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Preliminary evidence that the long-proboscid fly, *Philoliche gulosa*, pollinates *Disa karooica* and its proposed Batesian model *Pelargonium stipulaceum*

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Abstract

Long-proboscid flies have been documented as the primary pollinators in several long-tubed floral guilds in southern Africa. These specialized pollination systems have become models for studying floral mimicry, adaptation, and coevolution. In this study we document a previously unknown pollinator of *Pelargonium stipulaceum* (L.f.) Willd. (Geraniaceae) and *Disa karooica* Johnson & Linder, a species within the *Disa draconis* complex (Orchidaceae), and propose the occurrence of a new floral model-mimic system in the semiarid interior of South Africa. We find that *Philoliche gulosa* Wiedemann (Tabanidae) is a pollinator of the putative floral model, *Pelargonium stipulaceum* and its floral mimic, *Disa karooica*. We document similarities in floral spur/tube length, spectral reflectance, absence of scent, presence of nectar guides, and overlapping phenology and distribution in these two sympatric plant species. We use colour spectrum analyses to test the idea that divergence in flower colour in the *D. draconis* complex is driven by adaptation to model *Pelargonium* species of different colours.

Keywords: Batesian floral mimicry; *Disa draconis* complex (Orchidaceae); Geographic mosaic of coevolution; *Pelargonium*; Plant-pollinator interactions; Southern Africa; Speciation; Tabanidae

1. Introduction

Long-proboscid flies have been documented as primary pollinators in several long-tubed floral guilds in southern Africa. These specialized pollination systems have become models for studying floral mimicry, adaptation and coevolution (Johnson and Steiner, 1997; Manning and Goldblatt, 1997; Goldblatt and Manning, 2000a; Johnson, 2000; Johnson and Morita, 2006; Pauw et al., 2009). Long-proboscid fly pollination as defined by Goldblatt and Manning (2000a) is unique to two regions of the world: southern Africa and the Himalayan region. In southern Africa, long-proboscid flies have been estimated to be the primary pollinators of 25% of regional species of *Pelargonium* and approximately 10% of regional Iridaceae (Goldblatt and Manning, 2000a). Therefore, knowledge gained about these specialized plant-pollinator interactions may also have significant conservation implications.

The *Disa draconis* complex (Orchidaceae) is one of the best studied groups of long-proboscid fly pollinated plants (Johnson and Linder, 1995; Johnson and Steiner, 1997). The complex consists of four closely related taxa (Johnson and Linder, 1995) that produce long-spurred flowers in summer after the leaves have withered. All members of the group are thought to be pollinated by long-proboscid flies, and this has been established for three of the taxa: *Disa draconis* (L.f.) Sw. is pollinated by *Moegistorhynchus longirostris* Wiedemann (Nemestrinidae), and both subspecies of *D. harveiana* Lindl. are pollinated by *Philoliche rostrata* Linnaeus (Tabanidae). All three of these taxa are non-rewarding and rely on floral mimicry to attract pollinators (Johnson and Steiner, 1997). The pollination biology of the fourth taxon, *Disa karooica* Johnson & Linder, is the missing piece of the puzzle. This species occurs inland of the other three taxa and at higher altitude along the Roggeveld Escarpment that forms the arid eastern edge of the Cape Floristic Region.

Floral Batesian mimicry refers to the phenomenon in which non-rewarding plants (the mimic) imitate the floral morphology

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of rewarding plants (the model) to attract pollinators (Dafni, 1984; Roy and Widmer, 1999; Johnson et al., 2003). Several conditions must be met before Batesian mimicry can be established. These conditions are (1) the model and mimic overlap in distribution and phenology (2) the mimic resembles the model and thereby attracts the same pollinator(s) (3) the mimic has higher fitness when it co-occurs with the model and (4) the mimic occurs at a much lower frequency than the model (Roy and Widmer, 1999; Johnson et al. 2003). It has seldom been demonstrated that a putative mimic-model pair satisfies every one of these conditions (Johnson et al. 2003; Johnson and Morita, 2006).

In addition to providing general information about the phenomena of Batesian mimicry, groups of very closely related species, such as the *D. draconis* complex, provide an opportunity to study processes of divergence and speciation. Previous work by Johnson and Steiner (1997) provides evidence that divergence in floral spur length among the members of the *D. draconis* complex is driven by adaptation to pollinator species that differ in proboscis length. The members of the *D. draconis* complex also differ from one another in at least one non-structural feature, namely flower colour, which varies across taxa from mauve to cream and white. The possible role of pollinator-mediated selection and mimicry in driving this colour divergence has not been investigated.

In this study we determine the pollinator of *D. karooica*, and use floral trait measurements, distributional data and pollen load analysis to partially test the hypothesis that *D. karooica* is a Batesian mimic of a co-flowering nectar plant. We also use colour spectrum analyses to test the idea that divergence in flower colour in the *D. draconis* complex is driven by adaptation to model species of different colours.

2. Materials and methods

2.1. Study site

Field work was conducted in the winter-rainfall zone of the Roggeveld Karoo on the farm De Plaat near the town of Sutherland from 17–22 October 2008. Dominant plant species included the shrubs *Eriocephalus* spp., *Euryops lateriflorus*, *Dimorphotheca cuneata* and *Pteronia* spp. (all Asteraceae) and bunch grasses such as *Chaetobromus dregeanus* and *Ehrharta calycina* (Poaceae). *Disa karooica* was found growing at the base and within tussocks of grasses such as *C. dregeanus*. Plant species were identified using Goldblatt and Manning (2000b), Linder and Kurzweil (1999), Shearing (1994), and Van Der Walt and Vorster (1981). Vouchers have been deposited in the Stellenbosch University Herbarium.

2.2. Pollinators

Potential pollinators were captured with insect nets and checked for the presence of pollinaria. Pollinarium morphology was compared with that of pollinaria extracted from *D. karooica*. Blocks of fuchsin gel were used to remove other granular pollen from the same pollinators for examination under a compound microscope (Beattie, 1971). Pollen grains were compared with reference pollen collections from a potential model species. Reference collections were established from pollen extracted from voucher specimens of potential models. Proboscis measurements refer to non-extended mouthpart lengths (mm) of captured pollinators. Vouchers of pollinators have been deposited in the collections of the South African Museum.

2.3. Trait measurements

The farm was surveyed for the presence of potential model species. The total area surveyed was approximately fifteen hectares. From previous work in this pollination system (Johnson and Steiner, 1997; Manning and Goldblatt, 1997; Pauw et al., 2009), we assumed that the model would have an elongated floral tube and searched for any such species. Floral traits of *D. karooica* were compared with those of potential models. A one-way ANOVA was used to test for mean differences between tube/proboscis length in the model, the mimic and the pollinator. All statistical analyses were performed using the SPSS 12.0 (SPSS) Inc., Chicago, USA) statistical package (SPSS, 2003). The presence or absence of scent and nectar guides was determined by inspection in the field and laboratory. If present, the height of the nectar column inside the floral tube was measured in order to determine whether the nectar would be within reach of the proboscis of the potential pollinator. Flower colour was measured as spectral reflectance over the UV-visible range (300–700 nm), which was determined for three to five flowers of each species using an Ocean Optics (Dunedin, Florida, USA) USB4000 spectrometer and Ocean Optics PX-2 pulsed xenon light source . Readings were taken through a fiber-optic reflection probe (UV/VIS 400 µm) held at 45° approximately 5 mm from the surface of the dorsal petal. The replicate spectra for each species were averaged.

2.3. Trait divergence among related mimetic species

To test the hypothesis that adaptation to different models might be driving colour divergence in the *D. draconis* clade, colour spectra of *D. karooica* and its putative model were compared with spectra obtained for *D. draconis* and its putative model *Pelargonium longicaule* var. *longicaule* Jacq. (Geraniaceae). Flowers of this species pair were collected at Silverstoomstrand on 15 November 2008. Vouchers have been deposited in the Stellenbosch University Herbarium.

3. Results

3.1. Pollinators

Three long-proboscid horse flies belonging to the species *Philoliche gulosa* (two male, one female: Vouchers AP 429, 431-2) were captured mid-day while they hovered in the vicinity of the authors. One male caught near individuals of potential model and mimics had a pollinarium of *D. karooica*