



# Generalized food deception: colour signals and efficient pollen transfer in bee-pollinated species of *Eulophia* (Orchidaceae)

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Non-rewarding plants use a variety of ruses to attract their pollinators. One of the least understood of these is generalized food deception, in which flowers exploit non-specific food-seeking responses in their pollinators. Available evidence suggests that colour signals, scent and phenology may all play key roles in this form of deception. Here we investigate the pollination systems of five *Eulophia* spp. (Orchidaceae) lacking floral rewards. These species are pollinated by bees, notably *Xylocopa* (Anthophorinae, Apidae) or *Megachile* (Megachilidae) for the large-flowered species and anthophorid (Anthophorinae, Apidae) or halictid (Halictidae) bees for the small-flowered species. Spectra of the lateral petals and ultraviolet-absorbing patches on the labella are strongly contrasting in a bee visual system, which may falsely signal the presence of pollen to bees. All five species possess pollinarium-bending mechanisms that are likely to limit pollinator-mediated self-pollination. Flowering times extend over 3–4 months and the onset of flowering was not associated with the emergence of pollinators, some of which fly year round. Despite sharing pollinators with other plants and lacking rewards that would encourage fidelity, the *Eulophia* spp. exhibited relatively high levels of pollen transfer efficiency compared with other rewarding and deceptive orchids. We conclude that the study species employ generalized food deception and exploit food-seeking bees. © 2013 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2013, 171, 713–729.

**ADDITIONAL KEYWORDS:** bee pollination – generalized food deception – Hymenoptera – phenology – pollen mimicry – pollen transfer efficiency (PTE) – pollinia – pollinarium reconfiguration – *Xylocopa* – ultraviolet (UV).

## INTRODUCTION

As many as 8000 species of orchids (up to one third of all orchids; van der Pijl & Dodson, 1966; Ackerman, 1986) lack floral rewards and the majority of these are thought to employ generalized food deception (GFD) to attract pollinators (Jersáková, Johnson & Kindlmann, 2006). The mechanisms behind GFD are still poorly understood. It has been suggested that GFD would only work if insects were naïve and newly emerged (Dafni, 1986), which would explain why many species with GFD flower early in the season (Internicola, Bernasconi & Gigord, 2008), but there is now evidence that even experienced insects are

deceived by the flowers of these orchids (Gumbert & Kunze, 2001; Johnson *et al.*, 2003). GFD appears to require colour signals that trigger powerful food-seeking behaviour of pollinators, either because of innate responses or because of generalized associative conditioning in a community context.

Using the catalogue of known relationships between orchids and their pollinators published by van der Cingel (1995, 2001), Peter (2009) estimated that bees are involved in the pollination of 58% of non-autogamous orchid species that have been studied, which is similar to the estimate of Van der Pijl & Dodson (1966) of 60%, a figure which included wasps. Orchids that employ GFD most commonly exploit bee pollinators, which are the most widespread and important pollinators of plants (Faegri &

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van der Pijl, 1979). This may simply reflect the widespread availability of these insects, rather than any propensity by bees to be duped by flowers.

Bees visit flowers for a variety of rewards, notably nectar collected by both male and female bees as a carbohydrate source and pollen collected by females as a protein source to provision their nests (Proctor, Yeo & Lack, 1996). In orchids, pollen is almost never available as a reward, being bound up as pollinia (the only notable exception being members of Apostasioideae; Kocyan, 2010) and hence a number of orchid species produce pseudopollen as a possible substitute reward (Davies & Turner, 2004). Flowers frequently have strongly contrasting colours, including ultraviolet (UV). Hypotheses to explain these contrasting flower colours include the nectar guide hypothesis, pollinator conspecific mimicry hypothesis (marking resembles other insects already on the flowers) and the pollen and anther mimicry hypothesis (Heuschen, Gumbert & Lunau, 2005). This latter explanation holds that some plants have signals, typically in the centre of the flower, that advertise the presence of a pollen reward, pollen being strongly UV-absorbing (Lunau, 2000; Heuschen *et al.*, 2005). In contrast to generalized pollen mimicry, there is also evidence for more specific pollen mimicry, in which pollen-mimicking patches on the labella of deceptive orchids are a key component of resemblance in Batesian mimicry of the rewarding model species (Nilsson, 1983; Peter & Johnson, 2008).

Deceptive orchids must share pollinators with other rewarding species in the same community and the absence of rewards means that their pollinators do not show constancy (cf. Waser, 1986). The presence of pollinaria means that pollen is unlikely to be lost to the stigmas of non-orchid species, but it would still be expected that deceptive orchids should experience substantial pollen wastage because of pollinator infidelity. One measure of pollen wastage is pollen transfer efficiency (PTE), which can be estimated as the proportion of pollen removed from anthers that is subsequently deposited on conspecific stigmas. Orchids generally exhibit high levels of pollen transfer efficiency (Harder & Johnson, 2008), but this can also result in pollinaria removed from one flower being deposited on the next flower visited on the same plant (Johnson, Neal & Harder, 2005). It is thought that deceptive pollination in orchids can limit such pollinator-mediated self-pollination by discouraging pollinators from visiting many flowers on the same plant (Johnson, Peter & Agren, 2004); however rewardlessness normally comes with the expense of low fruit set (Neiland & Wilcock, 1998). In addition, post-removal pollinarium reconfiguration mechanisms were proposed by Darwin (1867) to limit geitonogamy in orchids. Peter & Johnson (2006b) tested Darwin's hypothesis and showed that in 18 out of 19

orchid and asclepiad species, pollinarium reconfiguration (including pollinarium bending, pollinia shrinking and anther cap retention) times exceed the visit times to the inflorescences by pollinators and hence should reduce facilitated self-pollination. Given the absence of rewards in GFD species, we expect visits to these species to be brief and consequently hypothesize that reconfiguration times of pollinaria of such GFD orchid species should be correspondingly rapid (cf. Peter & Johnson, 2006b).

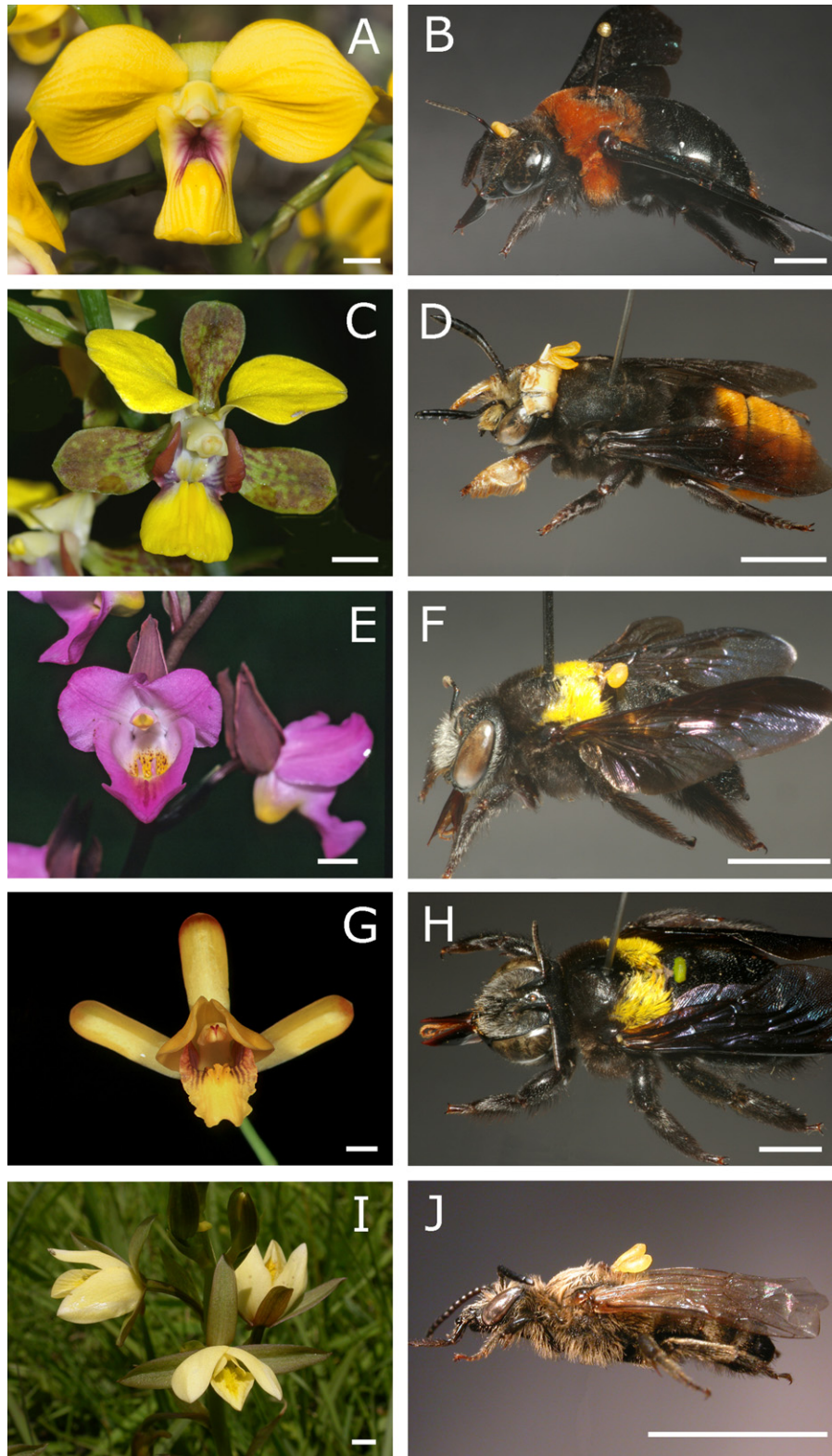
*Eulophia* R.Br. ex Lindl. is a large, primarily African genus of terrestrial epidendroid orchids. The pollination biology of this genus is not yet well known and, prior to our studies, only *Eulophia cristata* (Afzel. ex Sw.) Steud. pollinated by *Xylocopa* carpenter bees (Lock & Profita, 1975) and autonomously self-pollinating species from Zambia (Williamson, 1984) had had their pollination biology described. Subsequently, we have identified pollinators for a bee-pollinated Batesian mimic (Peter & Johnson, 2008); various beetle-pollinated species (Peter & Johnson, 2006a, 2009b) and a number of self-pollinating taxa (Peter & Johnson, 2009a). In addition, Jürgens *et al.* (2009) and Johnson *et al.* (2009) examined the breeding systems and pollination biology of *Eulophia alta* (L.) Fawc. & Rendle in the New World. In contrast, the small allied Cape genus, *Acrolophia* Pfitzer, includes some rewarding species, one of which is pollinated by small bees (Peter & Johnson, 2009c). Floral traits of many *Eulophia* spp. suggest that bee pollination is important in the genus. These traits include deep zygomorphic flowers that are mechanically strong and have a landing platform, with vivid colours, such as yellow and purple, often with nectar guides and a scent, which to the human nose is 'fresh' but not particularly strong (van der Pijl, 1961).

The aims of this paper were to document pollination systems in deceptive *Eulophia* spp., hypothesized on the basis of their floral traits to be bee pollinated, and to test predictions arising from the hypotheses related to generalized food deception, outlined above. Specifically, we asked: (1) whether floral spectral reflectance constitutes signals known to elicit food-seeking behaviour in bees; (2) whether the phenology of these species conform to predictions that GFD species flower early in the season and for long periods; (3) whether GFD in these species results in low levels of pollination success; and (4) whether pollen transfer efficiency was lower than in rewarding orchids.

## MATERIAL AND METHODS

### STUDY SPECIES AND SITES

*Eulophia speciosa* (R.Br. Ex Lindl.) Bolus (Fig. 1A; see also Supporting Information, Fig. S1A, B) has large



**Figure 1** Study species and their respective pollinators. *Eulophia speciosa* (A) is pollinated by *Xylocopa flavorufa* (B), *E. streptopetala* (C) is pollinated by large *Megachile* bees including *M. cincta* (D), *E. cucullata* (E) is pollinated by medium sized *Xylocopa* bees including *X. flavicollis* (F), *E. angolensis* (G) is pollinated by *X. flavicollis* (H) and *E. ovalis* subspecies *ovalis* (I) is pollinated by an unidentified species of *Lasioglossum* (Halictidae, J). Bar, 5 mm.

showy yellow flowers arranged in tall (1.5 m) inflorescences and is normally scentless to humans. It is a common species in South Africa, found along a narrow coastal strip (within approximately 1 km of the coast) growing in disturbed ground and stabilized beach sand. In the north of KwaZulu-Natal and in tropical Africa, the range of this species extends inland (Hall, 1965). Thirty-seven hours were spent observing pollinators in populations of this species at various sites (see also Supporting Information, Table S1).

*Eulophia streptopetala* Lindl. (Fig. 1C; see also Supporting Information, Fig. S2A, B) is a large and showy species with inflorescences reaching 1.5 m. A few flowers are open at a time and are unscented to the human nose. It is a common species growing in dense vegetation, such as the scrubby margins of forests. Its range in South Africa extends from near Port Elizabeth in the south through the eastern part of South Africa into tropical Africa (Hall, 1965). Sixteen hours were spent collecting pollinators in populations of this species.

*Eulophia cucullata* (Sw.) Steud. occurs in moist coastal grassland from near Durban in the south, northwards into tropical Africa. Flowering occurs mostly after fire, with inflorescences attaining c. 50 cm and bearing a few large, deep pink pouch-like flowers (Fig. 1E; see also Supporting Information, Fig. S3A, B) (Hall, 1965). The flowers of this species have a strong 'chemical' or 'plastic' scent (C. I. Peter, pers. observ.). Thirty-six hours were spent observing pollinators, primarily at the Amatikulu site.

*Eulophia angolensis* (Rchb.f.) Summerh. produces tall, many-flowered inflorescences that attain > 2 m in height. Flowers are densely packed on the inflorescences with all parts bright yellow (Fig. 1G; see also Supporting Information, Fig. S4A). The flowers are strongly and sweetly scented and neutral red staining indicated that the ridges on the labellum are the site of scent production (see also Supporting Information, Fig. S4B). *Eulophia angolensis* is found from the north-eastern parts of the former Transkei through the coastal parts of KwaZulu-Natal and thence inland to the Lowveld of Mpumalanga and into tropical Africa (Hall, 1965). Pollinator observations amounted to 22 h.

*Eulophia ovalis* Lindl. is a relatively small species with sparse inflorescences reaching c. 50 cm. Relatively few flowers (often only one) are open on an inflorescence at any one time. Sepals are pale brown to green, while the lateral petals and labellum are bright white to pale cream (Fig. 1I; see also Supporting Information, Fig. S5A, B). Two subspecies are currently recognized. This research focuses on subspecies *ovalis* found from the coast to the foot of the Drakensberg mountains and throughout the eastern parts of South Africa (Hall, 1965). There is no obvious

floral scent. Eighteen hours were spent observing flowers for pollinator.

For purposes of comparison, pollinarium reconfiguration time and spectral reflectance measurements were also made on a number of other *Eulophia* spp. including *E. aculeata* (L.f.) Spreng. subsp. *aculeata*, *E. aculeata* subsp. *huttonii* (Rolfe) A.V.Hall, *E. clitellifera* (Rchb.f.) Bolus, *E. leontoglossa* Rchb.f., *E. macowanii* Rolfe, *E. odontoglossa* Rchb.f., *E. petersii* (Rchb.f.) Rchb.f and *E. tuberculata* Bolus. Hall (1965) gave detailed descriptions of these species. No pollinators were identified for these species during field observations.

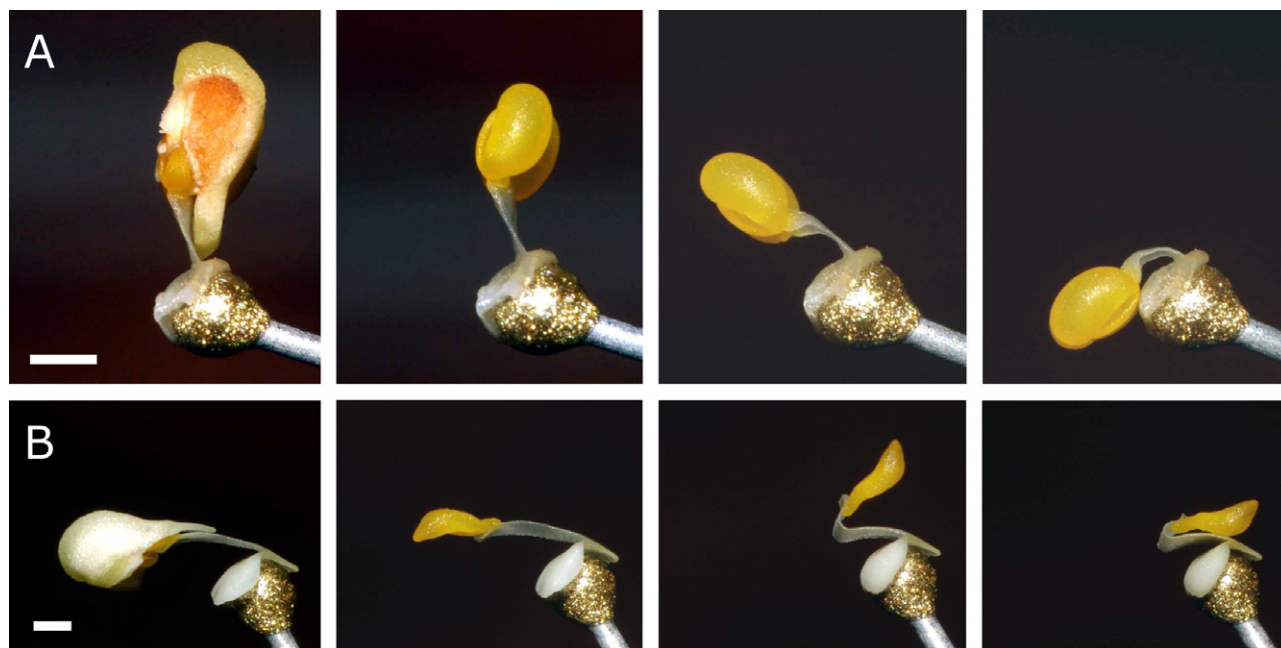
Flowers of all species listed above were dissected and inspected for the presence or absence of nectar using either a dissecting microscope or hand lens. Additional data on visitation rates and pollinarium reconfiguration for other *Eulophia* spp. were obtained from published studies (Peter & Johnson, 2006a, 2008, 2009b, c; Jürgens *et al.*, 2009; Peter, 2009). Autonomous self-pollinated species (Peter & Johnson, 2009a) were excluded.

#### POLLINATORS

As most *Eulophia* spp. are deceptive, visits by bee pollinators were rarely observed. Most bees bearing pollinaria were therefore collected while they were visiting nearby rewarding plants. Insects were sampled in the vicinity of flowering plants at each of the study sites and inspected for the presence of pollinaria. This is in contrast with the majority of the beetle-pollinated species (Peter & Johnson, 2006a, 2009b; Peter, 2009), for which most pollinators were collected directly on the orchid inflorescences.

The identity of the pollinaria borne by pollinators was readily confirmed on the basis of size, shape and reconfiguration orientation. *Eulophia speciosa* has larger pollinaria than any other co-occurring orchids with a characteristic bending reconfiguration that assists in its identification (Fig. 2). *Eulophia streptopetala* has fusiform pollinia that have not been observed in any other South African *Eulophia* spp. *Eulophia angolensis* and *E. cucullata* both have distinctive 'D'-shaped pollinia and short, broad stipes. However, the closest known populations of these two species are approximately 200 km apart. *Eulophia ovalis* co-occurs with a number of congeners, but the two subspecies of *E. clavicornis* Lindl. are obligately self-pollinating and have non-functional pollinaria that cannot be removed from the column (Peter & Johnson, 2009a), *E. zeyheriana* Sond. and *E. foliosa* Bolus have smaller pollinaria and *E. calanthoides* Schltr. has large pollinaria and flowers 1 month later than *E. ovalis*.

Insects-bearing pollinaria were killed in ethyl acetate killing jars with care taken to avoid dislodg-



**Figure 2** The two bending-type reconfiguration mechanisms identified so far in *Eulophia*. A, the less common *E. speciosa*-type reconfiguration bends from an upright position to a depressed position. B, the more common *E. streptopetala*-type of reconfiguration entails bending of the pollinarium from an initially depressed position through nearly 180° so that the pollinia are finally orientated in the opposite direction. Bar, 1 mm.

ing the pollinia (the viscidium glue is rapidly dissolved by ethyl acetate fumes), mounted and identified. Insect vouchers are lodged in the Albany Museum, Grahamstown. Plant vouchers are lodged in the University of KwaZulu-Natal (NU) and Schonland (GRA) herbaria.

#### POLLINARIUM RECONFIGURATION

Reconfiguration times of pollinaria removed from fresh flowers were recorded for most *Eulophia* spp. encountered (species given in Table 2). In species having a rapid bending mechanism, the end point of the reconfiguration is obvious and easily timed. In species with a slow bending action, rates of change of the angles of the pollinaria were determined with a protractor and plotted against time to determine the end points (see Peter & Johnson, 2006b, for details). We also investigated the possibility of anther cap retention in these species, as has been reported in other *Eulophia* spp. (Peter & Johnson, 2006a, 2009b).

#### BREEDING SYSTEMS

To establish the dependence of plants on pollinators, breeding system studies were conducted for *E. speciosa* and *E. angolensis*. In both cases, inflorescences were bagged to exclude pollinators and the flowers either self-pollinated, cross-pollinated with pollen

from other plants in the population or left untreated to test for autonomous self-pollination. Pollinations were conducted by hand. Fruit were left to mature and harvested shortly before dehiscence. Seeds were dispensed into Petri dishes, homogenized and then three randomly chosen samples of 50 seeds were scored for the presence or absence of embryos.

#### VISITATION RATES AND POLLEN TRANSFER EFFICIENCY (PTE)

Determination of PTE was based on pollinaria removal and deposition in ten to 641 flowers sampled from one to 149 plants in each population, and several populations were sampled for most species (see also Supporting Information, Table S2). In larger populations, a single flower was sampled from each of a large number of plants, whereas in smaller population several flowers were sampled per plant. The average number of pollinia deposited on stigmas was divided by the average number of pollinia removed (pollinaria multiplied by two, each pollinarium comprising two pollinia) and expressed as a percentage to determine PTE (Johnson *et al.*, 2005).

#### FLOWER COLOUR

Given the visual prominence of the labellum and the fact that it is the primary point of contact between

flower and pollinator in most species, we measured the colours of both lateral petal and labellum in these species to determine the contrast in colours of these two floral segments (cf. Peter & Johnson, 2008; Shuttleworth & Johnson, 2012). The orientation of the lateral petals of the study species differ between species. In some species the lateral petals are declinate, appressed to the column forming a tube (e.g. *E. angolensis*, Fig. 1G), whereas others have spreading lateral petals and the adaxial surface is presented to the pollinators (e.g. *E. speciosa*; Fig. 1A). We therefore measured either the adaxial or abaxial surface of the lateral petals (species details given in Table 5), depending on which surface is most likely to serve as a visual attraction in the approach phase of the visit by the pollinator.

Reflectance spectra of these floral segments were analysed quantitatively with an Ocean Optics spectrophotometer. Measured colours were summarized according to the model of Chittka (1992) as the species were suspected to be visited by bees and the trichromatic vision of Hymenoptera, which forms the basis of the model, seems to be phylogenetically conserved and widespread in insects (Briscoe & Chittka, 2001). This model uses the excitation of the three colour sensors in the eyes of bees (UV, blue and green) in response to flower colours under known illumination and against a green background to summarize measured reflectance spectra into a 'bee perception space'. Ultraviolet patterns of flowers of *E. speciosa*, *E. streptopetala*, *E. ovalis* and the long-spurred form of *E. parvilifera* were determined qualitatively using UV photography. Details of both the spectrophotometry and UV photography have been given by Peter & Johnson (2008).

#### FLOWERING PHENOLOGY

Collection dates of specimens bearing flowers included in the PRECIS database and specimens in GRA were used to determine flowering phenology of the five focal study species. Flowering dates were numbered from the first day of the year. Flight times of *Xylocopa* and *Megachile* pollinators were determined from Eardley (1983), the Albany Museum, Grahamstown (AMGS) collection and the collection of the first author. Sample sizes range between 33 and 199 and are given in the Supporting Information (Fig. S6).

## RESULTS

### POLLINATING INSECTS AND POLLINARIUM RECONFIGURATION

We collected bees bearing pollinaria or viscidia of all five study species (Table 1). *Eulophia speciosa* was found to be pollinated exclusively by large *Xylocopa*

*flavorufa* carpenter bees at several sites along the east coast of South Africa (Table 1). Pollinaria are attached between the antennae of this bee species (Figs 1B; see also Supporting Information, Fig. S1D). Most of the insects collected bearing pollinaria were in the vicinity of wild-collected plants growing in botanical gardens just outside the natural range of the plants. A few visits were observed to both these cultivated and wild plants, with *X. flavorufa* bees spending a number of seconds probing deeply into the flowers. Bees were observed to closely inspect a succession of inflorescences without visiting any flowers at Site 17. Smaller *Xylocopa* species (*X. caffra* and *X. flavicollis*) were common at most sites, but none of the many insects inspected bore pollinaria or viscidia (Table 1).

The reconfiguration mechanism of *E. speciosa* is distinctly different from the more common reconfiguration mechanism found in most other *Eulophia* spp. (*E. streptopetala*-type reconfiguration, see below). In *E. speciosa*-type reconfiguration, the freshly removed pollinarium is orientated with the pollinia perpendicular to the viscidium (Fig. 2A). The stipe then goes through a period of reconfiguration, bending forward so that the pollinia are orientated forward (comparable with unbent pollinaria in *E. streptopetala*-type reconfiguration, Fig. 2B). This reconfiguration takes 115.0 s (SE 1.5) (Table 2). The pollinaria of closely related *Eulophia clitelifera* and *Eulophia tuberculata* undergo this form of reconfiguration with average reconfiguration times of 32.3 s (SE 1.5) and 209.9 s (SE 16.0), respectively (Table 2).

*Eulophia streptopetala* was found to be pollinated by very large megachilid bees, including *Megachile cincta* (Fig. 1D), an unidentified *Megachile* sp. (see also Supporting Information, Fig. S2D) and *Megachile felina* (see also Supporting Information, Fig. S2E) (Table 1). Initially, a very large bee (*Megachile cincta*) bearing unusual pollinaria was collected on a yellow species of *Crotalaria* L. (Fabaceae) near Manzengwenya in Maputaland. It was not until four more *Megachile* bees were collected at Drummond in the KwaZulu-Natal midlands that the unusual elongated pollinia (Fig. 2B) were identified as belonging unambiguously to *E. streptopetala*. At the Drummond site, a *M. cincta* male was collected bearing four pollinaria, which had already had their pollinia deposited, and a complete pollinarium with pollinia orientated for deposition (Fig. 1D). Additional specimens (one *M. cincta* collected at Harare, Zimbabwe and one *M. felina* collected at the Blyde River, Mpumalanga, South Africa) bearing the distinctive *E. streptopetala* pollinaria were found in the collection of the Albany Museum, Grahamstown (AMGS).

The pollinaria of *E. streptopetala* attach to the posterior edge of the head of the bee. Initially, the pol-

**Table 1** Recorded bee-pollinators of *Eulophia* and *Acrolophia*

Pollinator	Pollinator family	Sex	Sites*	All insects sampled or observed	Number of sampled insects bearing pollinaria or (viscidia)	Number of insects observed approaching or visiting flowers
<i>E. speciosa</i>						
<i>Xylocopa flavorufa</i>	Xylocopinae, Apidae	9 F	1, 2, 3, 4, 17	38	8 (2)	21
<i>Xylocopa flavicollis</i>	Xylocopinae, Apidae	5 F, 1 M	3, 4, 9, 17	6	0	0
<i>Xylocopa caffra</i>	Xylocopinae, Apidae	3 F	2, 3, 17	3	0	0
<i>E. streptopetala</i>						
<i>Megachile cincta</i>	Megachilidae	4 M, 2 F	8, 10, 13, †	7	3 (1)	0
<i>Megachile felina</i>	Megachilidae	2 F	10, 13, ‡	5	2	0
<i>Megachile</i> sp. (CIP 3878)	Megachilidae	F	13	1	1	0
<i>E. cucullata</i>						
<i>Xylocopa flavicollis</i>	Xylocopinae, Apidae	Females	7, 8, 9	21	4 (2)	0
<i>Xylocopa hottentotta</i>	Xylocopinae, Apidae	3 M, 7 F	7, 8, 9	20	3	0
<i>E. angolensis</i>						
<i>Campsomesiella calebs</i>	Scoliidae	Female	5	1	1	1
<i>Xylocopa flavicollis</i>	Xylocopinae, Apidae	Females	5	14	3	10
<i>Xylocopa flavorufa</i>	Xylocopinae, Apidae	?	5	2	0	2
<i>E. ovalis subsp ovalis</i>						
<i>Lasioglossum</i> sp.	Halictidae	2 F, 3 M	12	5	1	0
<i>E. zeyheriana</i> (Peter & Johnson, 2008)						
<i>Lipotriches</i> sp	Halictidae	Males	6, 11	70 (46 collected)	34 of those collected	2
<i>E. parviflora</i> (long-spurred form) (Peter, 2009)	Anthophorinae, Apidae	Both sexes	14, 15	89	20	7 (9)
<i>Amegilla fallax</i>						
<i>E. cristata</i> (Lock & Profta, 1975)						
<i>Xylocopa olivacea</i>	Xylocopinae, Apidae	Not recorded	Southern Ghana	'A number'	'A number'	'A number'
<i>E. alta</i> (Jürgens <i>et al.</i> , 2009)						
At least 6 species of <i>Centris</i>	Apidae		3 sites Manaus, Brazil	735	-69	
<i>Xylocopa muscaria</i>	Xylocopinae, Apidae		3 sites Manaus, Brazil	187	-12	
<i>Megachile</i> sp.	Megachilidae		3 sites Manaus, Brazil	107	-7	
<i>Acrolophia cochlearis</i> (Peter & Johnson, 2009c)						
<i>Colletes claripes</i>	Colletidae	8 M (+9)§	1	8 (+9)§	8 (+9)§	8 (+9)§
<i>Colletes claripes</i>	Colletidae	2 F	1	2	0	2

\*Site numbers correspond with those in the Supporting Information (Table S1).

†One specimen housed in AMGS bearing pollinaria, collected at MacIlwaine, Salisbury (Harare).

‡One specimen housed in AMGS bearing pollinaria, collected in Blyde River, Mpumalanga.

§An additional nine male bees were observed bearing large pollinaria masses, patrolling and visiting the inflorescences, but were not collected.

**Table 2** Known reconfiguration modes and times for species of *Eulophia* and *Acrolophia*

Species	Reconfiguration mode*	Mimicry system	Reconfiguration time (mean)	SE	N
Bee-pollinated					
<i>Acrolophia cochlearis</i>	Bending, <i>speciosa</i> -type†	Rewarding	88.0	5.25	15
<i>Eulophia zeyheriana</i>	Bending, <i>streptopetala</i> -type‡	Batesian	43.2	3.4	23
<i>Eulophia speciosa</i>	Bending, <i>speciosa</i> -type	GFD	115	1.5	35
<i>Eulophia streptopetala</i>	Bending, <i>streptopetala</i> -type	GFD	106.0	19.3	30
<i>Eulophia cucullata</i>	Bending, <i>streptopetala</i> -type	GFD?	155.0	12.0	2
<i>Eulophia angolensis</i>	Bending, <i>streptopetala</i> -type	GFD	72.0	4.8	53
<i>Eulophia ovalis</i>	Bending, <i>streptopetala</i> -type	GFD	29.4	4.0	9
<i>Eulophia parviflora</i> (long-spurred form)	Bending, <i>streptopetala</i> -type§	GFD	48.3	3.6	113
<i>Eulophia alta</i>	No reconfiguration¶	Rewarding	–	–	–
Beetle-pollinated					
<i>Eulophia parviflora</i> (short-spurred form)	Bending, <i>streptopetala</i> -type§	GFD/rendezvous	107.7	5.5	41
<i>Eulophia ensata</i> (yellow form)	Anther-cap retention**	GFD/rendezvous	154.5	12.5	18
<i>Eulophia welwitschii</i>	Anther-cap retention**	GFD/rendezvous	222.9	25.6	18
<i>Eulophia foliosa</i>	Anther-cap retention††	GFD	511.7	36.6	24
Unknown pollinators					
<i>Eulophia clitellifera</i>	Bending, <i>speciosa</i> -type	Unknown	32.3	1.5	25
<i>Eulophia tuberculata</i>	Bending, <i>speciosa</i> -type	Unknown	209.9	16.0	7

\*Citations for previously published studies: †(Peter & Johnson, 2009c), ‡(Peter & Johnson, 2008), §(Peter, 2009), ¶(Jürgens *et al.*, 2009), \*\* (Peter & Johnson, 2009b), ††(Peter & Johnson, 2006a).

linia are orientated forwards on the long slender stipe (Fig. 2B). The stipe bends mid length, flipping the pollinia through nearly 180°, such that they are then orientated to point backwards and can thus be hooked into the stigmatic cavity (see relative orientation of pollinia and stigma in Figure 1 of Peter & Johnson, 2006b). As noted above, we term this *E. streptopetala*-type reconfiguration. On average, the reconfiguration of the pollinarium of this species takes 106.0 s (SE 19.3; Table 2). Visits by the pollinators to the flowers were not observed directly.

Pollinators of *E. cucullata* were collected near the southernmost limit of distribution of this species in Amatikulu Nature Reserve. This species is pollinated by medium-sized *Xylocopa* bees, including female *X. flavicollis* and male and female *X. hottentotta*. The pollinaria are attached dorsally to the posterior margin of the metathoracic segment of the pollinating carpenter bees. The stipe of the pollinarium in this species is short and broad, but undergoes a similar reconfiguration to that described for *E. streptopetala*. This reconfiguration takes 155 s (SE 12.0; Table 2). No visits to the flowers were observed despite the high visitation rates (Table 4).

*Eulophia angolensis* is pollinated by medium-sized *Xylocopa* bees, including *X. flavicollis*. Three of these bees bearing pollinia were collected while they visited

flowers of *E. angolensis* at Mpenjati on the KwaZulu-Natal south coast (Table 1). In addition, a very large scoliid wasp (*Campsomesiella calebs*) was collected following a visit to a flower. This insect had removed the pollinarium from the flower that it visited. As with *E. cucullata*, the pollinaria of *E. angolensis* are attached to the dorsal edge of the metathoracic segment between the wings of the bee. The pollinaria undergo a similar reconfiguration to that described for *E. streptopetala*. This reconfiguration takes 72.0 s on average (SE 4.8; Table 2). The duration of the observed visits to inflorescences were not recorded, but the few visits seen lasted less than 1 min.

A number of attempted visits by the large carpenter bee, *Xylocopa flavorufa* (see Fig 1B), were also observed (Table 1). In these instances, the bees approached and grappled the end of the flower and attempted to enter the flower. However, the two declinate lateral petals forming the tube into which the smaller bees crawl are too stiff to allow the entrance of such a large bee, effectively filtering out these bees as pollinators. The smaller *Xylocopa* bees and scoliid wasps that carried pollinaria can, however, easily enter the flowers.

Only one individual of a *Lasioglossum* sp. (Halictidae) bearing the pollinaria of *E. ovalis* subspecies *ovalis* (Fig. 1J) was collected at Wahroonga in the



**Table 3** Results of an experiment to determine the breeding system of *Eulophia speciosa* and *E. angolensis*. Values are overall percentages or means ( $\pm$  SE) with sample size in parentheses. Test statistics apply to comparison of self- and cross-pollinated treatments

	Unmanipulated control	<i>Eulophia speciosa</i>			Test statistic
		Cross-pollinated	Self-pollinated		
Percentage fruit set ( <i>N</i> )	0 (31)	90 (10)	73 (11)		$G = 0.12^{ns}$
Mean $\pm$ SE capsule and seed mass in grams ( <i>N</i> )	–	0.90 $\pm$ 0.15 (10)	0.64 $\pm$ 0.12 (11)		$t_{19} = 1.31^{ns}$
Mean $\pm$ SE seed mass in grams ( <i>N</i> )	–	0.27 $\pm$ 0.07 (10)	0.14 $\pm$ 0.02 (11)		$t_{19} = 1.28^{ns}$
Mean $\pm$ SE percentage of seeds with embryos ( <i>N</i> )	–	78.6 $\pm$ 3.9 (10)	39.7 $\pm$ 6.6 (11)		$t_{15} = 4.58^{\ddagger}$

	Unmanipulated control	<i>Eulophia angolensis</i>			Test statistic	Naturally pollinated flowers
		Cross-pollinated	Self-pollinated			
Per cent fruit set ( <i>N</i> )	0 (20)	83 (18)	82 (17)		$G = 0.001^{ns}$	n/r (24)
Mean $\pm$ SE capsule and seed mass in grams ( <i>N</i> )	–	0.64 $\pm$ 0.06 (15)	0.68 $\pm$ 0.07 (14)		$t_{33} = 0.3^{ns}$	0.67 (24)
Mean $\pm$ SE seed mass in grams ( <i>N</i> )	–	0.18 $\pm$ 0.03 (15)	0.19 $\pm$ 0.04 (14)		$t_{33} = 0.1^{ns}$	0.19 (24)
Mean $\pm$ SE percentage of seeds with embryos ( <i>N</i> )	–	16.1 $\pm$ 1.2 (15)	9.8 $\pm$ 1.9 (14)		$t_{26} = 3.3^{\dagger}$	17 (21)

\* $P < 0.05$ ,  $^{\dagger}P < 0.01$ ,  $^{\ddagger}P < 0.001$ .

n/r, not recorded; ns, not significant.

KwaZulu-Natal midlands. This bee was sheltering in *Gladiolus ecklonii* Lehm. flowers during inclement weather. Four other bees of the same species were collected at the same time, but without evidence of pollinaria or viscidia. Pollinarium reconfiguration in this species was more rapid than the other *Eulophia* spp. studied, taking just 29.4 s on average (SE 4.0; Table 2). A large number of bees and wasps were collected and inspected for pollinaria in the very large population of this species at Cobham in the southern Drakensberg. None of these Hymenoptera carried pollinaria.

None of the South African *Eulophia* spp. examined had evidence of nectar or any other obvious fluids in their flowers.

#### BREEDING SYSTEMS

There was little difference in the overall quantity of fruit resulting from cross- and self-pollination in *E. speciosa* and *E. angolensis* (Table 3). However, in both species, the quality of seeds produced from cross-pollinated capsules was significantly higher than that produced from self-pollination. There was no evidence of autonomous self-pollination and the rosetta of all South African *Eulophia* spp. included in this paper are entire, precluding autonomous contact between pollinia and the stigma (cf. Peter & Johnson, 2009a).

#### VISITATION RATES AND PTE

Flowers of all the study species and additional *Eulophia* and *Acrolophia* spp. included for comparison showed relatively low rates of visitation, pollination and pollinaria removal (Table 4; see also Supporting Information, Table S2) relative to other orchids for which data are available (e.g. Tremblay *et al.*, 2005). Pollen transfer efficiencies were highest in the rewarding *Acrolophia cochlearis* and lowest in the *Eulophia* spp. pollinated by small solitary bees (*E. ovalis* and the long-spurred form of *E. parviflora*). Species pollinated by larger bees had relatively high rates of PTE (13–25%; Table 4).

Species in which PTE has been examined in a number of populations show that PTE is variable. For example, in *E. speciosa* PTE ranged from 0% for a road side population growing at Kenton-on-Sea in the Eastern Cape to 45.5% for a large population growing in the Bayhead Natural Heritage Site adjacent to Durban Harbour, KwaZulu-Natal (Table 4).

#### FLOWER COLOUR

Of the five species examined here, the majority are yellow or cream to the human eye, with the fuchsia-coloured *E. cucullata* being the exception (Fig. 1E). The colour of all five species includes a UV component (Figs 3, 4).

**Table 4.** Summary of visitation rates and pollen transfer efficiencies in bee-pollinated species of *Eulophia*. Values in bold are means or grand means. Extended data set given in the Supporting Information (Table S2)

	Sites	Number of sampling dates	Number of inflorescences	Number of flowers	% flowers with removal	% flowers pollinated	% visited	Pollen transfer efficiency (PTE) %
<i>E. speciosa</i>	3, 4, 17	9	7–30	43–210	<b>12.0*</b>	<b>3.8*</b>	<b>15.3*</b>	<b>20.5*</b>
<i>E. streptopetala</i>	18, 19	2	1–26	10–200	<b>26.0</b>	<b>7.3</b>	<b>26.3</b>	<b>13.4</b>
<i>E. cucullata</i>	7	2	21–41	99–232	<b>70.9</b>	<b>31.4</b>	<b>65.9</b>	<b>24.7</b>
<i>E. angolensis</i>	5	3	5–23	89–446	<b>17.1</b>	<b>4.5</b>	<b>19.3</b>	<b>15.6</b>
<i>E. ovalis</i>	10	1	23	106	42.5	5.7	50.0	8.9
<i>E. zeyheriana</i>	6, 10, 21, 22	7	11–149	58–641	<b>42.1*</b>	<b>17.6*</b>	<b>47.1*</b>	<b>23.9*</b>
<i>E. parviflora</i>	14, 15, 23	10	6–36	28–213	<b>17.0*</b>	<b>1.9*</b>	<b>19.0*</b>	<b>4.4*</b>
<i>E. cristata</i>	1	1	10	203	32.5	2.0	33.0	6.1

\*Average represents grand means for each site based on different subsamples.

Perhaps the most striking feature of flower colour in most of the species examined is the contrast between the colours of the lateral petals and labellum in the bee colour space. This typically includes strongly UV-reflecting lateral petals (and sepals where these are petaloid) and a strongly UV-absorbing labellum or part of the labellum (Fig. 4). To humans, most lateral petals and labella in Figure 4 appear yellow. The majority (17/20) of species have petal colour loci in different colour segments to that of the labella (Fig. 3, Table 5). Distances between mean colour loci for petals and labella are 0.08 opponent units or greater (Table 5).

In many cases, the colour loci of the central labellum lobes fall in the bee green or blue–green segments of the colour hexagon. Seventeen of 20 species have labellar colour loci in either the green or blue–green segments of the colour hexagon (Fig. 3, Table 5).

#### FLOWERING PHENOLOGY

The five species flower mostly from late spring through the summer, with the earliest flowering exhibited by *E. cucullata*, which flowers in spring and early summer. In all species, peak flowering lasts between 3 and 4 months (see also Supporting Information, Fig. S6A). The large pollinators (*Xylocopa* and *Megachile*) fly throughout the year (see also Supporting Information, Fig. S6B), although some species (*M. cincta*, *M. felina* and *X. flavicollis*) are seldom collected in winter.

#### DISCUSSION

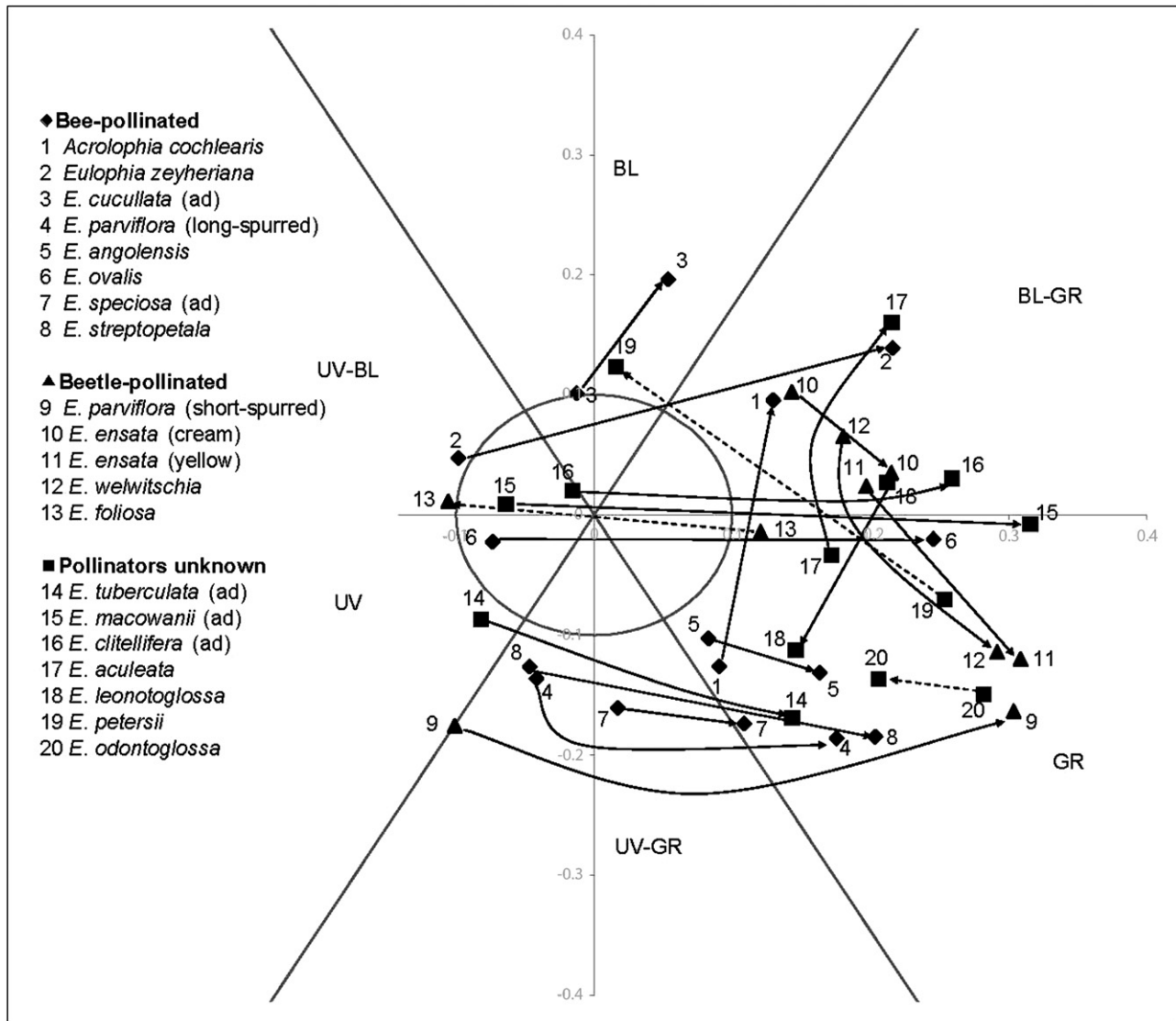
The data presented here and elsewhere (Lock & Profita, 1975; Peter & Johnson, 2008; Jürgens *et al.*,

2009; Peter, 2009) show that many *Eulophia* spp. are specialized for pollination by bees. However, in the absence of any obvious resemblance to, or consistent occurrence with, specific rewarding plants (C. I. Peter, pers. observ.), we infer that the five study species employ systems of generalized food deception.

Large *Xylocopa* bees seem to play a particularly important role in the pollination of *Eulophia* spp. with large showy flowers. These bees pollinated *E. speciosa*, *E. cucullata* and *E. angolensis* and have previously been reported as pollinators of *E. cristata* (Lock & Profita, 1975) and *E. horsfallii* (Martins, 2002). The traits of these *Xylocopa*-pollinated species are similar to those of other carpenter bee-pollinated angiosperms and include: large flowers, spacious enough to admit these large bees; and strong, heavy-tissued flowers, robust enough to withstand (in the words of van der Pijl, 1954a: 423) ‘rude insects and their rough foot work’. In addition, these species are said to have ‘well hidden nectar protected by closing mechanisms . . . to exclude unwanted visitors’; sexual organs brought into contact with the dorsal part of the insect; having pale unsaturated colours and producing ‘fresh’ scents (van der Pijl, 1954b: 559). The study species are all deceptive but differ in their spur morphology. Three species (*E. speciosa*, *E. streptopetala* and *E. ovalis*) have spurs or folds that simulate a nectar spur, whereas *E. angolensis* and *E. cucullata* have broad sac-like pouches.

The colours of the *Xylocopa*-pollinated *Eulophia* spp. are either bright yellow or bright pink. These two colours are common in other *Xylocopa*-pollinated plants in South Africa (C. I. Peter, pers. observ.), India (Solomon Raju & Purnachandra Rao, 2006) and the tropics (van der Pijl, 1954a, b, c).

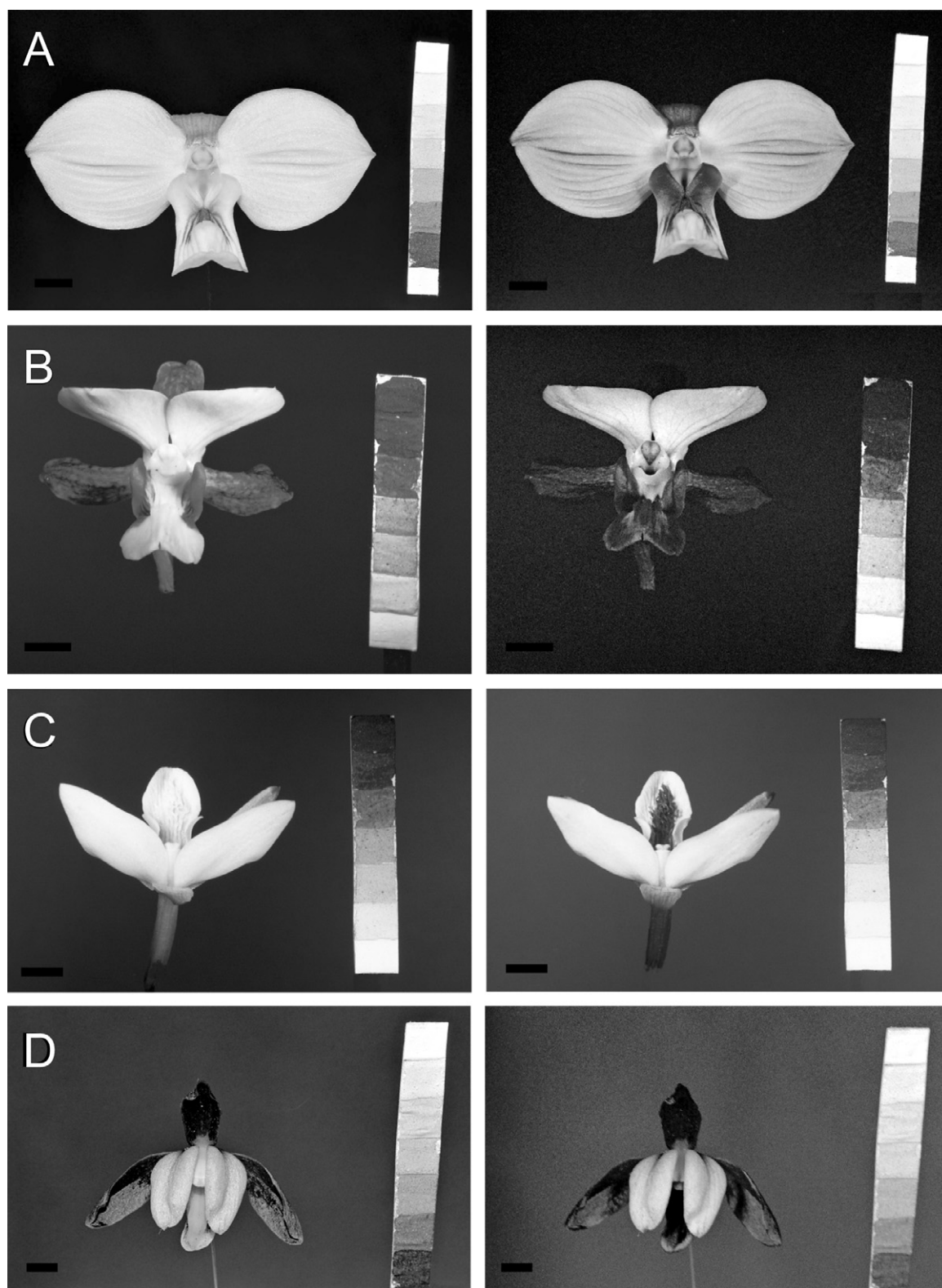
Colour loci of the textured labella of the majority of species are distinct from that of the lateral petals



**Figure 3** Comparisons of abaxial (or adaxial where appropriate, see Material and methods) petal colour (start of arrow) and central adaxial labellum colour (end of arrow) for common South African *Eulophia* spp. Dotted lines indicate species with labellar colour loci shifted to either blue or ultraviolet (UV)-blue segments.

(and coloured sepals in some species) and it is likely that bee pollinators can easily distinguish between these colours given the distance between colour loci (Dyer & Chittka, 2004, Table 5, cf. Giurfa, 2004). In the absence of specific model species, the UV-absorbing labellar patches found in these generalized food deceptive species are hypothesized to serve as generalized mimics of pollen-covered anthers. This is in contrast to 'specific' pollen mimicry by papillose patches on the labella of species such as *Cephalanthera rubra* (L.) Rich. (Nilsson 1983) and *E. zeyheriana* (Peter & Johnson 2008), which closely match the reflectance spectra of the pollen of the respective models in these two Batesian mimics.

Pollen is relatively uniform in colour, being either UV-absorbing yellow or white in the majority of angiosperms (corresponding to the GR or BL-GR segments in Fig. 3 and Table 5), possibly as a consequence of exine pigments that serve to protect DNA from UV damage (Heuschen *et al.*, 2005). Heuschen *et al.* (2005) suggested that the occurrence of uniform yellow, UV-absorbent flower centres in many unrelated lineages of angiosperms represents an unrecognized form of generalized pollen mimicry. In most cases, however, these markings in the centre of flowers are in close association with the anthers bearing the pollen, which serves as a reward to the pollinators, and so the yellow UV-absorbing colours of



**Figure 4** Human visual spectrum (400–700 nm; left column) and near ultraviolet (~360 nm to 400 nm; right column) images of flowers of bee-pollinated species including (A) *Eulophia speciosa*, (B) *E. streptopetala*, (C) *E. ovalis* and (D) *E. parviflora* (long-spurred form; Peter, 2009). The lateral petals and labellum of all species are yellow in the human visual spectrum. Bar, 5 mm.

**Table 5.** Comparison of petal and labellum colours in species of *Eulophia* and *Acrolophia*

No.*	Species	Pollination system†	Petal surface Measured§§	Petal colour‡‡	Labellum colour‡‡	Perceptual distance (petal to labellum)	N
1	<i>A. cochlearis</i>	Rewarding, colletid bees‡	ab	GR	BL-GR	0.22	1
2	<i>E. zeyheriana</i>	Batesian, halictid bees§	ad	UV-BL	BL-GR	0.33	22
3	<i>E. cucullata</i>	GFD, xylocopid bees	ad	BL	GR	0.25	2
4	<i>E. parviflora</i> (long-spurred form)	GFD, small anthophorid bees¶	ab	UV-GR	GR	0.40	34
5	<i>E. angolensis</i>	GFD, xylocopid bees	ab	GR	GR	0.09	2
6	<i>E. ovalis</i>	GFD, halictid bees	ab	UV	GR	0.32	6
7	<i>E. speciosa</i>	GFD, xylocopid bees	ad	UV-GR	GR	0.09	11
8	<i>E. streptopetala</i>	GFD, megachilid bees	ab	UV-GR	GR	0.26	3
9	<i>E. parviflora</i> (short-spurred form)	GFD/rendezvous, cetoniid beetles¶	ab	UV-GR	GR	0.22	30
10	<i>E. ensata</i> (cream)	GFD/rendezvous, cetoniid beetles¶	ab	BL-GR	BL-GR	0.10	3
11	<i>E. ensata</i> (yellow)	GFD/rendezvous, cetoniid beetles**	ab	BL-GR	GR	0.18	3
12	<i>E. welwitschii</i>	GFD/rendezvous, cetoniid beetles**	ab	BL-GR	GR	0.21	6
13	<i>E. foliosa</i>	GFD, elaterid beetles††	ab	GR	UV-BL	0.23	3
14	<i>E. tuberculata</i>	Unknown	ad	UV	GR	0.24	1
15	<i>E. macowanii</i>	Unknown	ad	UV-BL	GR	0.38	1
16	<i>E. clittellifera</i>	Unknown	ad	UV-BL	BL-GR	0.35	1
17	<i>E. aculeata</i>	Unknown	ab	GR	BL-GR	0.20	2
18	<i>E. leontoglossa</i>	Unknown	ab	BL-GR	GR	0.15	9
19	<i>E. petersii</i>	Unknown	ab	GR	BL	0.31	1
20	<i>E. odontoglossa</i>	Unknown	ad	GR	GR	0.08	2

\*Numbers correspond with those in Figure 3.

†Citations for previously published studies: ‡(Peter & Johnson, 2009c), §(Peter & Johnson, 2008), ¶(Peter, 2009), \*\* (Peter & Johnson, 2009b), ††(Peter & Johnson, 2006a).

‡‡Codes correspond with segments of the colour hexagon in Figure 3.

§§ad, adaxial surface; ab, abaxial surface.

the petals are a legitimate signal to pollinators, acting as 'pollen' guides to assist pollinators to find pollen rewards (Lunau *et al.*, 2006; Pohl, Watolla & Lunau, 2008). Given the absence of rewarding model species, and the fact that these species are deceptive, these 'pollen patches' on the labella (Fig. 4) probably serve as generalized mimics of pollen or anthers (*sensu* Heuschen *et al.* 2005) in these species, providing support for the idea that the presence of a pollen reward is critical for the attraction of bee pollinators.

In orchids that employ GFD, selection may favour early season flowering (to avoid competition with rewarding species or to exploit naïve insects) and extended flowering periods (to maximize opportunities for reproduction in the face of infrequent visits by experienced pollinators, at least in temperate species) (Internicola & Harder, 2012). The bee-pollinated *Eulophia* spp. studied here do indeed have long flowering times, with flowering lasting between 3 and 4 months (see also Supporting Information, Fig. S6A). However, relative to temperate northern hemisphere orchids with GFD (Internicola *et al.* 2008), these species flower relatively late in the season. The less seasonal climate and the solitary habit of *Xylocopa* and *Megachile* bees probably results in the emergence of naïve bees throughout the course of the very long season (see also Supporting Information, Fig. S6B), providing a mixture of naïve and experienced pollinators for these plants to exploit. In addition, all species, with the exception of some populations of *E. angolensis*, occur in sparse populations, with few individuals spread over large areas, such that they are probably infrequently encountered by the bees, reducing the opportunity for negative associative conditioning.

As a consequence of rapid decision making of bees encountering deceptive flowers, orchids with GFD should have rapid pollinarium reconfiguration times or no reconfiguration (e.g. Xiaohua *et al.*, 2012). Consistent with this prediction, reconfiguration times of small-flowered species pollinated by small halictid and anthophorid bees are generally < 50 s. However, the species pollinated by large bees had slower reconfiguration times, some even similar to that of beetle-pollinated species (Table 2). Although we were not able to record visit times of these large bees to individual inflorescences, the observed visits by *X. flavorufa* to *E. speciosa* were unexpectedly ponderous, perhaps because foraging decisions about the presence of pollen are slower than those involving the presence of nectar.

This study identifies three modes of pollinarium reconfiguration in *Eulophia*, including two modes of pollinarium bending, the *E. speciosa*-type reconfiguration found in a few species and the more common *E. streptopetala*-type reconfiguration (Fig. 2), as well

as anther cap retention (Peter & Johnson, 2006a, 2009b). Authors including Darwin (1867) and van der Pijl (1966) described a number of possible reconfiguration modes that may not be homologous with those described here. Both types of bending mechanisms have examples of rapid and slow bending pollinaria. Anther cap retention includes the slowest reconfiguration mechanism of these species (in the elaterid-pollinated *E. foliosa*), but the retention times of *E. ensata* Lindl. and *E. welwitschii* (Rchb.f.) Rolfe are comparable with the slowest of the species with a bending mechanism (Table 2).

As expected in deceptive species, the rates of pollen removal and deposition in most of these species is low (cf. Johnson *et al.*, 2004; Tremblay *et al.*, 2005; Jersáková & Johnson, 2006). However, they also tend to have relatively high pollen transfer efficiencies (Table 4) compared with other orchids (cf. Harder & Johnson, 2008). PTE in these GFD species is probably enhanced as a consequence of pollinaria being firmly glued to inaccessible parts of the pollinators (top of the head or dorsal part of the thorax) preventing loss via grooming and, counter-intuitively, as a consequence of being deceptive, which limits multiple visits and hence pollen layering, which can lead to whole clumps of pollinaria falling off the body of pollinators (cf. Johnson *et al.*, 2005; Harder & Johnson, 2008). In most cases (e.g. Fig. 1B), pollinating bees bore a single pollinarium. This is in contrast to the case in the rewarding *A. cochlearis*, in which pollinating bees accumulate large masses of pollinaria (Peter & Johnson, 2009c), and the Batesian mimic *E. zeyheriana*, in which a number of pollinators bore multiple pollinaria (Peter & Johnson, 2008). These latter two species do achieve very high PTE, but, at least in one population of *A. cochlearis*, this entails high levels of pollinator-mediated self-pollination (Peter & Johnson, 2009c).

Rates of PTE and other measures of visitation such as pollinarium removal and pollinium deposition in *Eulophia* are variable both within and between species (Table 4), probably as a consequence of stochastic fluctuations in foraging pollinator populations and the changes in abundance of surrounding rewarding species. PTE has rarely been tracked through the flowering period, but, where this has been done, there is variation between sample dates in rewarding species (Peter & Johnson, 2009c; Coombs & Peter, 2010; Coombs *et al.*, 2012). Coombs, Peter & Johnson (2009), however, showed that PTE was independent of population size in a rewarding asclepiad.

Data presented here are consistent with generalized food deception in these five species. This mode of deception remains poorly understood despite being the principle form of deception in the orchids (Jersáková *et al.*, 2006). Signalling of the presence of

pollen via labellum ornamentation and contrasting spectral reflectance appears to be widespread in this genus, and perhaps in orchids more generally, but the function of these labellum traits remains to be tested experimentally (but see Peter & Johnson, 2008). The overall importance of pollen vs. nectar signalling for attraction of *Xylocopa* and *Megachile* bees is also not clear. Although females predominated as pollinators, males carrying pollinaria were also captured on some species, suggesting that flowers are visited in some cases because they are perceived to have nectar. Other areas for future research include understanding the significance of the timing of anthesis in subtropical and tropical GFD orchids in relation to flowering of co-occurring nectar plants and the flight times of pollinators.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Figure S1.** (A) *Eulophia speciosa* produces large showy inflorescences made up of a number of large flowers (B). This species is pollinated by *Xylocopa flavorufa* (C) with the pollinaria being attached between the antennae of these bees (D).

**Figure S2.** (A) *Eulophia streptopetala* produces tall inflorescences with a succession of flowers (B), which have prominent yellow lateral petals and a yellow labellum with maroon side lobes. This species is pollinated by various species of Megachilidae including (C) *Megachile cincta*, (D) an unidentified *Megachile* sp. and (E) *M. felina*.

**Figure S3.** (A) The inflorescences of *Eulophia cucullata* are relatively few flowered, the flowers (B) being bright pink with a prominent yellow base to the sac-like spur. This species is pollinated by (C) *Xylocopa flavicollis* and (D) *X. hottentotta*, this latter specimen bearing only a viscidium.

**Figure S4.** (A) *Eulophia angolensis* produces large showy inflorescences with many open flowers. The flowers are sweetly scented, (B) staining with neutral red indicates that the three lamellae ridges of the labellum are the site of scent production. *Eulophia angolensis* is pollinated by *Xylocopa flavicollis* (C), with a scoliid wasp, *Campsomesiella calebs* (D) also being collected visiting the flowers.

**Figure S5.** The inflorescences of *Eulophia ovalis* subsp. *ovalis* are relatively few flowered, often with less than three open flowers per inflorescence at any one time (A, B). Only one individual of a species of *Lasioglossum* (Halictidae) has been collected bearing the pollinaria of this subspecies.

**Figure S6.** A, percentage of herbarium specimens (in PRE and GRA) in flower in each month of the year for the five study species. B, percentage of bee specimens collected in each month of the year, based on data in Eardley (1983), the collection in the Albany Museum, Grahamstown (AMGS) and the collection of the first author. Numbers in parentheses are sample sizes. Shading of bars for plant species do not correspond with those for pollinators.

**Table S1.** Study sites and study species. Square brackets are used in this table to distinguish natural populations from garden grown plants.

**Table S2.** Detailed visitation rates and pollen transfer efficiencies in bee-pollinated species of *Eulophia*.