

Pollinators can prefer rewarding models to mimics: consequences for the assumptions of Batesian floral mimicry

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Abstract Many members of the Orchidaceae offer no rewards to pollinators and attract them through deception. One common approach is to mimic the specific floral signals of co-flowering species that are rewarding (Batesian floral mimicry) to exploit their pollinators. We investigated two putative Batesian floral mimics, *Disa gladioliflora* subsp. *capricornis* and *Disa ferruginea*, within a single community to determine whether their pollinators are able to discriminate them from their models. Despite mimics being indistinguishable from models in terms of floral colour as perceived by pollinators, strong preference for model flowers was observed in both systems. We nevertheless recorded frequent pollen transfer in *D. gladioliflora* subsp. *capricornis*, suggesting pollination still occurs in the face of pollinator discrimination. Although the inability of pollinators to discriminate between models and mimics is often cited as a requirement for Batesian floral mimicry, we suggest that this need not be the case. Pollination by discriminating visitors may still lead to fruit set, with the most accurate mimics expected to have the highest fitness.

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Batesian floral mimicry may thus be best indicated when mimic fitness is positively correlated with its resemblance to model flowers.

Keywords Behaviour · Deceptiveness · *Disa* · Discrimination · Orchid · Pollinator learning

Introduction

While pollinators visit flowers in search of rewards such as nectar, pollen and oil, some animal pollinated flowers provide no rewards at all (Sprengel 1793). Instead they deceive their pollinators into transporting pollen through false advertising. By resembling other rewarding flowers, carrion or potential mates, rewardless species manipulate insects searching for nectar, brood sites or conspecific partners into pollinating their flowers (Dafni 1984). With approximately 30 % of all species being rewardless, the Orchidaceae are arguably the most successful group in terms of the number and proportion of deceitful species (Ackerman 1986; Jersáková et al. 2009). Because deceitfulness is often associated with high levels of pollinator specialization, it might play a role in the spectacular diversification of the Orchidaceae (Cozzolino and Widmer 2005). Pollinator specialization may facilitate the speciation process by promoting reproductive isolation between divergent forms (Ramsey et al. 2003), and by generating strong divergent selection on forms with different pollinators (Campbell et al. 1997).

The most common mode of deceit in plants is generalized food deception, where rewardless flowers exploit the innate foraging behaviours of pollinators in search of food (Jersáková et al. 2006). These flowers need not bear a strong resemblance to any rewarding flowers within their

community and can attract pollinators with generally attractive signals such as large, brightly coloured or strongly scented flowers. Often the duped pollinators are naïve or inexperienced and still in the process of learning which floral phenotypes are associated with rewards. These flowers often exhibit polymorphisms within populations that may retard the learning and recognition process of pollinators (Cropper and Calder 1990; Ferdy et al. 1998, but see Smithson et al. 2007). Generalized food deception is in fact only one end of a continuum of deceptive strategies with the other end occupied by highly specialized flowers that have been termed Batesian mimics (Brown and Kodric-Brown 1979).

Like classic Batesian mimicry, exemplified by edible mimetic butterflies that resemble distasteful models to avoid predation (Bates 1862), rare but rewardless flowers (mimics) benefit by their adaptive resemblance to more common rewarding floral phenotypes (models). Since learning can affect the preferences of animals, Batesian mimicry in predation and pollination is characterized by an assumption of negative frequency dependent selection (Fisher 1930; Anderson and Johnson 2006). As the mimic becomes more common relative to the model, mimic and model fitness may decline because the conditioning process of predators or pollinators is retarded. For instance, in predation the predators may not effectively learn to avoid aposematic colouration (Lindstrom et al. 1997), and in pollination the pollinators may take longer to learn that certain floral traits are rewarding (Dafni and Irvi 1981). The fitness of mimics may also decline as animals encounter them more regularly and learn to recognise them.

As a result, in floral mimicry systems that exploit the conditioned responses of animals to colour, scent or morphology, selection is expected to result in very strong resemblance between the mimic and its model so that it is difficult for pollinators to tell them apart. Several studies have made the association between Batesian floral mimicry and inability of pollinators to distinguish between mimics and models (Johnson 1994, 2000; Johnson et al. 2003a; Anderson et al. 2005). Consequently, the failure of pollinators to discriminate mimics from models has often been taken as a requirement for Batesian floral mimicry (Johnson 1994, 2000). Other criteria deemed necessary to confirm Batesian mimicry are sympatry and overlapping flowering times of the mimic and the model, shared pollinators, lower frequency of the mimic than the model, higher fitness of the mimic in the presence of the model and that deceptive traits should be phylogenetically derived (Johnson et al. 2003a).

One of the classic examples of Batesian floral mimicry is the South African orchid *Disa ferruginea* Sw. In the south western parts of the Cape Floristic Region, the

inflorescences of this rewardless plant are bright red in colour and bear a striking resemblance to nectar producing flowers of *Tritoniopsis triticea* (Burm.f.) Goldblatt, both pollinated by the butterfly *Aeroptes tulbaghia* (Johnson 1994). Here, the butterflies appear unable to distinguish the mimic from the model and visit both indiscriminately (Johnson 1994). In the eastern parts of the orchid's range, *T. triticea* seldom co-occurs with *D. ferruginea* and it bears orange flowers, which closely resemble the common co-occurring rewarding plant *Kniphofia uvaria* (L.) Hook., both also visited by *A. tulbaghia* (Newman et al. 2012).

These butterflies exhibit a preference for orange colours in the east where their primary food flowers are orange, and a preference for red in the west where their primary food flowers are red in colour (Newman et al. 2012). While it has been demonstrated that *D. ferruginea* and its models co-occur and share the same pollinator, it is not clear that *D. ferruginea* resembles its models more closely in floral colour than other species in its community. As similarity to a single model species or phenotype is expected under specialized Batesian mimicry it ideally requires a community approach to exclude the possibility of generalized food deception, where close resemblance to sympatric species is not expected. Within the eastern part of its range, it is also unknown whether the pollinator *A. tulbaghia* can distinguish between orange *D. ferruginea* and its putative eastern model *K. uvaria*.

Disa ferruginea occasionally occurs with another rewardless orchid in the east, *Disa gladioliflora* Burch. ex Lindl. subsp. *capricornis* (Rechb.f.) H.P.Linder (hereafter *D. gladioliflora* for brevity), both from *Disa* sect. *Stenocarpa* (Bytebier et al. 2008). Most species in sect. *Stenocarpa* are characterized by scentless flowers with medium to long spurs, typically varying from white to pink in colour with occasional dark blotches on a pale background. These characteristics are usually associated with long proboscis fly pollination (Goldblatt and Manning 2000) and almost all species with known pollinators in the section are pollinated by long proboscis flies (Johnson et al. 2015). The exceptions are the morphologically similar and very closely related *D. ferruginea* and *Disa porrecta* Sw., both of which are butterfly pollinated (Johnson et al. 1998). The pink *D. gladioliflora* was retrieved as the sister taxon to *D. ferruginea* in the most recent molecular phylogeny, although its precise relationship is not certain as *D. porrecta* was not sampled (Bytebier et al. 2008).

Presently, nothing is known about the pollination of *D. gladioliflora*, although it has often been noted to grow with and bear a strong resemblance to the abundant rewarding iris, *Tritoniopsis ramosa* (Eckl. ex Klatt) G.J.Lewis, which is visited by *Amegilla* bees (Manning and Goldblatt 2005). In this study we investigate a community where *D. gladioliflora* and *D. ferruginea* co-occur with their more

abundant models. Specifically, we test whether these two orchids conform to assumptions of Batesian floral mimicry, such as strong resemblance to a co-flowering model with which they share pollinators. While it is known that the eastern *D. ferruginea* shares pollinators with its model, *K. uvaria* (Newman et al. 2012), here we determine which insects visit *D. gladioliflora* and whether they also visit its putative model, *T. ramosa*. As present theory suggests pollinators should not be able to distinguish between a Batesian floral mimic and its model, we also determine whether pollinators of both orchids can discriminate between mimics and models.

Materials and methods

Study system: We investigated floral colour within a diverse plant community in South Africa (33.92901°; 20.86353E, altitude 1152 m). This community contained 14 flowering species at the time of fieldwork (February 2014 and 2015), including members of the Asteraceae, Asphodelaceae, Campanulaceae, Ericaceae, Iridaceae and Orchidaceae (see online Resource 1).

Floral similarity: Since floral colour is the primary component of deception in Batesian floral mimicry systems (Jersáková et al. 2012), we measured the reflectance spectra of all flowering species in the community. We picked between one and eight flowers on separate plants per species (mean = 5 per species). Floral colour was investigated by spectrophotometry with an Ocean Optics (Dunedin, FL, USA) USB 4500 spectrometer and a UV–vis 400 fibre optic reflection probe, held at 5 mm from the middle of the petal/tepal/floret surface at 45°. Multiple spectra per species were averaged before plotting in hymenopteran visual space (hereafter BeeSpace) to assess flower colour as perceived by bee visitors using the model of Chittka (1992). Similarly, we plotted the averaged spectra of species in dipteran visual space (hereafter FlySpace) to assess colour as perceived by fly visitors using a recent model to calculate fly photoreceptor excitations (Santer 2014).

Individual spectra of *D. gladioliflora* and *T. ramosa* were also plotted separately in BeeSpace and FlySpace ($n = 7$ each) to explore variation within and between these species that are likely involved in Batesian mimicry. As both these species bear striking nectar guides that may be important for pollinator attraction (Jersáková et al. 2012), we also analysed this floral colour trait with spectrophotometry. Other traits that may influence pollinator attraction in this system were also measured (inflorescence height above ground, inflorescence diameter, number of flowers, floral width, floral height, floral tube/spur length, nectar guide length and nectar concentration and volume)

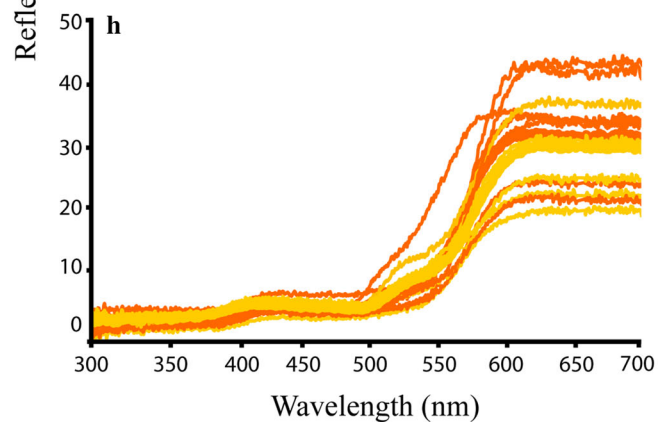
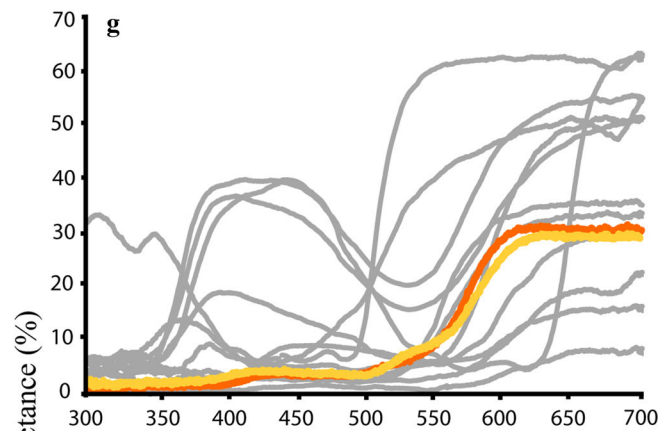
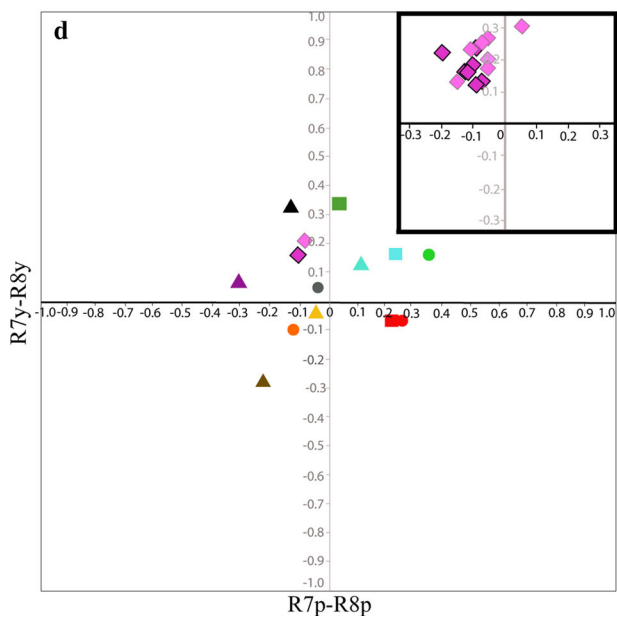
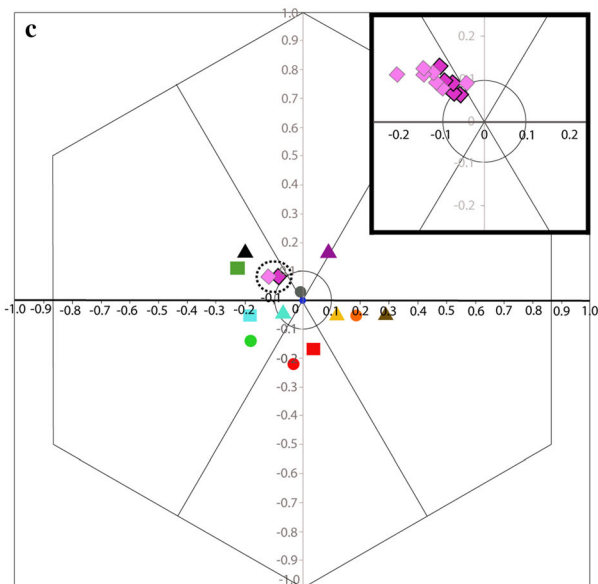
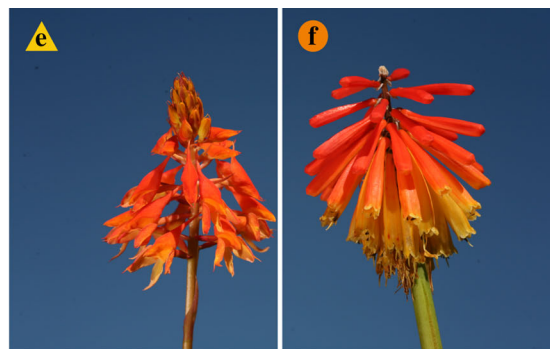
and compared between *D. gladioliflora* ($n = 15$) and *T. ramosa* ($n = 14$) with Mann–Whitney U-tests to explore morphological similarity between these species.

Pollinators of *D. gladioliflora*: To investigate which insects are likely pollinators and whether they are shared with its putative model *T. ramosa*, we recorded all floral visits during the course of the field work. This included observations of *T. ramosa* and *D. gladioliflora* where the observer was stationary, as well as periods spent walking among the populations of these two species. In total, approximately 105 h were spent observing pollinators of these two species. Representatives of visiting species were caught and killed with potassium cyanide fumes when possible to inspect them specifically for the presence of *D. gladioliflora* pollinaria.

Presentation experiments: We conducted paired choice experiments comprising a single inflorescence of *D. gladioliflora* and its proposed model *T. ramosa* ($n = 21$), to determine if they share pollinators and whether pollinators can discriminate between them. Fresh inflorescences were presented in watered vials spaced 30 cm apart at the start of each day and all visitor responses were recorded. These were divided into inspections (visitors inspect inflorescences, but do not alight on the flowers), visits (pollinators land on the flowers) and flower probes (when visitors could be seen foraging from the flowers). To ensure only virgin flowers were used, *D. gladioliflora* flowers were inspected for pollinaria removal and deposition prior to experiments. We trimmed inflorescences of *D. gladioliflora*/*T. ramosa* when necessary so each pair contained the same number of open flowers to control for small differences in display size (see Fig. 1a, b). Flowers of *D. gladioliflora* were inspected with a hand lens after every visit to determine if the visiting insect removed or deposited pollinaria, thus acting as an effective pollinator.

In addition, we also conducted a smaller choice experiment with *D. ferruginea* and its model *K. uvaria* ($n = 12$) to determine whether their shared pollinator, *A. tulbaghia*, can discriminate between them or not. Because the inflorescences of *K. uvaria* have many more flowers than *D. ferruginea* (see Fig. 1e, f for differences in floral display), we did not control for flower number in this pairing since pruning flowers from *K. uvaria* would produce a foreign and greatly reduced inflorescence phenotype that will likely influence pollinator attraction. The experimental layout for this paired comparison was identical to the previous experiment except that we did not check flowers for pollinaria deposition or removal as *A. tulbaghia* is already known to be the pollinator of both species (Newman et al. 2012).

Both paired choice experiments were observed for over 40 h and we analysed the visitation data with Generalized Estimating Equations (GEE), using the pair number as a repeated subject variable. An exchangeable correlation



- Community members
- ◆ *Disa gladioliflora*
 - ◆ *Tritoniopsis ramosa*
 - ▲ *Disa cardinalis*
 - *Erica versicolor*
 - ▲ *E. nudiflora*
 - *E. cruenta*
 - *E. steinbergiana*
 - ▲ *Disa ferruginea*
 - *Kniphofia uvaria*
 - *Euryops ericoides*
 - ▲ *Lobelia tomentosa*
 - ▲ *Stoebe ericoides*
 - *Syncarpha eximia*
 - *Watsonia fourcadei*

Fig. 1 **a** *Disa gladioliflora* and **b** *Tritoniopsis ramosa* and their floral colour similarity as perceived by hymenopteran pollinators in **c** BeeSpace and dipteran pollinators in **d** FlySpace. Note that they most closely resemble each other in the community within BeeSpace (dotted line) and how individuals overlap within each visual system (insets). **e** *D. ferruginea* and its model, **f** *Kniphofia uvaria*. As no Lepidopteran vision model is available, we illustrate **g** how their mean reflectance spectra most closely resemble each other in the community and **h** how closely their individual spectra overlap

matrix was selected with a Poisson distribution and a log link function. From this analysis we obtained the estimated marginal means and 95 % confidence intervals of responses made to each mimic and its model, which we back transformed from the log scale.

Results

Floral similarity: Average spectra plotted in BeeSpace revealed that while the community covered all colour regions of hymenopteran vision, *D. gladioliflora* and *T. ramosa* were most similar to each other (Fig. 1c). The Euclidian distance between their mean coordinates in BeeSpace was 0.04, which falls well below the 0.06 colour discrimination threshold of hymenopterans (Dyer and Chittka 2004). Plotting average spectra in FlySpace also revealed that *D. gladioliflora* and *T. ramosa* were most similar (Fig. 1d), although all colours within the same quadrant in FlySpace are perceived as the same by dipterans (Troje 1993). When plotting individual spectra for these two species in BeeSpace (Fig. 1c inset) and FlySpace (Fig. 1d inset) they were completely overlapping, indicating it would be difficult for hymenopterans and dipterans to distinguish between them by floral colour alone. Reflectance spectra of their nectar guides also revealed considerable overlap between model and mimic in both visual systems (Online Resource 2).

The length of nectar guides was similar between these species ($U = 87.0$, $df = 27$, $p = 0.43$), as were the number

of open flowers per inflorescence ($U = 101.5$, $df = 27$, $p = 0.15$). All other morphological traits differed significantly with *T. ramosa* exhibiting larger floral traits [inflorescence height ($U = 30$, $df = 27$, $p < 0.01$) and diameter ($U = 40$, $df = 27$, $p < 0.01$), flower width ($U = 19$, $df = 27$, $p < 0.01$), height ($U = 1$, $df = 27$, $p < 0.01$) and tube/spur length ($U = 23.5$, $df = 27$, $p < 0.01$)]. Nectar also differed between the two species as *D. gladioliflora* flowers did not contain nectar, whereas the nectar volume and concentration for *T. ramosa* were $1.3 \pm 0.6 \mu\text{l}$ and $13 \pm 3.5 \%$ (mean \pm SD) per flower, respectively.

The reflectance spectra of *D. ferruginea* and its model *K. uvaria* were also more similar to each other than to any other member in the community (Fig. 1g and h). Since there is no Lepidopteran colour vision model currently available, we could not investigate colour similarities between *D. ferruginea* and *K. uvaria* as perceived by their shared butterfly pollinator. Some butterflies have been found to exhibit tetrachromacy (Koshitaka et al. 2008), indicating that they likely perceive colour differently to trichromatic animals (Endler and Mielke 2005) like bees and flies.

Pollinators of *D. gladioliflora*: Insects observed visiting *D. gladioliflora* and *T. ramosa* throughout the duration of this study included *Amegilla* bees, bee flies and long proboscis flies (Table 1). Importantly, each visiting species was observed on both *D. gladioliflora* and *T. ramosa*. Most visitors had proboscis lengths that overlapped with the mean spur length of *D. gladioliflora* (11.24 ± 1.28 mm), except for the bee *A. fallax*. This species, however, was the only visitor to remove and deposit pollinaria during our presentation experiments and was the most regular visitor. This suggests that *A. fallax* is likely the dominant pollinator despite its slightly shorter proboscis, which may potentially improve pollen transfer as it will result in deeper probing on *D. gladioliflora* flowers. Captured specimens of *A. capensis* and the long proboscis fly *Prosoeca* sp. also carried *D. gladioliflora* pollinaria (Fig. 2) indicating that they are also likely pollinators.

Table 1 Insects observed visiting *Disa gladioliflora* and *Tritoniopsis ramosa* throughout the study, and pollinaria transferred during presentation experiments

Species	Order	Number captured	Mean proboscis length (mm) \pm SD	Visits to <i>Disa gladioliflora</i>	Visits to <i>Tritoniopsis ramosa</i>
<i>Amegilla capensis</i>	Hymenoptera	7	10.91 \pm 1.96*	3	47
<i>Amegilla fallax</i>	Hymenoptera	4	8.66 \pm 0.94	32	78
<i>Australoechus hirtus</i>	Diptera	6	10.43 \pm 1.99*	3	25
<i>Prosoeca</i> sp.	Diptera	4	13.94 \pm 3.22*	1	2
Pollinaria transferred in presentation experiments					
	Removed	Deposited			
<i>Amegilla fallax</i>	17	7			

* Indicates overlap with *D. gladioliflora* spur length

Fig. 2 The pollinators, *Amegilla capensis* (top) and *Prosoeca* sp. (bottom), next to a flower of *Disa gladioliflora*. Note the overlap in spur length and proboscis lengths and the pollinaria of *D. gladioliflora* attached midway along each pollinator's tongue. Scale bar = 1 cm

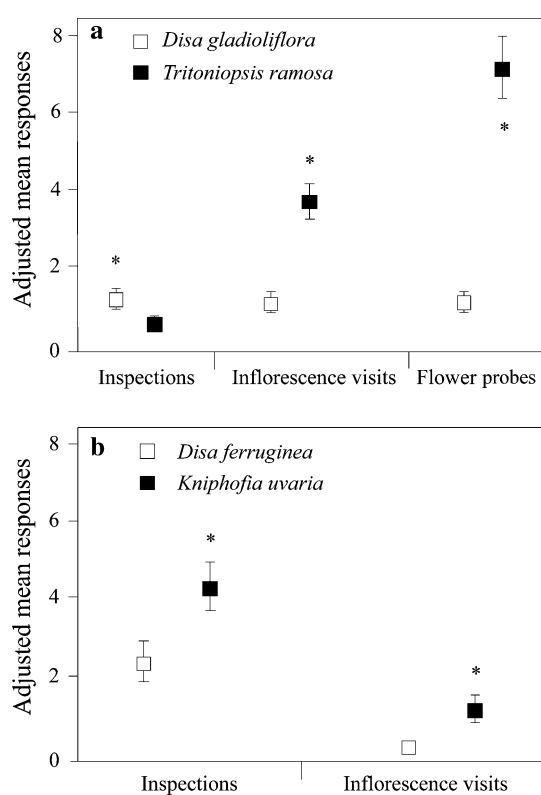


Fig. 3 Mean number of responses to mimics (white squares) and models (black squares) in **a** the *D. gladioliflora*–*T. ramosa* system, and **b** the *D. ferruginea*–*K. uvaria* system. Asterisk indicates significant differences between model and mimic at $p < 0.05$

Presentation experiments: The bee, *A. fallax*, made 39 responses to *D. gladioliflora* and 78 responses to *T. ramosa* in our choice experiments. All other responses were made by the bee fly, *Australoechus hirtus* (four to *D. gladioliflora* and eight to *T. ramosa*). Responses were analysed separately as inspections, visits or flower probes, which revealed that although *D. gladioliflora* received more

inspections than its model *T. ramosa*, (Wald $\chi^2 = 4.671$, $df = 1$, $p = 0.031$; Fig. 3a), this did not translate into more visits. Instead, more *T. ramosa* inflorescences were visited (Wald $\chi^2 = 36.852$, $df = 1$, $p < 0.001$) and more of its flowers were probed (Wald $\chi^2 = 54.547$, $df = 1$, $p < 0.001$). The butterfly, *A. tulbaghia*, made 30 responses to *D. ferruginea* and 66 responses to *K. uvaria*, and was the only visitor observed in this choice experiment. As butterflies were difficult to approach while foraging, we were unable to record the number of flowers probed per inflorescence. *A. tulbaghia* inspected the model *K. uvaria* more frequently than its mimic, *D. ferruginea*, (Wald $\chi^2 = 22.328$, $df = 1$, $p < 0.001$; Fig. 3b) and visited *K. uvaria* inflorescences more often (Wald $\chi^2 = 8.246$, $df = 1$, $p = 0.004$).

Discussion

Disa gladioliflora and its model, *T. ramosa*, are inseparable in floral colour as perceived by their shared pollinators. They also overlap greatly in the colour and length of their nectar guides and number of open flowers per inflorescence, all traits known to influence pollinator attraction (Thompson 2001; Jersáková et al. 2012). Lack of rewards confirmed that *D. gladioliflora* attracts its pollinators through deception. Both *D. ferruginea* and *D. gladioliflora* are thus rare and rewardless species that co-flower and share pollinators with their rewarding models. Both mimics also resemble their respective models most closely within the investigated community. These resemblances appear to be the products of unique, derived traits, as *D. gladioliflora* is the only pink species with red nectar guides in *Disa* sect. *Stenocarpa*, while *D. ferruginea* along with another butterfly pollinated mimic (*D. porrecta*) is the only species with orange flowers in this section.

The extraordinary floral similarity of these mimics to their respective models is thus the result of recent adaptive colour matching, providing convincing evidence for Batesian floral mimicry (Johnson et al. 2003a). Despite these numerous and clear indicators that *D. gladioliflora* and *D. ferruginea* are specialized Batesian floral mimics, their pollinators visit them less frequently than their respective models, raising some doubt about the criteria commonly used to distinguish between specialized Batesian floral mimicry and generalized food deception, which we discuss below.

Batesian floral mimicry or generalized food deception

The primary difference between these types of deception is that generalized food deception typically exploits innate foraging behaviours of pollinators and deceptive flowers are thus not required to resemble specific community members very strongly (Jersáková et al. 2009). In contrast, Batesian floral mimics exploit the positive learned association that pollinators have with rewarding species in the community, and consequently need to resemble specific sympatric models (Jersáková et al. 2009). These models can either be a single species (Johnson 1994, 2000) or a guild of phenotypically similar species (Brown and Kodric-Brown 1979; Papadopulos et al. 2013). Resembling rewarding models more closely than any other community member is thus a good indicator of Batesian floral mimicry. Another often used criterion for identifying Batesian floral mimicry is an inability of pollinators to discriminate between models and mimics (Johnson 1994; Jersáková et al. 2006, 2009).

Our data, however, show that pollinators in both systems discriminate against mimics and visit their rewarding models significantly more often. Using this criterion thus argues against Batesian mimicry in *D. ferruginea* and *D. gladioliflora*. However, in floral Batesian mimicry, pollinator discrimination may merely reflect their ability to learn, which is inherent in the assumption of negative frequency dependent selection in Batesian mimicry systems (Fisher 1930). Highly specialized pollination systems involving sexual mimicry are strongly characterized by pollinator learning, with pollinator responses to deceptive flowers decreasing with experience in both orchids (Gaskett et al. 2008) and non-orchids (de Jager and Ellis 2014). How long such learned avoidance lasts is not entirely certain. Studies on wasp pollinators of Australian sexually deceptive orchids suggest it may be less than 24 h (Whitehead and Peakall 2012). In deceptive food mimicry, it may be even shorter as the cost to pollinators of making a mistake is minimal.

Learning in Batesian mimicry has been shown to be slowest at low costs and low frequency of the mimic

(Lindstrom et al. 1997). Low costs of errors indicate that both experienced and inexperienced pollinators are likely to make mistakes when dealing with Batesian floral mimics that closely resemble their co-flowering models. Mimics will thus continue to be visited and pollinated despite an overall pattern of discrimination, as illustrated by frequent pollen transfer in our presentation experiments with *D. gladioliflora* and its model *T. ramosa*. Despite pollinators in our study discriminating between models and mimics, we therefore suggest that both *D. gladioliflora* and *D. ferruginea* are on the specialized Batesian side of the deceptive strategy continuum.

Another indicator that could help identify Batesian floral mimicry is a positive correlation between geographic proximity to the model and mimic fitness (Peter and Johnson 2008). Such correlations, however, can also occur in generalized food deception through facilitation or the magnet effect, where an abundance of rewarding plants increase local pollinator densities (Johnson et al. 2003b). Consequently, distinguishing between the mimic and the magnet effect may be difficult. We suggest that perhaps the strongest demonstration of Batesian floral mimicry may be a correlation between model-mimic trait similarity and mimic fitness. While we are not aware of any such study, a reciprocal translocation experiment by Newman et al. (2012) revealed that poor colour matching between the mimic *D. ferruginea* and its models resulted in reduced pollinator visits to the mimic. A decrease in pollinator visitation has also been reported when the inflorescence architecture of a mimic was manipulated to no longer match that of its model (Johnson et al. 2003a).

How do pollinators distinguish between species?

Interestingly, our results show that butterflies can discriminate between orange *D. ferruginea* and its orange model *K. uvaria*, where a previous study demonstrated the same species of butterfly is unable to distinguish between the red *D. ferruginea* and its red model, *T. triticea* (Johnson 1994). While this may suggest the two ecotypes occupy different positions on the generalization-specialization continuum, we propose they are equally specialized as each closely resembles a single model phenotype in their communities. Certain characteristics of the orange *K. uvaria* may simply make it more difficult to resemble (i.e. number of flowers). Laboratory experiments with bees have shown that employing multiple traits instead of a single trait can improve the discriminatory abilities of pollinators in a mimicry situation (Kunze and Gumbert 2001). For example, the effectiveness of visual cues for eliciting feeding responses from pollinators can be complemented by floral scent (Raguso and Willis 2002), which may help pollinators discriminate between morphologically similar flowers.

Floral scent has so far not been identified as an important trait in Batesian floral mimicry (Jersáková et al. 2012). *Tritoniopsis* flowers pollinated by long proboscid flies (Manning and Goldblatt 2005) and flowers pollinated by the butterfly *A. tulbaghia* (Johnson and Bond 1994) are unscented to the human nose, and both pollinators have often been recorded visiting plastic or paper model flowers without scent (Jersáková et al. 2012; Newman et al. 2012). Although bees might utilize scents that humans are not sensitive to, previous studies demonstrated that even when floral scent is present and differs between models and mimics, bees are easily deceived if mimics exhibit a similar floral colour to their models (Galizia et al. 2005), suggesting scent is unlikely used to distinguish models from mimics in these systems.

Field studies on rewarding *Oxalis* L. plants found that co-occurring species which are extremely similar in floral colour do not overlap in flower size, and that honeybees likely use size traits to reliably distinguish between these species (de Jager et al. 2011). Despite their colour similarity, *D. gladioliflora* and *T. ramosa* flowers differ significantly in size, including floral width, height and tube/spur length, which may promote discrimination by their pollinators. *Disa ferruginea* and its model, *K. uvaria*, also differed considerably in flower morphology, with *K. uvaria* exhibiting many small actinomorphic flowers and *D. ferruginea* fewer and wider zygomorphic flowers. The spur length of orange *D. ferruginea* in our community was also significantly shorter than the corolla length of *K. uvaria* (Johnson 1994). Pollinators may prefer larger flowers and inflorescences and likely employ such morphological differences to identify the model and visit it preferentially. This discrimination may only occur at close range to the flower, i.e. just prior to landing, which could explain why more inspections by bees to the mimic *D. gladioliflora* did not translate into more visits.

Although we did not specifically test it, pollinator decisions may possibly follow a hierarchy, where they first respond to floral colour, followed by size and shape at closer range. Such a hierarchy has been documented in a congeneric Batesian floral mimic pollinated by long proboscid flies (Jersáková et al. 2012). Interestingly, bee pollinators of sexually deceptive orchids that mimic female insect sex pheromones also exhibit hierarchies, responding to floral scent followed by floral colour at close range (Streinzer et al. 2009). Bee fly pollinators of sexually deceptive daisies, which mimic female insect morphology instead of sex pheromones, also exhibit hierarchies where pollinators respond firstly to morphology and secondly to floral scent (de Jager and Ellis 2012). Pollinators of *D. gladioliflora* and *D. ferruginea* may thus utilize secondary attractive traits such as flower size and shape to decide whether they visit a mimic, after initial colour attraction.

Another factor influencing pollinator decisions is the phenotype of recently visited flowers. Bee pollinators likely retain their experiences from only a few previous visits and not all visits made over their lifetime (Chittka et al. 1997). It has been well established that pollinators are more likely to switch to a new species when it is similar in floral colour to the previously visited species (Chittka et al. 1997; Gumbert and Kunze 2001; Johnson et al. 2003b; de Jager et al. 2011). These factors, in conjunction with the low cost of visiting mimics will all contribute to the pollination of rare rewardless mimics that resemble rewarding flowers, despite evidence of some pollinator discrimination. We suggest that incomplete discrimination by pollinators between models and mimics still represent Batesian mimicry if a single model phenotype is being mimicked, and we recommend that future studies should not rule out Batesian floral mimicry in systems where pollinators exhibit some discrimination.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interests.

Information on Electronic Supplementary Material

Online Resource 1. List of flowering plants in the community during fieldwork over two years.

Online Resource 2. Nectar guides of the mimic *Disa gladioliflora* and its model, *Tritoniopsis ramosa*, in BeeSpace and FlySpace.

References

- Ackerman J (1986) Mechanisms and evolution of food-deceptive pollination systems in orchids. *Lindleyana* 1:108–113
- Anderson B, Johnson SD (2006) The effects of floral mimics and models on each others' fitness. *Proc Roy Soc London Ser B Biol Sci* 273:969–974. doi:10.1098/rspb.2005.3401
- Anderson B, Johnson SD, Carbutt C (2005) Exploitation of a specialized mutualism by a deceptive orchid. *Amer J Bot* 92:1342–1349
- Bates HW (1862) Contribution to an insect fauna of the Amazon Valley. Lepidoptera: Heliconidae. *Trans Linn Soc London* 23:495–566
- Brown JH, Kodric-Brown A (1979) Convergence, competition and mimicry in a temperate community of hummingbird-pollinated flowers. *Ecology* 60:1022–1035
- Bytbeier B, Bellstedt DU, Linder HP (2008) A new phylogeny-based sectional classification for the large African genus *Disa*. *Taxon* 57:1233–1251

- Campbell DR, Waser NM, Melendez-Ackerman EJ (1997) Analyzing pollinator-mediated selection in a plant hybrid zone: hummingbird visitation patterns on three spatial scales. *Amer Naturalist* 149:295–315
- Chittka L (1992) The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. *J Comp Physiol A* 170:533–543
- Chittka L, Gumbert A, Kunze J (1997) Foraging dynamics of bumble bees: correlates of movements within and between plant species. *Behav Ecol* 8:239–249
- Cozzolino S, Widmer A (2005) Orchid diversity: an evolutionary consequence of deception? *Trends Ecol Evol* 20:487–494. doi:10.1016/j.tree.2005.06.004
- Cropper SC, Calder DM (1990) The floral biology of *Thelymitra epipactoides* (Orchidaceae), and the implications of pollination by deceit on the survival of this rare orchid. *Pl Syst Evol* 170:11–27. doi:10.1007/BF00937846
- Dafni A (1984) Mimicry and deception in pollination. *Annual Rev Ecol Syst* 15:259–278
- Dafni A, Irvi Y (1981) Floral mimicry between *Orchis israelitica* Baumann and Dafni (Orchidaceae) and *Bellavalia flexuosa* Boiss (Liliaceae). *Oecologia* 49:229–232
- De Jager ML, Ellis AG (2012) Gender-specific pollinator preference for floral traits. *Funct Ecol* 26:1197–1204. doi:10.1111/j.1365-2435.2012.02028.x
- De Jager ML, Ellis AG (2014) Costs of deception and learned resistance in deceptive interactions. *Proc Roy Soc London Ser B Biol Sci* 281:20132861
- De Jager ML, Dreyer LL, Ellis AG (2011) Do pollinators influence the assembly of flower colours within plant communities? *Oecologia* 166:543–553. doi:10.1007/s00442-010-1879-7
- Dyer A, Chittka L (2004) Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (*Bombus terrestris*) as a case study. *J Comp Physiol A* 190:105–114. doi:10.1007/s00359-003-0475-2
- Endler JA, Mielke PW (2005) Comparing entire colour patterns as birds see them. *Biol J Linn Soc* 86:405–431. doi:10.1111/j.1095-8312.2005.00540.x
- Ferdy J-B, Gouyon P-H, Moret J, Godelle B (1998) Pollinator behavior and deceptive pollination: learning process and floral evolution. *Amer Naturalist* 152:696–705
- Fisher RA (1930) The genetical theory of natural selection. Clarendon Press, Oxford, pp 164–167
- Galizia CG, Kunze J, Gumbert A et al (2005) Relationship of visual and olfactory signal parameters in a food-deceptive flower mimicry system. *Behav Ecol* 16:159–168. doi:10.1093/behco/arh147
- Gaskett A, Winnick C, Herberstein M (2008) Orchid sexual deceit provokes ejaculation. *Amer Naturalist* 171:E206–E212. doi:10.1086/587532
- Goldblatt P, Manning JC (2000) The long-proboscid fly pollination system in Southern Africa. *Ann Missouri Bot Gard* 87:146–170
- Gumbert A, Kunze J (2001) Colour similarity to rewarding model plants affects pollination in a food deceptive orchid, *Orchis boryi*. *Biol J Linn Soc* 72:419–433. doi:10.1006/bijl.2000.0510
- Jersáková J, Johnson SD, Kindlmann P (2006) Mechanisms and evolution of deceptive pollination in orchids. *Biol Rev Cambridge Philos Soc* 81:219–235. doi:10.1017/S1464793105006986
- Jersáková J, Johnson SD, Jürgens A (2009) Deceptive behaviour in plants. II. Food deception by plants: from generalized systems to specialized floral mimicry. In: Baluška F (ed) Plant-environment interactions. Springer, Berlin, Heidelberg, pp 223–246. doi:10.1007/978-3-540-89230-4
- Jersáková J, Jürgens A, Šmilauer P, Johnson SD (2012) The evolution of floral mimicry: identifying traits that visually attract pollinators. *Funct Ecol* 26:1381–1389. doi:10.1111/j.1365-2435.2012.02059.x
- Johnson S (1994) Evidence for Batesian mimicry in a butterfly-pollinated orchid. *Biol J Linn Soc* 53:91–104. doi:10.1006/bijl.1994.1062
- Johnson SD (2000) Batesian mimicry in the non-rewarding orchid *Disa pulchra*, and its consequences for pollinator behaviour. *Biol J Linn Soc* 71:119–132. doi:10.1006/bijl.1999.0430
- Johnson SD, Bond WJ (1994) Red flowers and butterfly pollination in the fynbos of South Africa. In: Arianoutsou M, Groves R (eds) Plant-animal interactions in mediterranean-type ecosystems. Kluwer, Academic publishers, Dordrecht, pp 137–148
- Johnson SD, Linder HP, Steiner KE (1998) Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). *Amer J Bot* 85:402–411
- Johnson SD, Alexandersson R, Linder HP (2003a) Experimental and phylogenetic evidence for floral mimicry in a guild of fly-pollinated plants. *Biol J Linn Soc* 80:289–304
- Johnson SD, Peter CI, Nilsson AL, Agren J (2003b) Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. *Ecology* 84:2919–2927
- Johnson SD, Bytebier B, Starker H (2015) Orchids of South Africa. *Struik Nature* 118–265
- Koshitaka H, Kinoshita M, Vorobyev M, Arikawa K (2008) Tetrachromacy in a butterfly that has eight varieties of spectral receptors. *Proc Roy Soc London Ser B Biol Sci* 275:947–954. doi:10.1098/rspb.2007.1614
- Kunze J, Gumbert A (2001) The combined effect of color and odor on flower choice behavior of bumble bees in flower mimicry systems. *Behav Ecol* 12:447–456
- Lindstrom L, Alantalo R, Mappes J (1997) Imperfect Batesian mimicry—the effects of the frequency and the distastefulness of the model. *Proc Roy Soc London Ser B Biol Sci* 264:149–153
- Manning JC, Goldblatt P (2005) Radiation of pollination systems in the Cape genus *Tritoniopsis* (Iridaceae: Crocoideae) and the development of bimodal pollination strategies. *Int J Pl Sci* 166:459–474
- Newman E, Anderson B, Johnson SD (2012) Flower colour adaptation in a mimetic orchid. *Proc Roy Soc London Ser B Biol Sci* 279:2309–2313. doi:10.1098/rspb.2011.2375
- Papadopulos AST, Powell MP, Pupulin F et al (2013) Convergent evolution of floral signals underlies the success of Neotropical orchids. *Proc Roy Soc London Ser B Biol Sci* 280:20130960. doi:10.1098/rspb.2013.0960
- Peter CI, Johnson SD (2008) Mimics and magnets: the importance of color and ecological facilitation in floral deception. *Ecology* 89:1583–1595
- Raguso RA, Willis MA (2002) Synergy between visual and olfactory cues in nectar feeding by naïve hawkmoths, *Manduca sexta*. *Anim Behav* 64:685–695. doi:10.1006/anbe.2002.4010
- Ramsey J, Bradshaw H, Schemske D (2003) Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution* 57:1520–1534
- Santer RD (2014) A colour opponent model that explains tsetse fly attraction to visual baits and can be used to investigate more efficacious bait materials. *PLOS Neglected Trop Diseases* 8:e3360. doi:10.1371/journal.pntd.0003360
- Smithson A, Juillet N, Macnair M, Gigord L (2007) Do rewardless orchids show a positive relationship between phenotypic diversity and reproductive success? *Ecology* 88:434–442
- Sprengel CK (1793) Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen. Vieweg
- Streinzner M, Paulus HF, Spaethe J (2009) Floral colour signal increases short-range detectability of a sexually deceptive orchid

to its bee pollinator. *J Exp Biol* 212:1365–1370. doi:[10.1242/jeb.027482](https://doi.org/10.1242/jeb.027482)

Thompson JD (2001) How do visitation patterns vary among pollinators in relation to floral display and floral design in a generalist pollination system? *Oecologia* 126:386–394. doi:[10.1007/S004420000531](https://doi.org/10.1007/S004420000531)

Troje N (1993) Spectral categories in the learning behaviour of blowflies. *Z Naturforsch* 48:96–104

Whitehead MR, Peakall R (2012) Short-term but not long-term patch avoidance in an orchid-pollinating solitary wasp. *Behav Ecol* 24:162–168. doi:[10.1093/beheco/ars149](https://doi.org/10.1093/beheco/ars149)