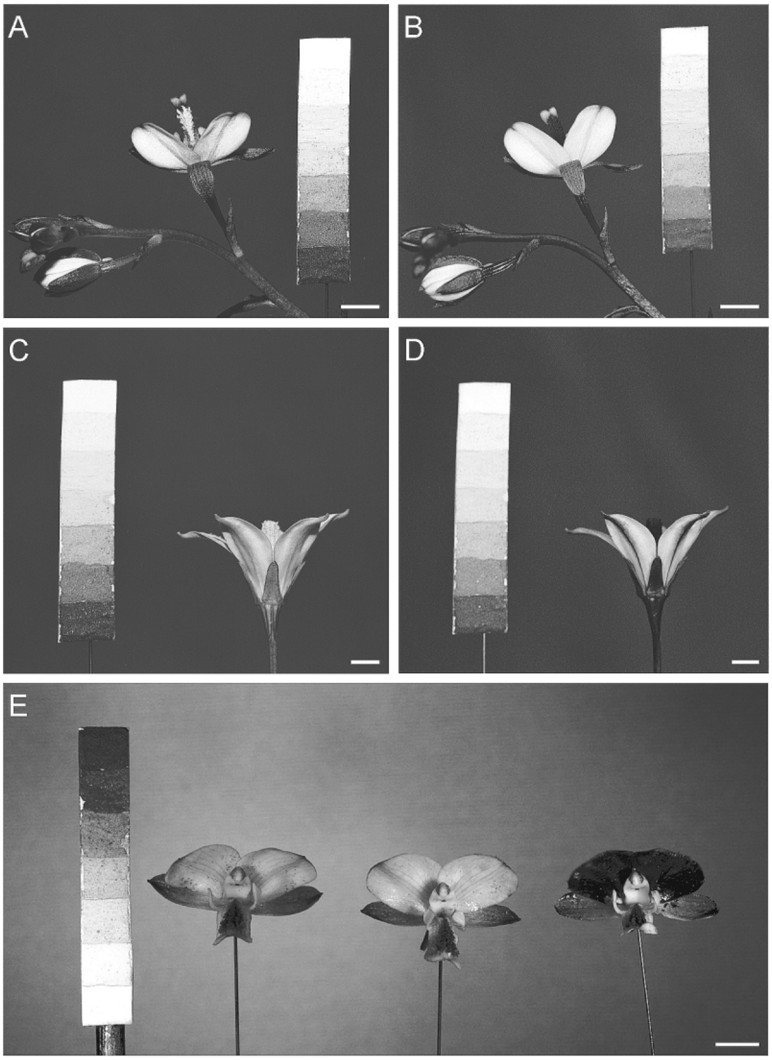

FIG. 4. Appearance of (A) *Eulophia zeyheriana* and (C) *Wahlenbergia cuspidata* flowers in the human visual spectrum. Appearance of (B) *E. zeyheriana* and (D) *W. cuspidata* flowers in the near-UV region. (E) Appearance of *E. zeyheriana* flowers with a UV-absorbent mixture (right) and with a control mixture (middle) in the near-UV region; the leftmost flower is an untreated control. Scale bars in each panel indicate 5 mm.

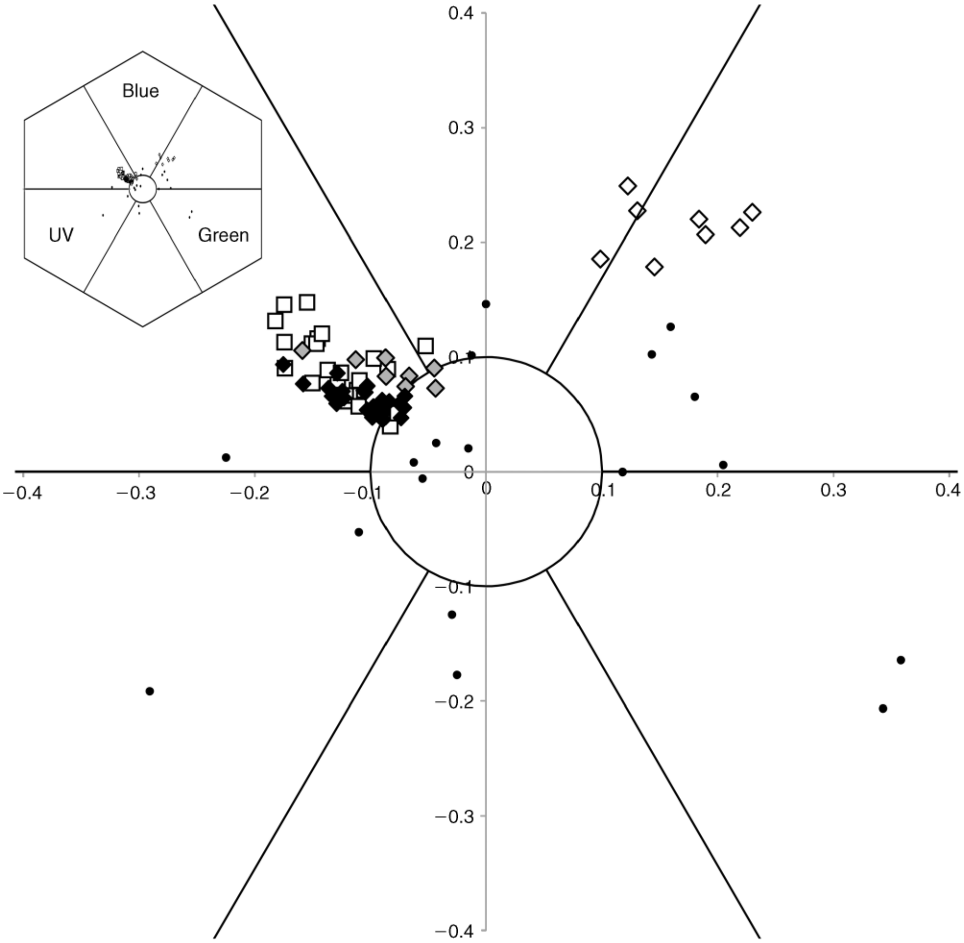

FIG. 5. Color loci of adaxial petal surfaces of individuals of *Eulophia zeyheriana* (solid diamonds) and *Wahlenbergia cuspidata* (open squares), as well as mean color loci for 19 other species of *Eulophia* (solid circles), calculated according to the Hexagon color model of bee vision (Chittka 1992). The color loci of *E. zeyheriana* petals modified by the addition of a UV-absorbent mixture (open diamonds) as well as *E. zeyheriana* petals coated with the control mixture (gray diamonds) are also shown. The inset shows segments and is labeled blue, green, and UV. Segments between these are combinations, e.g., blue-green, green-UV, UV-blue. The central circle indicates colors that are close to the green background and hence difficult for bees to perceive. The axes correspond to the excitation values of the two types of color opponent neurons (Chittka and Raine 2006).

Spectrophotometry confirmed these qualitative observations. The average reflective spectra of the petals of *E. zeyheriana* and *W. cuspidata* are similar and include reflectance in the near-UV region between 350 and 400 nm (Appendix D: Fig. D1A). The shape of the average reflectance curves is likewise similar when comparing the labellum of *E. zeyheriana* and the pollen presenter of *W. cuspidata*, although the overall brightness of the labellum of *E. zeyheriana* is higher and there is no UV reflectance from these parts of the flowers (Appendix D: Fig. D1B).

Analysis of individual spectra using the bee vision model of Chittka (1992) showed that adaxial petal colors fall in the blue-UV segment of the color hexagon and there is broad overlap of measurements made for *E. zeyheriana* and *W. cuspidata* (Fig. 5). The perceptual color distance between average loci of the two species is only 0.03 color opponent units. These colors are quite distinct from the average colors of a number of other southern African species of *Eulophia* including likely sister taxa (Fig. 5). The average distance of the color loci of the petals of different *Eulophia* species is 0.24 units, with a range of 0.09–0.56 units.

The color of *E. zeyheriana* labellae is similar to the pollen presenter of *W. cuspidata*, and there is some overlap of loci (Fig. 6), with a distance between the average loci of 0.06 units. A number of other species of *Eulophia* have similar-colored labellae with average loci grouping in the blue-green region of the color hexagon. The closest of these are two autogamous "varieties" of *Eulophia clavicornis* at 0.06 and 0.09 color opponent units. The average distance of the color loci of the labellae of different *Eulophia* species is 0.29 units, with a range of 0.06–0.69 units.

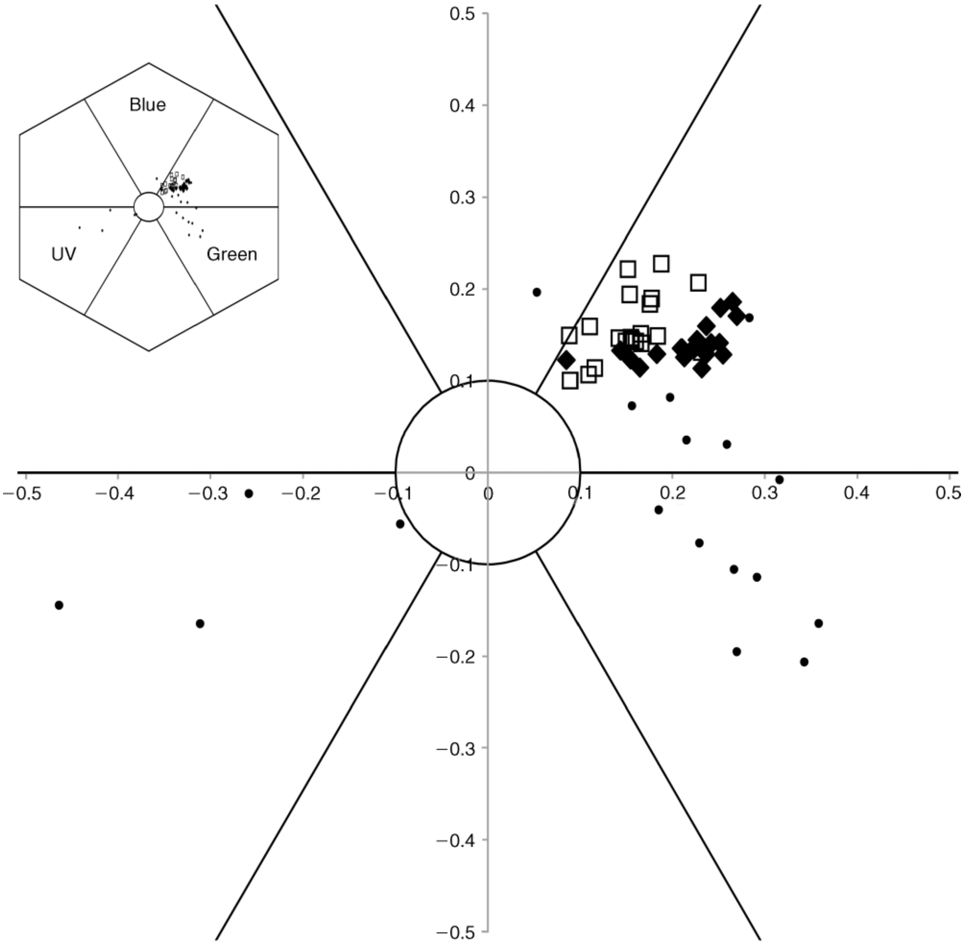

FIG. 6. Color loci of the labellae of *Eulophia zeyheriana* (solid diamonds) and pollen presenter of *Wahlenbergia cuspidata* (open squares). Mean color loci of the labellae of a number of other species of *Eulophia* are also included (solid circles). Color loci are calculated according to the Hexagon color model of bee vision (Chittka 1992). See Fig. 5 for additional details.

Color modifications

Qualitatively, the addition of the UV-absorbing treatment substantially alters the appearance of the lateral petals of the flower in the near-UV region of the spectrum (Fig. 4E) relative to the two controls. The manipulated control, painted with duck preen fat, appears slightly more reflective in the near-UV region when compared to the unmanipulated control (Fig. 4E). The colors of the manipulated controls are similar to those of unmanipulated *E. zeyheriana* petals and comparable to the petals of *W. cuspidata* with perceptual color distances of <0.04 units between average loci. In contrast, the colors of the petals treated with the UV-absorbing Parsol mixtures appear distinctly different from the average *W. cuspidata* and unmanipulated *E. zeyheriana* flowers (color opponent distance of 0.32 and 0.31 units, respectively) in the blue-green region of the color hexagon (Fig. 5).

Flowers with the UV-absorbent treatment applied had significantly lower rates of pollinarium removal and overall visitation than the treated and untreated controls. The two controls did not differ significantly from one another (Table 1). The UV-absorbent treatment also had lower rates of pollinia deposition than the two controls, although this was not significant.

Breeding systems

Eulophia zeyheriana is self-compatible, and capsules produced from selfing are comparable in mass to those resulting from outcrossing. However, the quality of seed resulting from self-pollination is significantly inferior to seed produced by outcrossing, with one-third fewer seeds with embryos per capsule (Appendix E: Table E1). While hand-pollination of flowers ($n = 33$) always resulted in fruit set, none of the bagged and unmanipulated flowers ($n = 16$) set fruit. This response to hand-pollination is significant ($G = 59.3$, $P < 0.0001$) and indicates that pollinator visits are required for fruit set in *E. zeyheriana*.

DISCUSSION

The results of this study indicate that the deceptive orchid *Eulophia zeyheriana* exploits a specialized inter-

TABLE 1. Pollination success of *Eulophia zeyheriana* flowers with modified UV reflectance.

Treatment	No. inflorescences	No. flowers	Proportion of pollinaria removed	Proportion of pollinia deposited	Proportion of flowers visited
Untreated control	16	43	0.30^{a}	0.16	0.33^{a}
Treated control	14	35	0.40^{a}	0.09	0.43^{a}
UV treatment	17	41	0.05^{b}	0.05	0.05^{b}
$F_{2,44}$		0.22	4.89	1.06	5.18
P		0.80	0.012	0.35	0.010

Notes: Values within a column that share the same letter are not significantly different (based on one-way ANOVA of inflorescence-level data, followed by Tukey multiple comparisons).

action between *Wahlenbergia cuspidata* and a single species of *Lipotriches* bee primarily by means of color signalling. Although flowers of the orchid differ morphologically from those of *W. cuspidata* (Fig. 1A–F), a large proportion of the bees caught carried pollen of both species, suggesting that they cannot easily distinguish the deceptive orchid from the rewarding *Wahlenbergia*.

The basis for the lack of discrimination by bees is probably that the color of the petals of the orchid is very similar to that of petals of the model in bee color space (Fig. 5), being only 0.03 units apart on average. There is evidence that bumble bees and honey bees cannot distinguish colors less than 0.06 units apart (Giurfa 2004, Dyer and Chittka 2004*a*, *b*). This is supported by our color modification treatments. The mean color distance of the manipulated controls was 0.03 units from the untreated flowers and 0.04 units from the *W. cuspidata* but the visitation rates to these flowers was not significantly different from that of the untreated flowers. In contrast, flowers that had the UV component of their flower colors experimentally removed (color opponent distance of 0.32 units to the model flowers) were effectively ignored by the pollinators and had very low visitation rates (Table 1).

By altering the color of the flowers, the mimicry is apparently disrupted in much the same way as altering the shape of inflorescences was shown to reduce visits by pollinators to a Batesian mimic in a fly-pollinated system (Johnson et al. 2003*a*). By contrast, we have found that manipulation of UV reflectance (using the same technique as described in this study) in the bee-pollinated generalized food deceptive orchid *Eulophia parviflora* actually resulted in significantly increased visitation (C. I. Peter and S. D. Johnson, *unpublished data*). Indeed, negative frequency-dependent selection on flower color has been predicted for generalized food deceptive systems (Smithson and Macnair 1997, Gigord et al. 2001, Lynn et al. 2005).

The phylogenetic relationships within the large genus *Eulophia* remains unresolved. However, *E. zeyheriana* is the only species in the genus with pale blue flowers having loci in the blue-UV segment of the bee color hexagon. This is in contrast to other congenerics with loci in the other five segments (Fig. 5). It is likely, therefore, that the floral coloration of *E. zeyheriana* is a derived trait in the phylogenetic sense (cf. Wanntorp 1983). This, considered together with the results of the spectral analyses and color manipulation experiments (Figs. 4 and 5, Table 1), suggests that the pale blue flower color in *E. zeyheriana* could be an adaptation for exploiting the relationship between *W. cuspidata* and its *Lipotriches* bee pollinator.

Besides the overall similarity of petal color between model and mimic, the labellum of *E. zeyheriana* may also mimic the pollen presenter of *W. cuspidata*. Heuschen et al. (2005) suggest that UV-absorbing patches on flowers may mimic the UV-absorbing signal of pollen, with UV absorption thought to be a result of flavonoid pigments that protect the pollen against bateria, fungi, and UV damage. Our data suggest a more specific case of pollen mimicry, with the white, UV-absorbing area on the labellum of *E. zeyheriana* mimicking the unusual pale blue to white pollen (and pollen presenter) of *W. cuspidata* (Fig. 1B, E, F). While the male bees that we observed would not be seeking out the pollen of *W. cuspidata* as a primary reward, the pollen presenter is visually a central feature of *W. cuspidata* flowers and is apparently used by many of the bees to orient in the flower to access nectar (Fig. 1H). Nilsson (1983) proposed a similar system with a patch of cream frills on the labellae of *Cephalanthera ruba* thought to mimic the pollen presenters of *Campanula* flowers.

Unlike the novel color of the petals of *E. zeyheriana*, the color of the pollen-mimicking patch on the labellum is similar to that found in closely related taxa. Similar yellow, UV-absorbing patches are found on labellae of a number of other species of *Eulophia* (Fig. 6, lower right segment) and may be an important component of generalized food deception in these species. This trait in *E. zeyheriana* is therefore most likely a preadaptation that has been enhanced by the rolling of the labellum to more closely resemble the pollen presenter of *W. cuspidata* (Figs. 1C and 5A, B).

Little is known about the biology of *Lipotriches* bees. Immelman and Eardley (2000) reported that some *Lipotriches* species collect grass pollen to provision their nests. They found that female *Lipotriches* bees emerged between 06:30 and 07:30 to forage for pollen (and presumably also nectar from plants other than grasses) and then sealed themselves into their nest with mud plugs by midday. Tchuenguem Fohouo et al. (2004) documented nearly identical foraging times for another grass-pollen-collecting *Lipotriches* from west Africa. It is puzzling that only male bees were observed on *Wahlenbergia* flowers, but as our observations usually commenced only mid-morning, we cannot firmly exclude the possibility that *Wahlenbergia* flowers (and those of the orchid) are also visited by female bees.

Batesian floral mimicry

Eulophia zeyheriana is from a lineage of deceivers: none of the species we have examined in this genus have rewards and most *Eulophia* species are pollinated by naive insects through generalized food deception (Peter and Johnson 2006*a*; C. I. Peter, *unpublished data*). These can be considered generalized exploiters as they exploit generalist mutualisms between pollinators and suites of plants.

Eulophia zeyheriana, in contrast, appears to be specialized to exploit the very close association between *W. cuspidata* and a single species of *Lipotriches* bee. This orchid cannot set seed without pollinator visits (Appendix E: Table E1), and this bee was the only insect species observed to carry its pollinaria. These observations, together with the close spectral matching (Fig. 5) and facilitated pollination at one site and in the translocation experiment (Figs. 2 and 3), are consistent with the initial hypothesis of Batesian floral mimicry. The hypothesis is further supported by the geographical distribution of the orchid, which is broadly congruent with that of *W. cuspidata* (Appendix A: Fig. A1), and the presence of *W. cuspidata* plants at all of the six sites where we have observed the orchid. Flowering times of the two species are very similar, although the orchids sometimes flower a few days earlier than the rewarding model (Appendix B: Fig. B1). This and the observation that pollination of orchids in at least one population did not depend strongly on proximity to *W. cuspidata* plants (Fig. 2) suggest that the orchids may, under some circumstances, attract *Lipotriches* bees that have not already been conditioned by visiting *W. cuspidata* flowers.

Facilitated pollination

There is increasing evidence that interactions among coexisting plants can be characterized by facilitation, rather than competition (e.g., Callaway 1995). As a result of pollinator conditioning, Batesian floral mimicry systems should be characterized by unidirectional facilitation of pollination success in mimics by their models (Johnson 1994), although this special case has received little attention in earlier literature on ecological facilitation (e.g., Callaway 1995). Facilitation of pollination among co-occuring plant species is not confined to mimicry systems, however, and may be a more general phenomenon, as in the "magnet species effect" (Thomson 1978), whereby local aggregation of pollinators around particularly rewarding plants, rather than pollinator conditioning per se, enhances the pollination of other plants in the close vicinity (cf. Laverty 1992).

We found that pollinaria removal and overall visitation of *E. zeyheriana* was significantly enhanced by proximity to *W. cuspidata* plants in one population (Gilboa), while in another population (Cobham) we did not find a significant relationship between these variables (Fig. 2). One plausible explanation for the lack of a significant facilitation effect in the Cobham population is simply that the removal of pollinaria from almost 100% of flowers at this site (Fig. 2), presumably because bees were particularly abundant, makes it difficult to use pollinaria removal rates to assess variation in actual visitation rates among plants (Fig. 2C). Further evidence for facilitation was obtained in an experiment in which plants were translocated either in or out of patches of *W. cuspidata* at the Gilboa site. In this experiment, pollinaria removal and overall visitation, but not pollen deposition, were significantly affected by proximity to *W. cuspidata* patches (Fig. 3). Rates of pollinaria removal were much higher than rates of pollen deposition in this experiment, which suggests either that most bees that visited the experimental inflorescences were not already carrying pollinaria or that pollinium deposition, even by bees already carrying pollinaria, occurs during a smaller fraction of visits than does pollinarium removal.

Other studies of deceptive orchids have yielded mixed evidence for the idea that pollination can be facilitated by co-occuring rewarding plants (Johnson 1994, Lammi and Kuitunen 1995, Alexandersson and Ågren 1996, Johnson et al. 2003*b*, Juillet et al. 2007). Facilitation of pollination by a rewarding species was evident in several populations of the Batesian floral mimic *Disa ferruginea* (Johnson 1994) and the generalized food deceptive orchid *Anacamptis morio* (Johnson et al. 2003*b*), while it was detectable in only one of three years in a study of the generalized food deceptive orchid *Calypso bulbosa* (Alexandersson and Ågren 1996).

In none of the above studies including the present one can facilitation be firmly ascribed to either pollinator conditioning (mimicry effect) or pollinator abundance (magnet effect). While our color manipulation data indicates that the resemblance between the model and the mimic is important for deception of pollinators, we don't yet know whether the color preferences of the bees are innate or learned. Furthermore, there could also be a magnet effect behind the enhancement of *E. zeyheriana* pollination by *W. cuspidata* at the Gilboa site (Fig. 2A), as *Lipotriches* bees appear to be concentrated around patches of *W. cuspidata*. Further work is required to unravel the precise contributions of the mimic and magnet effects to the ecological facilitation of pollination.

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APPENDIX A

A figure showing the distribution of *Eulophia zeyheriana* and *Wahlenbergia cuspidata* in eastern South Africa (*Ecological Archives* E089-094-A1).

APPENDIX B

A figure showing the overlap of flowering phenology of *Eulophia zeyheriana* with that of *Wahlenbergia cuspidata* (*Ecological Archives* E089-094-A2).

APPENDIX C

A table showing the mean pollen loads on *Lipotriches* bees at the Mount Gilboa and Cobham study sites (*Ecological Archives* E089-094-A3).

APPENDIX D

A figure showing the mean reflectance spectra of *Eulophia zeyheriana* and *Wahlenbergia cuspidata* flower parts (*Ecological Archives* E089-094-A4).

APPENDIX E

A table showing the results of a breeding system experiment for *Eulophia zeyheriana* (*Ecological Archives* E089-094-A5).