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# Flower colour adaptation in a mimetic orchid

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Although the tremendous variability in floral colour among angiosperms is often attributed to divergent selection by pollinators, it is usually difficult to preclude the possibility that floral colour shifts were driven by non-pollinator processes. Here, we examine the adaptive significance of flower colour in *Disa ferruginea*, a non-rewarding orchid that is thought to attract its butterfly pollinator by mimicking the flowers of sympatric nectar-producing species. *Disa ferruginea* has red flowers in the western part of its range and orange flowers in the eastern part—a colour shift that we hypothesized to be the outcome of selection for resemblance to different local nectar-producing plants. Using reciprocal translocations of red and orange phenotypes as well as arrays of artificial flowers, we found that the butterfly *Aeropetes tulbaghia*, the only pollinator of the orchid, preferred both the red phenotype and red artificial flowers in the west where its main nectar plant also has red flowers, and both the orange phenotype and orange artificial flowers in the east, where its main nectar plant has orange flowers. This phenotype by environment interaction demonstrates that the flower colour shift in *D. ferruginea* is adaptive and driven by local colour preference in its pollinator.

**Keywords:** Batesian mimicry; *Disa ferruginea*; ecological divergence; geographical colour variation; local adaptation; pollinator selection

## 1. INTRODUCTION

Angiosperms are characterized by extensive inter- and intra-specific variation in floral traits, many of which are heritable [1], under selection [2] and potentially important in the speciation process [3,4]. Although it is clear that the abiotic environment, herbivores and neutral processes can all play roles in generating floral divergence [5–9], divergence in floral form is most frequently ascribed to geographical mosaics or clines in pollinator composition—the ‘pollinator-shift’ or ‘Grant-Stebbins’ model of floral divergence [10,11]. Because different pollinators frequently have different functional morphologies and floral preferences, geographical variability in pollinator composition is likely to result in divergent selection pressures in allopatric populations. The importance of pollinator shifts in generating floral variation is supported by several lines of evidence, including the mapping of pollinators onto phylogenetic trees [12–14], pollination ecotypes [15,16], selection studies [17,18] and floral syndromes [19].

An extension of this idea is that geographical variation in the morphology or foraging preferences of a single pollinator (instead of shifts between pollinators) could provide the divergent selection pressures that lead to shifts in floral traits. One way that this could occur is through floral Batesian mimicry [20]. Floral Batesian mimicry is the adaptive resemblance of flowers of a non-rewarding plant species (mimic) to those of co-occurring rewarding plant species (model), where the function of this resemblance is to deceive pollinators into visiting the mimic. Deception is usually achieved through similarities in floral shape, scent

or colour [21–23]. Potential for floral divergence occurs if the non-rewarding plant coexists with different rewarding species across its geographical range. Partial evidence for this idea was obtained by Johnson [21], who showed that the non-rewarding South African orchid (*Disa ferruginea*) has allopatric red and orange floral morphs that are very similar in appearance to the red-flowered *Tritoniopsis triticea* (Iridaceae) and orange-flowered *Kniphofia uvaria* (Asphodelaceae), respectively. He demonstrated that the only pollinator of both deceptive orchid morphs is the large nymphalid butterfly *Aeropetes tulbaghia* that relies heavily on nectar from *T. triticea* and *K. uvaria*, making this a putative example of floral Batesian mimicry.

To test the hypothesis that flower colour in *D. ferruginea* is adaptive and driven by pollinator preference for the colours of rewarding flowers, we examined the following predictions: (i) spectral reflectances of the orchid should correspond geographically with those of its putative models; (ii) pollinators should exhibit preferences for flower colours of rewarding plants in the local community; and (iii) *Disa ferruginea* colour morphs translocated in their home site should be visited more often than *D. ferruginea* colour morphs translocated from elsewhere.

## 2. MATERIAL AND METHODS

To objectively assess whether the colour forms of *D. ferruginea* match their co-occurring models closely, we used spectrophotometry to measure the reflectance spectra (300–700 nm) of the two *D. ferruginea* colour forms and their respective models. Previous measurements of the flower colour of these plants [21] did not include the UV component. Plants used for colour analysis were collected from our study sites in the Langeberg (33°56′06″ S, 20°51′42″ E) and on Table Mountain (33°59′51″ S, 18°24′43″ E; figure 2). Measurements were

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taken with an Ocean Optics (Dunedin, FL, USA) USB 4500 spectrometer and a UV–vis 400 fibre optic reflection probe, held 5 mm away from the petal surface at 45°. Spectra were recorded from the inside of the lateral sepal in morphs of *D. ferruginea*, and from the upper lateral tepal in *T. triticea*. For *K. uvaria*, these were recorded from the outside of the perianth, at its centre. Five individuals per species/phenotype were sampled and the curves were averaged.

To further determine whether *D. ferruginea* colour forms associate geographically with their differently coloured putative models, we used data from herbarium records that include colour information. The point localities of all known *D. ferruginea* populations were mapped using geographical data from the Compton Herbarium, South African National Botanical Institute. Model localities were mapped only if they occupied the same 0.25° grid square and were within 100 m altitude of *D. ferruginea* populations within that grid. The association between model and mimic colours within the different grid squares was tested using Fisher's exact probability test. A grid square with both mimic colours, but just a single model was assumed to represent one matched and one mismatched site. A grid square with both model colours could not be included in the analysis owing to the non-independence of data.

To test whether the local colour forms of *D. ferruginea* reflect differences in local pollinator preferences, we performed reciprocal translocations between *D. ferruginea* populations in the Langeberg Mountains (orange morphs) and Table Mountain (red morphs), respectively. Pollinaria were removed from experimental plants to prevent local gene pool contamination. At each site, the emasculated red and orange orchid inflorescences of equal size were arranged in pairs in water-filled test tubes. These pairs were positioned at a height of 50 cm above the ground, with a 50 cm gap between pair members. Pairs were arranged in a rough circle around the observer, with at least 20 m between them. The performance of colour phenotypes in a foreign environment relative to that in their native environment was measured by recording the colour phenotype pair member first chosen by each approaching butterfly, as well as the total number of visits to the inflorescence.

Pollen deposition was not recorded, as there are spur-length differences between populations [21] that could potentially affect pollen deposition. As in most orchids [24,25], fruit set in *D. ferruginea* is generally pollen-limited and an increase in pollinator visits translates into increased fecundity [21], allowing us to use relative visitation rates as a proxy for fitness.

All pairs were observed between 07.00 and 10.00, and between 15.00 and 17.00 when pollinators were most active. At the end of each day, pair combinations were changed and the positions of the pairs swapped. Observations were made for 17 pairs of plants at the Langeberg site over 5 days, and 14 pairs on Table Mountain over 3 days.

To remove the possible effects of traits other than colour on pollinator preference, we repeated the experiment at each site using model inflorescences that approximated the shape of the orchid inflorescences. Upward pointing paper cones (height 13.5 cm, basal diameter 10 cm) matching the reflectance spectra (electronic supplementary material, figure S1) of the red and orange *D. ferruginea* phenotypes were placed in pairs on Table Mountain ( $n = 7$ ) and the Langeberg ( $n = 18$ ). Observations of pollinator behaviour were recorded as for the actual flowers; however, in addition

to butterflies landing on the cones, we also recorded inspection visits where butterflies circled within 30 cm of the paper cones.

Differences between sites in the proportion of first visits to red pair members were analysed with generalized linear models that considered a binomial distribution and logit link function [26], and were implemented in PASW (SPSS) v. 19.0 (IBM Inc.). Preferences for or against red pair members were assessed by considering whether the mean logits differed significantly from 0 (corresponding to an equal choice proportion of 0.5). The numbers of visits to pair members were analysed with generalized estimating equations that considered a Poisson distribution and a ln-link function, with colour and site as crossed factors. These models used an exchangeable correlation matrix to account for correlation among pair members, and score statistics to assess significance. For graphical representation of marginal (adjusted) mean and standard errors, we used back-transformed values, resulting in asymmetric standard errors.

### 3. RESULTS

The colours of red and orange *D. ferruginea* phenotypes closely match those of their sympatric models (figure 1a). Insects discriminate colours based on the wavelength differences of spectral peaks, but not differences in overall brightness/reflectance [27–29]. Here, the spectral peak for orange mimics and models starts at 550 nm, whereas the peak for red mimics and models starts at 600 nm. Herbarium data at the level of quarter degree grid squares suggest that the geographical distribution of the orange *D. ferruginea* morph is associated with the orange-flowered *K. uvaria*, whereas the red morph is associated with red-flowered *T. triticea* (figure 2). Colour matching occurred in five out of six localities with red models and all five localities with orange models ( $\chi^2 = 8.57$ ,  $p < 0.015$ ).

Measured as first approaches, butterflies significantly preferred *D. ferruginea* phenotypes that matched the colour of the model at each site ( $\chi^2 = 51.09$ ,  $p < 0.0001$ ; figure 1b). Inflorescences that matched the local model colour also received significantly more butterfly visits than those that did not (colour by site interaction:  $\chi^2 = 18.02$ ,  $p < 0.0001$ ; figure 1c). Similarly, butterfly choices for paper inflorescences reflected a preference for the colour that matched the rewarding plants at each site ( $\chi^2 = 24.2$ ,  $p < 0.0001$ ; figure 1b), and these received more visits than those that did not match the rewarding models (colour by site interaction:  $\chi^2 = 12.9$ ,  $p < 0.0001$ ; figure 1d).

### 4. DISCUSSION

This study demonstrates that the flower colour shift in *D. ferruginea* between the western and eastern parts of its distribution range is most likely an adaptation to the local colour preferences of its pollinator. Although intra-specific scent differences can play a role in pollinator choices [16], this is unlikely to be the case for *D. ferruginea* because butterflies choose between paper flowers using colour alone. Furthermore, models and mimics in this system have no scent discernible to the human nose.

The local colour matching of *D. ferruginea* to rewarding models is probably due to colour-based floral

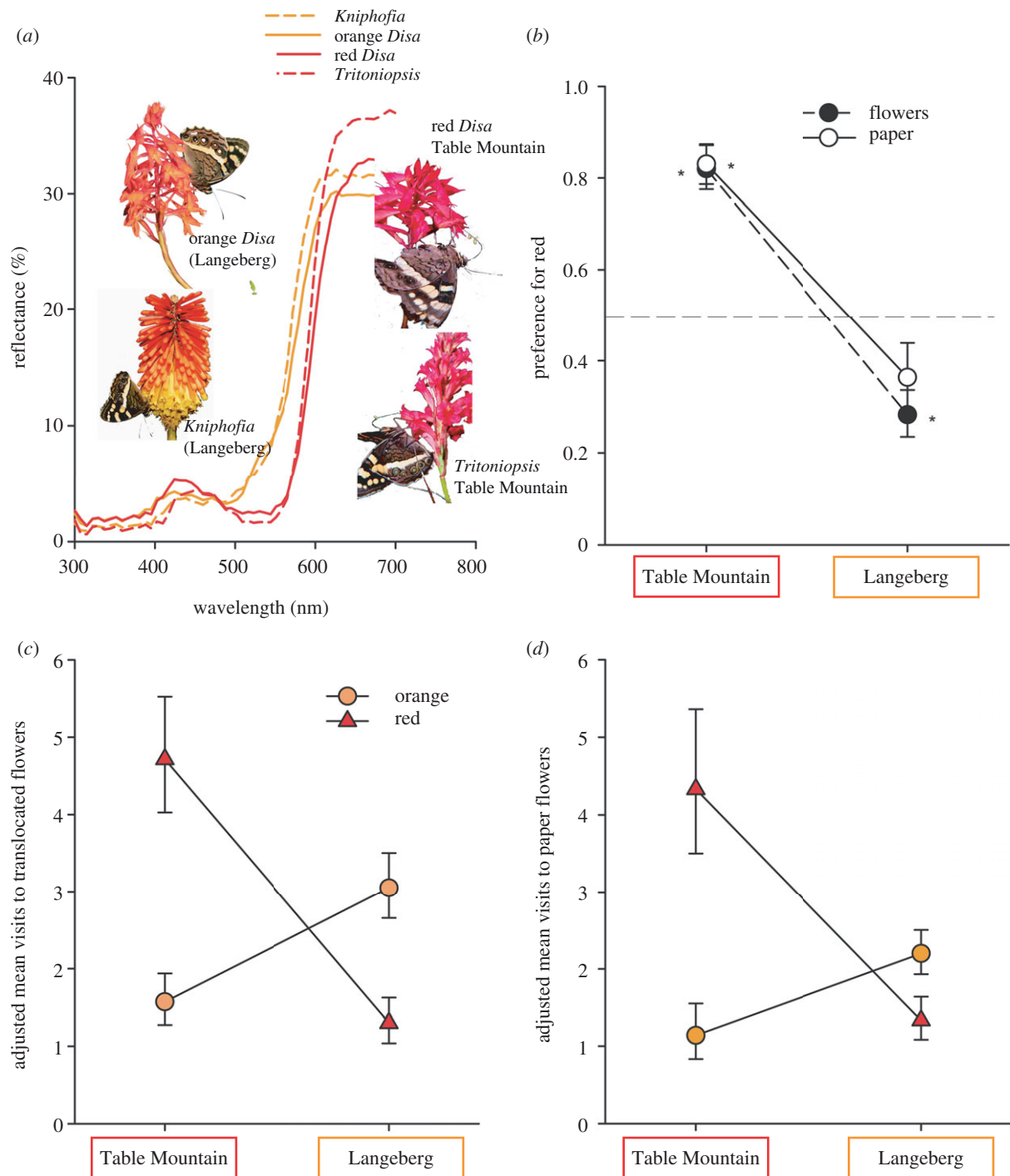


Figure 1. Butterfly responses to flower colour. (a) Reflectance spectra of deceptive red and orange *Disa ferruginea* mimics match those of their respective rewarding models. (b) Preference for red orchid phenotypes and paper flowers (over orange) in habitats with red (Table Mountain) and orange (Langeberg) rewarding flowers. Asterisks indicate means that differ significantly from 0.5 (no preference). (c) Numbers of visits to red and orange inflorescences and to (d) paper flowers at the Langeberg and Table Mountain sites.

Batesian mimicry, whereby a non-rewarding species evolves in response to pollinator preferences conditioned by rewarding model flowers. Butterflies could develop learned colour preferences either through positive experiences with rewarding flowers or negative experiences with non-rewarding flowers. In support of the former, there is compelling evidence to suggest that colour preferences of insect pollinators are readily conditioned by experience with rewarding flowers [30–32]. Traits of the mimics should thus track the traits of the model, but not the other way around (advergent evolution, *sensu* [20,32]).

Another explanation for model-mimic colour matching is that there is geographical variation in the innate preferences of the butterfly pollinators, which is then reflected in convergent evolution among unrelated plants. Unravelling the roles of learned as opposed to innate pollinator preferences in the evolution of colour similarity between plants represents a hurdle still to be overcome in order to distinguish between true Batesian mimicry (advergent evolution) and convergent evolution [22].

While spatial ‘race’ formation (mimicry rings) has frequently been described for animal mimicry systems [33],



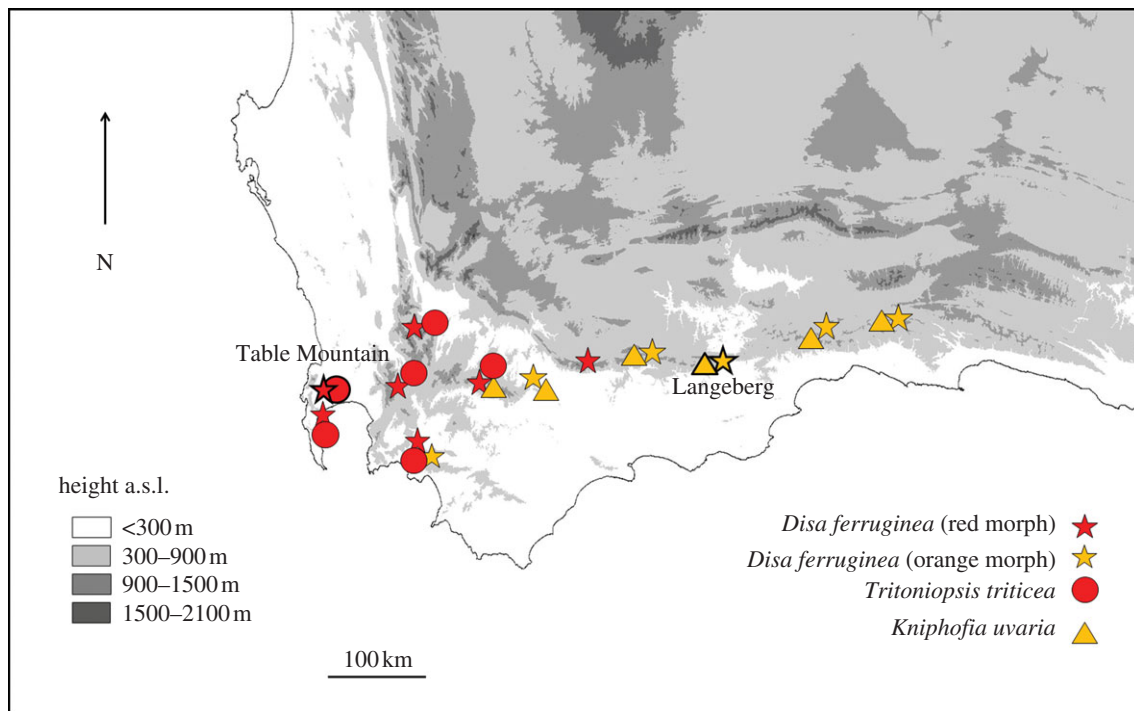


Figure 2. Herbarium records showing the geographical overlap of *Disa ferruginea* colour forms and rewarding model plant species in the Cape region of South Africa. Bold symbols indicate study sites.

the system described here may be the only documented example of such ‘race’ formation in a floral Batesian mimic. This is also one of the few studies that suggests that a single species of pollinator can drive floral variation; however, it is likely that this mechanism for driving floral divergence is under-reported because it is not as obvious as shifts in actual pollinator species. Other studies have suggested that geographical variation in morphology [33–38] or gender-specific foraging strategies [39,40] of a single pollinator can also drive floral divergence. Importantly, these studies imply that the current interpretation of the pollinator-shift model for explaining floral diversification is oversimplified in that pollinator choice or morphology are not species-level traits, but those that can vary between populations of the same pollinator [34–38]. Geographical variability and even temporal variability in pollinator preference is likely to be very common in the wild, because several studies have shown rapid learning in pollinators and that even innate preferences can be overruled by positive or negative learning experiences [30,31]. This makes it likely that geographical variation in pollinator preference can affect not only floral Batesian mimics but rewarding flowers as well. For example, Benitez-Vieyra *et al.* [41] show that the colour of the rewarding species *Turnera sidoides* varies geographically because it tracks the colour of the local Malvaceae floral community on which its bee pollinators are specialized.

Through tracking the colour of the local floral community, floral Batesian mimics as well as other rewarding flowers can potentially undergo colour shifts without pollinator shifts. This means that we should not simply dismiss the role of pollinators in driving floral variation in cases where no geographical differences in pollinator composition are found [42] and that the importance of pollinators in generating floral divergence may thus have been underestimated in the past.

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