

POLLINATION AND
GEOGRAPHICAL DIVERGENCE
IN FLOWER COLOUR OF THE
DROSERA CISTIFLORA
SPECIES COMPLEX

or

‘How the snottosie got its colours’

By

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A thesis submitted in conformity with the requirements for the degree of
Doctor of Philosophy

School of Life Sciences
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Abstract

The question of floral colour diversity among congeneric plants has intrigued evolutionary biologists since Lamarck—yet remains a hot topic amid the varied groundbreaking ecological insights that emerge to this day. I investigated potential causes of floral colour divergence in *Drosera cistiflora* sensu lato (Droseraceae), an insectivorous plant species complex which exhibits considerable variation over its geographical range in the Greater Cape Floristic Region of South Africa. Although several studies suggest that the foraging strategies of biotic pollination vectors can generate selective forces for floral trait diversification, no study has demonstrated unequivocally that pollinator-mediated selection is the core driver of shifts in flower colour. Indeed, selection by pollinating agents is not the sole possible explanation for floral colour disparity among populations, and other hypotheses, such as a role for edaphic factors, have been proposed as mechanisms modulating trends in flower colour. *D. cistiflora* s.l. is an exemplary study species complex for addressing these hypotheses as it displays remarkable heterogeneity in corolla colour—pink, purple,

red, white and yellow—both between and within populations, which occur across a range of soil types. My primary aim was to establish whether pollinators can explain spatial patterns of flower colour in the complex. The thesis is divided into the following chapters:

Chapter 1 is a general overview of the theory of pollinator-driven geographical divergence in floral traits, with a focus on flower colour, and includes a detailed account of the study species complex. Chapter 2 is an investigation into the breeding systems of *D. cistiflora* s.l., to assess whether floral attributes may reflect adaptations for allogamy. I discovered the complex to be highly pollinator-dependent for seed production with variable low autonomous selfing ability among floral colour forms and evidence for pollen limitation of fecundity. Chapter 3 examines associations between floral colour variation and the pollinating fauna and abiotic factors that may have played a role in the evolution of sympatric and allopatric floral colour forms. I show that respective forms are associated with geographically variable pollinator communities dominated by hopliine beetles (Coleoptera: Scarabaeidae: Hopliini), and that floral colour shifts appear to be largely independent of abiotic factors. These findings present a case for pollinator-mediated floral colour divergence in *D. cistiflora* s.l. In Chapter 4 I present the results of experiments using arrays (of models matched to *D. cistiflora* s.l. petal spectra and of reciprocally translocated flowering plants) that tested whether local pollinator discrimination among colours can explain geographical variation in flower colour. Analyses show that flower visitors discriminated significantly among models varying in colour and also among translocated flowers of *D. cistiflora* s.l. forms, and that the flower colour of the local *D. cistiflora* s.l. phenotype was generally favoured by insect visitors over introduced colours. Differential floral colour preferences and selection exhibited by polylectic pollinator communities across the range of study populations led me to conclude that the floral colour forms represent geographically divergent ecotypes adapted to broad pollinator assemblages. Chapter 5 specifically assesses the level of pollinator isolation between sympatric purple- and red-flowered *D. cistiflora* s.l. forms, to determine whether pollinators can maintain flower colour in the absence of macrogeographical barriers. Here, morphological observations and reproductive isolation indices demonstrate that strong pre-F₁ barriers to gene flow between the two forms are governed largely by floral isolation, through differences in flower colour and beetle pollinator preferences.

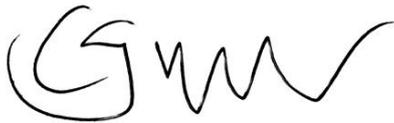
Evidence of early-acting postzygotic isolation is revealed by the significantly lower seed set from inter-colour hand cross-pollinations than from crosses within forms. Results support the taxonomic classification of endangered purple- and red-flowered *D. cistiflora* s.l. populations above the rank of form and thereby signal their unprecedented conservation need. The thesis thus provides new evidence for adaptive floral divergence driven by a generalist pollinating fauna and demonstrates how the study of pollination ecotypes may benefit red-listing and conservation of threatened plant populations with poorly understood taxonomic limits. Lastly, in Chapter 6, I offer a summary of my main findings and their evolutionary, taxonomic and conservation significance, and outline key areas for further research.

Preface

The research described in this thesis was carried out under the auspices of the School of Life Sciences, University of KwaZulu-Natal (Pietermaritzburg), on a part-time basis from May 2009 to December 2010, July 2011 to December 2015, and August 2016 to December 2019. Data were collected in 2009, 2010, 2011 and 2016. Research was conducted under the supervision of Professor Steven Johnson (University of KwaZulu-Natal) and Associate Professor Bruce Anderson (Stellenbosch University).

The thesis presents the original work of the author and has not been otherwise submitted (in part or in its entirety) to obtain any qualification. External contributions have been duly acknowledged in the text.

Signed:



.....
Caitlin Greta von Witt
(Candidate)



.....
Professor Steven Dene Johnson
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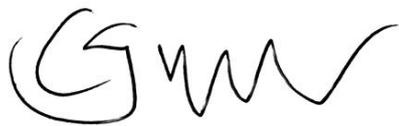
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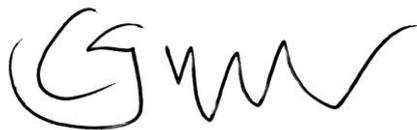
Publication 1

von Witt, C. G., Anderson, B. A., Durbach, I. N. & Johnson, S. D. (2019) Breeding systems of floral colour forms in the *Drosera cistiflora* species complex. Manuscript under revision.

Author contributions

CGvW, BA and SDJ conceived the ideas and designed methodology; CGvW collected the data; IND performed analyses with input from SDJ, and CGvW wrote the manuscript with guidance and assistance from SDJ and advice from SDJ and BA. All authors approved the manuscript for publication and agree to be accountable for all aspects of the work.

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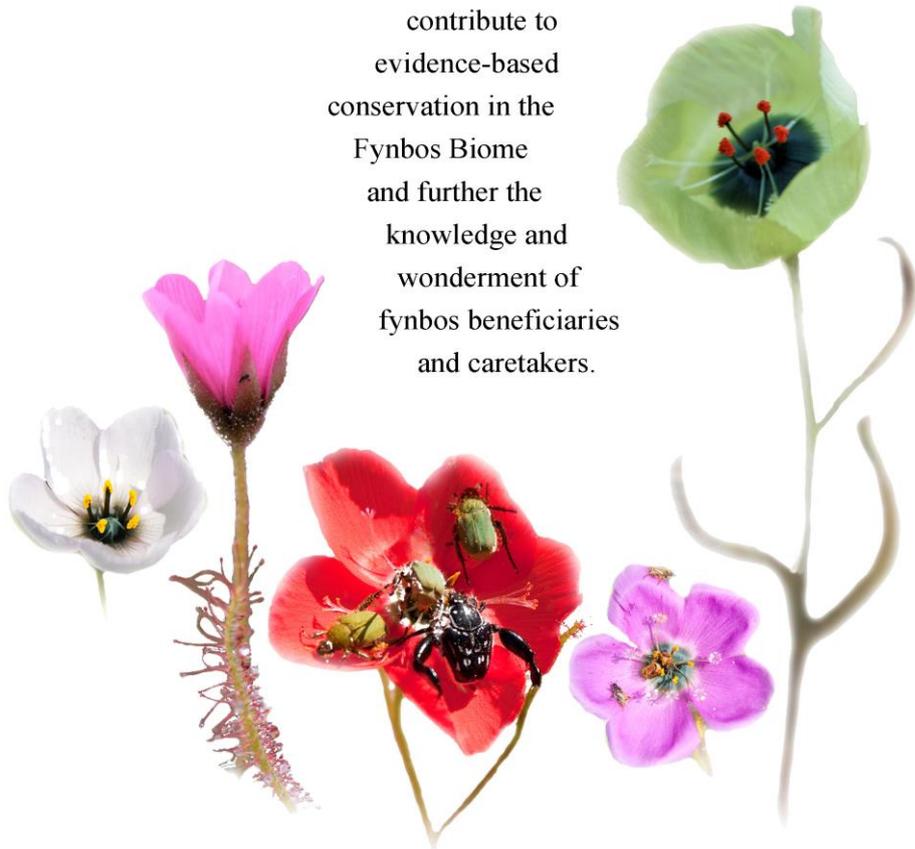


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Dedication

For fynbos people and wildlife

May this work
contribute to
evidence-based
conservation in the
Fynbos Biome
and further the
knowledge and
wonderment of
fynbos beneficiaries
and caretakers.





The hopline beetle *Omocrates* sp. visiting *Felicia tenella* (top) and purple-flowered *Drosera cistiflora* s.l. (above) at the same site near Darling



Pink-flowered *Drosera cistiflora* s.l. at Waylands, Darling

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This thesis would not have been possible without the support of others. I hope that the following testimonial demonstrates the extent of my appreciation for every contribution.

Firstly, I thank my supervisor, Prof. Steven Johnson, for his guidance and extensive input in this study; I am fortunate to have had such a distinguished teacher and am grateful for his patience and motivation. I am also thankful to my co-supervisor, Assoc. Prof. Bruce Anderson, for his vision for the study, ideas and candid comments.

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Dr Kim Steiner accompanied me in the field for most of the first season of my fieldwork. I learnt from his field expertise and enjoyed comparing notes, macro photos and miggie endurance strategies upon return from our respective study sites.

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I procured all the herbarium data from the Bolus and Compton Herbaria, and thank the respective curators Terry Trinder-Smith and Dr Koos Roux for permission to document *D. cistiflora* s.l. specimens. Edwina Marinus, Joy Tobin and Dr Anthony Magee of the Compton Herbarium subsequently accessioned my voucher specimens.

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I gratefully acknowledge the administrative support of Tanya Karalic and Tamlyn Skye (UKZN), as well as the attentive service provided by other institutional support staff involved, both at UKZN and SU.

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Amrei von Hase provided me with the 1:250 000 vegetation and geology layers I required for ARCVIEW GIS.

It is with respect and gratitude that I acknowledge the authors cited in this thesis. Their research has contributed greatly to my understanding of the subject and underpins many of the ideas, materials, methods and conclusions.

I pay tribute to the plants and insects that were sacrificed for the study and hope that any disturbance caused may be offset by a contribution to knowledge that improves conservation efforts.

Last, but by no means least, I extend my heartfelt appreciation to my parents Robert and Jane von Witt for nurturing my interest in natural science and for their constant support and unobtrusive encouragement; to my aunt Claire for sponsoring my computer; to Jaco Adendorff for the loan of a Nikon D70s kit and macro lens; to my dad for the German translations; to Shona, Cheryl, Janine, Carol and Audrey for their belief in me; to *all* my friends for cheering me on, but particularly to Robs, Ian, Theoni, Zanna, my brother Simon, Zooty, Lou, and to Doug and Edward who are sorely missed; to my ever-loyal dog Harry for his companionship while I stayed in Darling for my fieldwork, and to James, for his unfailing reassurance; I could not have done this without all of you.



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Table 3. Relative pollinator importance (%), i.e. percentage contribution per *Drosera cistiflora* s.l. floral colour form, of hopliine beetles (Coleoptera: Scarabaeidae: Hopliini) observed visiting *D. cistiflora* s.l., and of those trapped in model flowers and reciprocal translocations representing each floral colour form, in 2009 and 2010. Pollinator importance was calculated as the product of abundance in *D. cistiflora* s.l. flowers and average *D. cistiflora* s.l. pollen loads.

CHAPTER FIVE

Table 1. Morphological assessment of quantitative and qualitative characters of purple and red floral colour forms of *Drosera cistiflora* s.l. Means \pm SE in millimetres are provided for morphometric data, with sample sizes shown in parentheses and t-statistics and p-values reported for differences between mean values.

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Table 3. Individual components of reproductive isolation and their relative cumulative contributions to total isolation among purple and red *Drosera cistiflora* s.l. floral colour forms occurring in sympatry only (EI = 0), providing ranges for all defined habitat, phenological and pollinator isolation indices. A value of zero indicates a complete lack of isolating barriers and 1.00 indicates complete isolation. Relative cumulative contributions for each isolating barrier were calculated as percentages of the remainder from the previous barrier level (in order of effect in *D. cistiflora* s.l. life history), so that the sum of all contributions provides a value for total isolation between purple- and red-flowered *D. cistiflora* s.l. (shown in bold type).

Table 4. Individual components of reproductive isolation and their relative cumulative contributions to total isolation among purple and red *Drosera cistiflora* s.l. floral colour forms, for conservative choices of reproductive isolation indices defined for habitat, phenological and pollinator isolation. Here, ecogeographical isolation refers to isolation amidst the entire geographical range of purple- and red-flowered populations (involving sympatric and allopatric populations). A value of zero indicates a complete lack of isolating barriers and 1.00 indicates complete isolation. Relative cumulative contributions for each isolating barrier were calculated as percentages of the remainder from the previous barrier level (in order of effect in *D. cistiflora* s.l. life history), so that the sum of all contributions provides a value for total isolation between purple- and red-flowered *D. cistiflora* s.l. (shown in bold type).

Table 5. Individual components of reproductive isolation and their relative cumulative contributions to total isolation among purple and red *Drosera cistiflora* s.l. floral colour forms, providing ranges for all defined habitat, phenological and pollinator isolation indices. Ecogeographical isolation refers to isolation amidst the entire geographical range of purple- and red-flowered populations (involving sympatric and allopatric populations). A value of zero indicates a complete lack of isolating barriers and 1.00 indicates complete isolation. Relative cumulative contributions for each isolating barrier were calculated as percentages of the remainder from the previous barrier level (in order of effect in *D. cistiflora* s.l. life history), so that the sum of all contributions provides a value for total isolation between purple- and red-flowered *D. cistiflora* s.l. (shown in bold type).

CHAPTER SIX

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CHAPTER ONE

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CHAPTER ONE

GENERAL INTRODUCTION

This introduction provides a general overview of the theory of floral trait divergence, with a focus on flower colour, followed by a detailed account of the study species complex, *Drosera cistiflora* s.l., commonly known as the ‘snotrosie’. Finally, I outline the specific objectives of my thesis.

FLORAL TRAIT DIVERGENCE

A plant’s reproductive success may often depend on its ability to attract flower visitors; as a result, visitors that serve as pollinators can exert selection on floral traits that are alluring to them (Kay & Sargent 2009, Sletvold et al. 2016). Plant fitness may be enhanced by the effectiveness of pollinators during each visit, which gives rise to selection on floral traits that promote pollen receipt and export by pollinators (Trunschke et al. 2017). These dynamic traits include timing of anther dehiscence, anther position, pollen presentation, and stigma position, receptivity and morphology (Harder et al. 2001). Evidence that supports the theory that pollinators impose selection on floral traits has revealed: i) direct floral phenotypic adaptations to pollinators (e.g. Emms & Arnold 2000, Alexandersson & Johnson 2002, Hirota et al. 2013, Queiroz et al. 2015, Anderson et al. 2016, Sletvold et al. 2016, Fornoff et al. 2017); ii) convergent/advergent floral syndromes (e.g. Faegri & van der Pijl 1979, Fenster et al. 2004, Schiestl & Johnson 2013, Newman et al. 2014); iii) floral trait divergence consistent with pollinator shifts, shown by mapping of pollination systems onto phylogenetic trees (e.g. Johnson et al. 1998, Beardsley et al. 2003, Pérez et al. 2006, Smith et al. 2008, van der Niet & Johnson 2012, Valente et al. 2012), and iv) pollination ecotypes (e.g. Johnson 1997, Pérez-Barrales et al. 2007, Streisfeld & Kohn 2007, Anderson et al. 2010, Gómez et al. 2014, Newman et al. 2014, van der Niet et al. 2014, Parker et al. 2017).

Pollination ‘ecotypes’ (sensu Turesson 1922) may evolve in plant populations that exhibit local adaptation to pollinator assemblages across the geographical range of a species (Robertson & Wyatt 1990). This notion stems from the concept that pollinators can generate divergent selective pressures on floral traits in allopatric plant populations if there are spatial differences in pollinator assemblage or preference, consistent with the pollinator-shift model (q.v. Grant 1949; Grant & Grant 1965; Stebbins 1970; Whittall & Hodges 2007; Kay & Sargent 2009; Johnson 2010; Peter & Johnson 2014; van der Niet, Peakall & Johnson 2014; Ojeda et al. 2016; Gervasi & Schiestl 2017; Smith & Kriebel 2018), also termed the ‘Grant-Stebbins Model of Floral Divergence’ (sensu Johnson 2006). Although it is easy to imagine how spatial variation in the abundance of pollinators may result in divergent selection in plants with specialised pollination systems, it is less clear whether selection can suffice to generate divergence in more generalist plant taxa, where pollinators are diverse and often vary temporally. Contrary to conventional perception, Waser et al. (1996) showed that most plants display moderate to high levels of generalisation, leading to conflicting selection by pollinators (Ollerton 1996, Waser 1998, Ippolito et al. 2004). However, southern African plants exhibit substantially higher levels of specialisation than their American and European counterparts assessed by Waser et al. (Johnson & Steiner 2000, 2003; Ollerton et al. 2006; Anderson et al. 2014). Indeed, specialised adaptations of plants to their narrow guilds of pollinators are hypothesised to be one of the main drivers of speciation in the Greater Cape Floristic Region (Johnson 2010, Forest et al. 2014). Yet controversy abounds as compelling evidence also suggests that pollinators can drive evolution in plants with generalised pollination systems, where variation in the assemblage itself provides a selection mosaic. The model example by Gómez et al. (2008) suggested that phenotypic evolution and diversification of floral traits *can* occur in the generalist Mediterranean crucifer *Erysimum mediohispanicum* (Brassicaceae), where suites of diverse pollinating fauna selected for contrasting corolla shapes in different plant populations across the geographical range of the crucifer. Subsequently, Gómez et al. (2014) argued that *E. mediohispanicum* populations correspond to five pollination niches, which were found to be distributed in a phylogeographical mosaic. Here, correlation was demonstrated between the evolution of these pollination niches and floral traits, thus providing evidence for the presence of generalised pollination ecotypes.

Alternative studies have challenged the pollinator-shift model, suggesting that further hypotheses for floral divergence need to be investigated. In this context, appraisal of the South African spring annual daisy *Gorteria diffusa* (Asteraceae), which exhibits great diversity in floral morphology and ornamentation of the capitulum (rendering 14 discrete floral forms), found all of these forms to be primarily pollinated by a single species of *Megapalpus* bee fly, namely *Megapalpus capensis* (Diptera: Bombyliidae) [Ellis & Johnson 2009]. De Jager & Ellis (2011) subsequently suggested that the diverse array of floral phenotypic variation in *G. diffusa* may essentially be attributed to divergent selection imposed by the sexual behaviour of the fly on account of contrasting floral preferences of male and female flies. The most recent findings of Ellis et al. (2014) show that pollinator-mediated selection by mate-searching male flies influences integration of a suite of floral traits involved in pollinator attraction, positing that floral diversification may have arisen through variable selection along a gradient in levels of pollinator deception; although the ultimate reasons for the existence of this gradient remain elusive.

Studies of floral trait divergence motivated by pollinator explanations ought to account for biotic factors other than pollinators, including seed predators, herbivores, pathogens and nectar/pollen thieves; abiotic, such as edaphic, factors; physiological costs of flowers; genetic drift, and constraints on floral development (q.v. Herrera 1993, 1996; Galen 1999a&b; Frey 2004; Rey et al. 2005; Strauss & Whittall 2006; Ellis & Johnson 2009). In some cases pollinators might be the initial drivers of floral trait selection, but subsequent modifications may be ascribed to other selective agents (Herrera 1993, Strauss & Whittall 2006). For example, during years with abundant seed predators, selection in *Erysimum* is more likely a consequence of predator preferences than pollinator preferences (Gómez 2003). Thus, floral traits may effectively be a compromise between adaptations to pollinators and other agents.

Floral colour divergence

Floral colour shifts are thought to be one of the most common evolutionary transitions in the reproductive parts of plants (Rausher 2008) and numerous lines of evidence implicate pollinators as important selective agents for these transitions (e.g. Meléndez-

Ackerman & Campbell 1998, Newman et al. 2012, Hopkins & Rausher 2012, Tao et al. 2018, Streinzer et al. 2019). One of the strongest of these representations is that colour is often a key component of floral syndromes in guilds of unrelated plants sharing the same pollinator or class of pollinator, as is indicative of convergent evolution (Fenster et al. 2004). Nevertheless, the evolution of floral colour syndromes cannot necessarily be attributed to direct pollinator-mediated selection on flower colour (Rausher 2008). Accordingly, there is consensus that pollinators are not the only explanation for floral colour shifts and several other supported hypotheses have been proposed (Narbona & Wang et al. 2017).

Non-pollinator-driven floral colour divergence

Floral colour transitions have occasionally been attributed to spatial disparity in physical and/or chemical (abiotic) environmental conditions between floral colour forms, such as moisture (e.g. Schemske & Bierzychudek 2001), moisture and light (e.g. Arista et al. 2013) and moisture, light and nutrient (e.g. Ernst 1987) availability, as well as soil properties such as soil reaction/pH (Ito et al. 2009). For instance, the sepals of *Hydrangea macrophylla* (Hydrangeaceae) can vary in colour from blue to pink depending on the pH of the soils that support the plants (Ito et al. 2009). In such cases soils could determine flower colours, which in turn may affect the pollinators which are attracted to them. However, even if flower colour is associated with different soils, the actual causality may be indirect and not through plant physiology. One such example is that pollinator distributions may also be determined by edaphic factors. In this scenario, soils may determine pollinators, which circumstantially determine flower colours via selection.

Other non-pollinator-driven mechanisms that have been associated with the expression of floral colour transitions include genetic drift (q.v. Wright 1943a&b, 1978) and pleiotropy (q.v. Rausher & Fry 1993; Levin & Brack 1995; Simms & Bucher 1996; Fineblum & Rausher 1997; Armbruster 2002; Irwin et al. 2003; Coberly & Rausher 2003; Strauss & Whittall 2006; Carlson & Holsinger 2010, 2012).

Drift in the frequency of functional genes in a population probably rarely occurs in isolation of natural selection and is thus unconvincing as a primary explanation for floral colour shifts. To this end, Schemske & Bierzychudek (2001, 2007) provided strong evidence to refute Sewall Wright's (1943a&b) argument for random genetic drift as the basis for blue and white floral colour polymorphisms in *Linanthus parryae* (Polemoniaceae). Instead, their findings support the notion that natural selection maintains the polymorphism (q.v. Epling et al. 1960) and substantiate Mayr's (1965) proposal that "selective neutrality can be excluded almost automatically whenever polymorphisms or character clines are found in natural populations".

Pleiotropy has been postulated by multiple authors as an alternative to the pollinator-shift model with respect to flower colour, given that many of the enzymes involved in anthocyanin synthesis are also required for the synthesis of other flavonoid compounds. These may not only influence flower colour, but also other ecological and physiological traits (Rausher 2008). Here, floral pigmentation often correlates with vegetative pigmentation (Onslow 1925), where anthocyanins afford vegetative tissues photoprotective (Steyn et al. 2002) amongst other physiological functions and may promote the survival of plants amidst abiotic stresses (Strauss & Whittall 2006). For example, individuals of anthocyanin polymorphic plants with anthocyanin-pigmented flowers and vegetative tissues have been found to have greater survivorship and flower production than their non-pigmented correlates (Levin & Brack 1995). Further, anthocyanin pigments may confer a fitness advantage to plants under experimental conditions of drought (Warren & Mackenzie 2001). Non-activation of genes in anthocyanin pigment pathways may also affect flower colour, as seen in *Ipomoea purpurea* (Convolvulaceae), which has a low frequency of the white (*a*) allele as a result of a mutation blocking anthocyanin synthesis (Coberly & Rausher 2003). Here, white-flowered plants with a complete lack of anthocyanins demonstrated a reduced ability to survive heat and light stress when compared to pigmented plants (Coberly & Rausher 2003). Considering that the presence of anthocyanins in seedlings of *Clarkia unguiculata* (Onagraceae) determines petal colour in adult plants (Bowman 1987), it is even possible that floral colour transitions may be maintained via indirect selection for traits that may otherwise only have been retained in seedlings (Strauss & Whittall 2006).

There is now some evidence that pleiotropic effects can constrain the evolution of flower colour by opposing natural selection. Coberly & Rausher (2008) found that although pollinator-mediated natural selection favours the white (*a*) allele of *Ipomoea purpurea* (Fischer 1941), the effects of deleterious pleiotropy appear to counteract selection by pollinators and maintain the rarity of this allele. Genes controlling flower colour have additionally been shown to indirectly affect a plant's resistance to herbivory, which may counteract selective pressures by pollinators (Irwin et al. 2003, Frey 2004). This phenomenon can occur in a number of ways, through, for instance: i) discrimination of herbivores between flower colours (Vernon & Gillespie 1990, Gaum et al. 1994, Chyzik et al. 1995, Giamoustaris & Mithen 1996, Irwin et al. 2003); ii) associations between the synthesis of defensive plant compounds and floral pigments (Gyuláné et al. 1980, Simms & Bucher 1996, Fineblum & Rausher 1997, Irwin et al. 2003, Frey 2004), and iii) trade-offs between the allocation of resources for the synthesis of defensive plant compounds or floral pigments (Mole 1994).

Yet another consideration is that selection by seed predators can direct the evolution of flower colour. A classic case is the maintenance of the white/pink inflorescence colour polymorphism in 40% of *Protea* species (Proteaceae), which appears to be facilitated by selection associated with seed predators and negative trait pleiotropisms (Carlson & Holsinger 2010, 2012). A comprehensive study of *Protea aurea* established that these pink and white polymorphisms are maintained by the pleiotropic effects of selection through seed predation coupled with intrinsic differences in fecundity (Carlson & Holsinger 2012).

It is clear, therefore, that floral colour divergence cannot be attributed to pollinators alone, unless: i) such divergence is explicitly shown to be the immediate product of natural selection on floral colour forms (viz. affecting the frequency of genes directly expressing flower colour and not linked genes) and ii) a role for a definite selective agent other than pollinators can be excluded (Rausher 2008).

Pollinator-driven floral colour divergence

Diverse floral colour forms, both within and among populations, have long fascinated biologists (Kay 1978, Gigord et al. 2001). Given the colour vision of many flower visitors (Kevan & Baker 1983, Arnold 2010, Kelber & Osorio 2010, Renoult et al. 2017) and the synergistic colour signals of the flowers they pollinate (van der Kooi et al. 2019), along with frequent patterns of convergence in flower colour among plants pollinated by the same group of animals (Fenster et al. 2004, Newman et al. 2014), it has been widely speculated that these floral colour forms evolve through pollinator-mediated selection.

Moreover, there is now substantial evidence that pollinators discriminate between flower colours (e.g. Kay 1976; Hannan 1981; Wesselingh & Arnold 2000; Emms & Arnold 2000; Jones & Reithel 2001; Bradshaw & Schemske 2003; Streisfeld & Kohn 2005; Campbell et al. 2010, 2012; McGimpsey & Lord 2015). Under simplified experimental conditions, two species of bumblebee pollinators [*Bombus appositus* and *B. flavifrons* (Hymenoptera: Apidae)] discriminated between two floral colour forms of the snapdragon *Antirrhinum majus* (Plantaginaceae) [Jones & Reithel 2001]. Direct observations and time-lapse videos revealed that both native syrphid and introduced *Bombus* pollinators preferred white flowers—which were rewarding and receptive—over non-rewarding, unreceptive purple conspecifics of *Euphrasia dyeri* (Orobanchaceae) [McGimpsey & Lord 2015]. The variability of within-plant *E. dyeri* flowers, which rapidly changed colour from white to purple following pollination, thus appeared to yield a means for unpollinated flowers to be presented to their colour-discerning insect pollinators.

Evidence that pollinators elicit fitness differences among floral colour forms, and thereby impose selection on flower colour, is limited to only a few studies, namely Levin & Kerster (1967), Waser & Price (1981), Meléndez-Ackerman & Campbell (1998), Schemske & Bradshaw (1999), Gigord et al. (2001), Irwin & Strauss (2005), Campbell et al. (2012), Hopkins & Rausher (2012), Newman et al. (2012), Tao et al. (2018), and Streinzer et al. (2019). By painting *Ipomopsis tenuituba* (Polemoniaceae) and hybrid flowers red and comparing hybrid seed set to a control population, Meléndez-Ackerman & Campbell (1998) clearly showed that hummingbird pollinators

exert selection on flower colour and prefer red-flowered *I. aggregata* over white-flowered *I. tenuituba* in the hybrid zone. Notably, owing to restriction of the study to the hybrid zone, their results cannot be extrapolated to explain floral colour divergence between the two species. Gigord et al. (2001) found that the foraging preference of the bumblebee pollinators *Bombus lapidarius* and *B. terrestris* (Hymenoptera: Apidae) for rare floral colour forms of the European orchid *Dactylorhiza sambucina* (Orchidaceae) resulted in the maintenance of a purple–yellow floral colour polymorphism. This was achieved by pollinators enhancing fitness of the rare colour form through both the male and female components. Hopkins & Rausher (2012) demonstrated that reinforcing selection occurs on floral colour pigmentation as a result of non-random movement of certain butterfly pollinators (of the families Papilionidae and Hesperiiidae) in two sympatric *Phlox* (Polemoniaceae) species displaying light and dark pigmentation. Newman et al. (2012) found that local floral colour forms of the orchid *Disa ferruginea* (Orchidaceae) were favoured over novel forms by the butterfly *Aeroptes tulbaghia* (Lepidoptera: Nymphalidae). Fresh evidence by Tao et al. (2018), acquired through combined ecological and molecular evolutionary approaches, has shown that differential selection largely imposed by guilds of varying Apidae (bee) pollinators among two floral colour groups (namely pink and intermediate, and white) of the orchid *Spiranthes sinensis* (Orchidaceae) corresponds to the formation of well-defined clades for these groups. Here, selective pollinator colour preferences play a substantial role in maintaining the balanced polymorphism of the pink and white floral colour forms and appear to be inextricably linked to intraspecific reproductive isolation of the orchid.

The study by Newman et al. (2012) was notable for its use of both reciprocal translocations and model flowers to test whether the evolution of spatial discontinuity between orange- and red-flowered *D. ferruginea* may be attributed to divergent pollinator-mediated selection. Here, selection was imposed by a single pollinator species that was seemingly conditioned by the dominant flower colours in local communities. As yet, no study to my knowledge has conclusively demonstrated that generalist pollinators may influence floral colour expression, and the question remains whether allopatric floral colour forms can represent an adaptive response to differential pollinator assemblages.

STUDY SYSTEM

Genus Drosera

The carnivorous plant family Droseraceae comprises three genera, namely *Drosera* (commonly known as ‘sundews’), *Aldrovanda* and *Dionaea*, and has a cosmopolitan species distribution. *Drosera* includes over 150 species distributed in Australia, Africa, South and North America (Rivadavia et al. 2003), approximately 20 of which occur in South Africa (Fleischmann et al. 2008). Phylogenetic analyses indicate that the genus originated in Africa or Australia (Rivadavia et al. 2003).

The growth form in *Drosera* ranges from herbs to shrubs, with leaves covered in sticky glandular trichomes that trap and digest insects to supplement poor soil mineral nutrition. It is these mucilaginous glands for which *Drosera* is named, with the Greek root ‘*drosos*’, meaning ‘dewdrops’. Whilst the trapping mechanisms have been studied in depth, formal pollination research remains limited to a few studies, including that of Murza and Davis (2005), who studied the flowering phenology and pollination biology of *Drosera anglica* at two localities in mid-western Canada. *D. anglica* was found to be self-compatible and able to self-pollinate autonomously. Although flies (Diptera: Syrphidae) were determined to be the primary pollinators of the species, these were seldom observed and natural seed set occurred predominantly autonomously. Other pollination studies conducted in *Drosera* typically investigated pollinator-prey conflict, to determine whether these carnivorous plants have evolved mechanisms to reduce the incidence of pollinators being captured by leaf traps. Murza et al. (2006) recorded pollinator and prey assemblages in *Drosera anglica*, in which flowers are separated from basal trap leaves by a long flower stalk. Only one taxon (Thrips sp. larvae) was found to serve as both pollinator and prey. Measurements of selfing and outcrossing rates and pollinator/prey overlap in the morphologically differing *Drosera arcturi* and *Drosera spatulata* by Sciligo et al. (2007) showed *D. arcturi* to exhibit a high incidence of self-pollination in the form of pseudo-cleistogamy, prior and delayed selfing, whilst only delayed selfing was observed in *D. spatulata*. Pollinators were not observed in *D. spatulata* traps, which were consistently well separated from flowers; however, in *D. arcturi*, the same insect species often served as pollinator and prey. It was concluded that *D. arcturi* reproduced largely by selfing instead of outcrossing to reduce high

pollinator/prey conflict. Sciligo (2009) found three more New Zealand *Drosera* species, namely *D. stenopetala*, *D. auriculata* and *D. peltata*, to be capable of autonomous selfing. Selfing in *Drosera* (Table 1) was thus thought to reduce pollinator-prey conflict by alleviating pollen limitation. Anderson & Midgley (2001) detected an overall negative relationship between plant height and spatial separation of flowers and traps in over 50% of *Drosera* species globally (namely 17 South African and 36 Australian *Drosera* species); suggesting that the reduction of pollinator-prey conflict may be the exception, not the rule, in driving the evolution of long cymes. Anderson (2010) thereafter proposed that pollinator attraction, rather than pollinator-prey conflict, may be the selective force for long cymes, since a lack of pollinator-prey overlap was found in both the short-cymed *Drosera cistiflora* s.l. and long-cymed *Drosera pauciflora* s.l. and experimentally shortened plants received fewer visits by pollinators than taller plants. Jürgens et al. (2015) also showed that increased flower height improved pollinator visitation whilst flower-trap distance did not significantly influence the risk of pollinator trapping in models representing *D. arcturi* and *D. spatulata*. Here, pigmentation of trapping leaves played an important role in constraining pollinator capture, with the red pigment reducing pollinator attraction and thereby conferring pollinators with protection against trapping.

Table 1. Comparison of the presence of self-compatibility (SC) or self-incompatibility (SI) in *Drosera* species that have been tested (obtained from Sciligo 2009). *Drosera auriculata* is classified as SC and SI by reason of partial self-incompatibility.

Species	SC	SI	Study
<i>Drosera anglica</i>	*		Murza & Davis 2003
<i>Drosera arcturi</i>	*		Sciligo et al. 2007
<i>Drosera capillaris</i>	*		Essig 2013 (anecdotal)
<i>Drosera glanduligera</i>	*		Chen et al. 1997
<i>Drosera linearis</i>	*		Murza & Davis 2003
<i>Drosera nitidula subsp. omissa</i>	*		Chen et al. 1997
<i>D. peltata</i>	*		Sciligo 2009
<i>Drosera pulchella</i>	*		Chen et al. 1997
<i>Drosera rotundifolia</i>	*		Murza & Davis 2003
<i>D. spatulata</i>	*		Sciligo et al. 2007
<i>D. stenopetala</i>	*		Sciligo 2009
<i>Drosera tracyi</i>	*		Wilson 1995
<i>Drosera auriculata</i>	*	*	Chen et al. 1997 /Sciligo 2009
<i>Drosera bulbosa</i>		*	Chen et al. 1997
<i>Drosera bulbosa subsp. major</i>		*	Chen et al. 1997
<i>Drosera eneabba</i>		*	Chen et al. 1997
<i>Drosera erythrorhiza</i>		*	Chen et al. 1997
<i>Drosera gigantea</i>		*	Chen et al. 1997
<i>Drosera macrantha subsp. macrantha</i>		*	Chen et al. 1997
<i>Drosera manniana</i>		*	Chen et al. 1997
<i>Drosera menziesii subsp. basifolia</i>		*	Chen et al. 1997
<i>Drosera menziesii subsp. menziesii</i>		*	Chen et al. 1997
<i>Drosera orbiculata</i>		*	Chen et al. 1997
<i>Drosera radicans</i>		*	Chen et al. 1997
<i>Drosera rosulata</i>		*	Chen et al. 1997
<i>Drosera stolonifera subsp. compacta</i>		*	Chen et al. 1997
<i>Drosera stolonifera subsp. porrecta</i>		*	Chen et al. 1997
<i>Drosera stolonifera subsp. rupicola</i>		*	Chen et al. 1997
<i>Drosera tubaestylis</i>		*	Chen et al. 1997

The study species complex: Drosera cistiflora sensu lato

Drosera cistiflora s.l. is a species complex endemic to the fynbos and renosterveld vegetation of the winter-rainfall Greater Cape Floristic Region of South Africa. It is widely distributed in the Region, ranging east from Cape Town to Port Elizabeth and not far beyond Nieuwoudtville, in Namaqualand, in the north (Obermeyer 1970). Here *D. cistiflora* s.l. flowers in profusion in moist areas on slopes and flats in the austral springtime (Trinder-Smith et al. 2006), viz. August–October, with each flower lasting for only 1–3 days. The flowers resemble those of the genus *Cistus* (Cistaceae), from which the specific epithet ‘*cistiflora*’ is derived. *D. cistiflora* s.l. displays great variety in floral morphology with respect to petal pigmentation, where six corolla colours are known [namely pink, purple, red, salmon pink, white and yellow (Figure 3)], three of which are exceptionally rare and one (salmon pink) thought to be extinct in the wild (Eric Green 2008, pers. comm.). Populations have flowers either of mixed colours or, more commonly, are fixed for a single colour. The cymose inflorescences consist of 1–few large; 5-petalled; bisexual; actinomorphic, i.e. radially symmetrical, flowers (Obermeyer 1970) which are bowl-shaped, devoid of nectar (Goldblatt et al. 1998) and unscented to human olfaction (pers. obs). Apart from corolla colour, size and shape, there are other visible floral traits in *D. cistiflora* s.l. that may be adaptations for entomophily, where pollen is transferred from one flower to the stigma of another by an insect vector (q.v. Tcherkez 2004). These traits include dark centres; strong ‘herkogamy’ (sensu Axell 1869, quoted in Müller 1873), i.e. spatial separation of anthers and stigmas; fringed stigmas; pollen colour, and the raised presentation of anthers above the carpels (Figure 1). Tall stems (10–40cm) additionally raise the cymes above ground level, thereby promoting insect pollinator attraction (Anderson 2010) while allowing the plants to reach the light in areas where they become covered by the surrounding vegetation in their dormant phase (Obermeyer 1970).

Plants of *D. cistiflora* s.l. are perennial, with narrow, lanceolate rosulate and cauline leaves. Their thickened, fleshy roots act as underground storage organs for water and carbohydrates (Rivadavia 2000). The aerial parts disappear and the plants return to dormancy soon after the seeds are released, in accordance with drying of seasonal wetland substrates (pers. obs). Anthocyanin pigments are conspicuous in the stalked tentacle-like mucilaginous glands on the leaves (Ichiishi et al. 1999, Egan & van der

Kooy 2013), which are responsible for capturing and digesting prey. In addition to achieving pollinator protection (Jürgens et al. 2015), it is possible that, together with mucilage, anthocyanins in the trichomes act to reduce light and/or heat stress (Chalker-Scott 1999) to which the trichomes' large surface area may make them prone. Anthocyanins appeared to be present in the leaves of all *D. cistiflora* s.l. populations observed in this study, notwithstanding differences in flower colour (pers. obs).



Figure 1. A flower of the pink-flowered *Drosera cistiflora* s.l. displaying actinomorphy, a dark centre, contrasting petal and pollen colours, and spatial separation of anthers from the fringed stigmas

To date, there have been only two comprehensive pollination studies involving *D. cistiflora* s.l. (namely Goldblatt et al. 1998, Anderson 2010) and no previous study has determined whether the different flower colours are associated with different pollinator assemblages. Goldblatt et al. (1998) carried out a general investigation of pollination by hopline (monkey) beetles (Coleoptera: Scarabaeidae: Hopliini) in southern African petaloid geophytes, and suggested that *D. cistiflora* L. is primarily, and likely exclusively, pollinated by these beetles. While investigating the evolution of relatively

long cymes in *Drosera pauciflora* s.l. versus *D. cistiflora* s.l., Anderson (2010) affirmed the suggestion of Goldblatt et al. (1998) by finding that hopliine beetles appeared to be primary pollinators of *D. cistiflora* s.l. However, 42% of visitors were not hopliine beetles, and a single species of chrysomellid beetle comprised more than 18% of all visitors. Hopliine beetles are associated with flowers of a wide range of colours, with some evidence for differing colour preferences among species (Picker & Midgley 1996, Johnson & Midgley 2001, van Kleunen et al. 2007). Some hopliine species have a preference for red flowers and recent study of their visual systems suggests that they possess red-sensitive photoreceptors (Arnold 2010). Similarly, red, bowl-shaped, weakly scented flowers of the poppy guild in the East Mediterranean have been found to be primarily pollinated by scarabaeid beetles of the genus *Pygopleurus* (formerly included in *Amphicoma*) [Coleoptera: Glaphyridae], which chose odourless red flower models over other colours in experimental arrays (Dafni et al. 1990). Although cantharophily has classically been shown to be governed by scent production (Pellmyr & Thien 1986) over pigmentation, the aforementioned evidence has shown hopliine and *Pygopleurus* beetles to be chiefly attracted to visual cues such as ‘beetle marks’ (sensu Goldblatt et al. 1998) and colour, where colour is typically long-wavelength-reflecting (namely orange-red) for attraction of certain species of hopliine beetles (Picker & Midgley 1996, Johnson & Midgley 2001, Colville et al. 2002, van Kleunen et al. 2007) and *Pygopleurus* (Dafni et al. 1990). In stark contrast with their outer petal lobe colour, *D. cistiflora* s.l. populations have dark olive- or blue-green to grey floral centres (Figure 1), and similar patterns of colour contrasts have been shown to increase visitation by hopliine beetles in the irid geophytes *Sparaxis elegans*, *Hesperantha vaginata* and *Romulea monadelphae* (Iridaceae) on the Bokkeveld Escarpment (van Kleunen et al. 2007). Further contrasts are evident between *D. cistiflora* s.l. petal and pollen colours (Figure 1). Bernhardt (2000) designates such open bowl-shaped, odourless or faintly fragrant flowers displaying corolla pigmentation patterns frequently contrasting with pollen pigments to one of four modes of floral presentation in beetle-pollinated flowers: the Painted Bowl.

The easily observed Painted Bowl beetle pollination system with flowers bearing a range of corolla colours, equable dark centres, lack of floral nectar and odour, and typically large corolla size, alongside the abundance and wide geographical range of

populations (Figure 2), make *D. cistiflora* s.l. an ideal subject for study of floral colour shifts.

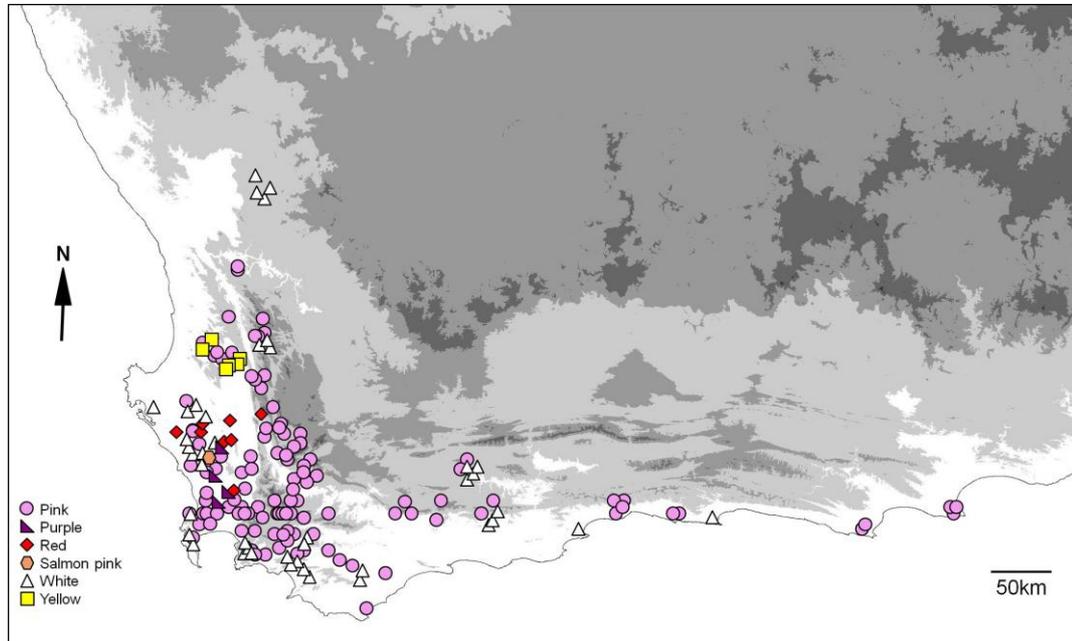


Figure 2. The geographical distribution of all known extant and extinct populations of *Drosera cistiflora* s.l. where corolla colour data has been formally recorded. Corolla colours include pink, white, red, yellow, purple and salmon pink (the orange shade of pink). Pink-flowered populations range from the Cape Peninsula to Port Elizabeth in the east, and white-flowered populations extend beyond Nieuwoudtville in the north. The greatest corolla colour diversity has been documented in the southwestern Cape, which is also the centre of *Drosera* species diversity in South Africa.

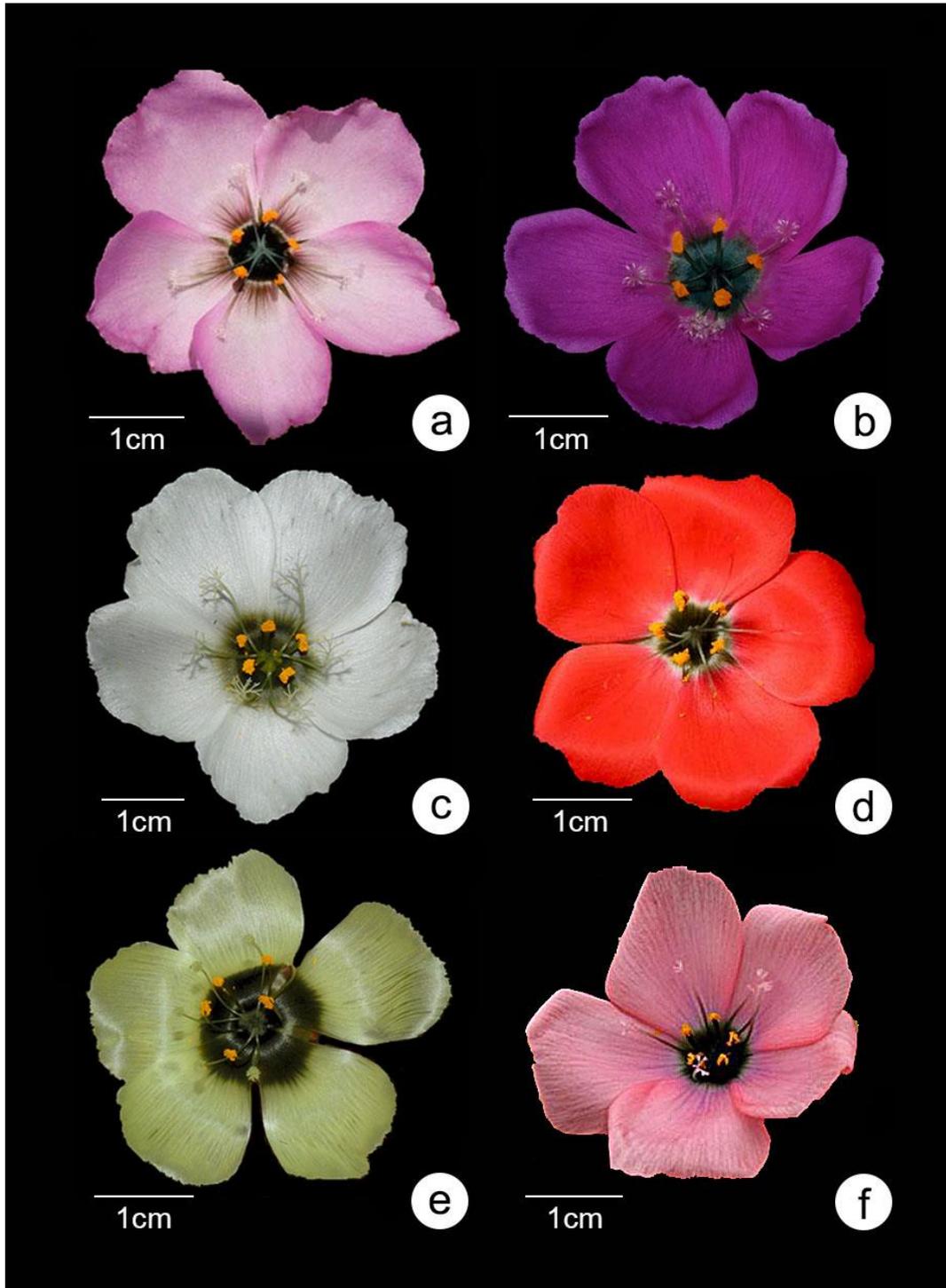


Figure 3. Corolla colours in the *Drosera cistiflora* species complex: pink (a), purple (b), white (c), red (d), yellow (e) and salmon pink (f). Photo (f) by Ignace Janssens.

Drosera cistiflora s.l. is one of few species complexes with a remarkably large range of corolla colours (Figure 3). The red- and yellow-flowered populations appear to have little variation within and between populations. Although red-flowered populations occur in sympatry with purple-flowered populations in two known locations, no individuals with intermediate colouration have been observed. Likewise, a site of sympatric red- and white-flowered populations is known, as well as one with both purple- and white-flowered populations, and another with pink- and purple-flowered populations, and none of these populations display visible floral colour intermediates. In contrast, populations of pink-flowered plants may frequently vary in flower colour from deep pink to white (Figure 4), indicating intermediate inheritance of flower colour through incomplete dominance in allele expression. However, flowers in most pink- and white-flowered populations are fixed for either end of the pink–white spectrum. Similarly, floral colour transitions may occur in the hybrid zone between two taxa. For example, the magenta-flowered *Antirrhinum majus* subsp. *pseudomajus* and yellow-flowered *Antirrhinum majus* subsp. *striatum* display unstable clines in floral colour variation between populations in the region where their ranges meet (Whibley et al. 2006).



Figure 4. Intrapopulation corolla colour variation of pink-flowered *Drosera cistiflora* s.l. in Darling, Western Cape (a) showing floral colour variation from pink (b) to white (e)

Drosera cistiflora L. is taxonomically classified within *Drosera* L. subgen. *Drosera* sect. *Ptycnostigma* Planch., largely using style morphology, a lack of stipules, thickened roots, corolla size and general habit (viz. the presence of cauline leaves) as delimitations (Schlauer 1996). The systematic considerations of Seine & Barthlott (1994), which are based on morphological, anatomical, palynological and cytotaxonomical studies, parallel this classification, and specifically recognise radially symmetrical marginal tentacles in the cauline leaves of *D. cistiflora* L., and a chromosome number of $2n = 60$ in section *Ptycnostigma*. However, more recently, phylogenetic analyses of DNA sequences of the chloroplast *rbcL* gene reveal the presence of two clades in *D. cistiflora* [sic], characterised by chromosome numbers $2n = 40$ and $2n = 60$ (Rivadavia et al. 2003, Figure 5). These findings might indicate the presence of polyploidy (q.v. Ramsey & Schemske 1998) within the species complex. Here, chromosome numbers (sourced from Kondo & Olivier 1979 and Hoshi & Kondo 1998) were obtained from only two sources of plant material (namely “South Africa” and “ca 11km west of Port Elizabeth”), the *D. cistiflora* [sic] *rbcL* gene sequence was derived from a single voucher specimen, and flower colour was not specified. The study by Rivadavia et al. (2003) may therefore underestimate the number of operational taxonomic units in *D. cistiflora* s.l.

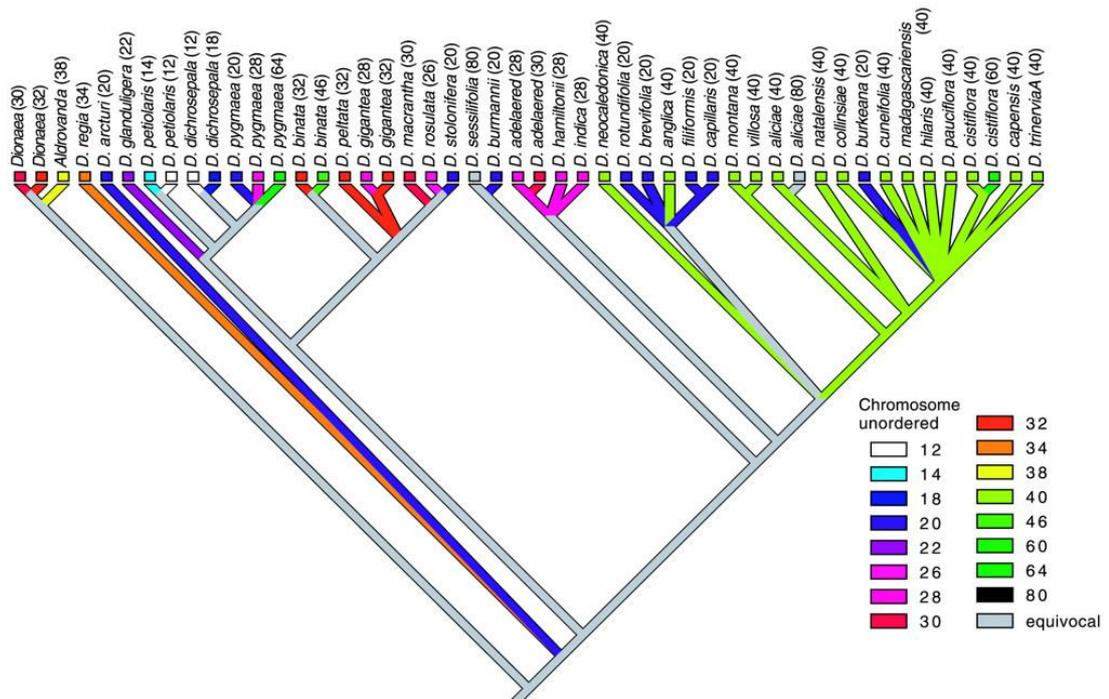


Figure 5. Strict consensus of the shortest trees obtained for *Drosera* using *rbcL* sequence data, onto which chromosome numbers have been mapped using MacClade (Maddison and Maddison 1992). More than one chromosome number has been reported for *Drosera cistiflora* [sic], with each chromosome number (indicated in parentheses) corresponding to a different operational taxonomic unit forming a clade. Reproduced from Rivadavia et al. (2003).

Preliminary observations of purple- and red- flowered populations suggest that morphological differences such as inflorescence positioning may be associated with floral colour disparity (Figure 6). Such differences were observed where these populations occurred in sympatry, suggesting a need for rigorous molecular work and further analyses of morphological characters in the *D. cistiflora* species complex.



Figure 6. Habitat of sympatric purple- and red-flowered *Drosera cistiflora* s.l. north of Darling, Western Cape (a) and close-up images showing morphological differences between purple- (b) and red-flowered (c) individuals. A lateral shoot arises at the junction of the stem and peduncle in purple-flowered plants (b) and is absent in red-flowered plants (c). Anthocyanins appear to be more abundant in the vegetative parts of red- (c) than purple-flowered (b) plants.

Although an extensive preliminary key to the genus was produced by Schlauer (1996), *Drosera* has not been comprehensively revised since Diels' (1906) classical monograph. Here, *D. cistiflora* L. was treated as having two varieties, namely *D. cistiflora* var. *δ. exilis* Diels and *D. cistiflora* var. *β. speciosa* (Presl) Diels (Table 2). Considering the variability of leaf size, shape, quantity, stem length, flower size and colour in the *D. cistiflora* species complex (Rivadavia 2000), a multitude of other varieties, and even some species-level splits, have been proposed (Table 2). The latter comprise *Drosera helianthemum* Planchon, *D. speciosa* Presl, *D. violacea* Willdenow, *D. zeyheri* Salter, *D. liniflora* Debbert, *D. rubripetala* Debbert, *D. coccipetala* Debbert and *D. variegata* Debbert. Of these, *D. liniflora*, *D. rubripetala*, *D. coccipetala* and *D. variegata* have been accepted (The Plant List 2013). It remains undecided whether *D. zeyheri* is a 'good' biological species or a diminutive growth form of *D. cistiflora* L., in which the enlarged basal rosette, shortened stem and scape represent adaptations to the high light conditions of open clearings or recently burnt areas where the plants appear (Obermeyer 1970). Additionally, although *D. rubripetala* is an accepted species name (Debbert 1991, The Plant List 2013), taxonomists remain dubious about whether it is a distinct species and seem hesitant to regard it as more than a synonym for *D. cistiflora* L. (e.g. Goldblatt & Manning 2000).

Table 2. *Drosera cistiflora* L.: synonyms and valid names. Brief descriptions and petal colour were obtained from type specimens and published material where available. Notes: * *Drosera zeyheri* Salter is not a formally accepted name (The Plant List 2013) and † *Drosera rubripetala* Debbert may also be regarded as a synonym of *D. cistiflora* L. (q.v. Goldblatt & Manning 2000).

<i>Drosera cistiflora</i> L. and synonyms	Coarse description, flowering time and locality	Petal colour	Type	Presently valid name	References
<i>Drosera cistiflora</i> L.	Plants to 30(–40)cm; >3 cauline leaves, lanceolate; inflorescence terminal; 1–few-flowered Aug.–Sep. Cape Peninsula to Namaqualand in the north and Port Elizabeth in the east	Pink, white or mauve	Locality, date and collector unknown (Lectotype; Linnaean Society of London Herbarium 398.6)	<i>Drosera cistiflora</i> L.	von Linnaeus 1760; de Candolle 1824; Diels 1906; Obermeyer 1970; Pers. obs
<i>Drosera cistiflora</i> var. <i>δ. exilis</i> Diels	Stems short, 5–15cm long; flowers much smaller than <i>D. cistiflora</i> L. Cape Hangklip and Riversdale	Whitish	Southwestern Cape; Riversdale; 1891/1893; C. Rust #627 (Type; Herbarium Berolinense)	<i>Drosera cistiflora</i> L.	Diels 1906
<i>Drosera cistiflora</i> var. <i>minutiflora</i> Eckl. et Zeyh.	Unknown (not described in Ecklon & Zeyher 1835)			<i>Drosera cistiflora</i> L.	The Plant List 2013; Schlauer 2014
<i>Drosera liniflora</i> Debbert	Stems 7–13cm tall, flowers 1–6, corolla small (1.5–2cm diam.); ovary and styles whitish; ovary 5x smaller than <i>D. cistiflora</i> L. Aug.–Sep. Found in wet montane slopes and rock walls in Bainskloof, Ceres and Tulbagh	Pale lilac, rarely white	Mountains near Ceres; Debbert #125 (Holotype; Botanische Staatssammlung München)	<i>Drosera liniflora</i> Debbert or <i>D. cistiflora</i> L.	Debbert 2002

<i>Drosera pauciflora</i> Banks ex DC. var. <i>minor</i> Sond.	Diminutive plants; all parts including flowers more slender than those of <i>D. pauciflora</i> Banks ex DC. Aug.–Sep. Western Cape	Pale rose or white	Caledon, “Zwarteberg”, wet places below the baths; C.F. Ecklon and C.L.P. Zeyher (Type; Biocentre Klein Flottbek and Botanical Garden, University of Hamburg)	<i>Drosera cistiflora</i> L.	Sonder 1859–1860; Salter 1940
<i>Drosera zeyheri</i> Salter	Diminutive plants to 9cm; leaves subrosulate, basal, oblong or oblanceolate, exstipulate; 0–3 basal, narrow cauline leaves; petals obovate; corolla approx. 3cm diam. Equivalent to <i>D. pauciflora</i> Banks ex DC. var. <i>minor</i> Sond. Aug.–Sep. Western Cape (white-flowered form restricted to Simonstown and Smitswinkel hills)	Pink or white	Caledon; Aug.; Zeyher #1920 (Isotype; Swedish Museum of Natural History, Department of Botany)	<i>Drosera zeyheri</i> * Salter or <i>D. cistiflora</i> L.	Salter 1940
<i>Drosera cistiflora</i> var. <i>zeyheri</i> (Salt.) Hort. Weiner				<i>Drosera cistiflora</i> L.	Schlauer 2014; Culham & Yesson 2018
<i>Drosera coccipetala</i> Debbert	Diminutive plants, short scapes (2–3cm long); occasionally 1–2 cauline leaves Aug.–Sep. Caledon or Tulbagh	Red or white	Caledon, on sandy loam soil; Debbert #127 (Holotype; Botanische Staatssammlung München)	<i>Drosera coccipetala</i> Debbert or <i>D. cistiflora</i> L.	Debbert 2002

<i>Drosera variegata</i> Debbert	Rosette leaves both prostrate and erect; cauline leaves (1–3) only in lowest quarter of stem; flower stem 7–15cm long; 1–2 (–3) flowers; corolla 3cm diam.; petals obovate	Pale violet, pale reddish or white	Cape Province, Ceres; Debbert #130 (Holotype; Botanische Staatssammlung München)	<i>Drosera variegata</i> Debbert or <i>D. cistiflora</i> L.	Debbert 2002
	Aug.–Sep.				
	Near Ceres and Tulbagh				
<i>Drosera cistiflora</i> var. 'Eitz'	Short stems with long, partially erect rosulate leaves; few, oblong cauline leaves located in basal third of stem	Mauve		<i>Drosera cistiflora</i> L.	Eric Green pers. comm. 2008
	Cederberg				
<i>Drosera cistiflora</i> 'Piketberg'	Plants to 20cm; linear rosulate and cauline leaves; cup-shaped corolla	Pale yellow, translucent	Rest, S of Piketberg, in sand; 1930; J. B. Gillett [Specimen labelled as <i>D. cistiflora</i> L. (flower colour: "yellow with black eye"); Compton Herbarium]	<i>Drosera cistiflora</i> L.	Pers. obs
	Aug.–Sep.				
	Piketberg				
<i>Drosera cistiflora</i> var. <i>a. alba</i> Thunb.	Flowers smaller than <i>D. cistiflora</i> L.	White, spotted at base		<i>Drosera cistiflora</i> L.	Thunberg 1818–1820; Sonder 1859–1860; Hooker 1890
<i>Drosera cistiflora</i> var. <i>alba</i> Sond.				<i>Drosera cistiflora</i> L.	The Plant List 2013; Schlauer 2014
<i>Drosera cistiflora</i> f. <i>alba</i> (Sond.) Hort. Weiner				<i>Drosera cistiflora</i> L.	Schlauer 2014

<i>Drosera speciosa</i> Presl	Smaller plants with fewer, narrower and less glandular leaves than typical <i>D. cistiflora</i> L.; fewer (typically 1–2) flowers “Blawberg”, Paardeneiland and Tygerberg, below 500ft	Vivid mauve	South Africa; 1838; Drege (Syntype; Royal Botanic Gardens, Kew)	<i>Drosera cistiflora</i> L.	Presl 1844; Planchon 1848; Sonder 1859–1860; Hooker 1890
<i>Drosera cistiflora</i> var. β . E. Meyer or <i>D. cistiflora</i> β . E. Meyer	All parts more slender than typical <i>D. cistiflora</i> L.; 1–2 flowers Equivalent to <i>D. speciosa</i> Presl “Blawberg”, Paardeneiland and Tygerberg, below 500ft	Vivid mauve, pale pink or white		<i>Drosera cistiflora</i> L.	Planchon 1848; Hooker 1890
<i>Drosera cistiflora</i> var. β . <i>rubra</i> Thunb.	Flowers larger than <i>D. cistiflora</i> L. Sep. In sandy areas near rivers “Bergrivier” and depressions “Saldanhabay” (“Cap.”)	Red, dark greenish base		<i>Drosera cistiflora</i> L.	Thunberg 1818–1820; Ecklon & Zeyher 1835
<i>Drosera cistiflora</i> var. β . <i>speciosa</i> (Presl) Diels	Stems short (8–16cm long), sparsely glandular; cauline leaves narrow; 1 (–2) flowers This variety appears to be a conflicting combinatio nova of the discrete taxon <i>D. speciosa</i> Presl. Aug. Cederberg, Darling, Hopefield surrounds, Malmesbury, Paarl and Piketberg	Magenta or scarlet	Leliefontein, Hopefield surrounds, Malmesbury; Aug. 1885; F. Bachmann #1014 (Type; Herbarium Berolinense)	<i>Drosera cistiflora</i> L.	Diels 1906
<i>Drosera rubripetala</i> Debbert	Rosette leaves linear; cauline leaves linear, acuminate; 1–2 (–3) flowers Aug.–Sep. Romansrivier	Burgundy	Romansrivier; 1991; Debbert # 129 (Holotype; Botanische Staatssammlung München)	<i>Drosera rubripetala</i> Debbert [†] or <i>D. cistiflora</i> L.	Debbert 1991

<i>Drosera cistiflora</i> var. <i>γ. multiflora</i> Eckl. et Zeyh. or <i>D. cistiflora γ.</i> <i>multiflora</i> Eckl. et Zeyh.	Plants robust; stem tall, 25– 40cm long, many-leaved; leaves rhombic; cymes many-flowered (2–6) Aug. Clanwilliam, Caledon and Rondebosch	Pale mauve or white	Cape of Good Hope; C. F. Ecklon #129 (Type; Botanic Garden and Botanical Museum Berlin- Dahlem, Freie Universität Berlin)	<i>Drosera cistiflora</i> L.	Ecklon & Zeyher 1835; Hooker 1890; Diels 1906
<i>Drosera</i> <i>helianthemum</i> Planch.	Tall, many-leaved, robust plants with rhombic leaves; 3–4(–6)-flowered Equivalent to <i>D. cistiflora</i> var. <i>γ. multiflora</i> Eckl. et Zeyh. Aug. Clanwilliam, Caledon and Rondebosch	Pale mauve or white	“Brackfontein”, Clanwilliam and “Klynrivier”, Caledon; Aug. 1835; C. F. Ecklon (Syntype; Royal Botanic Gardens, Kew)	<i>Drosera cistiflora</i> L.	Planchon 1848; Sonder 1859–1860; Hooker 1890
<i>Drosera violacea</i> Willd.	Plants 15–30cm; leaves linear-lanceolate; 1–2(–3) flowers; petals reflexed “Cap. Bona Spei”; Cape Town northern suburbs and Malmesbury District	Violet/ purple with dark blue– olive green metallic base	Cape of Good Hope, South Africa; 1803–1806; M.H.C. Lichtenstein (Specimen; Herbarium Willdenow in Herbarium Berlin)	<i>Drosera cistiflora</i> L.	Willdenow 1809; Sonder 1859–1860; Hooker 1890; Hamet 1907; Pers. obs
<i>Drosera cistiflora</i> var. <i>β. violacea</i> Thunb.	Possible equivalent to <i>D.</i> <i>violacea</i> Willd. with imprecise account of petal colour	Rosy, purple or red		<i>Drosera cistiflora</i> L.	Sonder 1859–1860; Hooker 1890
<i>Drosera cistiflora</i> var. <i>violacea</i> (Willd.) Sond.	Possible equivalent to <i>D.</i> <i>violacea</i> Willd.			<i>Drosera cistiflora</i> L.	The Plant List 2013; Schlauer 2014

<i>Drosera cistiflora</i> 'Purple West Coast'	Plants to 20cm; cauline leaves linear-lanceolate; flowers large; 1–2(–3) flowers; corolla crateriform; petals oblong-spathulate; lateral shoot frequently present on stem at base of peduncle Aug.–Sep. West Coast	Purple with blue-green metallic- iridescent base	<i>Drosera cistiflora</i> L.	Pers. obs
<i>Drosera cistifolia</i> L.	Possible typographic or reading error			Schlauer 2014; Culham & Yesson 2018
<i>Drosera cistoïdes</i> St.Hil.	Possible typographic or reading error			Prouvençal de Saint- Hilaire 1816; Schlauer 2014

Of those that have received taxonomic attention, the proposed *D. cistiflora* s.l. taxa examined in this thesis include *D. rubripetala* Debbert, *D. cistiflora* var. β . *rubra* Thunberg or *D. cistiflora* var. β . *speciosa* (Presl) Diels (red flowers); *D. violacea* Willdenow, *D. cistiflora* var. β . *violacea* Thunberg or *D. cistiflora* var. *violacea* (Willdenow) Sonder (purple flowers), and the typical *D. cistiflora* von Linnaeus (pink, mauve or white flowers).

For the scope of a pollination study relating to flower colour, I group *D. cistiflora* s.l. populations according to flower colour similarity and consider each group as a form. It should be noted that these forms are conjectural delineations of differing population groups and thus not circumscribed. Nevertheless, since the secondary taxonomic rank of form is used to characterise morphology and is not synonymous with clades in wild plants (Hamilton & Reichard 1992), this provisional classification offers a prudent approach that will not conflict with prospective taxonomic limits prescribed in the *D. cistiflora* species complex. Here, 'form' would seem a more appropriate treatment than 'morph' on account of geographical isolation between extant pink-, red- and yellow-flowered populations and the lack of evidence of prior contact zones (Figure 2), which

is at variance with the notion of polymorphism, where two or more morphs must occur in sympatry within their geographical range and thus have the potential to interbreed (Ford 1945). Further, despite corolla colour appearing to be the most obvious variable trait among groups of populations, additional morphological differences exist between these groups (Table 2, Figure 6). Without knowledge of pigment biosynthetic pathways and the potential pleiotropic effects of genes coding for pigment enzymes (Sobel & Streisfeld 2013) in *D. cistiflora* s.l., I cannot link all morphological differences to the expression of flower colour. In other words, I cannot be sure whether the *D. cistiflora* s.l. system represents a floral colour polymorphism (where morphs may have been lost over time, giving rise to the apparent spatial isolation of pink-, red- and yellow-flowered populations) or whether flower colour and other morphological differences are attributable to different gene pools among populations that may represent varieties, subspecies (or even species). The application of the ranks of subspecies and/or variety would, however, imply more certainty of infraspecific rank, specifically with respect to significant patterns of geographical, ecological or physiological isolation (subspecies) or lack thereof (varieties) [q.v. Clausen 1941]. Such taxonomic certainty shall remain beyond the ambit of this thesis and form classification thus appears to be the least presumptuous approach when considering *D. cistiflora* s.l. populations with different flower colours in the absence of genetic analyses.

Purple-flowered plants with reflexed petals [namely *D. violacea* Willdenow, *D. cistiflora* var. β , *violacea* Thunberg or *D. cistiflora* var. *violacea* (Willdenow) Sonder] and those with crateriform corollas (namely *D. cistiflora* ‘Purple West Coast’, Table 2) were grouped together as a single form based on striking similarity in petal colour: purple with a strongly metallic-iridescent blue-green base. *D. cistiflora* ‘Purple West Coast’ was however considered separately for reproductive isolation assessments that pertained specifically to purple-flowered plants with crateriform corollas. Likewise, the pink and white forms studied, although respectively similar in corolla colour and shape, may represent more than one genetic and/or evolutionary lineage.

The yellow floral colour form, which I have provisionally named *D. cistiflora* ‘Piketberg’, was rediscovered personally together with a local conservation group [Custodians of Rare and Endangered Wildflowers (CREW)] in Piketberg in 2008 (von Witt 2008), having previously been known from a 1930 specimen housed in the

Compton Herbarium as *Drosera cistiflora* L. One population of this form occurs within 1km of a typical pink-flowered form of *D. cistiflora* s.l. with no signs of introgression. The distinct corolla colour and cup-like shape additionally indicate that it warrants taxonomic investigation.

I treat *D. cistiflora* as a species complex for the purposes of this thesis given the absence of adequate taxonomic resolution by means of an extensive revision that considers molecular, morphological and ecological factors. The complex also includes diminutive and small-flowered ‘forms’ [namely *D. pauciflora* Banks ex de Candolle var. *minor* Sonder, *D. zeyheri* Salter, *D. cistiflora* var. *zeyheri* (Salter) Hort. Weiner, *D. coccipetala* Debbert, *D. variegata* Debbert, *D. cistiflora* var. ‘Eitz’, *D. cistiflora* var. *a. alba* Thunberg, *D. cistiflora* var. *β*. E. Meyer, *D. cistiflora* var. *δ. exilis* Diels, *D. cistiflora* var. *minutiflora* Ecklon et Zeyher and *D. liniflora* Debbert] and large and floriferous ‘forms’ (namely *D. cistiflora* var. *γ. multiflora* Ecklon et Zeyher and *D. helianthemum* Planchon) [Table 2] which were excluded from the study so that the ecology of flower colour could be consistently compared between populations. The study nonetheless examines all known extant floral colour forms of *D. cistiflora* s.l.: pink, purple, red, white and yellow.

SPECIFIC OBJECTIVES OF THIS THESIS

The main objective of this thesis was to investigate the potential for pollinator-driven floral colour divergence in the *Drosera cistiflora* species complex; I address this objective in Chapters 2–5, which are prepared for publication. Chapter 2 has been formatted for submission to *Plant Biology*.

The following describes the questions that are considered in each of the thesis chapters:

Chapter Two: *Breeding systems of floral colour forms in the Drosera cistiflora species complex*

In this chapter I ask whether *D. cistiflora* s.l. is self-incompatible and completely reliant on pollinator visits for seed production and whether seed set is naturally pollen-limited, since pollinator-mediated selection on secondary sexual traits is strongly affected by the degree to which seed production is pollen-limited (Knight et al. 2005). I also determine the overall dependence on pollinators and whether discrepancies in pollinator dependence exist between floral colour forms. Previous studies of *Drosera* pollination indicate that they reproduce largely by self-pollination (Murza et al. 2006, Sciligo et al. 2007, Sciligo 2009, Table 1), which may be attributed to the ephemeral nature of many of the plants (Cruden 1977, Lloyd & Webb 1986, Herrera et al. 2001, Sciligo 2009). However, I hypothesise that *D. cistiflora* s.l. has invested in attractive flowers because of complete or partial self-incompatibility and pollinator dependence.

Chapter Three: *Associations between soils, pollinators and spatial patterns of flower colour in the Drosera cistiflora species complex*

Here I investigate the associations between flower colours and pollinator assemblages in 2–4 (depending on population availability and number of flowering plants) populations of each floral colour form of *D. cistiflora* s.l. Should floral colour variation be an adaptation to different pollinating fauna, then I infer that pollinator groups in *D. cistiflora* s.l. populations of the same flower colour would be more alike than those in populations with different flower colours. To test this, I use multivariate statistical approaches to group populations according to pollinator composition and relative

pollinator importance, i.e. the product of their *D. cistiflora* s.l. pollen load and abundance on *D. cistiflora* s.l. flowers. I then determine whether these groups are similar in flower colour. I also consider whether certain abiotic factors such as soil chemistry, temperature, light and moisture may be involved in the expression of flower colour across the range of *D. cistiflora* s.l. If these factors drive floral colour divergence, then I expect to find floral colour modifications when conditions are adjusted as well as affiliations between the same flower colours and defined soil and vegetation types. Lastly, I assess whether geographical proximity could be associated with both similar flower colours and similar pollinating fauna simultaneously, in which case pollinator distributions may be geographically structured and unrelated to flower colour.

Chapter Four: *Local adaptation of flower colour in the Drosera cistiflora species complex: an assessment using model flowers and reciprocal translocations*

This study provides a detailed investigation into the role of pollinator-mediated selection in floral colour divergence in *D. cistiflora* s.l. I use model and live flower arrays to determine pollinator colour choice, if any, and to find out whether their choice matched the dominant local *D. cistiflora* s.l. flower colour. Thereafter I fit a model for pollinator colour choice; I do this for all pollinators and also the most important pollinators in isolation. If floral colour divergence in *D. cistiflora* s.l. is a result of pollinator preferences, then I predict that pollinators should choose local colours over introduced colours when presented with model and live flower colour arrays.

Chapter Five: *Reproductive isolation of sympatric floral colour forms in the Drosera cistiflora species complex*

Incipient speciation is typically set in motion through geographical and habitat differences between plant populations (q.v. van der Niet & Johnson 2009). However, the role of pollinator shifts in driving and/or maintaining floral radiation in sympatry, and their significance for taxonomy and conservation, may be underestimated. I thus examine the morphological differences and pre-F₁ reproductive barriers of ecogeography, habitat, flowering phenology, pollinator isolation and genetic incompatibility between two sympatric *D. cistiflora* s.l. floral colour forms (namely purple and red) to assess whether local adaptation to pollinator communities may act as

a primary driver of floral isolation. Here, I use reproductive isolation indices to assess the strength and relative contribution of each component towards total isolation, with a focus on pollinator isolation. If the colour preferences of pollinators drive incipient speciation in sympatry, the study of pollination ecotypes could facilitate the application of taxonomic rank beyond the scope of form and consequently aid the red-listing and conservation of such endangered and taxonomically obscure plant populations.

References

- Alexandersson, R. & Johnson, S. D. (2002) Pollinator-mediated selection on flower-tube length in a hawk moth-pollinated *Gladiolus* (Iridaceae). *Proceedings of the Royal Society B — Biological Sciences* **269**, 631–636.
- Anderson, B. (2010) Did *Drosera* evolve long scapes to stop their pollinators from being eaten? *Annals of Botany* **106** (4), 653–657.
- Anderson, B., Alexandersson, R. & Johnson, S. D. (2010) Evolution and coexistence of pollination ecotypes in an African gladiolus (Iridaceae). *Evolution* **64**, 960–972.
- Anderson, B., Allsopp, N., Ellis, A. G., Johnson, S. D., Midgley, J. J., Pauw, A. & Rodger, J. (2014) Biotic interactions. In N. Allsopp, J. F. Colville, A. G. Verboom & R. M. Cowling [Eds], *Ecology and evolution of fynbos: Understanding megadiversity*. Oxford University Press, Oxford, UK.
- Anderson, B. & Midgley, J. J. (2001) Food or sex; pollinator-prey conflict in carnivorous plants. *Ecology Letters* **4**, 511–513.
- Anderson, B., Pauw, A., Cole, W. W. & Barrett, S. C. H. (2016) Pollination, mating and reproductive fitness in a plant population with bimodal floral-tube length. *Journal of Evolutionary Biology* **29** (8), 1631–1642.
- Arista, M., Talavera, M., Berjano, R. & Ortiz, P. L. (2013) Abiotic factors may explain the geographical distribution of flower colour morphs and the maintenance of colour polymorphism in the scarlet pimpernel. *Journal of Ecology* **101** (6), 1613–1622.
- Armbruster, W. S. (2002) Can indirect selection and genetic context contribute to trait diversification? A transition-probability study of blossom-colour evolution in two genera. *Journal of Evolutionary Biology* **15** (3), 468–486.

- Arnold, S. E. J. (2010) *Flowers through insect eyes: the contribution of pollinator vision to the evolution of flower colour*. PhD Thesis, University of London, UK.
- Beardsley, P. M., Yen, A. & Olmstead, R. G. (2003) AFLP phylogeny of *Mimulus* section *Erythranthe* and the evolution of hummingbird pollination. *Evolution* **57**, 1397–1410.
- Bernhardt, P. (2000) Convergent evolution and adaptive radiation of beetle-pollinated angiosperms. *Plant Systematics and Evolution* **222** (1/4), 293–320.
- Bowman, R. N. (1987) Cryptic self-incompatibility and the breeding system of *Clarkia unguiculata* (Onagraceae). *American Journal of Botany* **74**, 471–476.
- Bradshaw, H. D. & Schemske, D. W. (2003) Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature* **426**, 176–178.
- Campbell, D. R., Bischoff, M., Lord, J. M. & Robertson, A. W. (2010) Flower colour influences insect visitation in alpine New Zealand. *Ecology* **91** (9), 2638–2649.
- Campbell, D. R., Bischoff, M., Lord, J. M. & Robertson, A. W. (2012) Where have all the blue flowers gone: Pollinator responses and selection on flower colour in New Zealand *Wahlenbergia albomarginata*. *Journal of Evolutionary Biology* **25** (2), 352–364.
- Carlson, J. E. & Holsinger, K. E. (2010) Natural selection on inflorescence colour polymorphisms in wild *Protea* populations: The role of pollinators, seed predators and inter-trait correlations. *American Journal of Botany* **97**, 934–944.
- Carlson, J. E. & Holsinger, K. E. (2012) Direct and indirect selection on floral pigmentation by pollinators and seed predators in a colour polymorphic South African shrub. *Oecologia* **171**, 905–919.
- Chalker-Scott, L. (1999) Environmental significance of anthocyanins in plant stress responses. *Photochemistry and Photobiology* **70** (1), 1–9.

- Chyzik, R., Klein, M., Ben-Dov, Y. & Cohen, A. (1995) Differential population density of western flower thrips in various flower colours of *Gladiolus*. *NATO ASI (Advanced Science Institutes) Series A: Life Sciences* **276**, 449–452.
- Clausen, R. T. (1941) On the use of the terms “subspecies” and “variety”. *Rhodora* **43** (509), 157–167.
- Coberly, L. C. & Rausher, M. D. (2003) Analysis of a chalcone synthase mutant in *Ipomoea purpurea* reveals a novel function for flavonoids: amelioration of heat stress. *Molecular Ecology* **12**, 1113–1124.
- Coberly, L. C. & Rausher, M. D. (2008) Pleiotropic effects of an allele producing white flowers in *Ipomoea purpurea*. *Evolution* **62**, 1076–1085.
- Colville, J. F., Picker, M. D. & Cowling, R. (2002) Species turnover of monkey beetles (Coleoptera: Scarabaeidae: Hopliini) along environmental and disturbance gradients in the Namaqualand region of the Succulent Karoo, South Africa. *Biodiversity and Conservation* **11**, 243–264.
- Cruden, R. W. (1977) Pollen-ovule ratios: A conservative indicator of breeding systems in flowering plants. *Evolution* **31**, 32–46.
- Culham, A. & Yesson, C. (2018) Droseraceae Database (version 0.1, Dec. 2008). In Y. Roskov, G. Ower, T. Orrell, D. Nicolson, N. Bailly, P. M. Kirk, T. Bourgoin, R. E. deWalt, W. Decock, A. de Wever, E. van Nieukerken, J. Zarucchi & L. Penev [Eds], *Species 2000 & ITIS Catalogue of Life*, 24th September 2018. Digital resource at www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden. ISSN 2405-8858.
- Dafni, A., Bernhardt, P., Shmida, A., Ivri, Y. Greenbaum, S., O’Toole, Ch. & Losito, L. (1990) Red bowl-shaped flowers: Convergence for beetle pollination in the Mediterranean region. *Israel Journal of Botany* **39** (1–2), 81–92.

Debbert, P. (1991) Einige neue arten der gattungen *Drosera* (Droseraceae) und *Pinguicula* (Lentibulariaceae). *Mitteilungen der Botanischen Staatssammlung München* **30**, 373–380.

Debbert, P. (2002) Einige neue *Drosera*-arten aus Südafrika (Droseraceae). *Linzer biologische Beiträge* **34** (1), 793–800.

de Candolle, A. (1824) “Droseraceae”. *Prodromus* **1**, 317–319.

de Jager, M. L. & Ellis, A. G. (2011) Intraspecific variation in pollinators as a driver of floral diversification within the sexually deceptive daisy *Gorteria diffusa*. *South African Journal of Botany* **77** (2), 523–524.

Diels, L. (1906) “Droseraceae”. In A. Engler [Ed.], *Das Pflanzenreich*, 1–136. Wilhelm Engelmann, Leipzig, Germany.

Ecklon, C. F. & Zeyher, C. (1835) *Enumeratio plantarum Africae australis extratropicae*. Perthes & Besser, Hamburg, Germany.

Egan, P. A. & van der Kooy, F. (2013) Phytochemistry of the carnivorous sundew genus *Drosera* (Droseraceae)—future perspectives and ethnopharmacological relevance. *Chemistry and Biodiversity* **10** (10), 1774–1790.

Ellis, A. G., Brockington, S. F., de Jager, M. L., Mellers, G., Walker, R. H. & Glover, B. J. (2014) Floral trait variation and integration as a function of sexual deception in *Gorteria diffusa*. *Philosophical Transactions of the Royal Society B: Biological Sciences* **369** (1649), 20130563.

Ellis, A. G. & Johnson, S. D. (2009) The evolution of floral variation without pollinator shifts in *Gorteria diffusa* (Asteraceae). *American Journal of Botany* **96**, 793–801.

Emms, S. K. & Arnold, M. L. (2000) Site-to-site differences in pollinator visitation patterns in a Louisiana iris hybrid zone. *Oikos* **91**, 568–578.

- Epling, C., Lewis, H. & Ball, F. M. (1960) The breeding group and seed storage: A study in population dynamics. *Evolution* **14**, 238–255.
- Ernst, W. H. O. (1987) Scarcity of flower colour polymorphism in field populations of *Digitalis purpurea* L. *Flora* **179** (3), 231–239.
- Faegri, K. & van der Pijl, L. (1979) *The principles of pollination ecology*. Pergamon Press, Oxford, UK.
- Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R. & Thomson, J. D. (2004) Pollination syndromes and floral specialisation. *Annual Review of Ecology and Evolution* **35**, 375–403.
- Fineblum, W. L. & Rausher, M. D. (1997) Do floral pigmentation genes also influence resistance to enemies? The W locus in *Ipomoea purpurea*. *Ecology* **78** (6), 1646–1654.
- Fischer, R. A. (1941) Average excess and average effect of gene substitution. *Annals of Eugenics* **11** (1), 53–63.
- Fleischmann, A., Gibson, R. & Rivadavia, F. (2008) *Drosera ericgreenii*, a new species from the fynbos of South Africa. *Bothalia* **38** (1), 141–159.
- Ford, E. B. (1945) Polymorphism. *Biological Reviews* **20** (2), 73–88.
- Forest, F., Goldblatt, P., Manning, J. C., Baker, D., Colville, J. F., Devey, D. S., Jose, S., Kaye, M. & Buerki, S. (2014) Pollinator shifts as triggers of speciation in painted petal irises (*Lapeirousia*: Iridaceae). *Annals of Botany* **113** (2), 357–371.
- Fornoff, F., Klein, A., Hartig, F., Benadi, G., Venjakob, C., Schaefer, H. M. & Ebeling, A. (2017) Functional flower traits and their diversity drive pollinator visitation. *Oikos* **126** (7), 1020–1030. doi:10.1111/oik.03869.

- Frey, F. M. (2004) Opposing natural selection from herbivores and pathogens may maintain floral-colour variation in *Claytonia virginica* (Portulacaceae). *Evolution* **58** (11), 2426–2437.
- Galen, C. (1999a) Flowers and enemies: Predation by nectar-thieving ants in relation to variation in floral form of an alpine wildflower, *Polemonium viscosum*. *Oikos* **85**, 426–434.
- Galen, C. (1999b) Why do flowers vary? The functional ecology of variation in flower size and form within natural plant populations. *Bioscience* **49**, 631–640.
- Gaum, W. G., Giliomee, J. H. & Pringle, K. L. (1994) Resistance of some rose cultivars to the western flower thrips, *Frankliniella occidentalis* (Thysanoptera, Thripidae). *Bulletin of Entomological Research* **84** (4), 487–492.
- Gervasi, D. D. L. & Schiestl, F. P. (2017) Real-time divergent evolution in plants driven by pollinators. *Nature Communications* **8**, 14691.
- Giamoustaris, A. & Mithen, R. (1996) The effect of flower colour and glucosinolates on the interaction between oilseed rape and pollen beetles. *Entomologia Experimentalis et Applicata* **80** (1), 206–208.
- Gibson, R. (2006) The stunning red-flowered *Drosera cistiflora* in the wild and in cultivation. *Carnivorous Plant Newsletter* **35** (3), 70–73.
- Gigord, L. D. B., Macnair, M. R. & Smithson, A. (2001) Negative frequency-dependent selection maintains a dramatic flower colour polymorphism in the rewardless orchid *Dactylorhiza sambucina* (L.) Soð. *Proceedings of the National Academy of Sciences of the United States of America* **98** (11), 6253–6255.
- Goldblatt, P., Bernhardt, P. & Manning, J. C. (1998) Pollination of petaloid geophytes by monkey beetles (Scarabaeidae: Rutelinae: Hopliini) in Southern Africa. *Annals of the Missouri Botanical Garden* **85**, 215–230.

Goldblatt, P. & Manning, J. C. (2000) Cape plants: A conspectus of the Cape flora of South Africa. *Strelitzia* **9**. National Botanical Institute, Pretoria.

Gómez, J. M. (2003) Herbivory reduces the strength of pollinator-mediated selection in the Mediterranean herb *Erysimum mediohispanicum*: Consequences for plant specialisation. *The American Naturalist* **162**, 242–256.

Gómez, J. M., Bosch, J., Perfectti, F., Fernández, J. D., Abdelaziz, M. & Camacho, J. P. M. (2008) Spatial variation in selection on corolla shape in a generalist plant is promoted by the preference patterns of its local pollinators. *Proceedings of the Royal Society B — Biological Sciences* **275**, 2241–2249.

Gómez, J. M., Muñoz-Pajares, A. J., Abdelaziz, M., Lorite, J. & Perfectti, F. (2014) Evolution of pollination niches and floral divergence in the generalist plant *Erysimum mediohispanicum*. *Annals of Botany* **113** (2), 237–249.

Grant, V. (1949) Pollination systems as isolating mechanisms in angiosperms. *Evolution* **3**, 82–97.

Grant, V. & Grant, K. A. (1965) *Flower pollination in the Phlox family*. Columbia University Press, New York, USA.

Gyuláné, L., Peter, T. & Gyuláné, V. (1980) Some results in poppy *Papaver somniferum* breeding 1. Breeding of winter poppy. *Herba hungarica* **19**, 45–54.

Hamet, R. (1907) Observations sur le genre *Drosera*. *Bulletin de la Société Botanique de France* **54**, 78–79.

Hamilton, C. W. & Reichard, S. H. (1992) Current practice in the use of subspecies, variety, and forma in the classification of wild plants. *Taxon* **41** (3), 485–498.

Hannan, G. L. (1981) Flower colour polymorphism and pollination biology of *Platystemon californicus* Benth. (Papaveraceae). *American Journal of Botany* **68** (2), 233–243.

Harder, L. D., Williams, N. M., Jordan, C. Y. & Nelson, W. A. (2001) The effects of floral design and display on pollinator economics and pollen dispersal. *In* L. Chittka & J. D. Thomson [Eds], *Cognitive ecology of pollination: Animal behaviour and floral evolution*, 297–317. Cambridge University Press, Cambridge, UK.

Herrera, C. M. (1993) Selection on floral morphology and environmental determinants of fecundity in a hawk-moth-pollinated violet. *Ecological Monographs* **63**, 251–275.

Herrera, C. M. (1996) Floral traits and plant adaptation to insect pollinators: A devil's advocate approach. *In* D. G. Lloyd & S. C. H. Barrett [Eds], *Floral biology: Studies on floral evolution in animal-pollinated plants*, 65–87. Chapman & Hall, New York, USA.

Herrera, C. M., Sánchez-Lafuente, A. M., Medrano, M., Guitián, J., Cerda, X., Rey, P. (2001) Geographical variation in autonomous self-pollination levels unrelated to pollinator service in *Helleborus foetidus* (Ranunculaceae). *American Journal of Botany* **88**, 1025–1032.

Hirota, S. K., Nitta, K., Suyama, Y., Kawakubo, N., Yasumoto, A. A. & Yahara, T. (2013) Pollinator-mediated selection on flower colour, flower scent and flower morphology of *Hemerocallis*: Evidence from genotyping individual pollen grains on the stigma. *PLoS ONE* **8** (12), e85601. doi:10.1371/journal.pone.0085601.

Hooker, J. D. (1890) *Drosera cistiflora*. *Curtis's Botanical Magazine* **46**, 7100.

Hopkins, R. & Rausher, M. D. (2012) Pollinator-mediated selection on flower colour allele drives reinforcement. *Science* **335**, 1090–1092.

Hoshi, Y. & Kondo, K. (1998) A chromosome phylogeny of the Droseraceae by using CMA-DAPI fluorescent banding. *Cytologia* **63**, 329–339.

Ichiishi, S., Nagamitsu, T., Kondo, Y., Iwashina, T., Kondo, K. & Tagashira, N. (1999) Effects of macro-components and sucrose in the medium on *in vitro* red-colour pigmentation in *Dionaea muscipula* Ellis and *Drosera spatulata* Labill. *Plant Biotechnology* **16** (3), 235–238.

- Ippolito, A., Fernandes, G. W. & Holtsford, T. P. (2004) Pollinator preferences for *Nicotiana alata*, *N. forgetiana*, and their F1 hybrids. *Evolution* **58**, 2634–2644.
- Irwin, R. E. & Strauss, S. Y. (2005) Flower colour microevolution in wild radish: Evolutionary response to pollinator-mediated selection. *The American Naturalist* **165** (2), 225–237.
- Irwin, R. E., Strauss, S. Y., Storz, S., Emerson, A. & Guibert, G. (2003) The role of herbivores in the maintenance of a flower colour polymorphism in wild radish. *Ecology* **84** (7), 1733–1743.
- Ito, D., Shinkai, Y., Kato, Y., Kondo, T. & Yoshida, K. (2009) Chemical studies on different colour development in blue- and red-coloured sepal cells of *Hydrangea macrophylla*. *Bioscience, Biotechnology and Biochemistry* **73** (5), 1054–1059.
- Johnson, S. D. (1997) Pollination ecotypes of *Satyrium hallackii* (Orchidaceae) in South Africa. *Botanical Journal of the Linnean Society* **123**, 225–235.
- Johnson, S. D. (2006) Pollinator-driven speciation in plants. In L. D. Harder & S. C. H. Barrett [Eds], *The ecology and evolution of flowers*, 296–306. Oxford University Press, Oxford, UK.
- Johnson, S. D. (2010) The pollination niche and its role in the diversification and maintenance of the southern African flora. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365** (1539), 499–516.
- Johnson, S. D., Linder, H. P. & Steiner, K. E. (1998) Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). *American Journal of Botany* **85**, 402–411.
- Johnson, S. D. & Midgley, J. J. (2001) Pollination by monkey beetles (Coleoptera: Scarabaeidae: Hopliini): Do colour and dark centres of flowers influence alighting behaviour? *Environmental Entomology* **30** (5), 861–868.

- Johnson, S. D. & Steiner, K. E. (2000) Generalisation versus specialisation in plant pollination systems. *Trends in Ecology and Evolution* **15**, 140–143.
- Johnson, S. D. & Steiner, K. E. (2003) Specialised pollination systems in southern Africa: Review article. *South African Journal of Science* **99** (7&8), 345–348.
- Jones, K. N. & Reithel, J. S. (2001) Pollinator-mediated selection on a flower colour polymorphism in experimental populations of *Antirrhinum* (Scrophulariaceae). *American Journal of Botany* **88** (3), 447–454.
- Jürgens, A., Witt, T., Sciligo, A. & El-Sayed, A. M. (2015) The effect of trap colour and trap–flower distance on prey and pollinator capture in carnivorous *Drosera* species. *Functional Ecology* **29** (8), 1026–1037.
- Kay, Q. O. N. (1976) Preferential pollination of yellow-flowered morphs of *Raphanus raphanistrum* by *Pieris* and *Eristalis* spp. *Nature* **261** (5557), 230–232.
- Kay, Q. O. N. (1978) The pollination of flowers by insects. In A. J. Richards [Ed.], *Linnean Society Symposium Series* 6, 175–190. Academic, London, UK.
- Kay, K. M. & Sargent, R. D. (2009) The role of animal pollination in plant speciation: Integrating ecology, geography, and genetics. *Annual Review of Ecological Systems* **40**, 637–656.
- Kelber, A. & Osorio, D. (2010) From spectral information to animal colour vision: Experiments and concepts. *Proceedings of the Royal Society B — Biological Sciences* **277** (1688), 1617–1625.
- Kevan, P. G. & Baker, H. G. (1983) Insects as flower visitors and pollinators. *Annual Review of Entomology* **28**, 407–453.

- Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mitchell, R. J. & Ashman, T. L. (2005) Pollen limitation of plant reproduction: Pattern and process. *Annual Review of Ecology Evolution and Systematics* **36**, 467–497.
- Kondo, K. & Olivier, M. C. (1979) Chromosome numbers of four species of *Drosera* (Droseraceae). *Annals of the Missouri Botanical Garden* **66**, 584–587.
- Levin, D. A. & Brack, E. T. (1995) Natural selection against white petals in phlox. *Evolution* **49**, 1017–1022.
- Levin, D. A. & Kerster, H. W. (1967) Natural selection for reproductive isolation in *Phlox*. *Evolution* **21** (4), 679–687.
- Lloyd, D. G. & Webb, C. J. (1986) The avoidance of interference between the presentation of pollen and stigmas in angiosperms. 1. Dichogamy. *New Zealand Journal of Botany* **24**, 135–162.
- Maddison, W. P. & Maddison, D. R. (1992) *MacClade: Analysis of phylogeny and character evolution*. Sinauer, Sunderland, Massachusetts, USA.
- Mayr, E. (1965) *Animal species and evolution*. Belknap Press of Harvard University Press, Cambridge, Massachusetts, USA.
- McGimpsey, V. J. & Lord, J. M. (2015) In a world of white, flower colour matters: A white–purple transition signals lack of reward in an alpine *Euphrasia*. *Austral Ecology* **40**, 701–708.
- Meléndez-Ackerman, E. J. & Campbell, D. R. (1998) Adaptive significance of flower colour and inter-trait correlations in an *Ipomopsis* hybrid zone. *Evolution* **52** (5), 1293–1303.

- Minnaar, C., de Jager, M. L. & Anderson, B. (2019) Intraspecific divergence in floral tube length promotes asymmetric pollen movement and reproductive isolation. *New Phytologist*. doi:10.1111/nph.15971.
- Mole, S. (1994) Trade-offs and constraints in plant-herbivore defense theory—a life-history perspective. *Oikos* **71** (1), 3–12.
- Müller, H. (1873) *Die befruchtung der blumen durch insekten und die gegenseitigen anpassungen beider: Ein beitrag zur erkenntniss des ursächlichen zusammenhanges in der organischen natur*. Engelmann, Leipzig, Germany.
- Murza, G. L. & Davis, A. R. (2005) Flowering phenology and reproductive biology of *Drosera anglica*. *Botanical Journal of the Linnean Society* **147** (4), 417–426.
- Murza, G. L., Heaver, J. R. & Davis, A. R. (2006) Minor pollinator-prey conflict in the carnivorous plant, *Drosera anglica*. *Plant Ecology* **184**, 43–52.
- Narbona, E. & Wang, H., Ortiz, P., Arista, M. & Imbert, E. (2017). Flower colour polymorphism in the Mediterranean Basin: Occurrence, maintenance and implications for speciation. *Plant Biology* **20**, Suppl 1. doi:10.1111/plb.12575.
- Newman, E., Anderson, B. & Johnson, S. D. (2012) Flower colour adaptation in a mimetic orchid. *Proceedings of the Royal Society B — Biological Sciences* **279**, 2309–2313.
- Newman, E., Manning, J. & Anderson, B. (2014) Matching floral and pollinator traits through guild convergence and pollinator ecotype formation. *Annals of Botany* **113** (2), 373–384.
- Obermeyer, A. A. (1970) Droseraceae. *Flora of Southern Africa* **13**, 187–201.

Ojeda, D. I., Valido, A., Fernández de Castro, A. G., Ortega-Olivencia, A., Fuertes-Aguilar, J., Carvalho, J. A. & Santos-Guerra, A. (2016) Pollinator shifts drive petal epidermal evolution on the Macaronesian Islands bird-flowered species. *Biology Letters* **12** (4). ISSN 1744-9561.

Ollerton, J. (1996) Reconciling ecological processes with phylogenetic patterns: The apparent paradox of plant-pollinator systems. *Journal of Ecology* **84**, 767–769.

Ollerton, J., Johnson, S. D. & Hingston, A. B. (2006) Geographical variation in diversity and specificity in pollination systems. In N. M. Waser & J. Ollerton [Eds], *Plant-pollinator interactions: from specialisation to generalisation*. University of Chicago Press, USA.

Onslow, M. W. (1925) *The anthocyanin pigments of plants*. Cambridge University Press, London, UK.

Parker, A. J., Williams, N. M. & Thomson, J. D. (2017) Geographic patterns and pollination ecotypes in *Claytonia virginica*. *Evolution* **72** (1), 202–210.

Pellmyr, O. & Thien, L. B. (1986) Insect reproduction and floral fragrances: Keys to the evolution of the angiosperms? *Taxon* **35** (1), 76–85.

Pérez, F., Arroyo, M. T. K., Medel, R. & Hershkovitz, M. A. (2006) Ancestral reconstruction of flower morphology and pollination systems in *Schizanthus* (Solanaceae). *American Journal of Botany* **93**, 1029–1038.

Pérez-Barrales, R., Arroyo, J. & Armbruster, W. S. (2007) Differences in pollinator faunas may generate geographic differences in floral morphology and integration in *Narcissus papyraceus* (Amaryllidaceae). *Oikos* **116** (11), 1904–1918.

Peter, C. I. & Johnson, S. D. (2014) A pollinator shift explains floral divergence in an orchid species complex in South Africa. *Annals of Botany* **113** (2), 277–288.

Picker, M., Griffiths, C. & Weaving, A. (2004) *Field guide to insects of South Africa*. Struik, Cape Town.

Picker, M. & Midgley, J. J. (1996) Pollination by monkey beetles (Coleoptera: Scarabaeidae: Hopliini): flower and colour preferences. *African Entomology* **4** (1), 7–14.

Planchon, J. E. (1848) Sur la famille des Droséracées—revisio systematica Droseeorum. In H. Milne Edwards, Ad. Brongniart & J. Decaisne [Eds], *Annales des Sciences Naturelles Botanique Série 3* (9), 185–207. Victor Masson, Paris, France.

Presl, K. B. (1844) *Botanische bemerkungen*. Hofbuchdruckerei von Gottlieb Haase Söhne, Prague, Czech Republic.

Prouvençal de Saint-Hilaire, A. (1816) *Mémoire sur les plantes auxquelles on attribue un placenta central libre et sur la nouvelle famille des Paronychiées*. L'Imprimerie de A. Belin, Paris, France.

Queiroz, J. A., Quirino, Z. G. M. & Machado, I. C. (2015) Floral traits driving reproductive isolation of two co-flowering taxa that share vertebrate pollinators. *AoB PLANTS* **7**, plv127. doi:10.1093/aobpla/plv127.

Ramsey, J., Bradshaw, H. D. & Schemske, D. W. (2003) Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution* **57** (7), 1520–1534.

Ramsey, J. & Schemske, D. W. (1998) Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* **29**, 467–501.

Rausher, M. D. (2008) Evolutionary transitions in floral colour. *International Journal of Plant Sciences* **169**, 7–21.

Rausher, M. D. & Fry, J. D. (1993) Effects of a locus affecting floral pigmentation in *Ipomoea purpurea* on female fitness components. *Genetics* **134**, 1237–1247.

- Renoult, J. P., Kelber, A. & Schaefer, H. M. (2017) Colour spaces in ecology and evolutionary biology. *Biological Reviews of the Cambridge Philosophical Society* **92** (1), 292–315.
- Rey, P. J., Herrera, C. M., Guitián, J., Cerdá, X., Sánchez-Lafuente, A. M., Medrano, M. & Garrido, J. L. (2005) The geographic mosaic in predispersal interactions and selection on *Helleborus foetidus* (Ranunculaceae). *Journal of Evolutionary Biology* **19** (1), 21–34.
- Rivadavia, F. (2000) Endless carnivorous plants in Cape Town, South Africa. *Carnivorous Plant Newsletter* **29** (2), 42–25.
- Rivadavia, F., Kondo, K., Kato, M. & Hasebe, M. (2003) Phylogeny of the sundews, *Drosera* (Droseraceae), based on chloroplast rbcL and nuclear 18S ribosomal DNA sequences. *American Journal of Botany* **90** (1), 123–130.
- Robertson, J. L. & Wyatt, R. (1990) Evidence for pollination ecotypes in the yellow-fringed orchid, *Platanthera ciliaris*. *Evolution* **44**, 121–133.
- Salter, T. M. (1940) Plantae novae Africanae: Series XV. *The Journal of South African Botany* **6**, 165–175.
- Schemske, D. W. & Bierzychudek, P. (2001) Evolution of flower colour in the desert annual *Linanthus parryae*: Wright revisited. *Evolution* **55**, 1269–1282.
- Schemske, D. W. & Bierzychudek, P. (2007) Spatial differentiation for flower colour in the desert annual *Linanthus parryae*: Was Wright right? *Evolution* **61**, 2528–2543.
- Schemske, D. W. & Bradshaw, H. D., Jr (1999) Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proceedings of the National Academy of Sciences of the United States of America* **96** (21), 11910–11915.
- Schiestl, F. P. & Johnson, S. D. (2013) Pollinator-mediated evolution of floral signals. *Trends in Ecology and Evolution* **28** (5), 307–315.

- Schlauer, J. (1996) A dichotomous key to the genus *Drosera* L. (Droseraceae). *Carnivorous Plant Newsletter* **25**, 67–88.
- Schlauer, J. (2014) Nomenclatural synopsis of carnivorous phanerogamous plants. *World Carnivorous Plant List*. <http://www.omnisterra.com/botany/cp/data/jans.db>. Accessed on 20 September 2018.
- Sciligo, A. R. (2009) *Food or sex: which would you choose? Pollinator-prey conflict and reproductive assurance in New Zealand Drosera*. PhD Thesis. Lincoln University, New Zealand.
- Sciligo, A. R., Sullivan, J. J., Jesson, L. K., Witt, T., Jürgens, A., Hale, R. J. & Newstrom, L. (2007) *Is selfing more advantageous than sex in Drosera due to the pollinator-prey conflict?* ESA/SER Joint Meeting, San Jose McEnergy Convention Centre, California.
- Seine, R. & Barthlott, W. (1994) Some proposals on the infrageneric classification of *Drosera* L. *Taxon* **43**, 583–589.
- Simms, E. L. & Bucher, M. A. (1996) Pleiotropic effects of flower-colour intensity on herbivore performance on *Ipomoea purpurea*. *Evolution* **50**, 957–963.
- Sletvold, N., Trunschke, J., Smit, M., Verbeek, J. & Ågren, J. (2016) Strong pollinator-mediated selection for increased flower brightness and contrast in a deceptive orchid. *Evolution* **70** (3), 716–724.
- Smith, S. D., Ané, C. & Baum, D. A. (2008) The role of pollinator shifts in the floral diversification of *Iochroma* (Solanaceae) *Evolution* **62** (4), 793–806.
- Smith, S. D. & Kriebel, R. (2018) Convergent evolution of floral shape tied to pollinator shifts in Iochrominae (Solanaceae). *Evolution* **72** (3), 688–697.
- Sobel, J. M. & Streisfeld, M. A. (2013) Flower colour as a model system for studies of plant evo-devo. *Frontiers in Plant Science* **4**, 321. doi:10.3389/fpls.2013.00321.

Sonder, O. W. (1859–1860) Droseraceae. In W. H. Harvey & O. W. Sonder [Eds], *Flora Capensis I (Ranunculaceae to Connaraceae)*, 75–79. Hodges, Smith, and Co., Dublin & A. S. Robertson, Cape Town.

Stebbins, G. L. (1970) Adaptive radiation of reproductive characteristics in angiosperms, I: Pollination mechanisms. *Annual Review of Ecological Systems* **1**, 307–326.

Steyn, W. J., Wand, S. J., Holcroft, M. & Jacobs, G. (2002) Anthocyanins in vegetative tissues: A proposed unified function in photoprotection. *New Phytologist* **155** (3), 349–361.

Strauss, S. Y. & Whittall, J. B. (2006) Non-pollinator agents of selection on floral traits. In L. D. Harder & S. C. H. Barrett [Eds], *Ecology and evolution of flowers*, 120–138. Oxford University Press, Oxford, UK.

Streinzer, M., Roth, N., Paulus, H. F. & Spaethe, J. (2019) Colour preference and spatial distribution of glaphyrid beetles suggest a key role in the maintenance of the colour polymorphism in the peacock anemone (*Anemone pavonina*, Ranunculaceae) in Northern Greece. *Journal of Comparative Physiology*. doi:10.1007/s00359-019-01360-2.

Streisfeld, M. A. & Kohn, J. R. (2005) Contrasting patterns of floral and molecular variation across a cline in *Mimulus aurantiacus*. *Evolution* **59** (12), 2548–2559.

Streisfeld, M. A. & Kohn, J. R. (2007) Environment and pollinator-mediated selection on parapatric floral races of *Mimulus aurantiacus*. *Journal of Evolutionary Biology* **20**, 122–132.

Tao, Z. B., Ren, Z. X., Bernhardt, P., Liang, H., Li, H. D., Zhao, Y. H., Wang, H. & Li, D. Z. (2018) Does reproductive isolation reflect the segregation of colour forms in *Spiranthes sinensis* (Pers.) Ames complex (Orchidaceae) in the Chinese Himalayas? *Ecology and Evolution* **00**, 1–15. doi:10.1002/ece3.4067.

Tcherkez, G. (2004) *Flowers: Evolution of the floral architecture of angiosperms*. Science Publishers, New Hampshire, USA.

The Plant List (2013) Version 1.1. Published on the Internet, <http://www.theplantlist.org/>.

Thunberg, C. P. (1818–1820) *Flora Capensis I*. Gerhardum Bonnierum, Hafniae, Denmark.

Trinder-Smith, T., Maytham Kidd, M. & Anderson, F. (2006) *Wild flowers of the Table Mountain National Park*. Botanical Society of South Africa, Cape Town.

Trunschke, J., Sletvold, N. & Ågren, J. (2017) Interaction intensity and pollinator-mediated selection. *New Phytologist* **214**, 1381–1389.

Turesson, G. (1922) The species and the variety as ecological units. *Hereditas* **3**, 100–113.

Valente, L. M., Manning, J. C., Goldblatt, P. & Vargas, P. (2012) Did pollination shifts drive diversification in southern African *Gladiolus*? Evaluating the model of pollinator-driven speciation. *The American Naturalist* **180** (1), 83–98. doi:10.1086/666003.

van der Kooi, C. J., Dyer, A. G., Kevan, P. G. & Lunau, K. (2019) Functional significance of the optical properties of flowers for visual signalling. *Annals of Botany* **123** (2), 263–276.

van der Niet, T. & Johnson, S. D. (2009) Patterns of plant speciation in the Cape Floristic Region. *Molecular Phylogenetics and Evolution* **51**, 85–93.

van der Niet, T. & Johnson, S. D. (2012) Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends in Ecology and Evolution* **27** (6), 353–361.

- van der Niet, T., Peakall, R. & Johnson, S. D. (2014) Pollinator-driven ecological speciation in plants: New evidence and future perspectives. *Annals of Botany* **113** (2), 199–211.
- van der Niet, T., Pirie, M. D., Shuttleworth, A., Johnson, S. D. & Midgley, J. J. (2014) Do pollinator distributions underlie the evolution of pollination ecotypes in the Cape shrub *Erica plukenetii*? *Annals of Botany* **113** (2), 301–316.
- van Kleunen, M., Nänni, I., Donaldson, J. S. & Manning, J. C. (2007) The role of beetle marks and flower colour on visitation by monkey beetles (Hopliini) in the Greater Cape Floral Region, South Africa. *Annals of Botany* **100** (7), 1483–1489.
- Vernon, R. S. & Gillespie, D. R. (1990) Spectral responsiveness by *Frankliniella occidentalis* (Thysanoptera, Thripidae) determined by trap catches in greenhouses. *Environmental Entomology* **19** (5), 1229–1241.
- von Linnaeus, C. (1760) *Plantae Rariores Africanae* **9**, 85.
- von Witt, C. G. (2008) Inspector CREWseau: Botanical detectives at work. *Veld & Flora* **94** (4), 188–191.
- Warren, J. & Mackenzie, S. (2001) Why are all colour combinations not equally represented as flower colour polymorphisms? *New Phytologist* **151**, 237–241.
- Waser, N. M. (1998) Pollination, angiosperm speciation, and the nature of species boundaries. *Oikos* **81**, 198–201.
- Waser, N. M., Chittka, L. Price, M. V., Williams, N. M. & Ollerton, J. (1996) Generalisation in pollination systems and why it matters. *Ecology* **77**, 1043–1060.
- Waser, N. M. & Price, M. V. (1981) Pollinator choice and stabilising selection for flower colour in *Delphinium nelsonii*. *Evolution* **35** (2), 376–390.

Wesselingh, R. A. & Arnold, M. L. (2000) Pollinator behaviour and the evolution of Louisiana iris hybrid zones. *Journal of Evolutionary Biology* **13** (2), 171–180.

Whibley, A. C., Langlade, N. B., Andalo, C., Hanna, A. I., Bangham, A., Thébaud, C. & Coen, E. (2006) Evolutionary paths underlying flower colour variation in *Antirrhinum*. *Science* **313**, 963–966.

Whittall, J. B. & Hodges, S. A. (2007) Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature* **447**, 706–709.

Willdenow, C. L. (1809) *Enumeratio Horti Regii Botanici Berolinensis part I: Enumeratio plantarum Horti Regii Botanici Berolinensis*. Real Schools Bookstore, Berlin, Germany.

Wright, S. (1943a) Isolation by distance. *Genetics* **28**, 114–138.

Wright, S. (1943b) An analysis of local variability of flower colour in *Linanthus parryae*. *Genetics* **28**, 139–156.

Wright, S. (1978) *Evolution and the genetics of populations, volume 4: Variability within and among natural populations*. University of Chicago Press, Chicago, USA.

CHAPTER TWO

BREEDING SYSTEMS OF FLORAL COLOUR FORMS IN THE *DROSERA CISTIFLORA* SPECIES COMPLEX

Abstract

Strong pollinator-mediated selection and high vulnerability to mutualism failure are both more likely when plants are reliant on pollinator visits for seed production. Consequently, variation in plant breeding systems has implications for evolutionary ecology and conservation of populations. Here I evaluate pollinator contribution to fecundity, pollinator dependence, self-compatibility and pollen limitation in the *Drosera cistiflora* species complex (Droseraceae). *Drosera cistiflora* s.l. comprises insectivorous, perennial plants endemic to fynbos and renosterveld vegetation of the Greater Cape Floristic Region (GCFR) of South Africa. The complex includes five floral colour forms (namely pink, purple, red, white and yellow), some of which are known to be pollinated by beetles. Open pollination and controlled hand-pollination experiments were conducted in 15 populations of *D. cistiflora* s.l. (2–4 populations per colour form) to test whether the colour forms vary in their degree of self-compatibility and their ability to produce seeds through autonomous self-fertilisation. Yellow-flowered populations appeared to be highly to fully self-incompatible, and hand cross-pollination in all other colour forms also resulted in greater seed set than self-pollination, suggesting that these populations may be partially self-incompatible, or suffer from inbreeding depression. Pollinator contribution to fecundity was high overall; seed set resulting from autonomous selfing was extremely low, and pollinator dependence indices were high, in all populations. Since hand cross-pollination and hand self-pollination respectively resulted in greater seed set than open pollination and

autonomous self-pollination in 13 of the 15 populations, I inferred that fecundity is generally pollen-limited. The pollen limitation and high pollinator dependence typical of *D. cistiflora* s.l., but unusual among *Drosera* species worldwide, suggest that pollinators are likely to mediate strong selection on attractive traits such as floral colour. They also suggest that these populations, many of which are rare and threatened, are likely to be vulnerable to local extinction if mutualisms were to collapse indefinitely.

Introduction

Pollinator-mediated selection through the female component of reproductive fitness is likely to be strongest if seed production is limited by the availability or efficiency of pollinators (Sandring & Ågren 2009, Trunschke et al. 2017). Although animal pollinators are known to visit approximately 87.5% of plant species worldwide (Ollerton et al. 2011) and 88% of the Cape flora (Koutnik 1987, Anderson et al. 2014), and reduced visitation may directly compromise plant fecundity (Lundgren et al. 2016), it is unclear how reliant these plants are on pollinators and how frequently seed production is limited by pollen receipt. Breeding system experiments using controlled hand-pollinations are consequently important for unravelling the degree of ecological dependence of plants on pollinator visits. Such information is of conservation significance since pollinator-dependent species are vulnerable to local extirpation of pollinators (Bond 1994, Pauw 2007). Given the context of the current ‘Anthropocene’ (sensu Crutzen & Stoermer 2000), this is of particular concern in ‘biodiversity hotspots’ (sensu Mittermeier et al. 1998) and fire-prone environments (Brown et al. 2017) such as the Cape Floristic Region (q.v. Rebelo 1987, Anderson et al. 2014).

Plants that are fully dependent on pollinators are more likely to exhibit pollen limitation (Larson & Barrett 2000), which refers to the reduction of a plant’s reproductive success as a result of insufficient quantity or quality of pollen (Ashman et al. 2004). Quantity limitation is governed by pollinator availability and efficiency as well as post-pollination aspects of seed production (viz. all events that take place after pollination until a mature fruit is formed), and quality limitation depends on the difference in embryo survival that occurs as a result of the quality of pollen received (if self- or incompatible pollen is received) [Snow 1994, Ashman et al. 2004, Aizen & Harder 2007].

Pollen limitation could result in strong selection to increase visitation rates that enhance seed set in animal-pollinated plants. Typically, pollen-limited plants rely on floral modes of attraction and pollinator rewards such as colour, display size or shape, and nectar concentrations and volumes, to ensure pollination success (Primack 1978, Lloyd

1985, Herrera 1996, Ashman et al. 2004, Ashman & Morgan 2004, Knight et al. 2005, Sciligo 2009, Sletvold et al. 2010). Pollen limitation may consequently result in selection on mechanisms that enhance the efficiency of pollen transfer to the stigma, thereby playing an important role in shaping floral phenotype through the selection for secondary sexual traits that favour more effective pollinators (Kiester et al. 1984, Knight et al. 2005). In contrast, primarily self-pollinating species usually limit resource investment in floral display by having small flowers (Guerrant 1989, Dart et al. 2012) and fewer pollen grains per ovule (Cruden 1977, Strauss & Whittall 2006).

Pollen limitation is shown experimentally if fruit and/or seed set in hand-pollinated flowers is greater than in naturally pollinated flowers (Bierzychudek 1981, Young & Young 1992, Ashman et al 2004). This would imply that pollen limitation, rather than resource limitation, is acting through the female component of fitness, since fruit or seed set would not increase with added pollen if resources were not sufficiently adequate to bring about maturation of the additional fertilised ovules (Bateman 1948, Ashman et al. 2004). This expectation comprises a fundamental element of the sexual selection theory in plant reproductive ecology and evolution (Bateman 1948, Ashman et al. 2004).

Even if pollen is not limiting, it is still possible that selection on floral traits may be operating through male fitness. According to Bateman's principle, male fitness is usually limited by the number of matings achieved, whilst female fitness is usually limited by the amount of resources available for reproduction. Nonetheless, if male fitness is limited by matings because of inadequate pollen export, then it is likely that female fitness will also be limited by matings rather than resources (Burd 1994). Siring success is however difficult to measure in most angiosperms, and so most studies only determine whether selection is operating through female fitness.

Strong selection by pollinators, because of pollen limitation combined with phenotype-dependent fitness differences among individuals, may accelerate speciation rates when pollinators vary geographically (Kay & Sargent 2009), as suggested by a correlation between high levels of pollen limitation and species richness (Vamosi et al. 2006). To this end, Anderson et al. (2014) examined the extent of pollinator limitation in the Greater Cape Floristic Region (GCFR) to evaluate whether pollen limitation may have

contributed to the high floral diversity of the Region. Although they found many species to be heavily reliant on pollinators, and a small but noteworthy amount to self autonomously, no firm links between pollen limitation and floral diversity could be drawn since available data represented only 55 species from 12 families. Two additional meta-analyses, one focussed on the Brazilian Atlantic rainforest (q.v. Wolowski et al. 2014) and another on the GCFR (q.v. Rodger & Ellis 2016) did not find evidence that these biodiverse regions were characterised by high levels of pollen limitation.

Whilst pollen limitation usually results in selection for generalised pollination systems, it may facilitate the evolution of specialised relationships between plants and a small subset of possible flower visitors (Johnson & Bond 1997, Fenster et al. 2004). Many Cape plants have developed such specialised pollinator relationships (Steiner 1987). However, an apparent paradox among plants is that many species with specialised pollination systems also possess mechanisms for selfing (Wessinger & Kelly 2018). For example, the specialist iris *Lapeirousia anceps* (Iridaceae) is visited almost exclusively by the long-proboscid fly *Moegistorhynchus longirostris* (Diptera: Nemestrinidae), but *L. anceps* is a facultative selfer that is capable of producing seeds in the absence of this pollinator (Pauw 2004). The orchid *Disa draconis* s.l. (Orchidaceae) is also pollinated by *M. longirostris* (Johnson & Steiner 1997) but has a stronger ecological dependence on the fly as it is self-compatible and fully allogamous, i.e. dependent on pollen vectors. The association between specialisation and selfing can often be explained in terms of reproductive assurance where the ability to self may be strongly selected for in pollinator-dependent species where specialisation increases the risks of pollination failure (Fenster & Martén-Rodríguez 2007), especially if they are short-lived and dependent on seed for recruitment (Bond 1994, Geerts & Pauw 2012). Consequently, if pollinators are absent or ineffective, pollen limitation may be reduced by selection for autogamy or facultative selfing which results in mixed mating systems (Fryxell 1957, Barrett & Eckert 1990, Vogler & Kalisz 2001, Barrett 2003, Ashman et al. 2004, Sciligo 2009). Selfing will thus confer an immediate fitness advantage if ‘inbreeding depression’ (sensu Wright 1977), i.e. the reduction in fitness of the progeny of closely related individuals, is not prohibitive (Lloyd 1992, Barrett & Harder 1996, Herrera et al. 2001, Barrett 2002, Elle & Carney 2003, Kalisz & Vogler 2003, Moeller & Geber 2005, Hodgins & Barrett 2006, Sciligo 2009, Rodger & Johnson 2013). In

general, plants with brief lifespans and those that otherwise inhabit unstable environments are most likely to exhibit selfing strategies (Cruden 1977, Lloyd & Webb 1986, Herrera et al. 2001, Sciligo 2009).

Such selfing strategies include: i) autonomous selfing, which occurs spontaneously without the aid of a pollinator, and may take place in flowers which remain closed because of poor environmental conditions, in buds prior to opening, in open flowers, or during floral closure (through delayed autonomous selfing, which follows anthesis, when the stigma and anthers may come into contact as a result of floral movement) [Kearns & Inouye 1993]; ii) vector-mediated self-pollination (or facilitated selfing), which relies on a pollinating vector to move pollen within a flower (Lloyd 1992), and may enable reproduction if outcrossing and autonomous selfing are not possible (Lloyd 1979, Anderson et al. 2003, Sciligo 2009), and iii) autogamy, i.e. selfing that occurs within a flower, which may occur either by facilitation or autonomously, and may involve pollen from the same flower—or pollen from a different flower, either on the same plant or on a genetically identical individual (geitonogamy, q.v. Snow et al. 1996, Barrett 2003). Delayed autonomous selfing may mitigate some of the costs associated with inbreeding by utilising pollen and ovules which were not going to contribute towards reproduction through outcrossing (Barrett & Eckert 1990, Vogler & Kalitz 2001, Barrett 2003, Ashman et al. 2004, Sciligo 2009).

Here I investigate the breeding systems of the *Drosera cistiflora* species complex, to determine how different colour forms within the complex vary in terms of pollinator dependence, pollen limitation and their ability to self-pollinate through autonomous and facilitated selfing mechanisms. I use the term ‘breeding system’ (sensu Neal & Anderson 2005), i.e. “anatomical/morphological and physiological aspects of individuals and populations” that influence the potential for selfing, rather than ‘mating system’ (sensu Neal & Anderson 2005), i.e. “genetic relatedness and pairings between individuals”, since the study investigates the selfing potential in the species complex and does not examine actual rates of selfing versus outcrossing between individuals.

Drosera cistiflora s.l. belongs to the cosmopolitan carnivorous plant family Droseraceae. Although little is known about pollination in *Drosera*, some previous studies suggest that species in the genus often have a very low dependence on

pollinators for reproduction with many being facultatively autogamous and reproducing primarily through self-pollination (q.v. Murza & Davis 2005; Murza et al. 2006; Sciligo et al. 2007; Sciligo 2009; Cross et al. 2018; Table 1, Chapter 1). Flowers are nectarless with pollen as the only reward (Murza & Davis 2003, Cross et al. 2018) and a variety of different flower visitors have been recorded including bees, flies and beetles (q.v. Wilson 1995, Murza & Davis 2005, Murza et al. 2006, Anderson 2010). Low pollinator dependence is often associated with habitat ephemerality (Cruden 1977, Lloyd & Webb 1986, Herrera et al. 2001), and many *Drosera* species are reliant on seasonal wetlands for active growth with only a small window of opportunity for outcrossing to take place (Sciligo 2009). Only one monophyletic lineage of *Drosera* is known to have xenogamous members with self-incompatibility (Chen et al. 1997). Several authors (namely Sciligo et al. 2007, Sciligo 2009, Jürgens et al. 2011) have argued that selfing in *Drosera* is adaptive because it may reduce the conflict generated by capturing pollinators. However, most studies suggest that pollinator capture is unlikely to be an important selective force in *Drosera* (q.v. Anderson & Midgley 2001, Murza et al. 2006, Anderson 2010). One of these studies (namely Anderson & Midgley 2001), a meta-analysis on *Drosera* pollinator-prey conflict, found that there was no evidence to suggest that *Drosera* species had evolved adaptations to specifically resolve this putative conflict, and the other (namely Anderson 2010) found that *D. cistiflora* s.l. never captures its pollinators. The ability of numerous *Drosera* species to reproduce asexually (via stolons, older leaves that take root when they touch the ground, scale leaves or gemmae in pygmy sundews, and/or tuber offshoots, q.v. D'Amato 1998) may make many of them even less reliant on pollinators in the short term.

I hypothesised that, unlike the small-flowered *Drosera* species which typically self-pollinate, the large-flowered forms in the *D. cistiflora* species complex reflect high pollinator dependence. Plants in this complex are insectivorous perennials endemic to the fynbos and renosterveld vegetation of the GCFR of South Africa. They display great variety in corolla colour, with five known extant floral colour forms: pink, purple, red, white and yellow. Two existing pollination studies involving *D. cistiflora* s.l. show that the species complex may be primarily pollinated by hopliine beetles (Coleoptera: Scarabaeidae: Hopliini) [q.v. Goldblatt et al. 1998, Anderson 2010]. As is typical of hopliine beetle-pollinated flowers, these are showy, nectarless, odourless and bowl-shaped. In addition to colour, visible floral traits that may be adaptations to allogamy

include spatial separation of anthers and stigmas; fringed stigmas and raised presentation of anthers above the carpels (Figure 1, Chapter 1). Despite a report that plants can reproduce vegetatively, with plantlets developing from the succulent rootstock to form a clonal group (Gibson 2006), my experience is that clusters usually comprise groups of solitary plants and individuals may frequently be separated from conspecifics by a metre or more, indicating that recruitment occurs mainly or exclusively via seeds. To date, it has been unknown whether the various colour forms of *D. cistiflora* s.l. are self-compatible and, if so, whether any self autonomously. It is also unknown whether natural fecundity of the various forms is pollen-limited. Showy flowers, which are otherwise costly to the plant in both energy requirements and herbivory risk (Leege & Wolfe 2002, Sletvold & Grindeland 2008, de Jager & Ellis 2014), are only likely to be maintained by selection if they promote cross-pollination. I test this hypothesis by investigating the effects of pollination treatments on the female component of fitness in five floral colour forms of *D. cistiflora* s.l. By doing so, I can establish whether pollinators may be associated with floral colour divergence in the species complex.

Materials and Methods

Study sites

I selected 12 study sites that represent 15 populations of five floral colour forms of *Drosera cistiflora* s.l., three of which occurred in sympatry at three respective sites. The sampling included four populations of the pink-flowered form, two of the purple-flowered form (one with reflexed petals and one with crateriform corollas), three of the red-flowered form, three of the white-flowered form and three of the yellow-flowered form (Table 1). Voucher specimens for each population studied are housed in the Compton Herbarium (Table 1).

Table 1. Flower colour, site name, GPS location and number of *Drosera cistiflora* s.l. flowers in the 15 study populations of 2009, with respective Compton Herbarium and/or Kirstenbosch National Botanical Garden Collections Nursery accession numbers. Precise locality information has been omitted owing to the sensitive nature of these populations and their vulnerability to overcollection.

<i>Drosera cistiflora</i> s.l. flower colour	Site name and locality	Latitude	Longitude	Accession number(s)	Number of plants observed flowering in 2009
Pink	Darling 1	-33.4	18.4	1483809	222
Pink	Darling 2	-33.4	18.4	1483807 & 2019/5	> 250
Pink	Durbanville	-33.8	18.6	1483814	<100
Pink	Riverlands Nature Reserve	-33.5	18.6	1483811	187
Purple	Darling 3	-33.3	18.5	1483816 & 2019/1	190
Purple	Durbanville	-33.8	18.6	1483810	38
Red	Darling 3	-33.3	18.5	1483801	210
Red	Darling 4	-33.2	18.3	1483806	> 250
Red	Darling 5	-33.2	18.3	1483805 & 2019/2	> 250
White	Darling 4	-33.2	18.3	1483802	19
White	Darling 6	-33.4	18.4	2019/6	> 250
White	Darling 7	-33.2	18.3	1483804	> 250
Yellow	Piketberg 1	-32.8	18.8	1483812 & 2019/4	> 250
Yellow	Piketberg 2	-32.7	18.7	1483815	> 250
Yellow	Piketberg 3	-32.7	18.7	1483813	220

Breeding system experiments

To determine the degree of self-compatibility and capacity for autonomous self-fertilisation in *D. cistiflora* s.l., four treatments—(i) bagged only, (ii) open pollination, (iii) hand self-pollination and (iv) hand cross-pollination—were applied to a minimum of eight plants per treatment (mean = 11.1, range = 8–21) per population. The ‘bagged only’ treatment was applied to test for autonomous selfing. For this treatment, a fine bridal veil mesh bag was suspended over a wire frame and placed over an unmanipulated *D. cistiflora* s.l. plant with a marked flower in bud stage to ensure that pollinators could not access it. The bag was removed after the flower had wilted. The open pollination treatment was applied to test for natural seed set, where pollinators had access to flowers throughout their development. These flowers were not bagged, hand-pollinated or manipulated in any way. The hand self-pollination treatment was applied to test for self-incompatibility. Here, a marked flower was bagged in bud and when the bud opened, the receptive stigma was pollinated using ripe pollen from the same flower (flowers are strongly herkogamous, but not completely protandrous). The flower was re-bagged to prevent subsequent pollinator-mediated contamination and the bag was removed after the flower had wilted. The hand cross-pollination treatment was applied as a positive control. These flowers were emasculated in the bud phase prior to anther dehiscence, before being bagged to exclude pollinators. Once receptive, the stigma was pollinated using a donor from at least five metres away to avoid treating with pollen from within a possible genet, i.e. clonal group. The plant was re-bagged and the bag was removed once the flower had wilted. Experiments were conducted in 2009 and included a total of 335 bagged plants and 255 plants observed for natural seed set. In all treatments, only a single flower was used per plant. Sample size per treatment group is given in Supplementary Table 1.

Purple-flowered plants in Durbanville had anthers that appeared to be only partially formed and produced little to no pollen, and for this reason only natural seed set was recorded in this population (Supplementary Figure 1). Likewise, there were too few fully formed white flowers at Darling 4 for treatments (i), (iii) and (iv) to be performed.

For all treatments, mature seed capsules were collected and seeds [which were dark brown/black in colour, ovoid-ellipsoid and subacuminate in shape, and approximately

0.5mm long, with an irregularly colliculate surface pattern (pers. obs)] counted under a dissecting microscope approximately 20 days later. Wind-damaged exclusion-bagged plants were omitted.

Pollination indices

Empirical mean values of seed set per flower were used to calculate all pollination indices to obtain independent pollinator contribution to fecundity, pollinator dependence, self-incompatibility and pollen limitation indices, for each *D. cistiflora* s.l. population and for each floral colour form over all populations (Table 1). White-flowered populations at Darling 4, and pink and purple populations at Durbanville were excluded since only their natural seed set could be assessed.

Pollinator contribution to fecundity (PCF)

To obtain a metric for PCF, I compared seed set from the ‘bagged only’ treatment (i), that tested for autonomous self-pollination, with natural seed set (ii). This provides an indication of the pollinator contributions to natural seed set by assessing the degree of natural pollination without artificial pollen supplementation.

I computed PCF as:

$$PCF = 1 - \frac{\text{seed set through autonomous self - pollination}}{\text{seed set through open pollination}}$$

PCF varies from zero to one, with a total lack of pollinator contribution yielding a value of zero and a value of one defining maximum PCF.

Pollinator dependence (PD)

The PD index compared seed set from the ‘bagged only’ treatment (i) with that arising from hand cross-pollination (iv).

PD was computed as:

$$PD = 1 - \frac{\textit{seed set through autonomous self - pollination}}{\textit{seed set through hand cross - pollination}}$$

Here, a value of zero indicates independence from pollinators, whilst a value of one indicates high dependence on pollinators. PD is modified from the commonly presented ‘Index of Autofertility (AF)’ (sensu Lloyd & Schoen 1992, q.v. Anderson et al. 2014).

Self-incompatibility (SI)

The degree of self-compatibility was determined by comparing seed set from the hand cross-pollination treatment (iv) to seed set from the hand self-pollination treatment (iii) [sensu Lloyd 1965].

An index for SI was calculated as:

$$SI = 1 - \frac{\textit{seed set through hand self - pollination}}{\textit{seed set through hand cross - pollination}}$$

Self-compatibility varies from zero to one and decreases as the index approaches a value of one (complete self-incompatibility).

Pollen limitation (PL)

PL was measured by comparing natural seed set through open pollination (ii) with seed set after hand cross-pollination (iv) [modified from Larson & Barrett 2000], as follows:

$$PL = 1 - \frac{\textit{natural seed set}}{\textit{seed set through hand cross - pollination}}$$

Pollen limitation is expressed as a number between zero and one, where complete pollen limitation equates to a value of one whilst a lack of pollen limitation yields a value of zero.

Confidence intervals for the overall indices per colour form were calculated using a bootstrap procedure in which the mean values, together with ratios and indices derived from the means, were computed for each of 1000 resampled datasets. Bootstrap datasets were generated so that the same numbers of flowers were drawn from each treatment group at each population as was observed in the actual populations. Owing to differing sample sizes of treated plants between populations of the same colour form, overall means for each colour form were weighted by sample size. The overall mean seed set for each treatment per colour form was generated as the sum of all weighted population means: $(\sum_{i=1}^P w_i x_i) / \sum_{i=1}^P w_i$, where w_i is the sample size in population i ($i = 1, \dots, P$), and x_i is the mean seed set per sample in population i ($i = 1, \dots, P$).

Statistical analyses

Data were analysed using generalised linear mixed models (GLMM) implemented in R (R Core Team 2018). The number of flowers setting fruit in a *D. cistiflora* s.l. population was assumed to be binomially distributed. Probability of setting fruit was modelled as being dependent on treatment, floral colour form, and population. The explanatory variables thus include categorical fixed effects for treatment and flower colour and a random effect for population. Note that for a study of plant genetic factors such as this, population (rather than site) is the appropriate random effect level. As a result, two different colour populations occurring at the same site (at Darling 3 and Durbanville) are treated as separate levels rather than pooled into a common one. The linear dependency between the probability of setting fruit and explanatory variables was specified using a logit link function. The number of seeds per fruit was overdispersed relative to the expectation of a Poisson distribution (the variance exceeded the mean), and was thus modelled using a negative binomial distribution where the mean was dependent on treatment, floral colour form, and population. The explanatory variables thus included categorical fixed effects for treatment and flower colour and a random effect for population. Linear dependency was specified using a log link function. Following model fitting, the Tukey method was used to adjust for multiple comparisons in post hoc tests. Marginal means were obtained by back-transformation of values from the linear scale, resulting in asymmetrical standard errors. In cases in which GLMMs for seed set did not run because of lack of variance

(e.g. no fruits set for certain combinations of colour form and treatment effect), a single value of one seed was substituted (q.v. Zuur et al. 2009), which also makes the test more conservative (Johnson et al. 2019).

Results

Breeding system experiments

Overall, fruit and seed production varied between treatment conditions [fruit: $\chi^2(3) = 101.79$, $p < 0.001$; seed: $\chi^2(3) = 123.22$, $p < 0.001$] and *Drosera cistiflora* s.l. floral colour forms [fruit: $\chi^2(4) = 32.80$, $p < 0.001$; seed: $\chi^2(4) = 20.74$, $p < 0.001$], with a significant interaction effect between treatment condition and floral colour form for seed production ($\chi^2 = 73.20$, $p < 0.001$) [Figure 1a&b].

The effects of pollinator exclusion varied among floral colour forms, with purple- and yellow-flowered forms showing almost no fruit production after pollinator exclusion, as opposed to ca 25% of flowers setting fruit following this treatment in the pink-, red- and white-flowered forms (Figure 1a). However, seed set arising from autonomous self-pollination was very low across all colour forms (Figure 1b). Overall, significant differences ($p < 0.001$) in the marginal (model-adjusted) mean proportion of flowers that set fruit were found between the autonomous self-pollination [mean = 0.07, 95% confidence interval (0.03, 0.16)] and open pollination [mean = 0.63, 95% confidence interval (0.46, 0.78)] treatments. Additionally, significantly ($p < 0.001$) fewer seeds were set per fruit through autonomous selfing [mean = 28.47, 95% confidence interval (18.19, 44.54)] than open pollination [mean = 149.82, 95% confidence interval (105.98, 211.79)], with these differences being driven by significant differences in red ($z = 6.50$, $p < 0.001$), white ($z = 4.49$, $p < 0.001$) and pink ($z = 4.04$, $p < 0.001$) floral colour forms (other colours: $z < 0.17$, $p > 0.998$) [Supplementary Table 1]. Evidence is thus provided for high pollinator contribution to fecundity in *D. cistiflora* s.l.

Hand cross-pollinated flowers showed almost 100% fruit set in the case of the pink-, red- and white-flowered forms and ca 60% fruit set for the purple- and yellow-flowered forms. Autonomous self-pollination yielded lower levels of fruit set to hand cross-pollination across all forms (Figure 1a, Supplementary Table 1). Here, fruit set in hand cross-pollinated flowers [mean = 0.94, 95% confidence interval (0.84, 0.98)] was significantly ($p < 0.001$) greater than that from autonomous self-pollination [mean =

0.07, 95% confidence interval (0.03, 0.16)]. Similarly, seed set per fruit after autonomous self-pollination [mean = 28.47, 95% confidence interval (18.19, 44.54)] was significantly ($p < 0.001$) less than after hand cross-pollination [mean = 259.22, 95% confidence interval (184.40, 364.38)] (Figure 1b, Supplementary Table 1), with these differences being significant for all floral colour forms (all $z > 3.61$, all $p < 0.002$), thereby suggesting that *D. cistiflora* s.l. is strongly pollinator-dependent.

Across all floral colour forms, fruit set in hand cross-pollinated flowers [mean = 0.94, 95% confidence interval (0.84, 0.98)] was significantly ($p = 0.002$) greater than that from hand self-pollination [mean = 0.70, 95% confidence interval (0.48, 0.85)] (Figure 1a, Supplementary Table 1). Seed set per fruit after hand self-pollination [mean = 159.81, 95% confidence interval (110.69, 230.72)] was significantly ($p = 0.003$) less than after hand cross-pollination [mean = 259.22, 95% confidence interval (184.40, 364.38)] overall (Figure 1b, Supplementary Table 1), with these differences being driven by significant differences in pink- and yellow-flowered forms (pink: $z = 3.47$, $p = 0.003$; yellow: $z = 3.20$, $p = 0.007$; other colours: $z < 1.03$, $p > 0.735$). Partial self-incompatibility and/or strong inbreeding depression are thereby indicated in *D. cistiflora* s.l.

Levels of natural fruit set in open-pollinated flowers were similar to those following hand self-pollination (Figure 1a, Supplementary Table 1). Natural fruit set and seed set per fruit were particularly low for the purple- and yellow-flowered forms (Figure 1a&b, Supplementary Table 1). In purple-flowered forms at Durbanville, only two plants out of twenty that were open-pollinated produced fruit, and only one seed was found in each fruit. Low fruit and seed set may be associated with abnormal pollen production in this population (Supplementary Figure 1).

Mean fruit set was significantly ($p < 0.001$) higher in hand cross-pollinated flowers [mean = 0.94, 95% confidence interval (0.84, 0.98)] than in open-pollinated flowers [mean = 0.63, 95% confidence interval (0.46, 0.78)] (Figure 1a, Supplementary Table 1). Hand cross-pollinated flowers also set significantly ($p < 0.001$) more seed per fruit [mean = 259.22, 95% confidence interval (184.40, 364.38)] than those in open pollination treatments [mean = 149.82, 95% confidence interval (105.98, 211.79)] (Figure 1b, Supplementary Table 1), suggesting that *D. cistiflora* s.l. may be pollen-

limited. These differences were driven by significant differences in purple ($z = 6.18$, $p < 0.001$), yellow ($z = 4.40$, $p < 0.001$) and pink ($z = 3.96$, $p < 0.001$) floral colour forms (other colours: $z < 0.82$, $p > 0.845$).

Across all treatments, purple- and yellow-flowered forms had significantly ($p < 0.011$) lower proportion fruit set than pink-, red- and white-flowered forms, and produced significantly ($0.004 < p < 0.025$) fewer seeds per fruit than red- and white-flowered forms (Figure 1a&b), with pink-flowered forms occupying an intermediate position in the case of seed set.

Post hoc tests for the significant differences between treatment conditions and *D. cistiflora* s.l. floral colour forms, as discussed here, are provided in Supplementary Tables 2, 3, 4 and 5.

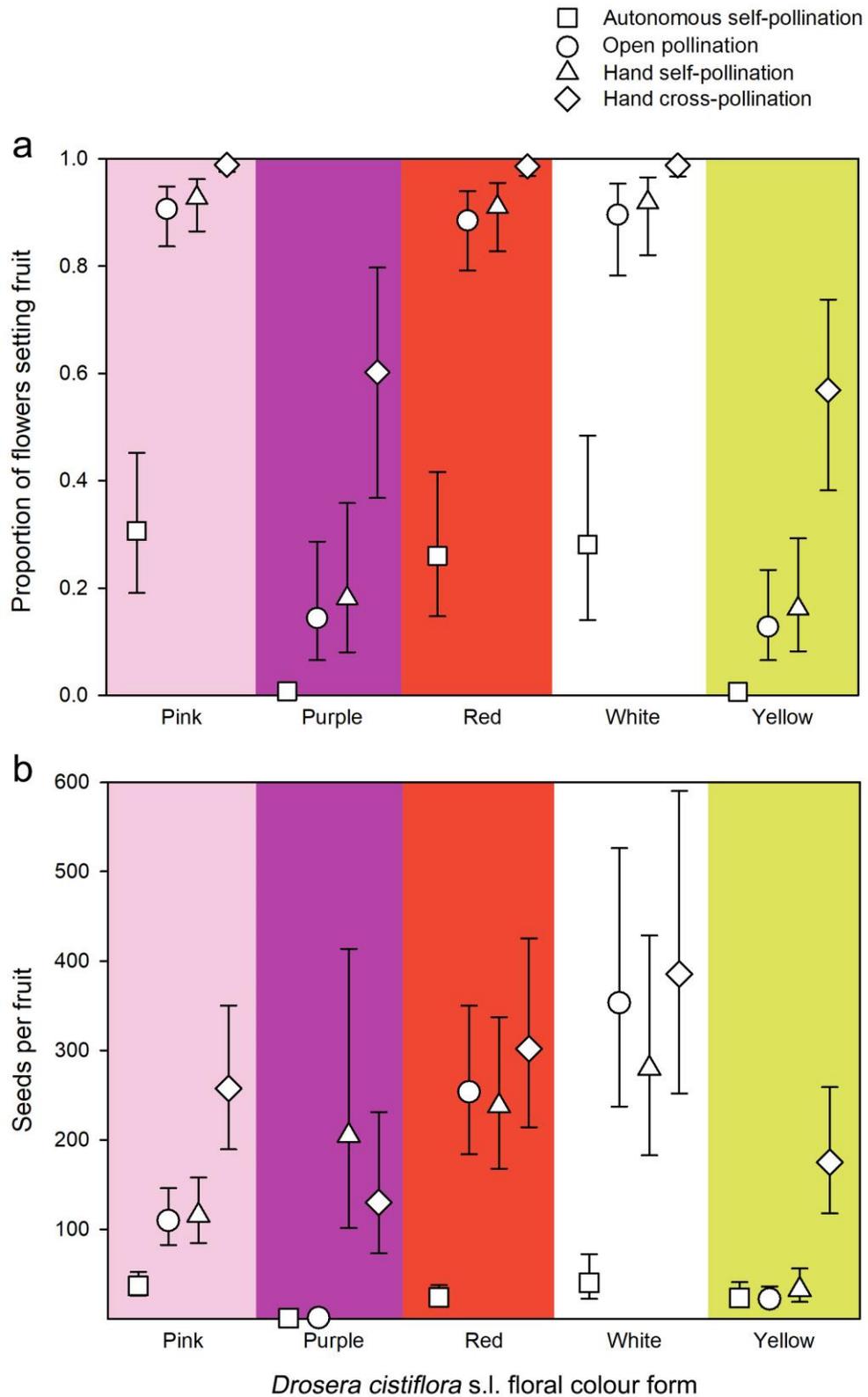


Figure 1. Comparison of the effects of autonomous self-pollination, open pollination, hand self-pollination and hand cross-pollination in each *Drosera cistiflora* s.l. floral colour form. Values represent marginal model mean (and asymmetrical standard error) proportions of flowers setting fruit (a) and number of seeds per fruit (b).

Pollination indices

Separate GLMMs were fitted for fruit set and seed set outcomes. Attempts to model both simultaneously suffered from estimation problems relating to overdispersion caused by the introduction of extra zeros induced by observations where no fruit was set. Pollination indices based on empirical means allow me to illustrate variation in both fruit and seed set in overall floral colour forms (Table 2) and between populations (Tables 3–6) with a single set of indices.

Table 2. Overall indices of pollinator contribution to fecundity (PCF), pollinator dependence (PD), self-incompatibility (SI) and pollen limitation (PL) for the five floral colour forms of *Drosera cistiflora* s.l. Indices for each floral colour form were calculated using empirical means weighted by sample size. PCF values for the yellow-flowered form were indeterminate, since seed set from both autonomous self-pollination and open pollination equal zero in Piketberg 2 (see main text for full explanation). 95% confidence intervals are shown in parentheses.

<i>Drosera</i> <i>cistiflora</i> s.l. floral colour form	PCF	PD	SI	PL
Pink	0.67 (0.51, 0.86)	0.89 (0.81, 0.94)	0.53 (0.37, 0.65)	0.66 (0.42, 0.72)
Purple	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	0.41 (-0.54, 1.00)	1.00 (1.00, 1.00)
Red	0.98 (0.96, 1.00)	0.98 (0.97, 1.00)	0.32 (-0.03, 0.57)	0.07 (-0.22, 0.28)
White	0.96 (0.90, 1.00)	0.97 (0.92, 1.00)	0.29 (0.06, 0.49)	0.19 (-0.05, 0.39)
Yellow	Indet. (-5.76, 1.00)	0.90 (0.67, 1.00)	0.82 (0.53, 1.00)	0.92 (0.82, 0.98)

Pollinator contribution to fecundity (PCF)

Indices of overall PCF for each floral colour form, calculated using means weighted according to population sample sizes, ranged from 0.67 to 1.00 among floral colour forms (Table 2). PCF was highest in white, red and purple floral colour forms in an ascending order of magnitude (Table 2). The indeterminate PCF index associated with

yellow-flowered forms (Table 2) is a function of the indeterminate PCF of the Piketberg 2 population. Here, no seed was set through either autonomous self-pollination or open pollination, thus $PCF = 1 - \frac{0}{0}$ (assuming $\frac{0}{0} = x$, then $0 = x \cdot 0$ which is true for any x value, hence PCF cannot be determined). Site-specific PCF indices (Table 3) for yellow-flowered populations at Piketberg 1 (0.40) and Piketberg 3 (1.00) however show that the yellow-flowered form exhibits some degree of PCF in reality.

Table 3. Pollinator contribution to fecundity (PCF) indices and empirical means \pm SE of seed set from autonomous self-pollination and open pollination for each *Drosera cistiflora* s.l. population, where $PCF = 1 - \frac{\text{seed set through autonomous self-pollination}}{\text{seed set through open pollination}}$. Sample size (n) indicates number of plants treated (one flower per plant). Indeterminate refers to the instance where seed set from both autonomous self-pollination and open pollination equal zero.

<i>Drosera cistiflora</i> s.l. flower colour and site	Autonomous self-pollination	n	Open pollination	n	PCF
Pink (Darling 1)	7.50 \pm 4.12	8	102.54 \pm 26.76	11	0.93
Pink (Darling 2)	68.13 \pm 19.64	15	196.56 \pm 39.55	16	0.65
Riverlands NR	2.38 \pm 1.31	8	36.90 \pm 3.16	10	0.94
Purple (Darling 3)	0.00 \pm 0.00	10	0.10 \pm 0.10	20	1.00
Red (Darling 3)	2.31 \pm 2.31	13	180.48 \pm 10.91	21	0.99
Red (Darling 4)	0.00 \pm 0.00	8	445.00 \pm 33.44	20	1.00
Red (Darling 5)	8.64 \pm 4.98	14	159.33 \pm 14.78	15	0.95
White (Darling 6)	26.62 \pm 18.34	8	506.00 \pm 60.73	15	0.95
White (Darling 7)	0.00 \pm 0.00	8	184.00 \pm 35.34	20	1.00
Yellow (Piketberg 1)	27.20 \pm 24.83	10	45.00 \pm 20.72	10	0.40
Yellow (Piketberg 2)	0.00 \pm 0.00	8	0.00 \pm 0.00	20	Indet.
Yellow (Piketberg 3)	0.00 \pm 0.00	8	0.29 \pm 0.17	21	1.00

Pollinator dependence (PD)

PD indices indicated that all floral colour forms are highly dependent on pollinators for seed set and that autonomous selfing contributes little to reproductive output (Table 2). Pink-flowered forms had the lowest dependence on pollinators (0.89) and purple-flowered forms were completely dependent on pollinators (1.00) [Table 2]. Similarly, at the population level, no population had a PD index of less than 0.83 (Table 4).

Table 4. Pollinator dependence (PD) indices and empirical means \pm SE of seed set from autonomous self-pollination and hand cross-pollination treatments for each *Drosera cistiflora* s.l. population, where $PD = 1 - \frac{\text{seed set through autonomous self-pollination}}{\text{seed set through hand cross-pollination}}$. Sample size (n) indicates number of plants treated (one flower per plant).

<i>Drosera cistiflora</i> s.l. flower colour and site	Autonomous self-pollination	n	Hand cross- pollination	n	PD
Pink (Darling 1)	7.50 \pm 4.12	8	356.25 \pm 36.79	8	0.98
Pink (Darling 2)	68.13 \pm 19.64	15	394.44 \pm 62.88	18	0.83
Riverlands NR	2.38 \pm 1.31	8	76.88 \pm 40.89	8	0.97
Purple (Darling 3)	0.00 \pm 0.00	10	137.50 \pm 29.32	8	1.00
Red (Darling 3)	2.31 \pm 2.31	13	276.67 \pm 59.38	12	0.99
Red (Darling 4)	0.00 \pm 0.00	8	363.75 \pm 84.81	8	1.00
Red (Darling 5)	8.64 \pm 4.98	14	233.75 \pm 31.50	8	0.96
White (Darling 6)	26.62 \pm 18.34	8	501.25 \pm 68.23	8	0.95
White (Darling 7)	0.00 \pm 0.00	8	296.25 \pm 42.00	8	1.00
Yellow (Piketberg 1)	27.20 \pm 24.83	10	206.25 \pm 38.36	8	0.87
Yellow (Piketberg 2)	0.00 \pm 0.00	8	43.75 \pm 37.13	8	1.00
Yellow (Piketberg 3)	0.00 \pm 0.00	8	71.25 \pm 46.73	8	1.00



Figure 2. The hopliine beetle *Omocrates* sp. (Coleoptera: Scarabaeidae: Hopliini) visiting the highly pollinator-dependent purple *Drosera cistiflora* s.l. floral colour form at Darling 3

Self-incompatibility (SI)

White, red, purple and pink floral colour forms were partially self-incompatible, with SI indices ranging from 0.29 (white) to 0.53 (pink) [Table 2]. Yellow-flowered forms displayed high levels of self-incompatibility (0.82), since plants in only one out of three populations set seed from self-pollen, and hand self-pollination yielded lower seed set than hand cross-pollination in all yellow-flowered populations. The degree of self-incompatibility varied substantially among populations of different flower colours [range: 0.18 (pink, Riverlands NR) to 1.00 (yellow, Piketberg 2 & 3), Table 5]. Self-incompatibility also varied between populations of the same flower colour, particularly between pink-flowered populations. For example, the SI index of 0.18 at Riverlands NR suggests good selfing capability, whilst another pink-flowered population had an SI index of 0.87, suggesting poor selfing capability (Table 5).

Table 5. Self-incompatibility (SI) indices and empirical means \pm SE of seed set from hand self-pollination and hand cross-pollination treatments for each *Drosera cistiflora* s.l. population, where $SI = 1 - \frac{\text{seed set through hand self-pollination}}{\text{seed set through hand cross-pollination}}$. Sample size (n) indicates number of plants treated (one flower per plant).

<i>Drosera cistiflora</i> s.l. flower colour and site	Hand self- pollination	n	Hand cross- pollination	n	SI
Pink (Darling 1)	46.25 \pm 7.30	8	356.25 \pm 36.79	8	0.87
Pink (Darling 2)	244.67 \pm 31.20	15	394.44 \pm 62.88	18	0.38
Riverlands NR	63.00 \pm 21.73	8	76.88 \pm 40.89	8	0.18
Purple (Darling 3)	81.25 \pm 50.44	8	137.50 \pm 29.32	8	0.41
Red (Darling 3)	200.00 \pm 56.66	12	276.67 \pm 59.38	12	0.28
Red (Darling 4)	201.25 \pm 84.80	8	363.75 \pm 84.81	8	0.45
Red (Darling 5)	183.75 \pm 45.86	8	233.75 \pm 31.50	8	0.21
White (Darling 6)	335.00 \pm 40.71	8	501.25 \pm 68.23	8	0.33
White (Darling 7)	231.25 \pm 62.18	8	296.25 \pm 42.00	8	0.22
Yellow (Piketberg 1)	58.12 \pm 37.56	8	206.25 \pm 38.36	8	0.72
Yellow (Piketberg 2)	0.00 \pm 0.00	8	43.75 \pm 37.13	8	1.00
Yellow (Piketberg 3)	0.00 \pm 0.00	8	71.25 \pm 46.73	8	1.00

Pollen limitation (PL)

PL indices ranged from 0.07 in red-flowered forms (least pollen-limited) to 1.00 in purple-flowered forms (complete pollen limitation). Fecundity of pink-, purple- and yellow-flowered forms was the most pollen-limited (Table 2). Despite plenty of variance in pollen limitation between floral colour forms (Table 2), variance within each form was low (Table 6). Some populations had slightly negative values for this metric, suggesting higher natural seed set than when flowers were hand-crossed. Slight negative values may reflect no real difference from zero. Alternatively, these could result from the fact that hand-pollinated flowers were bagged after crosses were made; hence open-pollinated flowers may have had more mating opportunities than hand-pollinated flowers in some instances.

Table 6. Pollen limitation (PL) indices and empirical means \pm SE of seed set from open pollination and hand cross-pollination treatments for each *Drosera cistiflora* s.l. population, where $PL = 1 - \frac{\text{natural seed set}}{\text{seed set through hand cross-pollination}}$. Sample size (n) indicates number of plants treated (one flower per plant).

<i>Drosera cistiflora</i> s.l. flower colour and site	Open pollination	n	Hand cross- pollination	n	PL
Pink (Darling 1)	102.54 \pm 26.76	11	356.25 \pm 36.79	8	0.71
Pink (Darling 2)	196.56 \pm 39.55	16	394.44 \pm 62.88	18	0.50
Riverlands NR	36.90 \pm 3.16	10	76.88 \pm 40.89	8	0.52
Purple (Darling 3)	0.10 \pm 0.10	20	137.50 \pm 29.32	8	1.00
Red (Darling 3)	180.48 \pm 10.91	21	276.67 \pm 59.38	12	0.35
Red (Darling 4)	445.00 \pm 33.44	20	363.75 \pm 84.81	8	-0.22
Red (Darling 5)	159.33 \pm 14.78	15	233.75 \pm 31.50	8	0.32
White (Darling 6)	506.00 \pm 60.73	15	501.25 \pm 68.23	8	-0.01
White (Darling 7)	184.00 \pm 35.34	20	296.25 \pm 42.00	8	0.38
Yellow (Piketberg 1)	45.00 \pm 20.72	10	206.25 \pm 38.36	8	0.78
Yellow (Piketberg 2)	0.00 \pm 0.00	20	43.75 \pm 37.13	8	1.00
Yellow (Piketberg 3)	0.29 \pm 0.17	21	71.25 \pm 46.73	8	1.00

Discussion

This study demonstrates that all floral colour forms of *Drosera cistiflora* s.l. have high pollinator contribution to fecundity (Table 3) and are highly dependent on pollinators for seed production (Tables 2 & 4). Floral colour forms range from partially self-compatible to almost completely self-incompatible (Tables 2 & 5, Fig. 1). In addition, fecundity in most forms appears to be strongly pollen-limited (Tables 2 & 6). Below I discuss the ecological and evolutionary significance of these four features of the breeding systems of *D. cistiflora* s.l. floral colour forms, with reference to pollination indices for individual forms. Results contrast starkly with the general view that *Drosera* species are often capable selfers with low levels of dependence on pollinators (cf. Murza et al. 2006; Sciligo et al. 2007; Sciligo 2009; Cross et al. 2018).

Pollinator contribution to fecundity and pollinator dependence

The partial genetic self-incompatibility and spatial separation of the anthers and stigmas of *D. cistiflora* s.l. plants are likely to be contributing factors to their dependence on pollinators. Relatively high natural seed set, in combination with low seed set from autonomous selfing, indicates high PCF in red (98%), white (96%) and pink (67%) floral colour forms (Tables 2 & 3). Although PD indices indicate complete pollinator dependence in two yellow-flowered populations, namely Picketberg 2 & 3 (Table 4), exceptionally low natural seed set was also recorded here (Table 3). The higher seed set after hand-crosses (Table 4) suggests a shortage of effective pollinators in both of these yellow-flowered populations and the possibility of a mutualism collapse. Correspondingly, unusually low pollinator visitation was observed in these two yellow-flowered populations (pers. obs), which was unexpected given the pristine, unfragmented nature of the landscape. It is uncertain why pollinators in these populations have performed so poorly and whether this situation is temporary or more permanent. It is possible that very recent fires (seven months prior to flowering) adversely affected pollinator communities through mortality or shortages of food plants for insects (Dafni et al. 2013). Pollinator abundance may also be influenced by low soil

nutrient levels in the sandstone fynbos vegetation type at Piketberg 2 & 3. Here, sandstone soils are thought to generate plant unpalatability, which in turn may support low insect numbers (Cottrell 1985, Anderson et al. 2014). The risks of pollinator failure in *D. cistiflora* s.l. are mitigated in the short-term by the fact that the plants are perennial and can reproduce vegetatively (q.v. Bond 1994). However, sexual reproduction is considered important for the long-term persistence of species (Wuerth et al. 2018), with persisting reproductive success usually depending on pollinators to ensure outcrossing (Pauw & Bond 2011). Remarkably low natural seed set was also found in the two purple-flowered populations (Supplementary Table 1) and purple-flowered plants at Darling 3 had low fecundity after hand-crossing (Table 4). Since the appearance of important visitors to purple flowers at Darling 3 (Figure 2) occurred approximately two weeks after the population was treated, poor natural seed set in this population may have been influenced by premature timing of the experiment rather than a recent mutualism collapse. Another explanation is that infertility may be the mechanism behind overall low seed set in these purple- and yellow-flowered populations, which is evidenced at Durbanville by the lack of pollen production by purple flowers (Supplementary Figure 1). Infertility could occur as a result of inbreeding depression (Eckert 2001, Wilcock & Neiland 2002, Charlesworth & Willis 2009, Rodger & Johnson 2013, Barrett 2015, Evans et al. 2017) or alternatively as a result of a lack of vigour of F₁ and/or subsequent filial generations (Mayr 1992, Johansen-Morris & Latta 2006) produced through hybridisation of sympatrically growing floral colour forms. Both purple-flowered populations were habitat-fragmented, suggesting that quantity (Buza et al. 2000) and/or quality (Aguilar et al. 2019) of progeny may be reduced through inbreeding; furthermore, these populations grew at two of three sites in this study which had sympatric *D. cistiflora* s.l. floral colour forms where introgression was possible. Whilst I did not observe plants in these populations with intermediate floral colour phenotypes, their mode of colour inheritance is not known; consequently, the lack of apparently intermediate forms cannot exclude the possibility of introgression. Further investigation into these potential mechanisms is required.

Self-incompatibility

The significantly greater fruit set after hand cross-pollination than hand self-pollination in all *D. cistiflora* s.l. floral colour forms (Figure 1a, Supplementary Table 2) suggests that *D. cistiflora* s.l. may possess partial self-incompatibility. Hand self-pollination also resulted in lower seed set per fruit than cross-pollination in all floral colour forms except purple; however these results for purple-flowered forms were not significant (Supplementary Table 4) and empirical means for seed set show evidence of partial self-incompatibility in this population (Table 5). Evidence of higher fruit and seed set after hand self-pollination than autonomous selfing in all populations (Figure 1, Supplementary Table 1) suggests that selfing largely relied on facilitation by pollinating vectors. The SI index for yellow-flowered forms was particularly high (82%, Table 2), with two populations at Piketberg appearing to be completely self-incompatible (Table 5).

Apparent incompatibility does not however preclude the possibility that plants may be self-compatible with high levels of early-acting inbreeding depression. However, seed set through inbreeding in self-compatible plants typically results in deleterious recessive alleles being exposed and selected against more often than would occur in self-incompatible plants (Gigord et al. 1998). The consequent purging of genetic load renders inbred self-compatible plants less likely to experience inbreeding depression in successive generations, e.g. Busch (2005). This scenario is plausible in most *D. cistiflora* s.l. floral colour forms, where seed set from selfing may stem from ‘leaky’ or partial incompatibility, resulting in seed setting even when individuals with the same incompatibility alleles are interbred (Reinartz & Lez 1994, Gigord et al. 1998). Although the scope of purging may depend on the plant species concerned (Byers & Waller 1999), Crnokrak & Barrett (2002) found a widespread reduction in the cost of inbreeding with purging in their review of data from studies of 13 plant species. Flower size, which may be affiliated with self-infertility, has been inversely associated with purging in *Collinsia parviflora* (Scrophulariaceae) [Kennedy & Elle 2008]. The putative higher genetic load in self-incompatible plants than their autogamous counterparts (Barrett & Charlesworth 1991) thus means that the yellow-flowered *D. cistiflora* s.l. populations may be prone to inbreeding depression on account of self-incompatibility. This may be pronounced with population bottlenecks and pollination

failure. Self-incompatible, pollinator-dependent plants also have a high likelihood of pollen limitation and fecundity impairment following pollinator failure because their own pollen does not contribute to fertilisation (Larson & Barrett 2000). The low pollinator visitation observed in these yellow-flowered populations thus renders them particularly vulnerable to advanced inbreeding depression. Although SI could not be assessed because of pollen paucity, the low female fitness after open pollination as well as the floral malformations of the small population of white-flowered forms at Darling 4 and large-flowered purple *D. cistiflora* s.l. forms at the severely habitat-fragmented Durbanville population may be evidence of inbreeding depression. On the whole, although inbreeding depression may occur in *D. cistiflora* s.l. in certain instances, SI indices of floral colour forms may generally be attributed to partial incompatibility. Comparisons of progeny vigour from experimentally selfed versus outcrossed plants would yield a more conclusive assessment of inbreeding depression in *D. cistiflora* s.l.

Sexual selection generally favours plant taxa with some degree of self-incompatibility (Raduski et al. 2012), and partial or complete self-incompatibility in *D. cistiflora* s.l. floral colour forms may thus play a role in the evolution of their many bright colours and sizable flowers (32–70mm, pers. obs) that are aberrant for *Drosera*. In combination with a lack of pollinator/prey species overlap in *D. cistiflora* s.l. (q.v. Anderson 2010), findings of self-incompatibility contrast with the notion that selfing mechanisms have evolved to reduce pollinator/prey conflict in *Drosera* (q.v. Sciligo et al. 2007, Sciligo 2009, Jürgens et al. 2011). However, they support the idea that elongated cymes promote pollinator visitation (q.v. Anderson 2010, where elevated *D. cistiflora* s.l. flowers received more visits than flowers of shortened plants in experimental manipulations) by marketing the plants' reproductive parts to pollinators in the presence of pollinator dependence.

Pollen limitation

All *D. cistiflora* s.l. floral colour forms displayed some degree of pollen limitation (Table 2), and PL indices were particularly high in pink-, purple- and yellow-flowered forms (Table 6). This indicates that seed set is considerably limited by a paucity of cross-pollen reaching the stigmas. It is therefore expected that there should be strong

selection acting on traits that make these plants more attractive to pollinators or enhance the efficiency of pollen transfer (Knight et al. 2005). Although the flowers of *D. cistiflora* s.l. are odourless and nectarless, their large size, raised height, bowl shape, showy colours and conspicuous orange-yellow anthers may make them easily visible. Floral characters such as these are commonly found in hopliine beetle-pollinated flowers (q.v. van Kleunen et al. 2007), where it has been proposed that the beetles visit the flowers in search of food and mates (Goldblatt et al. 1998) in response to visual rather than olfactory cues (Steiner 1998). Adaptations to hopliine beetle pollination in *D. cistiflora* s.l. may underlie the absence of pollinator-prey conflict in these insectivorous plants. This is conceivable because the beetles' relatively large size and armoured morphology (hard exoskeleton and elytra) may confer protection against trapping by mucilaginous glands, in contrast to smaller soft-bodied pollinators which may be trapped more easily (q.v. Zamora 1999).

Usually pollen limitation is calculated by comparing natural seed set with that arising from supplemental cross-pollination of open flowers (Larson & Barrett 2000, Knight et al. 2006) and is conventionally confirmed when plants with pollen supplementation produce more seed than control plants (Bierzychudek 1981, Ashman et al. 2004, Knight et al. 2005). Since my hand-pollinated flowers were bagged, they were protected from seed predators, whilst the open-pollinated flowers were not bagged and thus exposed to seed predators. Consequently, if seed predators account for lower seed production in open-pollinated flowers, then pollen limitation may be overestimated in this study. Thus, although the particularly high PL indices in purple- and yellow-flowered *D. cistiflora* s.l. forms could be attributed to low natural seed set stemming from pollinator insufficiency, they may alternatively proceed from seed predation on unbagged open-pollinated flowers. This is however unlikely, since minimal seed predation was observed when dissecting fruits and this did not vary in an obvious manner between treatments. Factors contributing to pollinator paucity in these floral colour forms may also have analogously affected seed predators.

Pollen delivery through natural pollination may be more gradual than through hand cross-pollinations; my bagged hand cross-pollinations may thus have experienced more intense pollen competition and/or stigma clogging (q.v. Ashman et al. 2004), which may be detrimental and lead to an underestimation of PL.

Supplemental hand-pollination with pure cross-pollen can also be considered unnatural and artificially boost seed set (Aizen & Harder 2007). By comparing natural seed set with seed set in both self- and cross-pollinated flowers (Figure 1b, Supplementary Table 1), I could determine if pollen limitation is due only to the quantity of pollen or if pollen quality also matters. The significantly greater seed set through hand cross-pollination than open pollination in *D. cistiflora* s.l. floral colour forms overall (Supplementary Table 2) indicates that seed set may be limited by pollen quantity (q.v. Aizen & Harder 2007). However, hand cross-pollinations transferred pure intraspecific cross-pollen only whilst pollinators usually deposit pollen mixtures which can include self-pollen, cross-pollen (Thomson 2001), and pollen from other plant species (Fang & Huang 2013). My bagging of hand cross-pollination treatments may have further enhanced these differences (q.v. Ashman et al. 2004). Open pollination also did not usually result in more seed than hand-selfing experiments (Figure 1b, Supplementary Table 1), suggesting that much of it could potentially be the result of facilitated selfing. The general increase in hand-crossed seed set relative to natural and hand-selfing (Figure 1b, Supplementary Table 1) is further evidence that fecundity is also being limited by pollen quality. If the timing and rate of pollen delivery in hand cross-pollinations were inopportune, the added pollen load may have impeded effective ovule fertilisation (Ashman et al. 2004). Here, pollen quality rather than quantity would be the primary factor resulting in improved seed set in *D. cistiflora* s.l. In summary, pollen quality as well as quantity limitation appear to be acting in *D. cistiflora* s.l., thereby further emphasising the importance of effective pollinator movement in ensuring the fecundity of each floral colour form. Rodger & Ellis (2016) accordingly found evidence suggesting that pollen quality was an important contributor towards pollen limitation in pollinator-dependent plants in the GCFR.

Considering uncertainties around the effects of seed predation on open-pollinated flowers; pollen competition resulting from application of a single large cross-pollen dose; lack of data on ovule numbers, and supplementation with pure cross-pollen, it follows that conclusions regarding pollen limitation in this manuscript are inferential only. Nevertheless, since the effects of these factors appear to be small, it is likely that many *D. cistiflora* s.l. populations are indeed pollen-limited.

Conclusions

Striking differences in breeding system biology among the floral colour forms included in this study suggest that these forms differ in more than just floral colour pigmentation. Strong pollinator dependence, partial self-incompatibility and inferred pollen limitation also suggest that colour may be under pollinator-mediated selection and that floral colour variation could reflect adaptations to different pollinators. Further experimental research is required to test the adaptive significance of floral colour variation and whether pollinators may be contributing towards apparent divergent evolutionary trajectories within the *D. cistiflora* complex. The several examples of co-occurring floral colour forms (namely purple and red, purple and pink, purple and white, and red and white), without the formation of obvious fertile hybrids, suggest that some of these forms deserve recognition at the species level. Moreover, preliminary findings show reduced seed set after inter- vs intra-colour hand-crossing between sympatric purple- and red-flowered forms, incompatibility of sympatric pink- and purple-flowered forms, and unidirectional incompatibility of red- with white-flowered forms in sympatry (viz. white-flowered forms did not set seed with red flower pollen donors and seed set of red-flowered forms was reduced with white flower pollen donors). These examples indicate that future research into reproductive isolation barriers and molecular phylogenies may also provide useful insights into the taxonomic and affiliated conservation statuses of rare plant forms.

From a conservation management perspective, there is a need to study the environmental basis for pollination failure. Statistical models that include measurements of fragment size, population size, population density, population isolation, vegetation type, post-fire successional stage, soil fertility, pollinator abundance and diversity, and urbanisation offer promise to explain patterns of natural seed set in *D. cistiflora* s.l. floral colour forms. Such information would supplement the limited existing research on the role of pollinators in long-term maintenance of habitat integrity and may improve comprehension of the requirements for effective environmental management strategies.

References

- Aguilar, R., Cristóbal-Pérez, E. J., Balvino-Olvera, F. J., de Jesús Aguilar-Aguilar, M., Aguirre-Acosta, N., Ashworth, L., Lobo, J. A., Martén-Rodríguez, S., Fuchs, E. J., Sanchez-Montoya, G., Bernardello, G. & Quesada, M. (2019) Habitat fragmentation reduces plant progeny quality: A global synthesis. *Ecology Letters* **22** (7), 1163–1173.
- Aizen, M. A. & Harder, L. D. (2007) Expanding the limits of the pollen-limitation concept: Effects of pollen quantity and quality. *Ecology* **88**, 271–281.
- Anderson, B. (2010) Did *Drosera* evolve long scapes to stop their pollinators from being eaten? *Annals of Botany* **106** (4), 653–657.
- Anderson, B., Allsopp, N., Ellis, A. G., Johnson, S. D., Midgley, J. J., Pauw, A. & Rodger, J. (2014) Biotic interactions. In N. Allsopp, J. F. Colville, A. G. Verboom & R. M. Cowling [Eds], *Ecology and evolution of fynbos: Understanding megadiversity*. Oxford University Press, Oxford, UK.
- Anderson, B. & Midgley, J. J. (2001) Food or sex; pollinator-prey conflict in carnivorous plants. *Ecology Letters* **4**, 511–513.
- Anderson, B., Midgley, J. J. & Stewart, B. A. (2003) Facilitated selfing offers reproductive assurance: A mutualism between a hemipteran and carnivorous plant. *American Journal of Botany* **90**, 1009–1015.
- Ashman, T. L., Knight, T. M., Steets, J. A., Amarasekare, P., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mazer, S. J., Mitchell, R. J., Morgan, M. T. & Wilson, W. G. (2004) Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. *Ecology* **85** (9), 2408–2421.
- Ashman, T. L. & Morgan, M. T. (2004) Explaining phenotypic selection on plant attractive characters: Male function, gender balance or ecological context? *Proceedings of the Royal Society B — Biological Sciences* **271**, 553–559.

- Barrett, S. C. H. (2002) The evolution of plant sexual diversity. *Nature Reviews: Genetics* **3**, 273–284.
- Barrett, S. C. H. (2003) Mating strategies in flowering plants: The outcrossing-selfing paradigm and beyond. *Philosophical Transactions of the Royal Society B: Biological Sciences* **358**, 991–1004.
- Barrett, S. C. H. (2015) Influences of clonality on plant sexual reproduction. *Proceedings of the National Academy of Sciences of the United States of America* **112**, 8859–8866.
- Barrett, S. C. H. & Charlesworth, D. (1991) Effects of a change in the level of inbreeding on the genetic load. *Nature* **352**, 522–524.
- Barrett, S. C. H. & Eckert, C. G. (1990) Variation and evolution of mating systems in seed plants. In S. Kawano [Ed.], *Biological approaches and evolutionary trends in plants*, 229–254. Academic Press, London, UK.
- Barrett, S. C. H. & Harder, L. D. (1996) Ecology and evolution of plant mating. *Trends in Ecology and Evolution* **11** (2), 73–79.
- Bateman, A. J. (1948) Intra-sexual selection in *Drosophila*. *Heredity* **2**, 349–368.
- Bierzychudek, P. (1981) Pollinator limitation of plant reproductive effort. *The American Naturalist* **117**, 838–840.
- Bond, W. J. (1994) Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical Transactions of the Royal Society B: Biological Sciences* **344**, 83–90.
- Brown, J., York, A., Christie, F. & McCarthy, M. (2017) Effects of fire on pollinators and pollination. *Journal of Applied Ecology* **54**, 313–322.

- Burd, M. (1994) Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *The Botanical Review* **60**, 83–112.
- Busch, J. W. (2005) Inbreeding depression in self-incompatible and self-compatible populations of *Leavenworthia alabamica*. *Heredity* **94**, 159–165.
- Buza, L., Young, A. & Thrall, P. (2000) Genetic erosion, inbreeding and reduced fitness in fragmented populations of the endangered tetraploid pea *Swainsona recta*. *Biological Conservation* **93** (2), 177–186.
- Byers, D. L. & Waller, D. M. (1999) Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annual Review of Ecology and Systematics* **30**, 479–513.
- Charlesworth, D. & Willis, J. H. (2009) The genetics of inbreeding depression. *Nature Reviews: Genetics* **10**, 783–796.
- Chen, L., James, S. H. & Stace, H. M. (1997) Self-incompatibility, seed abortion and clonality in the breeding systems of several western Australian *Drosera* species (Droseraceae). *Australian Journal of Botany* **45**, 191–201.
- Cottrell, C. B. (1985) The absence of coevolutionary associations with Capensis floral element plants in the larval/plant relationships of southwestern Cape butterflies. *Transvaal Museum Monographs* **4**, 115–124.
- Crnokrak, P. & Barrett, S. C. H. (2002) Perspective: Purging the genetic load: A review of the experimental evidence. *Evolution* **56** (12), 2347–2358.
- Cross, A. T., Davis, A. R., Fleischmann, A., Horner, J. D., Jürgens, A., Merritt, D. J., Murza, G. L. & Turner, S. R. (2018) Reproductive biology and pollinator-prey conflicts. In A. M. Ellison & L. Adamec [Eds], *Carnivorous plants: Physiology, ecology, and evolution*, 294–313. Oxford University Press, New York, USA.

- Cruden, R. W. (1977) Pollen-ovule ratios: A conservative indicator of breeding systems in flowering plants. *Evolution* **31**, 32–46.
- Crutzen, P. J. & Stoermer, E. F. (2000) The Anthropocene. *Global Change Newsletter* **41**, 17–18.
- D'Amato, P. (1998) *The savage garden: Cultivating carnivorous plants*. Ten Speed Press. Berkeley, California, USA.
- Dafni, A., Izhaki, I. & Ne'eman, G. (2013) The effect of fire on biotic interactions in Mediterranean Basin ecosystems: Pollination and seed dispersal. *Israel Journal of Ecology and Evolution* **58** (2), 235–250.
- Dart, S. R., Samis, K. E., Austen, E. & Eckert, C. G. (2012) Broad geographic covariation between floral traits and the mating system in *Camissoniopsis cheiranthifolia* (Onagraceae): Multiple stable mixed mating systems across the species' range? *Annals of Botany* **109**, 599–611.
- de Jager, M. L. & Ellis, A. G. (2014) Floral polymorphism and the fitness implications of attracting pollinating and florivorous insects. *Annals of Botany* **113**, 213–222.
- Eckert, C. G. (2001) The loss of sex in clonal plants. *Evolutionary Ecology* **15**, 501–520.
- Elle, E. & Carney, R. (2003) Reproductive assurance varies with flower size in *Collinsia parviflora* (Scrophulariaceae). *American Journal of Botany* **90**, 888–896.
- Evans, T. M., Cavers, S., Ennos, R., Vanbergen, A. J. & Heard, M. S. (2017) Florally rich habitats reduce insect pollination and the reproductive success of isolated plants. *Ecology and Evolution* **7**, 6507–6518.
- Fang, Q. & Huang, S. (2013) A directed network analysis of heterospecific pollen transfer in a biodiverse community. *Ecology* **94**, 1176–1185.

- Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R. & Thomson, J. D. (2004) Pollination syndromes and floral specialisation. *Annual Review of Ecology and Evolution* **35**, 375–403.
- Fenster, C. B. & Martén-Rodríguez, S. (2007) Reproductive assurance and the evolution of pollination specialisation. *International Journal of Plant Sciences* **168**, 215–228.
- Fryxell, P. A. (1957) Mode of reproduction of higher plants. *Botanical Review* **23** (3), 135–233.
- Geerts, S. & Pauw, A. (2012) The cost of being specialised: Pollinator limitation in the endangered geophyte *Brunsvigia litoralis* (Amaryllidaceae) in the Cape Floristic Region of South Africa. *South African Journal of Botany* **78**, 159–164.
- Gibson, R. (2006) The stunning red-flowered *Drosera cistiflora* in the wild and in cultivation. *Carnivorous Plant Newsletter* **35** (3), 70–73.
- Gigord, L., Lavigne, C. & Shykoff, J. A. (1998) Partial self-incompatibility and inbreeding depression in a native tree species of La Réunion (Indian Ocean). *Oecologia* **117**, 342–352.
- Goldblatt, P., Bernhardt, P. & Manning, J. C. (1998) Pollination of petaloid geophytes by monkey beetles (Scarabaeidae: Rutelinae: Hopliini) in Southern Africa. *Annals of the Missouri Botanical Garden* **85**, 215–230.
- Guerrant, E. O. Jr (1989) Early maturity, small flowers and autogamy: A developmental connection? In J. H. Bock & Y. B. Linhart [Eds], *The evolutionary ecology of plants*, 61–84. Westview Press, Boulder, Colorado, USA.
- Herrera, C. M. (1996) Floral traits and plant adaptation to insect pollinators: A devil's advocate approach. In D. G. Lloyd & S. C. H. Barrett [Eds], *Floral biology: Studies on floral evolution in animal-pollinated plants*, 65–87. Chapman & Hall, New York, USA.

- Herrera, C. M., Sánchez-Lafuente, A. M., Medrano, M., Guitián, J., Cerda, X., Rey, P. (2001) Geographical variation in autonomous self-pollination levels unrelated to pollinator service in *Helleborus foetidus* (Ranunculaceae). *American Journal of Botany* **88**, 1025–1032.
- Hodgins, K. A. & Barrett, S. C. H. (2006) Female reproductive success and the evolution of mating-type frequencies in tristylous populations. *New Phytologist* **171**, 569–580.
- Johansen-Morris, A. D. & Latta, R. G. (2006) Fitness consequences of hybridisation between ecotypes of *Avena barbata*: Hybrid breakdown, hybrid vigour, and transgressive segregation. *Evolution* **60**, 1585–1595.
- Johnson, S. D. & Bond, W. J. (1997) Evidence for widespread pollen limitation of fruiting success in Cape wildflowers. *Oecologia* **109**, 530–534.
- Johnson, S. D., Butler, H. C. & Robertson, A. W. (2019) Breeding systems in *Cyrtanthus* (Amaryllidaceae): variation in self-sterility and potential for ovule discounting. *Plant Biology*. ISSN 1435-8603.
- Johnson, S. D. & Steiner, K. E. (1997) Long-tongued fly pollination and evolution of floral spur length in the *Disa draconis* complex (Orchidaceae). *Evolution* **51**, 45–53.
- Jürgens, A., Sciligo, A. R., Witt, T., El-Sayed, A. M. & Suckling, M. (2011) Pollinator-prey conflict in carnivorous plants. *Biological reviews* **87** (3), 602–615.
- Kalisz, S. & Vogler, D. W. (2003) Benefits of autonomous selfing under unpredictable pollinator environments. *Ecology* **84** (11), 2928–2942.
- Kay, K. M. & Sargent, R. D. (2009) The role of animal pollination in plant speciation: Integrating ecology, geography, and genetics. *Annual Review of Ecological Systems* **40**, 637–656.

Kearns, C. A. & Inouye, D. W. (1993) *Techniques for pollination biologists*. University Press of Colorado, Boulder, Colorado, USA.

Kennedy, B. F. & Elle, E. (2008) The inbreeding depression cost of selfing: Importance of flower size and population size in *Collinsia parviflora* (Veronicaceae). *American Journal of Botany* **95** (12), 1596–1605.

Kiester, A. R., Lande, R. & Schemske, D. W. (1984) Models of coevolution and speciation in plants and their pollinators. *The American Naturalist* **124**, 220–243.

Knight, T. M., Steets, J. A. & Ashman, T. L. (2006) A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. *American Journal of Botany* **93**, 271–277.

Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mitchell, R. J. & Ashman, T. L. (2005) Pollen limitation of plant reproduction: Pattern and process. *Annual Review of Ecology Evolution and Systematics* **36**, 467–497.

Koutnik, D. (1987) Wind pollination in the Cape flora. In A. G. Rebelo [Ed.], A preliminary synthesis of pollination biology in the Cape flora, 126–133. *National Scientific Programmes Unit: CSIR, SANSP Report* **141**, 265.

Larson, B. M. H. & Barrett, S. C. H. (2000) A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society* **69** (4), 503–520.

Leege, L. M. & Wolfe, L. M. (2002) Do floral herbivores respond to variation in flower characteristics in *Gelsemium sempervirens* (Loganiaceae), a distylous vine? *American Journal of Botany* **89**, 1270–1274.

Lloyd, D. G. (1965) Evolution of self-compatibility and racial differentiation in *Leavenworthia* (Cruciferae). *Contributions from the Gray Herbarium of Harvard University* **195**, 3–134.

- Lloyd, D. G. (1979) Some reproductive factors affecting the selection of self-fertilisation in plants. *The American Naturalist* **113** (1), 67–79.
- Lloyd, D. G. (1985) Progress in understanding pollination systems in New Zealand. *New Zealand Journal of Botany* **23**, 707–722.
- Lloyd, D. G. (1992) Self- and cross-fertilisation in plants. II. The selection of self-fertilisation. *International Journal of Plant Sciences* **153** (3), 370–380.
- Lloyd, D. G. & Schoen, D. J. (1992) Self- and cross-fertilisation in plants. I. Functional dimensions. *International Journal of Plant Sciences* **153** (3), 358–369.
- Lloyd, D. G. & Webb, C. J. (1986) The avoidance of interference between the presentation of pollen and stigmas in angiosperms. 1. Dichogamy. *New Zealand Journal of Botany* **24**, 135–162.
- Lundgren, R., Totland, Ø. & Lázaro, A. (2016) Experimental simulation of pollinator decline causes community-wide reductions in seedling diversity and abundance. *Ecology* **97** (6), 1420–1430.
- Mayr, E. (1992) A local flora and the Biological Species Concept. *American Journal of Botany* **79**, 222–238.
- Mittermeier, R. A., Myers, N., Thomsen, J. B., Da Fonseca, G. A. B. & Olivieri, S. (1998) Biodiversity hotspots and major tropical wilderness areas: Approaches to setting conservation priorities. *Conservation Biology* **12**, 516–520.
- Moeller, D. A. & Geber, M. A. (2005) Ecological context of the evolution of self-pollination in *Clarkia xantiana*: Population size, plant communities and reproductive assurance. *Evolution* **59**, 786–799.
- Murza, G. L. & Davis, A. R. (2003) Comparative flower structure of three species of sundew (Droseraceae: *Drosera anglica*, *D. linearis* and *D. rotundifolia*) in relation to breeding system. *Canadian Journal of Botany* **81**, 1129–1142.

- Murza, G. L. & Davis, A. R. (2005) Flowering phenology and reproductive biology of *Drosera anglica*. *Botanical Journal of the Linnean Society* **147** (4), 417–426.
- Murza, G. L., Heaver, J. R. & Davis, A. R. (2006) Minor pollinator-prey conflict in the carnivorous plant, *Drosera anglica*. *Plant Ecology* **184**, 43–52.
- Neal, P. R. & Anderson, G. J. (2005) Are ‘mating systems’ ‘breeding systems’ of inconsistent and confusing terminology in plant reproductive biology? Or is it the other way around? *Plant Systematics and Evolution* **250**, 173–185.
- Ollerton, J., Winfree, R. & Tarrant, S. (2011) How many flowering plants are pollinated by animals? *Oikos* **120**, 321–326.
- Pauw, A. (2004) *Variation in pollination across a fragmented landscape at the Cape of Africa*. PhD thesis. University of Cape Town.
- Pauw, A. (2007) Collapse of a pollination web in small conservation areas. *Ecology* **88** (7), 1759–1769.
- Pauw, A. & Bond, W. J. (2011) Mutualisms matter: Pollination rate limits the distribution of oil-secreting orchids. *Oikos* **120**, 1531–1538.
- Primack, R. B. (1978) Variability in New Zealand montane and alpine pollinator assemblages. *New Zealand Journal of Ecology* **1**, 66–73.
- R Core Team (2018) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, <http://www.R-project.org/>.
- Raduski, A. R., Haney, E. B. & Igic, B. (2012) The expression of self-incompatibility in angiosperms is bimodal. *Evolution* **66** (4), 1275–1283.
- Rebelo, A. G. [Ed.] (1987) Preliminary synthesis of pollination biology in the Cape flora. *National Scientific Programmes Unit: CSIR, SANSP Report* **141**, 265.

- Reinartz, J. A., & Les, D. H. (1994) Bottleneck-induced dissolution of self-incompatibility and breeding system consequences in *Aster furcatus* (Asteraceae). *American Journal of Botany* **81**, 446–455.
- Rodger, J. G. & Ellis, A. G. (2016) Distinct effects of pollinator dependence and self-incompatibility on pollen limitation in South African biodiversity hotspots. *Biology Letters* **12**, 20160253. doi:10.1098/rsbl.2016.0253.
- Rodger, J. G. & Johnson, S. D. (2013) Self-pollination and inbreeding depression in *Acacia dealbata*: Can selfing promote invasion in trees? *South African Journal of Botany* **88**, 252–259.
- Sandring, S. & Ågren, J. (2009) Pollinator-mediated selection on floral display and flowering time in the perennial herb *Arabidopsis lyrata*. *Evolution* **63** (5), 1292–1300.
- Sciligo, A. R. (2009) *Food or sex: which would you choose? Pollinator-prey conflict and reproductive assurance in New Zealand Drosera*. PhD Thesis. Lincoln University, New Zealand.
- Sciligo, A. R., Sullivan, J. J., Jesson, L. K., Witt, T., Jürgens, A., Hale, R. J. & Newstrom, L. (2007) *Is selfing more advantageous than sex in Drosera due to the pollinator-prey conflict?* ESA/SER Joint Meeting, San Jose McEnery Convention Centre, California, USA.
- Sletvold, N. & Grindeland, J. M. (2008) Floral herbivory increases with inflorescence size and local plant density in *Digitalis purpurea*. *Acta Oecologica* **34**, 21–25.
- Sletvold, N., Grindeland, J. M. & Ågren, J. (2010) Pollinator-mediated selection on floral display, spur length and flowering phenology in the deceptive orchid *Dactylorhiza lapponica*. *New Phytologist* **188**, 385–392.
- Snow, A. A. (1994) Postpollination selection and male fitness in plants. *The American Naturalist* **144**, 869–883.

- Snow, A. A., Spira, T. P., Simpson, R. & Klips, R. A. (1996) The ecology of geitonogamous pollination. *In* D. G. Lloyd & S. C. H. Barrett [Eds] *Floral biology*. Springer, Boston, Massachusetts, USA.
- Steiner, K. E. (1987) Breeding systems in the Cape flora. *In* A.G. Rebelo [Ed.], A preliminary synthesis of pollination biology in the Cape flora, 126–133. *National Scientific Programmes Unit: CSIR, SANSP Report* **141**, 265.
- Steiner, K. E. (1998) Beetle pollination of peacock moraeas (Iridaceae) in South Africa. *Plant Systematics and Evolution* **209**, 47–65.
- Strauss, S. Y. & Whittall, J. B. (2006) Non-pollinator agents of selection on floral traits. *In* L. D. Harder & S. C. H. Barrett [Eds], *Ecology and evolution of flowers*, 120–138. Oxford University Press, Oxford, UK.
- Thomson, J. D. (2001) Using pollination deficits to infer pollinator declines: Can theory guide us? *Conservation Ecology* **5**. <http://www.consecol.org/vol5/iss1/art6>.
- Trunschke, J., Sletvold, N. & Ågren, J. (2017) Interaction intensity and pollinator-mediated selection. *New Phytologist* **214**, 1381–1389.
- Vamosi, J. C., Knight, T. M., Steets, J. A., Mazer, S. J., Burd, M. & Ashman, T. L. (2006) Pollination decays in biodiversity hotspots. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 956–961.
- van Kleunen, M., Nänni, I., Donaldson, J. S. & Manning, J. C. (2007) The role of beetle marks and flower colour on visitation by monkey beetles (Hopliini) in the Greater Cape Floral Region, South Africa. *Annals of Botany* **100**, 1483–1489.
- Vogler, D. W. & Kalisz, S. (2001) Sex among the flowers: The distribution of plant mating systems. *Evolution* **55**, 202–204.
- Wessinger, C. A. & Kelly, J. K. (2018) Selfing can facilitate transitions between pollination syndromes. *The American Naturalist* **191**, 582–594.

Wilcock, C. & Neiland, R. (2002) Pollination failure in plants: Why it happens and when it matters. *Trends in Plant Science* **7**, 270–277.

Wilson, P. (1995) Pollination in *Drosera tracyi*: Selection is strongest when resources are intermediate. *Evolutionary Ecology* **9**, 382–396.

Wolowski, M., Ashman, T. L. & Freitas, L. (2014) Meta-analysis of pollen limitation reveals the relevance of pollination generalisation in the Atlantic forest of Brazil. *PLoS ONE* **9**, e89498. doi:10.1371/journal.pone.0089498.

Wright, S. (1977) *Evolution and the genetics of populations. Vol. 3. Experimental results and evolutionary deductions*. University of Chicago Press, Chicago, Illinois, USA.

Wuerth, D. G., Eusemann, P., Trouillier, M., Buras, A., Burger, A., Wilmking, M., Roland, C. A., Juday, G. P. & Schnittler, M. (2018) Environment drives spatiotemporal patterns of clonality in white spruce (*Picea glauca*) in Alaska. *Canadian Journal of Forest Research* **48**, 1577–1586.

Young, H. J. & Young, T. P. (1992) Alternative outcomes of natural and experimental high pollen loads. *Ecology* **73**, 639–647.

Zamora, R. (1999) Conditional outcomes of interactions: The pollinator-prey conflict of an insectivorous plant. *Ecology* **80** (3), 786–795.

Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A. & Smith, G. M. (2009) *Mixed effects models and extensions in ecology with R*. Springer-Verlag, New York, USA.

CHAPTER THREE

ASSOCIATIONS BETWEEN SOILS, POLLINATORS AND SPATIAL PATTERNS OF FLOWER COLOUR IN THE *DROSERA CISTIFLORA* SPECIES COMPLEX

Abstract

Floral colour shifts are thought to be one of the most common evolutionary transitions in plants, with several lines of evidence proposing pollinators as important selective agents for these transitions. However, pollinators may not be the sole explanation for shifts in flower colour. I test two alternative hypotheses: i) that floral colour variation reflects plastic responses to differences in abiotic factors and ii) that floral colour divergence occurs as a result of geographical pollinator mosaics. I studied 2–4 populations of each of the five floral colour forms of the pollinator-dependent South African perennial *Drosera cistiflora* sensu lato (Droseraceae) in the Greater Cape Floristic Region. Flowers of this species complex are actinomorphic and devoid of nectar and odour, making them ideal for a study specific to colour. By mapping herbarium records of *D. cistiflora* s.l. flower colours, I determined the spatial distribution of floral colour forms and established their vegetation types and supporting soil structure. Soil composition, temperature, light and moisture do not appear to play a significant role in colour determination, since no flower colours are specific to a single soil or vegetation type and visible colour traits were maintained in common-garden and soil switching experiments. Instead there is an overall association between flower colour and the local composition of generalist pollinator assemblages. Based on pollinator data, red- and yellow-flowered populations formed distinct clusters in multivariate space, whilst pink- and white-flowered populations appear to have more

variable pollinator compositions and did not form discrete clusters. There were only two sufficiently large extant populations of the purple floral colour form for study and consequently insufficient statistical power to make inferences about the differences or similarities of their pollinator communities. Although the patterns observed suggest that flower colour may be an adaptation to pollinators, this does not preclude the possibility that populations with the same flower colour share pollinators because of their geographical proximity rather than as an adaptive response to pollinating fauna. Floral colour divergence may thus be non-adaptive or a by-product of selection on other traits, but future examination of pollinator colour preferences should shed light on causality in terms of spatial patterns of flower colours and pollinators.

Introduction

Pollinator-mediated selection for floral traits such as morphology, reward, scent, patterning, overall display and colour is considered a major driver of floral divergence (e.g. Grant & Grant 1965, Faegri & van der Pijl 1979, Johnson 1997, Johnson et al. 1998, Emms & Arnold 2000, Alexandersson & Johnson 2002, Beardsley et al. 2003, Fenster et al. 2004, Huber et al. 2005, Pérez et al. 2006, Streisfeld & Kohn 2007, Anderson et al. 2010, van der Niet & Johnson 2012, Newman et al. 2012, Schiestl & Johnson 2013, Liu & Huang 2013, Sun et al. 2014, van der Niet et al. 2014, Forest et al. 2014, Newman et al. 2014, Anderson et al. 2016, Parachnowitsch et al. 2018, Minnaar et al. 2019). It follows that spatial differences in pollinator assemblage or preference may generate divergent selective pressures among plant populations, as explained by the pollinator-shift model (Grant 1949; Grant & Grant 1965; Stebbins 1970; Johnson 2006; Kay & Sargent 2009; van der Niet, Peakall & Johnson 2014; Gervasi & Schiestl 2017; Smith & Kriebel 2018). For example, Sun et al. (2014) found that differing floral phenotypes across a large altitudinal gradient in the rewarding orchid *Gymnadenia odoratissima* (Orchidaceae) were associated with local adaptation to different lepidopteran pollinator guilds. It is less clear whether selection may generate divergence in more generalist plants owing to putative conflicting selection imposed by different pollinators (Ollerton 1996, Waser 1998, Ippolito et al. 2004). However, Gómez et al. (2008, 2014) demonstrated that phenotypic evolution and diversification can occur in corolla shape without specialisation in the generalist crucifer *Erysimum mediohispanicum* (Brassicaceae), where corolla shape varies in response to communities of diverse pollinators across a geographical range.

Flower colour often appears to be an important syndrome trait shared between guilds of plants pollinated by the same species (Fenster et al. 2004). Consequently, floral colour divergence may occur as a response to selective pressures by specific pollinators. Indeed, there is now some compelling evidence thereof, albeit limited to few studies (e.g. Waser & Price 1981, Meléndez-Ackerman & Campbell 1998, Schemske & Bradshaw 1999, Gigord et al. 2001, Irwin & Strauss 2005, Newman et al. 2012, Hopkins & Rausher 2012, Tao et al. 2018, Streinzer et al. 2019). Waser & Price (1981)

found that sympatric floral colour polymorphism in *Delphinium nelsonii* (Ranunculaceae) was maintained through discrimination against (and ensuing reduction in seed set in) the rare ‘albino’ flowers by hummingbird and bumblebee pollinators. They attributed this to the seemingly greater difficulty experienced by pollinators in extracting nectar from albino flowers over blue flowers, whereby the loss of nectar guides accompanying pigment loss in albino flowers gave rise to a “visual confusion” effect, delaying pollinator orientation at these flowers. Discrimination against albino flowers was inferred to be motivated by pollinators maximising net rate of energy intake during foraging bouts. Array experiments and genetic markers involving *Mimulus* species (Phrymaceae) and their hybrids have also demonstrated flower colour discrimination by both hummingbirds and bees, and further linked pollinator preferences to genes with large phenotypic effects, specifically for petal pigmentation and nectar volume (Schemske & Bradshaw 1999). By using arrays of both model and reciprocally translocated flowers, Newman et al. (2012) attributed allopatric floral colour shifts to local pollinator colour preferences in the western and eastern ranges of *Disa ferruginea* (Orchidaceae). Here, floral colour divergence between ecotypes of the orchid was attributed to geographically variable colour preferences of a single specialist butterfly pollinator.

Although these studies suggest that pollinators can be very important selective agents in floral colour transitions, and even the main driving force behind these shifts, no study has demonstrated unequivocally that different assemblages of pollinators are the agents of selection behind allopatric floral colour shifts. Several other hypotheses with similar levels of support have been proposed (Narbona et al. 2017). Some of these suggest that floral colour shifts are nonadaptive, and occur as a result of genetic drift (q.v. Wright 1931, 1943a&b, 1978) or the (indirect) consequence of pleiotropic effects of genes relating to physiological or vegetative adaptation to environmental conditions (q.v. Rausher & Fry 1993, Levin & Brack 1995, Warren & Mackenzie 2001, Armbruster 2002, Armbruster et al. 2002, Strauss & Whittall 2006). For example, the anthocyanin pigment may confer abiotic stress tolerance to seedlings, and, since its presence in seedlings can also determine flower colour (Bowman 1987, Strauss & Whittall 2006), floral colour transitions may be maintained through selection on seedling traits. Similarly, the plant genus *Acer* (Sapindaceae) has red and purple flowers that evolved in lineages where anthocyanins are present in leaves, whilst pale-green or yellow

flowers evolved in lineages without anthocyanins in leaves (Armbruster 2002). Alternatively, flower colour genes may have pleiotropic effects on water use physiology, which in turn may result in geographical structuring of flower colours, as presented by Schemske & Bierzychudek (2007) in their study of *Linanthus parryae* (Polemoniaceae). Further association between abiotic factors and floral colour shifts was shown by Arista et al. (2013), who found that the mechanism of selection in blue and red floral morphs of *Lysimachia arvensis* (Primulaceae) appears to be related to morph fitness differences in a geographical pattern of differing moisture and light availability. New evidence suggests that elevational segregation of flower colour in *Anemone pavonina* (Ranunculaceae) may be linked to glaphyrid beetle colour preferences, but does not yet preclude the potential effects of temperature, UV radiation and soils on plant pigmentation (Streinzer et al. 2019). Floral colour divergence may also be maintained through the pleiotropic effects of flower colour genes on herbivory (Irwin et al. 2003). Seed predators have additionally been shown to play a role in the evolution of flower colours, specifically in the genus *Protea* (Proteaceae) [Carlson & Holsinger 2010, 2012].

Lastly, floral colour shifts may represent plastic responses to differing edaphic conditions such as geographical soil mosaics, as determined by variation in physical and/or chemical components of the soil (Ito et al. 2009). For example, the effects of soil pH on flower colour are seen in *Hydrangea macrophylla* (Hydrangeaceae), which can vary in flower colour from blue to pink. Although the mechanism for this floral colour change is not completely understood, it has been determined that hydrangeas cultivated in acidic soil have blue sepals because Al^{3+} is soluble in acidic soil and can be absorbed and transported to the sepals, where it combines with anthocyanin to produce a blue colour. Al^{3+} is insoluble in neutral and weakly alkaline solutions, and the sepals turn red in these instances owing to the presence of unchelated anthocyanin pigments (Ito et al. 2009, Schreiber et al. 2010).

It has become clear, therefore, that studies of floral colour transitions should include an integrated consideration of selection by pollinators, seed predators and nectar thieves, as well as edaphic factors; physiological costs of flowers; genetic drift, and genetic constraints on floral evolution (Herrera 1996, Galen 1999a&b, Ellis & Johnson 2009).

Drosera cistiflora s.l. is a perennial, pollinator-dependent, insectivorous plant species complex endemic to the fynbos of the Greater Cape Floristic Region of South Africa. It has a range of floral colour forms: pink, purple, red, white and yellow (Trinder-Smith et al. 2006, pers. obs). Flowers are large, bisexual, actinomorphic, bowl-shaped, cymose (Codd et al. 1970) and devoid of nectar (Goldblatt et al. 1998) or odour (pers. obs), the latter two features adding to the desirability of the system for a selection study based on floral colour traits only.

Although the purple, red and yellow flower colours can be regarded as discrete floral colour forms, since there do not appear to be intermediate forms, pink- and white-flowered forms may vary in a quantitative manner across a pink-white gradient within populations. Entire populations of solely pink- or white-flowered forms also exist in isolation. In the rare instances when pink and purple, purple and red, purple and white, or red and white flowers co-occur, there are no apparent intermediate floral colour forms.

The results of two existing pollination studies involving *D. cistiflora* s.l. posit that the plants appear to be pollinated primarily by hopliine beetles (Coleoptera: Scarabaeidae: Hopliini) [Goldblatt et al. 1998, Anderson 2010]. Moreover, study of the breeding systems of the species complex (von Witt et al. unpublished) presented evidence of high pollinator contribution to fecundity and pollinator dependence, partial self-incompatibility, and pollen limitation in all floral colour forms. Not only do these attributes suggest that pollinators may be associated with floral trait selection, but their implications for fitness make it possible that the plants may respond more readily to selection by pollinators than to conflicting selection by abiotic factors or non-pollinator biotic factors. Conversely, predominantly self-pollinating species may be more responsive to non-pollinator selective agents (Strauss & Whittall 2006).

This study tests two alternative hypotheses: i) floral colour variation in *D. cistiflora* s.l. is a manifestation of plastic responses to allopatric abiotic factors and ii) flower colour diverges as a result of geographical pollinator mosaics. If floral colour divergence is driven by differences in components of the physical environment, then I predict that respective populations with the same flower colour should occur in similar soil and vegetation types, and that flower colour may shift when abiotic conditions are altered. Similarly, if floral colour variation is driven by selection by pollinators, then I expect

that respective populations with the same flower colour may have similar suites of insect pollinators, and that respective populations with different flower colours will have different pollinator assemblages.

Materials and Methods

Study sites

Study sites were chosen to represent extant populations of *Drosera cistiflora* s.l. (Table 1). Of the 16 populations studied, four were pink-flowered, two purple, four red, three white and three yellow. Here, more than 3000 individual plants were documented (Table 1). Although four populations of the purple-flowered form have been recorded, only two were found with sufficient flowers for study in 2009 and 2010. Whilst all *D. cistiflora* s.l. flower colours were locally dominant within each site, purple was not the dominant *D. cistiflora* s.l. flower colour overall at the two sites examined for this floral colour form. It should also be noted that the two purple-flowered study populations were grouped according to similarity in petal colour, but one population had reflexed petals and the other had consistently crateriform flowers (Table 2, Chapter 1).

Table 1. Population number; site name and locality description; flower colour; respective Compton Herbarium and Kirstenbosch National Botanical Garden Collections Nursery voucher accession numbers (where available); GPS location, and number of *Drosera cistiflora* s.l. flowers studied in 2009 and 2010 in all 16 study populations. Precise locality information has been omitted owing to the sensitive nature of these populations and their vulnerability to overcollection.

Population number	Site name and locality description	Flower colour	Voucher accession number	Latitude	Longitude	Number of plants observed flowering in 2009	Number of plants observed flowering in 2010
1	Darling 1	Pink	1483809	-33.4	18.4	222	205
2	Darling 2	Pink	1483807 and 2019/5	-33.4	18.4	> 250	> 250
3	Rawsonville	Pink		-33.7	19.4	190	N/a
4	Riverlands Nature Reserve	Pink	1483811	-33.5	18.6	187	N/a
5	Darling 3	Purple	1483816 and 2019/1	-33.3	18.5	190	179
6	Durbanville	Purple	1483810	-33.8	18.6	38	24
7	Darling 3	Red	1483801	-33.3	18.5	210	> 250
8	Darling 4	Red	1483806	-33.2	18.3	> 250	> 250
9	Darling 5	Red	1483805 and 2019/2	-33.2	18.3	> 250	> 250
10	Darling-Yzerfontein	Red		-33.3	18.3	55	50
11	Betty's Bay	White		-34.4	18.9	N/a	49
12	Darling 6	White	2019/6	-33.4	18.4	> 250	> 250
13	Darling 7	White	1483804	-33.2	18.3	> 250	204
14	Piketberg 1	Yellow	1483812 and 2019/4	-32.8	18.8	> 250	> 250
15	Piketberg 2	Yellow	1483815	-32.7	18.7	> 250	> 250
16	Piketberg 3	Yellow	1483813	-32.7	18.7	220	200

Geographical distribution of floral colour forms

To determine whether there is any geographical pattern in flower colour distribution, I mapped the spatial pattern of all records of historical populations of *D. cistiflora* s.l. in South Africa according to flower colour wherever this was documented. Records were obtained from specimens housed in the Compton and Bolus Herbaria; data collected by the Custodians of Rare and Endangered Wildflowers (CREW) programme, and field excursions carried out in my personal capacity. These were mapped using ARCVIEW GIS 3.2.

Spectrophotometric measurements of flower colour

I determined the spectral reflectance over the UV–visible range (300–700 nm) of a sample of 5–16 petals from two populations of each of the pink and purple *D. cistiflora* s.l. floral colour forms; three of yellow and white, and four of red. To do so, I used an Ocean Optics (Dunedin, FL, USA) S2000 spectrophotometer and Ocean Optics DT-mini deuterium tungsten halogen light source (200–1100 nm). In all cases petals were obtained from the flowers of separate plants. I took reflectance readings from the outer section of the petals by placing the fibre optic reflection probe (UV/VIS 400 μ m) at a 45° angle and approximately 5mm from the surface of the petal. Populations sampled comprised: Darling 1 and 2 (pink-flowered form); Darling 3 and Durbanville (purple-flowered form); Darling 3, 4 and 5, and Darling-Yzerfontein (red-flowered form); Betty's Bay, and Darling 6 and 7 (white-flowered form), and Piketberg 1, 2 and 3 (yellow-flowered form).

Flower colour and the physical environment

Soil types and underlying geology

To investigate the potential for edaphic endemism in *D. cistiflora* s.l. floral colour forms, I determined the underlying geology and soil structure of populations of each

floral colour form by overlaying the 1:250 000 2010 geology layer from the Western Cape Department of Agriculture onto point locality data of *D. cistiflora* s.l. populations with recorded flower colours (Supplementary Table 6).

Common-garden experiment and reciprocal soil transplants

A common-garden and soil switching experiment was conducted to test whether differences in soil chemistry and altered environmental conditions influenced flower colour. Samples of three plants in bud from one population of each floral colour form were potted in soils from each of the other floral colour forms, moved from their native sites, and observed for changes in flower colour (Supplementary Figure 2). Soil from a site where *D. cistiflora* s.l. did not occur was also added, as well as a control; the latter entailing plants being potted in their natural soil. Plants and soils were obtained from Darling 2 (granite and granodiorite soils supporting the pink-flowered form); Darling 3 (loam and sandy loam soils; purple-flowered form); Darling 5 (loam and sandy loam soils; red-flowered form); Darling 6 (granite and granodiorite soils; white-flowered form), and Piketberg 1 (grit and greywacke soils; yellow-flowered form). Clay soils collected from The Towers Farm, Darling were used for potting plants in soil from a site where *D. cistiflora* s.l. was not present.

Plants were potted at the beginning of the flowering season in 2010 and observations made until flowering ceased at the end of each season, up until 2013. All experimental plants were kept in common environmental conditions at all times, and these common conditions were altered when the experiment was transferred from Darling to the Kirstenbosch National Botanical Garden Collections Nursery greenhouse of the South African National Biodiversity Institute after the flowering season in 2010. The plants were thus exposed to changes in soil temperature, light and moisture availability when they were removed from their native sites and also during the course of the experiment. Flower observations were continued during the flowering seasons of 2011, 2012 and 2013. Here, all floral colour estimations were qualitative allocations to pink, purple, red, white and yellow categories and were made by the human eye.

Vegetation types

Considering that plant communities share similar abiotic environmental conditions, they may act as a surrogate for overall abiotic factors to be compared between *D. cistiflora* s.l. populations. Thus, to assess whether populations of the same *D. cistiflora* s.l. floral colour form are associated with similar plant communities, I established the vegetation type of each population by overlaying the 1:250 000 vegetation layer (Mucina & Rutherford 2006) onto point locality data for extant populations of all *D. cistiflora* s.l. floral colour forms (Supplementary Table 6).

Flower colour and pollinators

Pollinator composition

To determine whether the flower colour of a *D. cistiflora* s.l. population was associated with the pollinator community, flower visitors were observed in 16 populations of five floral colour forms in 2009 and 2010 on sunny, windless days during periods of peak pollinator activity: 09h30–15h00. Each site measured approximately 50m x 50m. Where possible, 250 flowers were randomly checked for the presence of pollinators. Where fewer than 250 flowers were present, all flowers in the population were checked (Table 1) and observation numbers standardised across populations. The abundance and kinds of flower visitors were noted and at least one voucher specimen of each visitor was captured for identification. Insects that came into contact with floral reproductive parts were considered as visitors, with the exception of the florivorous lunate blister beetle *Hycleus lunatus* (Coleoptera: Meloidae: Meloinae: Mylabrini) [Supplementary Figure 3]. Individual insects were killed by freezing and kept in separate vials to avoid pollen contamination. All insects were identified to family or subfamily, and genus and species where possible. Insect collections are housed at Stellenbosch University.

Pollinator importance

If flower colour is the product of adapting to the most effective pollinating fauna at a site, then pollen loads may provide an indication of the importance of the pollinators

associated with each colour. The potential importance of each species of flower visitor was calculated as the product of its relative abundance as a visitor to *D. cistiflora* s.l. flowers within a population and the average number of *D. cistiflora* s.l. pollen grains that it carried. Pollen grains were counted under a dissecting microscope for 1–12 (median = 5) individuals (in some cases fewer than 5 individuals were captured) of all observed insect visitors and classified as *D. cistiflora* s.l. pollen or ‘other’. *Drosera cistiflora* s.l. pollen grains were identified by comparison with a reference set of microscope slide preparations of pollen grains made from *D. cistiflora* s.l. and co-occurring plants at all study sites. Relative pollinator importance (RPI) was calculated as the percentage contribution of each pollinator to the overall pollinator importance in a single *D. cistiflora* s.l. floral colour form.

If pollinators discriminate among floral colour forms, or if pollinators are localised and impose selection for particular flower colours, then I would expect that populations of the same flower colour will be visited by similar assemblages of insects. This was tested with Multidimensional scaling (MDS) plots of the Bray-Curtis dissimilarity of pollinator species composition in *D. cistiflora* s.l. floral colour forms. Two analyses were performed, one using relative pollinator abundances in each *D. cistiflora* s.l. population and another using an estimate of the importance of each pollinator species (as the product of relative abundance and average pollen loads). Permutation tests for pollinating fauna were applied using ANOSIM implemented in PRIMER.

Geographical structuring of plant-pollinator communities

Geographical proximity of populations and the effect on pollinator/flower colour similarity

The relationship between geographical proximity of *D. cistiflora* s.l. floral colour forms and the similarity of pollinator communities was assessed to determine whether similarities in pollinator assemblages within floral colour forms could simply be a product of geographically determined floral colour form clumping in relation to pollinator distributions.

To analyse whether there is geographical structuring of *D. cistiflora* s.l. populations and associated pollinator communities and whether this relates to flower colour, pairwise distances between each population were calculated using ARCVIEW GIS 3.2, and a geographical distance matrix was produced. An MDS Mantel test (Manly 1991) implemented in R regressed pairwise geographical distance with the Bray-Curtis similarity index of the pollinator community composition of each population pair. Three separate analyses were performed, using data for: i) all population pairs; ii) respective population pairs with different flower colours only, and iii) respective population pairs with the same flower colour only.

Results

Geographical distribution of floral colour forms

Using herbarium and rare plant database records, locality data were found for 168 *Drosera cistiflora* s.l. populations of known flower colour in South Africa. Sites of these populations had a range spanning 685 km from west to east and 390 km from north to south. The majority of sites comprised either pink (108 sites: 64.3% of all sites) or white (41 sites: 24.4%) floral colour forms. These two forms were found to be randomly distributed and widespread throughout the entire range of the species complex. The remaining floral colour forms were restricted to few populations that appeared to be clumped relatively close together (Figure 1).

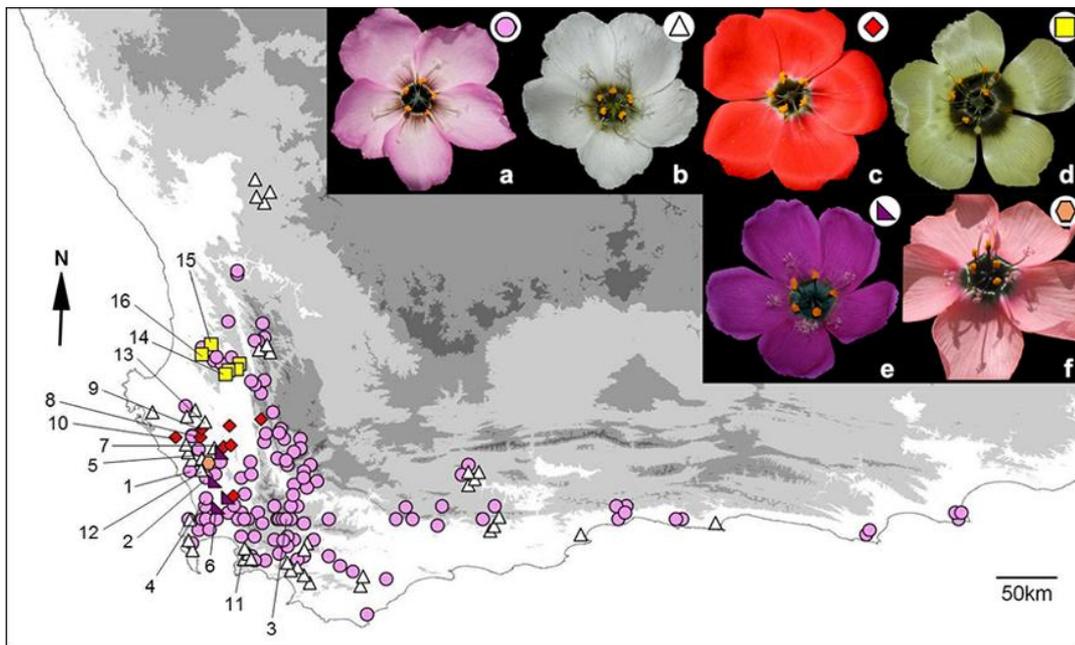


Figure 1. The geographical distribution of all known extant and extinct populations of *Drosera cistiflora* s.l. where corolla colour data has been recorded. Recorded flower colours in decreasing order of frequency are pink (a), white (b), red (c), yellow (d), purple (e) and salmon pink (f). Populations studied (Table 1) are labelled numerically. Photo (f) by Rob Maharajh.

Many of the *D. cistiflora* s.l. populations designated pink had both pink- and white-flowered individuals, frequently with a colour continuum spanning pink to white. However, no evidence of pink-flowered individuals was found in populations designated as white. In the central part of the range, clusters of three additional floral colour forms were found close to the pink- and white-flowered populations. Six yellow-flowered populations (3.6% of total sites) were found within 40 km of each other. To the south of these, nine red-flowered populations (5.3% of total sites) occurred within 83 km of each other, and further south, four purple-flowered populations (2.4% of total sites) were found within 38.5 km of each other. Two of the red-flowered populations co-occurred with purple-flowered individuals and another one co-occurred with white-flowered individuals. Purple-flowered populations also co-occurred with pink- or white-flowered populations at two respective sites. Here, different flower colours appeared to be discrete (with no intermediates observed in zones where the distribution ranges of different forms overlapped).

Spectrophotometric measurements of flower colour

The floral colour spectra of the different forms suggested that pink and white are not entirely discrete colours and that they may form a continuum. However, purple, red and yellow spectra were distinctly different from all other colours and did not blend into one another (Figure 2).

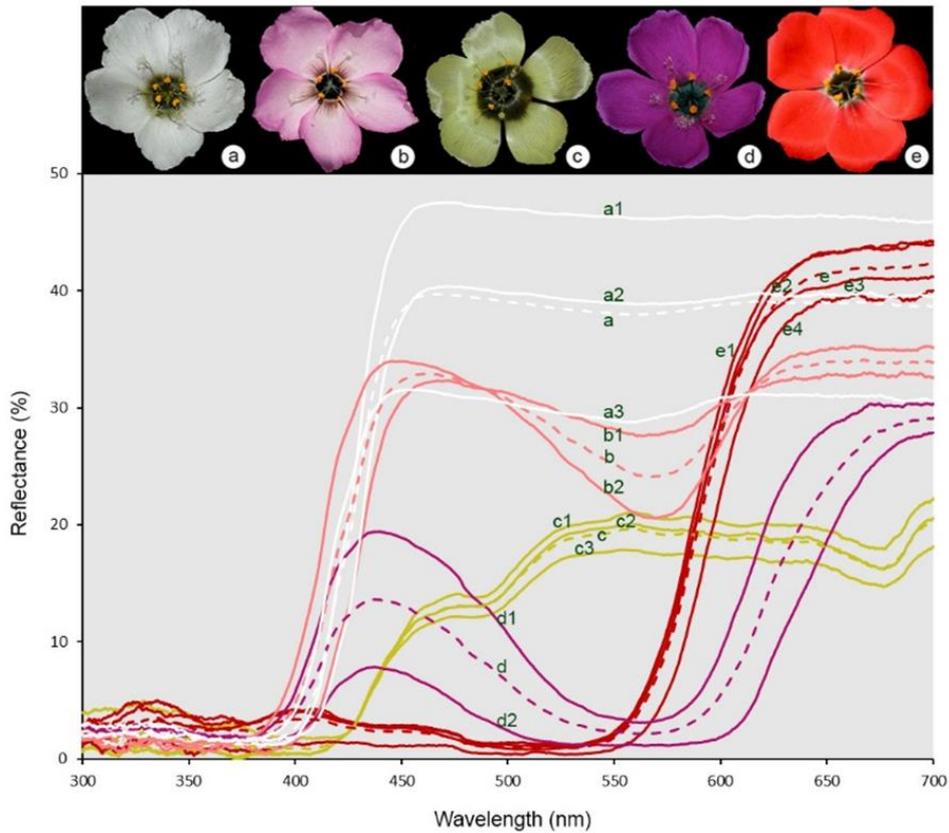


Figure 2. Spectrophotometer readings over the UV–visible range (300–700 nm) of the outer region of the petals of all five *Drosera cistiflora* s.l. floral colour forms. Readings were obtained from 5–16 flowers from two populations of each of the pink (b) and purple (d); three populations of white (a) and yellow (c), and four of red (e) *D. cistiflora* s.l. floral colour forms. Populations sampled comprised: Darling 7 (a1), Darling 6 (a2) and Betty’s Bay (a3) [white-flowered form]; Darling 2 (b1) and Darling 1 (b2) [pink-flowered form]; Piketberg 1 (c1), Piketberg 2 (c2) and Piketberg 3 (c3) [yellow-flowered form]; Darling 3 (d1) and Durbanville (d2) [purple-flowered form], and Darling 3 (e1), Darling 4 (e2), Darling 5 (e3) and Darling-Yzerfontein (e4) [red-flowered form]. Average readings for each floral colour form are distinguished by dashed lines.

Flower colour and the physical environment

Soil types and underlying geology

Associated attribute information of the 1:250 000 2010 geology layer from the Western Cape Department of Agriculture, which was overlaid onto available point locality data, showed populations of *D. cistiflora* s.l. to occur on 21 soil types. No floral colour forms were edaphic endemics as all occurred on two or more soil types. Pink- and white-flowered forms were found on the most diverse range of soil types, reflecting the much larger distribution range of these two floral colour forms. There was also considerable overlap of soil types between populations with different flower colours. For example, pink, purple, red and white flowers could all be found on loam soils and pink, purple and white flowers could all be found on sandy soils (Table 2, Supplementary Table 6).

Table 2. Percentage occurrence of the five *Drosera cistiflora* s.l. floral colour forms on different soil types. Geology code descriptions are provided in Supplementary Table 6.

Geology code	Occurrence (%)				
	Pink	Purple	Red	White	Yellow
Dr	5.1				
Dv	7.7				
Dw	2.6				
NCa	2.6			8.3	
NCd	10.3		16.7	25.0	
NCs	5.1				
Nm		50.0	16.7	8.3	
Nn	2.6				
Np					66.7
Npo	23.1				
Nt	2.6				
Og					33.3
Os				8.3	
Qf	2.6				
Qg	7.7	25.0	66.7	16.7	
Qgg	2.6				
QI				8.3	
Qs	18.0	25.0		8.3	
Qt	5.1				
Sg	2.6				
Ss				16.7	

Common-garden experiment and reciprocal soil transplants

Drosera cistiflora s.l. flower colour did not appear to be physiologically responsive to changes in soil chemistry, temperature, light or moisture, since no change in colour was observed by the human eye in any of the 90 potted plants (18 per colour form) with switched substrates and altered environmental conditions within a single flowering season in 2010 (Supplementary Figure 2).

The plants collected in 2010, which were subsequently kept in cultivation at the Kirstenbosch National Botanical Garden Collections Nursery, produced stems and leaves in 2011, but did not flower. In August and September 2012 and 2013, pink,

purple, red and white flowers were observed on plants of these respective floral colour forms and no floral colour variation away from the original flower colour was observed in the human visual spectrum.

Vegetation types

Each *D. cistiflora* s.l. floral colour form was found in more than one vegetation type and most of these vegetation types supported more than one floral colour form. For example, Atlantis Sand Fynbos and Swartland Granite Renosterveld support pink, purple, red and white floral colour forms; Swartland Shale Renosterveld supports pink, purple and yellow floral colour forms, and both red- and white-flowered forms also occur in Hopefield Sand Fynbos. The distribution of *D. cistiflora* s.l. flower colours was therefore not synonymous with that of specific plant communities and their associated abiotic environmental conditions (Table 3).

Occurrence according to detailed vegetation types (Supplementary Table 6) is outlined as follows: pink-flowered forms occur in a minimum of 15 vegetation types, with the majority of plants found in Swartland Granite Renosterveld (20.5% of pink-flowered populations), Breede Shale Renosterveld (12.8%), Swartland Shale Renosterveld (12.8%) and Atlantis Sand Fynbos (10.3%); purple-flowered forms occur in Atlantis Sand Fynbos (50%), Swartland Granite Renosterveld (25%) and Swartland Shale Renosterveld (25%); red-flowered forms appear in Sand Fynbos (66.7% Hopefield Sand Fynbos and 16.7% Atlantis Sand Fynbos) and Swartland Granite Renosterveld (16.7%); white-flowered forms emerge in at least six vegetation types, including Swartland Granite Renosterveld (25%), Atlantis Sand Fynbos (25%), Hopefield Sand Fynbos (16.7%), Kogelberg Sandstone Fynbos (16.7%), Bokkeveld Sandstone Fynbos (8.3%) and Overberg Sandstone Fynbos (8.3%), and yellow-flowered forms only occur in Piketberg Sandstone Fynbos (66.7%) and Swartland Shale Renosterveld (33.3%).

Table 3. Percentage occurrence of the five *Drosera cistiflora* s.l. floral colour forms in broad vegetation categories. Specific vegetation types are provided in Supplementary Table 6.

Broad vegetation category	Occurrence (%)				
	Pink	Purple	Red	White	Yellow
Alluvium fynbos	10.3				
Granite fynbos	2.6				
Granite renosterveld	20.5	25.0	16.7	25.0	
Quartzite fynbos	2.6				
Sand fynbos	10.3	50.0	83.3	41.7	
Sandstone fynbos	7.7			33.3	66.7
Shale fynbos	7.7				
Shale renosterveld	35.9	25.0			33.3
Silcrete renosterveld	2.6				

Flower colour and pollinators

Pollinator composition

A total of 28 insect pollinator species from 12 families were observed in *D. cistiflora* s.l. flowers in 2009 and 2010 (Table 4). Each floral colour form was pollinated by more than one insect species, with the total species number observed per form in ascending order of magnitude as: 6 (purple), 8 (red), 10 (yellow), 11 (white) and 14 (pink).

There was an overall relationship between flower colour and pollinator community (global $R = 0.53$, $p = 0.003$, stress value = 0.03) [Figure 3]. In particular, red- and yellow-flowered populations formed distinct clusters on the basis of pollinator composition. Red-flowered populations grouped together and had statistically different pollinating fauna to yellow- ($R = 1.00$, $p = 0.03$), pink- ($R = 0.54$, $p = 0.03$) and white-flowered ($R = 0.39$, $p = 0.06$) populations. The group formed by yellow-flowered populations had statistically different pollinating fauna to red- ($R = 1.00$, $p = 0.03$) and pink-flowered ($R = 0.46$, $p = 0.03$) populations. Populations of pink and white floral colour forms appeared to have variable pollinator compositions and did not form discrete clusters. Given that there were only two purple-flowered populations, there was not enough statistical power to report on the pollinating fauna of this flower colour.

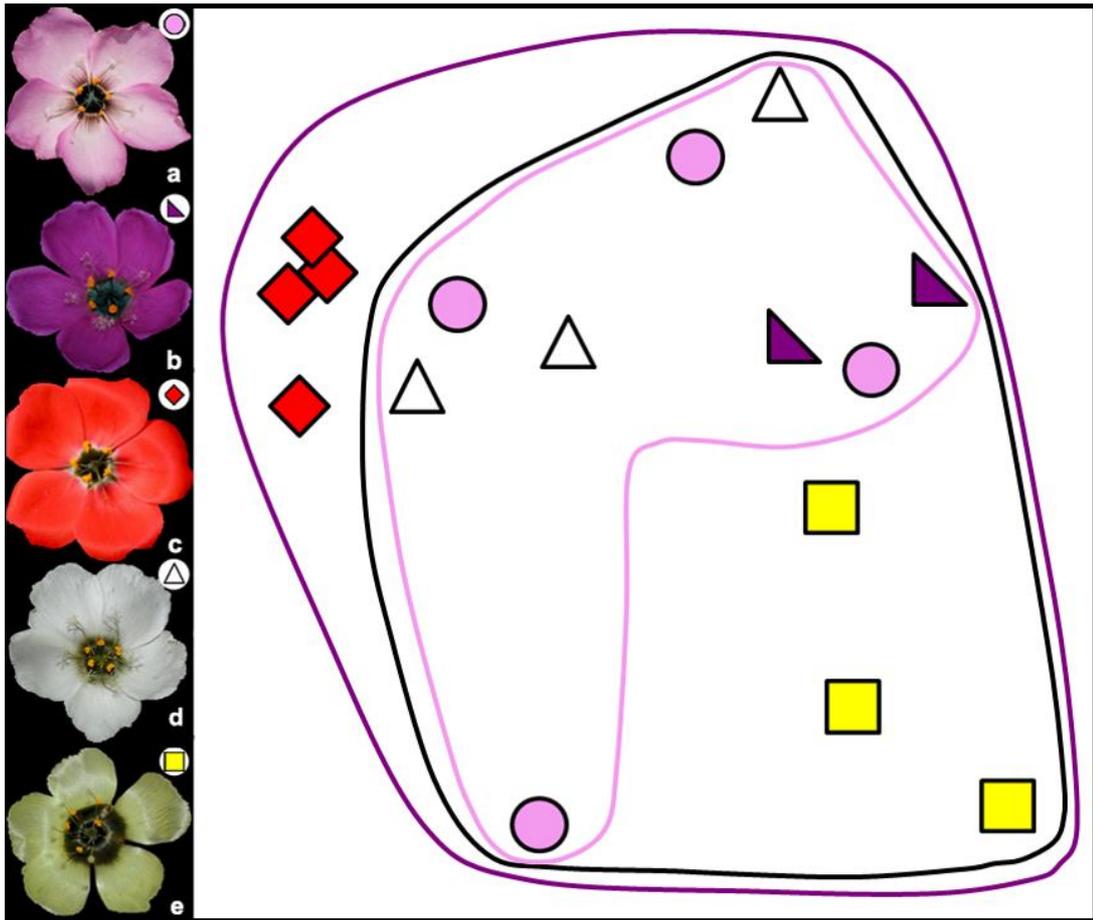


Figure 3. Multidimensional scaling (Bray-Curtis similarity index) plot grouping *Drosera cistiflora* s.l. populations of pink (a), purple (b), red (c), white (d) and yellow (e) floral colour forms according to relative abundance of different pollinator species (global $R = 0.53$, $p = 0.003$, stress value = 0.03). Populations that are close together share similar pollinator communities whilst those that are far apart have different pollinator communities. Significant differences at the level of $p < 0.06$ in the pollinating fauna of population floral colour forms are denoted using coloured rings. Images and corresponding symbols differentiate *D. cistiflora* s.l. flower colours.

Pollinator importance

Hopliine beetles (Coleoptera: Scarabaeidae: Hopliini) were the primary pollinators of purple, red, white and yellow *D. cistiflora* s.l. floral colour forms (Table 4, Figure 4, Supplementary Tables 8 & 9), with relative importance (RPI) per floral colour form in descending order of magnitude as follows: red (99.7%), white (91.7%), purple (80.4%), yellow (71.8%) and pink (31.8%). Species assemblages of hopliine beetles differed largely between colours. Beetles of the families Meloidae and Tenebrionidae were also important pollinators of pink-flowered forms, with RPI of 50.5% and 13.4%

respectively, and soft-winged flower beetles (Melyridae) were of importance in yellow-flowered forms (RPI: 21.8%). Notably, one species of hopliine beetle, *Lepisia rupicola* spec., had particularly high relative importance in red- (RPI: 88.7%), white- (RPI: 87.1%) and pink-flowered (RPI: 29.6%) populations, but was absent from yellow-flowered populations (Table 4). All *D. cistiflora* s.l. insect visitors were found to be polylectic (viz. not specific to *D. cistiflora* s.l.), carrying pollen from other plant species in the local environment.

Clustering weighted by relative pollinator importance did not alter the associations between pollinator assemblage and flower colour (global $R = 0.44$, $p = 0.001$, stress value = 0.03), so that red- and yellow-flowered populations still formed discrete groups. Yellow-flowered populations were significantly different from those of red- ($R = 1.00$, $p = 0.03$) and pink-flowered ($R = 0.53$, $p = 0.03$) populations, and, similarly, red-flowered populations had significantly different pollinating fauna to yellow- ($R = 1.00$, $p = 0.03$) and pink-flowered ($R = 0.35$, $p = 0.06$) populations; although pollinator compositions of pink- and white-flowered populations were more variable.

Table 4. Relative importance (RPI) values (%) of insect pollinators observed visiting each floral colour form of *Drosera cistiflora* s.l. in 2009 and 2010. Pollinator importance was calculated as the product of abundance in *D. cistiflora* s.l. flowers and average *D. cistiflora* s.l. pollen loads (Supplementary Tables 8 & 9). Relative importance was calculated as the percentage contribution of each pollinator to the overall pollinator importance in each *D. cistiflora* s.l. floral colour form. The pollinator group with the highest RPI value for each floral colour form is indicated in bold type. Insects are identified to family, and genus and species where possible. Superscript H denotes hopliine beetles (Coleoptera: Scarabaeidae: Hopliini).

Flower visitor species, genus and/or family	<i>Drosera cistiflora</i> s.l. floral colour form				
	Pink	Purple	Red	White	Yellow
<i>Anisochelus inornatus</i> (Scarabaeidae) ^H					16.537
<i>Anisonyx</i> sp. (Scarabaeidae) ^H				4.359	
<i>Anisonyx</i> cf. <i>ursus</i> (Scarabaeidae) ^H	2.298	2.040		0.198	
Ceratopogonidae	0.033	0.093	0.002	0.040	0.054
<i>Chasme decora</i> (Scarabaeidae) ^H			10.648		
<i>Chasme</i> sp. (Scarabaeidae) ^H			0.070		
Chrysomellidae	0.313		0.007	0.019	
Empididae (species 2)	0.040				
<i>Heterochelus</i> sp. (species 1) [Scarabaeidae] ^H					1.228
<i>Lepisia rupicola</i> spec. (Scarabaeidae) ^H	29.550	15.184	88.689	87.133	
<i>Lepithrix</i> sp. (Scarabaeidae) ^H					46.806
Lyganidae				0.121	
Megachilidae	0.502			0.318	
Meloidae	50.514			4.525	
Melyridae (species 1)	0.050				
Melyridae (species 2)	1.020			0.807	21.825
Muscidae	0.234				
<i>Omocrates</i> sp. (species 1) [Scarabaeidae] ^H		63.173			
<i>Peritrichia</i> sp. (species 1) [Scarabaeidae] ^H					6.240
<i>Peritrichia</i> sp. (species 2) [Scarabaeidae] ^H					0.991
<i>Platycheilus lupinus</i> (Scarabaeidae) ^H			0.289		
Scarabaeidae	1.339				0.286
Tabanidae (species 1)			0.276		
Tabanidae (species 2)					3.376
Tenebrionidae (species 1)	13.352	19.199		2.457	2.657
Tenebrionidae (species 2)			0.020		
Tenebrionidae (species 3)	0.660			0.023	
Thomisidae	0.096	0.311			

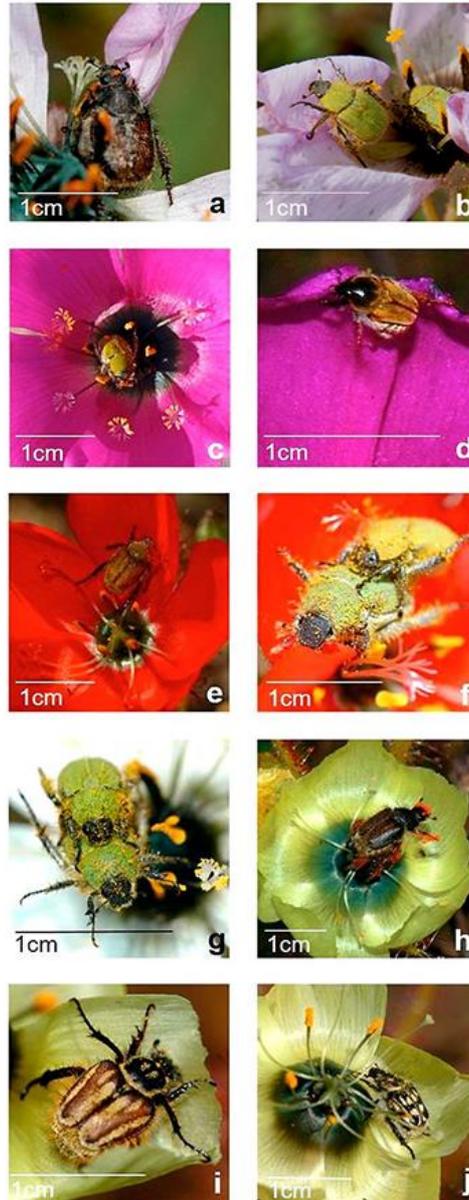


Figure 4. Hopliine beetle (Coleoptera: Scarabaeidae: Hopliini) pollinators in all five floral colour forms of *Drosera cistiflora* s.l. (a–j). *Lepisia rupicola* spec. (b, c, f & g) emerged as an important pollinator of pink- (b), purple- (c), red- (f) and white-flowered (g) forms. *Omodontes* sp. (d) was abundant in purple-flowered forms and *Chasmodon decora* (e) was only apparent in red flowers. *Heterochelus* sp. (h), *Lepithrix* sp. (i) and *Peritrichia* sp. (j) are shown visiting yellow flowers. Photo (h) by Dr Kim Steiner.

Geographical structuring of plant-pollinator communities

Geographical proximity of populations and the effect on pollinator/flower colour similarity

There was a significant negative relationship between pairwise geographical proximity of *D. cistiflora* s.l. populations and the pairwise similarity of the pollinating fauna (Mantel $Z = 92484$, $p < 0.00001$), viz. populations that were close together shared similar pollinators whilst those that were geographically distant had more dissimilar pollinating fauna compositions. This was true irrespective of whether analyses included all flower colour data (Figure 5a), pairs of different flower colours only (Figure 5b), or pairs with the same flower colour only (Figure 5c).

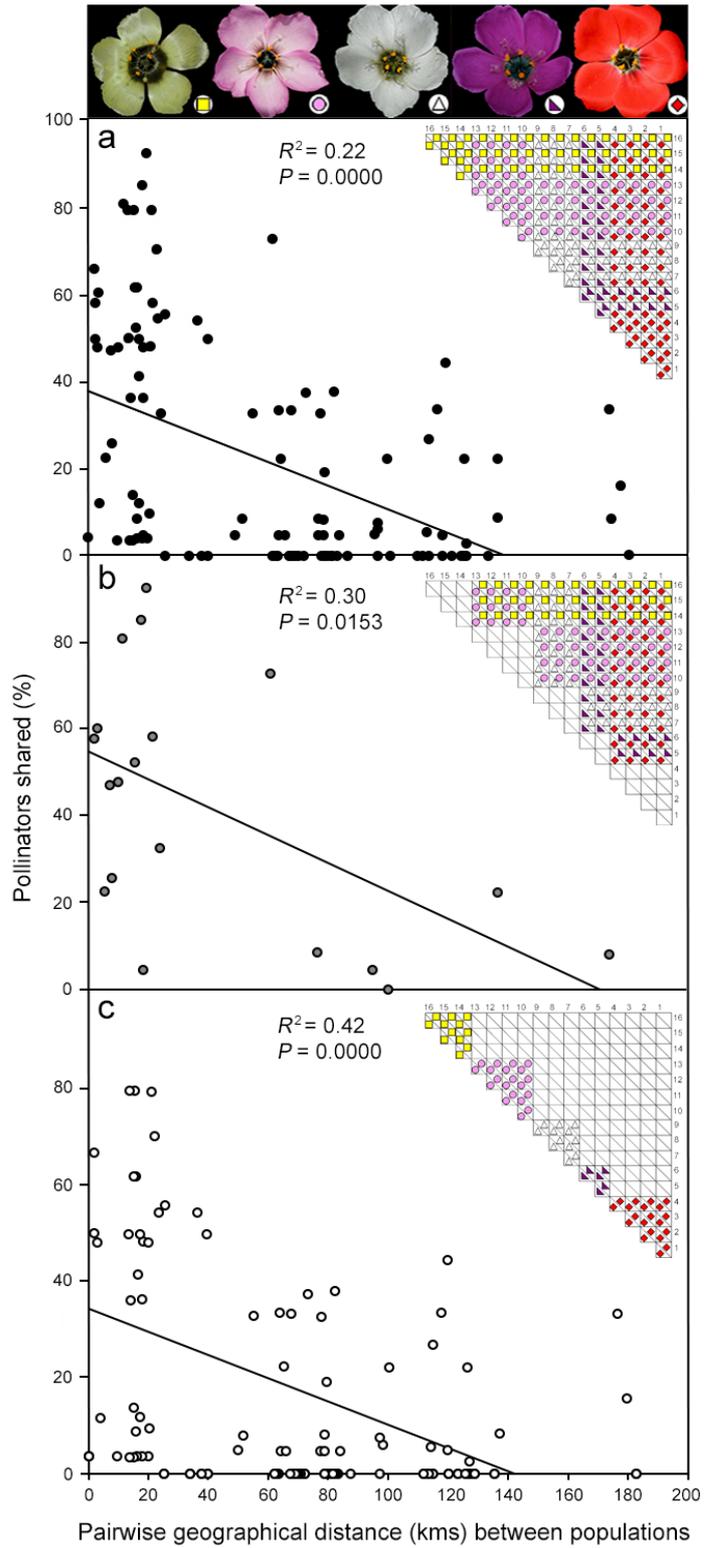


Figure 5. Mantel correlations between pairwise geographical distances and pairwise Bray-Curtis similarities of pollinating fauna in *Drosera cistiflora* s.l. populations, for all populations (a), populations with different flower colours only (b) and populations with the same flower colour only (c). Inset matrices show populations 1–16 and flower colours for which correlations are plotted.

Discussion

Findings of this study refute my first hypothesis, namely that floral colour variation in *Drosera cistiflora* s.l. is a manifestation of plastic responses to abiotic factors, and lend support to my second hypothesis, that flower colour has diverged as a result of geographical pollinator mosaics. Here I discuss these results, as well as the potentially confounding effect of pollinator distributions and geographical proximity of floral colour forms.

Floral colour divergence and abiotic factors

In view of the absence of edaphic and vegetation type endemism in all *D. cistiflora* s.l. floral colour forms, and given that vegetation types may serve as a proxy for multiple abiotic factors such as soil chemistry, temperature, light and moisture availability, flower colour did not appear to be a manifestation of a physiological response to components of the physical environment. Yellow, red and purple *D. cistiflora* s.l. floral colour forms were however more specialised in their habitats than other floral colour forms (perhaps because there were so few populations), since they occurred in only two or three broad vegetation categories and soil types. The latter habitats nonetheless also supported at least two other floral colour forms.

There was an overall association of floral colour forms with pollinator communities, and red- and yellow-flowered forms diverged significantly in pollination niche. The maintenance of flower colour in both soil switching and common-garden experiments (with seedling representatives of all floral colour forms) further supports a genetic basis for floral divergence. Evidence thus excludes the probability that floral colour forms reflect plastic responses to abiotic factors and points to a potential role for pollinator-driven floral phenotypic diversification.

Pollinator-driven floral colour divergence

All *D. cistiflora* s.l. floral colour forms, whether they occurred together at the same site, or apart at different sites, had coinciding flowering phenologies, and there was an overall association of forms with generalist pollinator communities. Local adaptation to pollination environments is an important component of adaptive floral diversification (Grant & Grant 1965). In particular, the discrete red and yellow *D. cistiflora* s.l. floral colour forms were associated with distinct geographical assemblages of pollinators (Figure 3) that may be directly selecting for these flower colours, in congruence with the pollinator-shift model (q.v. Grant 1949; Grant & Grant 1965; Stebbins 1970; Johnson 2006, 2010; Kay & Sargent 2009; van der Niet, Peakall & Johnson 2014; Smith & Kriebel 2018).

Hairy, and hence pollen-trapping, hopliine beetles (Picker et al. 2004, pers. obs) were found to be the primary pollinators of purple, red, white and yellow *D. cistiflora* s.l. floral colour forms. In particular, a ‘principal pollinator’ (sensu Stebbins 1974), i.e. that with the highest importance value overall, namely the hopliine beetle *Lepisia rupicola* spec., was shared by red- and white-flowered forms, and was also a frequent and effective pollinator of pink- and purple-flowered forms. Even so, each floral colour form was visited by other polylectic, diverse pollinating fauna comprising multiple insect families, many of which were pooled among forms. Assemblages of pollinators thus constituted shared components but differed on the whole. The least generalisation was displayed by red- and yellow-flowered forms, which nonetheless also respectively shared 37.5% and 40% of pollinator species with other forms. Generalised pollination systems such as these may prove advantageous in conditions where pollinator availability is unpredictable (Waser et al. 1996, Burns et al. 2019). Habitat ephemerality, in combination with partial self-incompatibility and pollinator dependence may accordingly have favoured the development of pollination system generalisation in *D. cistiflora* s.l.

Floral colour shifts in *D. cistiflora* s.l. may represent adaptations to entire community compositions of pollinators, since pollinator assemblages differed in their entirety but also contained largely overlapping components. This would be consistent with the assertion of Gómez et al. (2008, 2014) that floral phenotype may diversify across the

range of a species in response to suites of generalist pollinators. Similarly, findings of generalised pollination and spatial variation in visitor assemblage in *Calochortus* (Liliaceae) suggested that lineages may have been moving through a spatiotemporal mosaic of pollinators over evolutionary time (Dilley et al. 2000). Here the authors surmised that colour patterns, amongst other floral traits, have diverged through the historical accumulation of floral modifications that have been selected for by the suites of pollinators to which they appeal.

Geographical structuring of plant-pollinator communities

By virtue of the significant relationships between geographical proximity of *D. cistiflora* s.l. populations and pollinator assemblages (Figure 5), it is possible that geographically close plant populations may have similarities in pollinating fauna because of geographical structuring of pollinator distributions across the landscape, and not necessarily that they evolved flower colours in response to selection by similar pollinating fauna. In other words, if a plant occurs within a certain geographical region, it may have similar pollinators to other plants within that region simultaneously, irrespective of flower colour.

Pollinator compositions also differed between populations of different floral colour forms in sympatry, where separation by distance was minimal. Further, the observation of a significant reduction in female fitness in the purple-flowered form relative to respective sympatric pink- and red-flowered forms (von Witt et al. unpublished) provides indirect evidence that these floral colour forms may attract different pollinators. Although these results may simply reflect intrinsic differences in breeding systems among floral colour forms, sympatric purple- and red-flowered forms examined at Darling 3 exhibited high pollinator contribution to fecundity, pollinator dependence and partial self-incompatibility (von Witt et al. unpublished) in combination with shifts in pollinator assemblages (Supplementary Table 7). Whilst these findings suggest a role for selection through pollinator partitioning (e.g. Liu & Huang 2013) in the maintenance of floral colour divergence, only Darling 3 had sufficiently large sympatric populations of different floral colour forms for study.

Fitness differences may also be the outcome of disadvantageous alleles becoming fixed through genetic drift (Futuyma 2009), or pleiotropic effects of non-pollinator biotic factors (e.g. Carlson & Holsinger 2012). In variable pink *D. cistiflora* s.l. floral colour forms without well-defined pollinator assemblages there appeared to be a random shift in the frequencies of shades of flower colour. This may reflect neutral divergence in gene frequencies arising from drift in the presence of weak and conflicting selection by generalist pollinators. Here, intrapopulation analyses of performance and pollinator colour choice experiments differentiating between varying shades of flower colours may determine the relative importance of drift and natural selection.

Should pollinator distributions be geographically structured and not fundamentally related to flower colour in *D. cistiflora* s.l., the possibility remains that geographical patterns of flower colour may be a consequence of the pleiotropic effects of genes involved in adaptation to the local abiotic environment. In the example of *Linanthus parryae*, although blue and white floral colour forms occurred in close proximity, these forms exhibited an abrupt change in frequency in accordance with a shallow ravine (Schemske & Bierzychudek 2007). The use of GIS software in my study enabled broad comparisons of abiotic factors to be made between *D. cistiflora* s.l. floral colour forms with non-overlapping distribution ranges. It did not, however, allow for the examination of habitat differences on a microspatial scale in the three study sites where the distribution ranges of floral colour forms (namely pink and purple, purple and red, and red and white) coincided, nor where pink and white flowers varied along a colour continuum from pink to white within populations.

It is therefore not currently possible to distinguish cause and effect from current data about geographical patterns of pollinating fauna and flower colour in *D. cistiflora* s.l. Given that shifts in pollination systems have frequently been associated with parallel shifts in soil types (e.g. Patterson & Givnish 2003, Goldblatt & Manning 2006), it is plausible that future examination of *D. cistiflora* s.l. pollinator biogeography may indeed find pollinator assemblages to be determined by edaphic and/or other abiotic factors. If pollinators are additionally found to select for flower colour, then floral colour divergence may subsequently proceed as ‘consequent radiation’ (sensu Patterson & Givnish 2003) via an indirect association of plants with the soils and/or other physical components of the environment supporting their pollinators.

Conclusions

My results show a pattern linking pollinator communities and flower colour, and as such the only current explanation for the expression of *D. cistiflora* s.l. floral colour divergence that has supporting data is that colour variation is pollinator-driven. Studies of pollinator vision have detailed evidence for at least three photoreceptor types in certain species of hopliine beetles (Arnold 2010), and such confirmation of specific colour perception in these beetles upholds preliminary findings of local colour preferences of pollinators in the *D. cistiflora* complex (Supplementary Table 7).

In addition to pollinator colour choice experiments, further work should include study of *D. cistiflora* s.l. pigment biosynthetic pathways and their pleiotropic potential, and pleiotropic effects of non-pollinator biotic agents such as herbivores, pollen thieves and seed predators. Requisite molecular study of *D. cistiflora* s.l. plant populations may ultimately isolate genetic differences between floral colour forms and determine whether these are associated with pollinator shifts.

References

- Alexandersson, R. & Johnson, S. D. (2002) Pollinator-mediated selection on flower-tube length in a hawk moth-pollinated *Gladiolus* (Iridaceae). *Proceedings of the Royal Society B — Biological Sciences* **269**, 631–636.
- Anderson, B. (2010) Did *Drosera* evolve long scapes to stop their pollinators from being eaten? *Annals of Botany* **106** (4), 653–657.
- Anderson, B., Alexandersson, R. & Johnson, S. D. (2010) Evolution and coexistence of pollination ecotypes in an African *Gladiolus* (Iridaceae). *Evolution* **64**, 960–972.
- Anderson, B., Pauw, A., Cole, W. W. & Barrett, S. C. H. (2016) Pollination, mating and reproductive fitness in a plant population with bimodal floral-tube length. *Journal of Evolutionary Biology* **29** (8), 1631–1642.
- Arista, M., Talavera, M., Berjano, R. & Ortiz, P. L. (2013) Abiotic factors may explain the geographical distribution of flower colour morphs and the maintenance of colour polymorphism in the scarlet pimpernel. *Journal of Ecology* **101** (6), 1613–1622.
- Armbruster, W. S. (2002) Can indirect selection and genetic context contribute to trait diversification? A transition-probability study of blossom-colour evolution in two genera. *Journal of Evolutionary Biology* **15** (3), 468–486.
- Armbruster, P., Hutchinson, R. A. & Cotgreave, P. (2002) Factors influencing community structure in a South American tank bromeliad fauna. *Oikos* **96**, 225–234.
- Arnold, S. E. J. (2010) *Flowers through insect eyes: The contribution of pollinator vision to the evolution of flower colour*. PhD Thesis. Queen Mary, University of London, UK.

- Beardsley, P. M., Yen, A. & Olmstead, R. G. (2003) AFLP phylogeny of *Mimulus* section *Erythranthe* and the evolution of hummingbird pollination. *Evolution* **57**, 1397–1410.
- Bowman, R. N. (1987) Cryptic self-incompatibility and the breeding system of *Clarkia unguiculata* (Onagraceae). *American Journal of Botany* **74**, 471–476.
- Burns, J. H., Bennett, J. M., Li, J., Xia, J., Arceo-Gómez, G., Burd, M., Burkle, L. A., Durka, W., Ellis, A. G., Freitas, L., Rodger, J. G., Vamosi, J. C., Wolowski, M., Ashman, T. L., Knight, T. M. & Steets, J. A. (2019) Plant traits moderate pollen limitation of introduced and native plants: A phylogenetic meta-analysis of global scale. *New Phytologist* **223** (4), 2063–2075.
- Carlson, J. E. & Holsinger, K. E. (2010) Natural selection on inflorescence colour polymorphisms in wild *Protea* populations: The role of pollinators, seed predators and inter-trait correlations. *American Journal of Botany* **97**, 934–944.
- Carlson, J. E. & Holsinger, K. E. (2012) Direct and indirect selection on floral pigmentation by pollinators and seed predators in a colour polymorphic South African shrub. *Oecologia* **171**, 905–919.
- Codd, L. E., de Winter, B. & Killick, D. J. B. (1970) *Flora of Southern Africa* **13**. National Botanic Gardens, Kirstenbosch, Cape Town.
- Dilley, J. D., Wilson, P. & Mesler, M. R. (2000) The radiation of *Calochortus*: Generalist flowers moving through a mosaic of potential pollinators. *Oikos* **89** (2), 209–222.
- Ellis, A. G. & Johnson, S. D. (2009) The evolution of floral variation without pollinator shifts in *Gorteria diffusa* (Asteraceae). *American Journal of Botany* **96**, 793–801.
- Emms, S. K. & Arnold, M. L. (2000) Site-to-site differences in pollinator visitation patterns in a Louisiana iris hybrid zone. *Oikos* **91**, 568–578.

Faegri, K. & van der Pijl, L. (1979) *The principles of pollination ecology*. Pergamon Press, Oxford, UK.

Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R. & Thomson, J. D. (2004) Pollination syndromes and floral specialisation. *Annual Review of Ecology and Evolution* **35**, 375–403.

Forest, F., Goldblatt, P., Manning, J. C., Baker, D., Colville, J. F., Devey, D. S., Jose, S., Kaye, M. & Buerki, S. (2014) Pollinator shifts as triggers of speciation in painted petal irises (*Lapeirousia*: Iridaceae). *Annals of Botany* **113** (2), 357–371.

Futuyma, D. J. (2009) Genetic drift: Evolution at random. In D. J. Futuyma, *Evolution*. Sinauer Associates, Sunderland, Massachusetts, USA.

Galen, C. (1999a) Flowers and enemies: Predation by nectar-thieving ants in relation to variation in floral form of an alpine wildflower, *Polemonium viscosum*. *Oikos* **85**, 426–434.

Galen, C. (1999b) Why do flowers vary? The functional ecology of variation in flower size and form within natural plant populations. *Bioscience* **49**, 631–640.

Gervasi, D. D. L. & Schiestl, F. P. (2017) Real-time divergent evolution in plants driven by pollinators. *Nature Communications* **8**, 14691.

Gigord, L. D. B., Macnair, M. R. & Smithson, A. (2001) Negative frequency-dependent selection maintains a dramatic flower colour polymorphism in the rewardless orchid *Dactylorhiza sambucina* (L.) Soò. *Proceedings of the National Academy of Sciences of the United States of America* **98** (11), 6253–6255.

Goldblatt, P., Bernhardt, P. & Manning, J. C. (1998) Pollination of petaloid geophytes by monkey beetles (Scarabaeidae: Rutelinae: Hopliini) in Southern Africa. *Annals of the Missouri Botanical Garden* **85**, 215–230.

Goldblatt, P. & Manning, J. C. (2006) Radiation of pollination systems in the Iridaceae of sub-Saharan Africa. *Annals of Botany* **97** (3), 317–344.

Gómez, J. M., Bosch, J., Perfectti, F., Fernández, J. D., Abdelaziz, M. & Camacho, J. P. M. (2008) Spatial variation in selection on corolla shape in a generalist plant is promoted by the preference patterns of its local pollinators. *Proceedings of the Royal Society B — Biological Sciences* **275**, 2241–2249.

Gómez, J. M., Muñoz-Pajares, A. J., Abdelaziz, M., Lorite, J. & Perfectti, F. (2014) Evolution of pollination niches and floral divergence in the generalist plant *Erysimum mediohispanicum*. *Annals of Botany* **113** (2), 237–249.

Grant, V. (1949) Pollination systems as isolating mechanisms in angiosperms. *Evolution* **3**, 82–97.

Grant, V. & Grant, K. A. (1965) *Flower pollination in the Phlox family*. Columbia University Press, New York, USA.

Herrera, C. M. (1996) Floral traits and plant adaptation to insect pollinators: A devil's advocate approach. In D. G. Lloyd & S. C. H. Barrett [Eds], *Floral biology: Studies on floral evolution in animal-pollinated plants*, 65–87. Chapman & Hall, New York, USA.

Hopkins, R. & Rausher, M. D. (2012) Pollinator-mediated selection on flower colour allele drives reinforcement. *Science* **335**, 1090–1092.

Huber, F. K., Kaiser, R., Sauter, W. & Schiestl, F. P. (2005) Floral scent emission and pollinator attraction in two species of *Gymnadenia* (Orchidaceae). *Oecologia* **142** (4), 564–575.

Ippolito, A., Fernandes, G. W. & Holtsford, T. P. (2004) Pollinator preferences for *Nicotiana alata*, *N. forgetiana*, and their F1 hybrids. *Evolution* **58**, 2634–2644.

- Irwin, R. E. & Strauss, S. Y. (2005) Flower colour microevolution in wild radish: Evolutionary response to pollinator-mediated selection. *The American Naturalist* **165** (2), 225–237.
- Irwin, R. E., Strauss, S. Y., Storz, S., Emerson, A. & Guibert, G. (2003) The role of herbivores in the maintenance of a flower colour polymorphism in wild radish. *Ecology* **84** (7), 1733–1743.
- Ito, D., Shinkai, Y., Kato, Y., Kondo, T. & Yoshida, K. (2009) Chemical studies on different colour development in blue- and red-coloured sepal cells of *Hydrangea macrophylla*. *Bioscience, Biotechnology and Biochemistry* **73** (5), 1054–1059.
- Johnson, S. D. (1997) Pollination ecotypes of *Satyrium hallackii* (Orchidaceae) in South Africa. *Botanical Journal of the Linnean Society* **123**, 225–235.
- Johnson, S. D. (2006) Pollinator-driven speciation in plants. In L. D. Harder & S. C. H. Barrett [Eds], *The ecology and evolution of flowers*, 296–306. Oxford University Press, Oxford, UK.
- Johnson, S. D. (2010) The pollination niche and its role in the diversification and maintenance of the southern African flora. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365** (1539), 499–516.
- Johnson, S. D., Linder, H. P. & Steiner, K. E. (1998) Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). *American Journal of Botany* **85**, 402–411.
- Kay, K. M. & Sargent, R. D. (2009) The role of animal pollination in plant speciation: Integrating ecology, geography, and genetics. *Annual Review of Ecological Systems* **40**, 637–656.
- Levin, D. A. & Brack, E. T. (1995) Natural selection against white petals in phlox. *Evolution* **49**, 1017–1022.

- Liu, C. Q. & Huang, S. Q. (2013) Floral divergence, pollinator partitioning and the spatiotemporal pattern of plant-pollinator interactions in three sympatric *Adenophora* species. *Oecologia* **173**, 1411–1423.
- Manly, B. F. J. (1991) *Randomisation and Monte Carlo methods in biology*. Chapman & Hall, New York, USA.
- Meléndez-Ackerman, E. J. & Campbell, D. R. (1998) Adaptive significance of flower colour and inter-trait correlations in an *Ipomopsis* hybrid zone. *Evolution* **52** (5), 1293–1303.
- Minnaar, C., de Jager, M. L. & Anderson, B. (2019) Intraspecific divergence in floral tube length promotes asymmetric pollen movement and reproductive isolation. *New Phytologist*. doi:10.1111/nph.15971.
- Mucina, L. & Rutherford, M. C. [Eds] (2006) The vegetation of South Africa, Lesotho and Swaziland. *Strelitzia* **19**. South African National Biodiversity Institute, Pretoria.
- Narbona, E. & Wang, H., Ortiz, P., Arista, M. & Imbert, E. (2017). Flower colour polymorphism in the Mediterranean Basin: Occurrence, maintenance and implications for speciation. *Plant Biology* **20**, Suppl 1. doi:10.1111/plb.12575.
- Newman, E., Anderson, B. & Johnson, S. D. (2012) Flower colour adaptation in a mimetic orchid. *Proceedings of the Royal Society B — Biological Sciences* **279**, 2309–2313.
- Newman, E., Manning, J. & Anderson, B. (2014) Matching floral and pollinator traits through guild convergence and pollinator ecotype formation. *Annals of Botany* **113** (2), 373–384.
- Ollerton, J. (1996) Reconciling ecological processes with phylogenetic patterns: The apparent paradox of plant-pollinator systems. *Journal of Ecology* **84**, 767–769.

- Parachnowitsch, A. L., Manson, J. S. & Sletvold, N. (2018) Evolutionary ecology of nectar. *Annals of Botany* **123** (2), 247–261. doi:10.1093/aob/mcy132.
- Patterson, T. B. & Givnish, T. J. (2003) Geographic cohesion, chromosomal evolution, parallel adaptive radiations, and consequent floral adaptations in *Calochortus* (Calochortaceae): Evidence from a cpDNA phylogeny. *New Phytologist* **161** (1), 253–264.
- Pérez, F., Arroyo, M. T. K., Medel, R. & Hershkovitz, M. A. (2006) Ancestral reconstruction of flower morphology and pollination systems in *Schizanthus* (Solanaceae). *American Journal of Botany* **93**, 1029–1038.
- Picker, M., Griffiths, C. & Weaving, A. (2004) *Field guide to insects of South Africa*. Struik, Cape Town.
- Rausher, M. D. & Fry, J. D. (1993) Effects of a locus affecting floral pigmentation in *Ipomoea purpurea* on female fitness components. *Genetics* **134**, 1237–1247.
- Schemske, D. W. & Bierzychudek, P. (2007) Spatial differentiation for flower colour in the desert annual *Linanthus parryae*: Was Wright right? *Evolution* **61**, 2528–2543.
- Schemske, D. W. & Bradshaw, H. D., Jr (1999) Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proceedings of the National Academy of Sciences of the United States of America* **96** (21), 11910–11915.
- Schiestl, F. P. & Johnson, S. D. (2013) Pollinator-mediated evolution of floral signals. *Trends in Ecology and Evolution* **28** (5), 307–315.
- Schreiber, H. D., Swink, A. M. & Godsey, T. D. (2010) The chemical mechanism for Al³⁺ complexing with delphinidin: A model for the bluing of hydrangea sepals. *Journal of Inorganic Biochemistry* **104** (7), 732–739.
- Smith, S. D. & Kriebel, R. (2018) Convergent evolution of floral shape tied to pollinator shifts in Iochrominae (Solanaceae). *Evolution* **72** (3), 688–697.

Stebbins, G. L. (1970) Adaptive radiation of reproductive characteristics in angiosperms, I: Pollination mechanisms. *Annual Review of Ecological Systems* **1**, 307–326.

Stebbins, G. L. (1974) *Flowering plants: Evolution above the species level*. Harvard University Press, USA.

Strauss, S. Y. & Whittall, J. B. (2006) Non-pollinator agents of selection on floral traits. In L. D. Harder & S. C. H. Barrett [Eds], *Ecology and evolution of flowers*, 120–138. Oxford University Press, Oxford, UK.

Streiner, M., Roth, N., Paulus, H. F. & Spaethe, J. (2019) Colour preference and spatial distribution of glaphyrid beetles suggest a key role in the maintenance of the colour polymorphism in the peacock anemone (*Anemone pavonina*, Ranunculaceae) in Northern Greece. *Journal of Comparative Physiology*. doi:10.1007/s00359-019-01360-2.

Streisfeld, M. A. & Kohn, J. R. (2007) Environment and pollinator-mediated selection on parapatric floral races of *Mimulus aurantiacus*. *Journal of Evolutionary Biology* **20**, 122–132.

Sun, M., Gross, K. & Schiestl, F. P. (2014) Floral adaptation to local pollinator guilds in a terrestrial orchid. *Annals of Botany* **113** (2), 289–300.

Tao, Z. B., Ren, Z. X., Bernhardt, P., Liang, H., Li, H. D., Zhao, Y. H., Wang, H. & Li, D. Z. (2018) Does reproductive isolation reflect the segregation of colour forms in *Spiranthes sinensis* (Pers.) Ames complex (Orchidaceae) in the Chinese Himalayas? *Ecology and Evolution* **00**, 1–15. doi:10.1002/ece3.4067.

Trinder-Smith, T., Maytham Kidd, M. & Anderson, F. (2006) *Wild flowers of the Table Mountain National Park*. Botanical Society of South Africa, Cape Town.

van der Niet, T. & Johnson, S. D. (2012) Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends in Ecology and Evolution* **27** (6), 353–361.

- van der Niet, T., Peakall, R. & Johnson, S. D. (2014) Pollinator-driven ecological speciation in plants: New evidence and future perspectives. *Annals of Botany* **113** (2), 199–211.
- van der Niet, T., Pirie, M. D., Shuttleworth, A., Johnson, S. D. & Midgley, J. J. (2014) Do pollinator distributions underlie the evolution of pollination ecotypes in the Cape shrub *Erica plukenetii*? *Annals of Botany* **113** (2), 301–316.
- von Witt, C. G., Anderson, B. A., Durbach, I. N. & Johnson, S. D. (2019) Breeding systems of floral colour forms in the *Drosera cistiflora* species complex. Manuscript under revision.
- Warren, J. & Mackenzie, S. (2001) Why are all colour combinations not equally represented as flower-colour polymorphisms? *New Phytologist* **151**, 237–241.
- Waser, N. M. (1998) Pollination, angiosperm speciation, and the nature of species boundaries. *Oikos* **81**, 198–201.
- Waser, N. M., Chittka, L. Price, M. V., Williams, N. M. & Ollerton, J. (1996) Generalisation in pollination systems and why it matters. *Ecology* **77**, 1043–1060.
- Waser, N. M. & Price, M. V. (1981) Pollinator choice and stabilising selection for flower colour in *Delphinium nelsonii*. *Evolution* **35** (2), 376–390.
- Wright, S. (1931) Evolution in Mendelian populations. *Genetics* **28**, 114–138.
- Wright, S. (1943a) Isolation by distance. *Genetics* **28**, 114–138.
- Wright, S. (1943b) An analysis of local variability of flower colour in *Linanthus parryae*. *Genetics* **28**, 139–156.
- Wright, S. (1978) *Evolution and the genetics of populations, volume 4: Variability within and among natural populations*. University of Chicago Press, Chicago, USA.

CHAPTER FOUR

LOCAL ADAPTATION OF FLOWER COLOUR IN THE *DROSERA CISTIFLORA* SPECIES COMPLEX: AN ASSESSMENT USING MODEL FLOWERS AND RECIPROCAL TRANSLOCATIONS

Abstract

Colour preferences of pollinators have been proposed as the main driver of flower colour evolution in plants, but few field-based studies have fully tested this theory. Plant species that consist of geographically structured floral colour forms are ideal for testing whether divergent selection is mediated through a mosaic of pollinators with varying colour preferences. *Drosera cistiflora* sensu lato (Droseraceae) is a species complex of pollinator-dependent perennials with flowers that are devoid of nectar and odour and which vary geographically in colour, thus rendering an exemplary system for the assessment of pollinator-mediated selection on flower colour. *Drosera cistiflora* s.l. floral colour forms are associated with the composition of pollinator assemblages, but not with soil properties. I used 812 arrays of model flowers in 16 populations and 48 translocated flower arrays in six populations of the five *D. cistiflora* s.l. floral colour forms to test whether the pollinators of each form prefer locally dominant *D. cistiflora* s.l. flower colours over introduced colours. *Drosera cistiflora* s.l. floral colour forms and models matching the flower colour of the local population were more likely overall to be visited by pollinators than were novel floral colour forms and models that did not match. Models of flower colours matched to local populations generally had

significantly improved odds of receiving at least one visit and also received more visits when entire community compositions of pollinators were considered. Diverse assemblages of hopliine beetles, the primary pollinators of *D. cistiflora* s.l., showed an overall preference for red, white and yellow model colours that matched local populations. These findings support the novel concept that pollinator communities may collectively generate locally divergent selective forces on flower colour and suggest that *D. cistiflora* s.l. floral colour forms are geographically divergent generalised pollination ecotypes, viz. that populations are locally adapted to a generalised pollination environment across their geographical range in the Greater Cape Floristic Region.

Introduction

Flower colour of plant species can vary among populations (Kay 1978, Gigord et al. 2001) and floral colour adaptation can occur as a result of selection mediated by pollinators (Waser 1983, Kevan & Baker 1983, Schemske & Bradshaw 1999, Hopkins & Rausher 2012, Newman et al. 2012, Tao et al. 2018). It is well known that pollinators can have strong colour preferences (Ilse & Vaidya 1956, McCall & Primack 1992, Chittka 1992, McGimpsey & Lord 2015, van der Kooi et al. 2019), and these can reflect either innate (Real 1981, Giurfa et al. 1995, Lunau & Maier 1995) or learned preferences (Menzel & Erber 1978; Chittka et al. 1992, 1999; Dyer & Chittka 2004) or a combination of both (Weiss 1997, Gumbert 2000, Chittka et al. 2003). However, most studies of pollinator colour preferences have been carried out in laboratory settings (e.g. Troje 1993, Smithson & McNair 1997a&b, Chittka 1997, Jones & Reithel 2001, Briscoe & Chittka 2001, Dyer et al. 2007, Morante & Desplan 2008, Lunau 2014) rather than being field-based (e.g. Epperson & Clegg 1987; Dafni et al. 1990; Picker & Midgley 1996; Johnson & Midgley 2001; Campbell et al. 2010, 2012; Newman et al. 2012; Streinzer et al. 2019).

In the Grant-Stebbins model of pollinator-driven diversification, plant species diverge when they adapt to local pollinator assemblages (q.v. Grant 1949, Grant & Grant 1965, Stebbins 1970, Johnson 2006, Kay & Sargent 2009, van der Niet et al. 2014). According to this hypothesis, geographical structure in the distributions of pollinators with varying colour preferences can explain pollination ecotypes with floral colour divergence among populations. Alternatively, the variation in flower colour could represent an outcome of drift whereby different colours become fixed in different populations (Wright 1931, 1943a&b, 1978). A third possibility is pleiotropy, whereby, for example, enzymes involved in anthocyanin synthesis are also required for the synthesis of other flavonoid compounds (Strauss & Whittall 2006). Thus, selection on other ecological and physiological traits could also influence flower colour even in the absence of selection by pollinators (Rausher 2008).

In Chapter 3 of this thesis I demonstrated an association between pollinator communities and floral colour variation in the *Drosera cistiflora* species complex, which comprises insectivorous perennials endemic to the Greater Cape Floristic Region of South Africa. These associations between pollinators and flower colour were particularly evident for red- and yellow-flowered *D. cistiflora* s.l. populations which had distinctive assemblages of pollinators. Moreover, floral colour forms in *D. cistiflora* s.l. were not restricted to particular soil or vegetation types and were not altered by common-garden and soil switching experimentation, thereby excluding the possibility that flower colour manifests as a response to local soil chemistry and other abiotic components of the environment. This suggests a potential role for pollinators as the agents of selection in flower colour in the species complex. On the other hand, distinct pollinator assemblages were not found in pink- and white-flowered forms, and since flowers of these forms did not appear to be fixed for either flower colour in several populations, genetic drift may play a role in confounding natural selection in these populations.

Although earlier studies (namely Goldblatt et al. 1998, Anderson 2010) suggested that hopliine beetles are the primary pollinators of *D. cistiflora* s.l., my observations showed that the floral colour forms are visited by diverse pollinating fauna comprising multiple insect families. Whilst hopliine beetles do emerge as the most important of these pollinator groups, it is evident that *D. cistiflora* s.l. has a pollination system which is more generalised than previously realised. This does not preclude strong divergent selection by pollinators as shown by Gómez et al. (2008, 2014) in their studies of the generalist crucifer *Erysimum mediohispanicum* (Brassicaceae).

Drosera cistiflora s.l. is pollinator-dependent and appears to have pollen-limited fecundity (von Witt et al. unpublished). This means that plants with flower colours that are attractive to local pollinators should be favoured by selection through both female and male components of fitness. To test whether pollinators of *D. cistiflora* s.l. vary in their colour preferences, I conducted choice experiments using model and real flowers varying in colour. I predicted that pollinators would exhibit a preference for the flower colour of the local phenotype with which they are associated. Floral colour preference by flower visitors does not lead to selection unless the animal is also an effective pollinator (q.v. Jones & Reithel 2001). Accordingly, I used pollen loads of insects as a

measure of their pollination effectiveness and hence likely contribution to selection on flower colour.

Materials and Methods

Study Sites

Study sites were chosen from herbarium records of *Drosera cistiflora* s.l. populations (Table 1, Chapter 3). The 16 populations comprised four that were pink-flowered, four red, three white, three yellow and two purple, where these represented the locally dominant flower colours. Only two of the rare purple-flowered populations were found flowering sufficiently for study in 2009 and 2010. Each site measured approximately 50m x 50m. Voucher specimens for *D. cistiflora* s.l. floral colour forms were deposited in the Compton Herbarium (Table 1, Chapter 3).

Colour choice experiments

To determine whether pollinators of *D. cistiflora* s.l. vary in their colour preferences, I conducted choice experiments in populations of all *D. cistiflora* s.l. floral colour forms using arrays of model and real flowers representing each floral colour form. The experiment thus attempted to isolate pollinator choice with respect to floral colour traits only and, by consolidating all *D. cistiflora* s.l. flower colours in a single array, to account for the genetic background that may potentially influence the phenotypic expression of floral colour forms.

The arrays of both model and translocated real flowers of all five *D. cistiflora* s.l. flower colours were placed randomly in study populations. Model flower experiments were carried out in all study sites (Table 1, Chapter 3) between August and October 2009 and 2010, and reciprocal translocations were performed at Darling 2 (pink-flowered form); Darling 3 (purple- and red-flowered forms); Darling 6 and Darling 7 (white-flowered form), and Piketberg 1 (yellow-flowered form) between August and October 2010. All experiments were directed on sunny, windless days during periods of peak pollinator activity: 09h30–15h00.



Figure 1. Two arrays of model flowers (representing all five *Drosera cistiflora* s.l. flower colours) randomly placed amongst purple- and red-flowered forms at Darling 3. These *D. cistiflora* s.l. forms were dominant over three co-occurring *Drosera* species: i) white-flowered *D. trinervia*; ii) a creamy white-flowered, undescribed *Drosera* species, and iii) mauve-flowered *D. pauciflora* s.l. (*D. pauciflora* s.l. is visible in the foreground of this image).

Model flower arrays

Flower colour is necessary for pollinator attraction in most pollination systems, but the colours that humans perceive may differ remarkably to those seen through the eyes of an insect (Arnold 2010). Thus, in order to reduce the effect of human observational bias, I took readings of the floral spectral reflectance over the UV–visible range (300–700 nm) of the outer region of petals for all five *D. cistiflora* s.l. floral colour forms. UV reflection was found to be negligible. Coloured matt acrylic paints for model flower colours were matched as closely as possible to the petal readings (pink: Plascon R3-B2-1; purple: Plascon P7-B1-1; red: Dulux 19YR13/558; white: Dulux white, and yellow: Dulux 60YY79/367) [Figure 2]. I used an Ocean Optics (Dunedin, FL, USA) S2000 spectrophotometer and Ocean Optics DT-mini deuterium tungsten halogen light source (200–1100 nm), and placed the fibre optic reflection probe (UV/VIS 400 μm) at a 45° angle approximately 5mm above the surface of the painted card. The model colours did not offer a precise match to the actual *D. cistiflora* s.l. flower colours (Figure 2), but their consistency across all sites studied offered pollinators with a standard choice of colours that represented the closest alternative to real flowers that I could obtain.

Model flowers were constructed using paper discs (8cm diameter) attached to a central trap in the form of black photographic film canisters half filled with water. The black film canisters mimicked the dark centres of the real flowers, which have been shown to increase hopliine visitation rates in Irid geophytes on the Bokkeveld Escarpment (van Kleunen et al. 2007). However, since these dark centres were consistent in all colours in an array, the only varying choice offered to beetles (and all insect visitors) would pertain to peripheral petal colour. The traps were attached to wire stems that were raised to a height of 12cm above ground, in keeping with the stem length of *D. cistiflora* s.l.

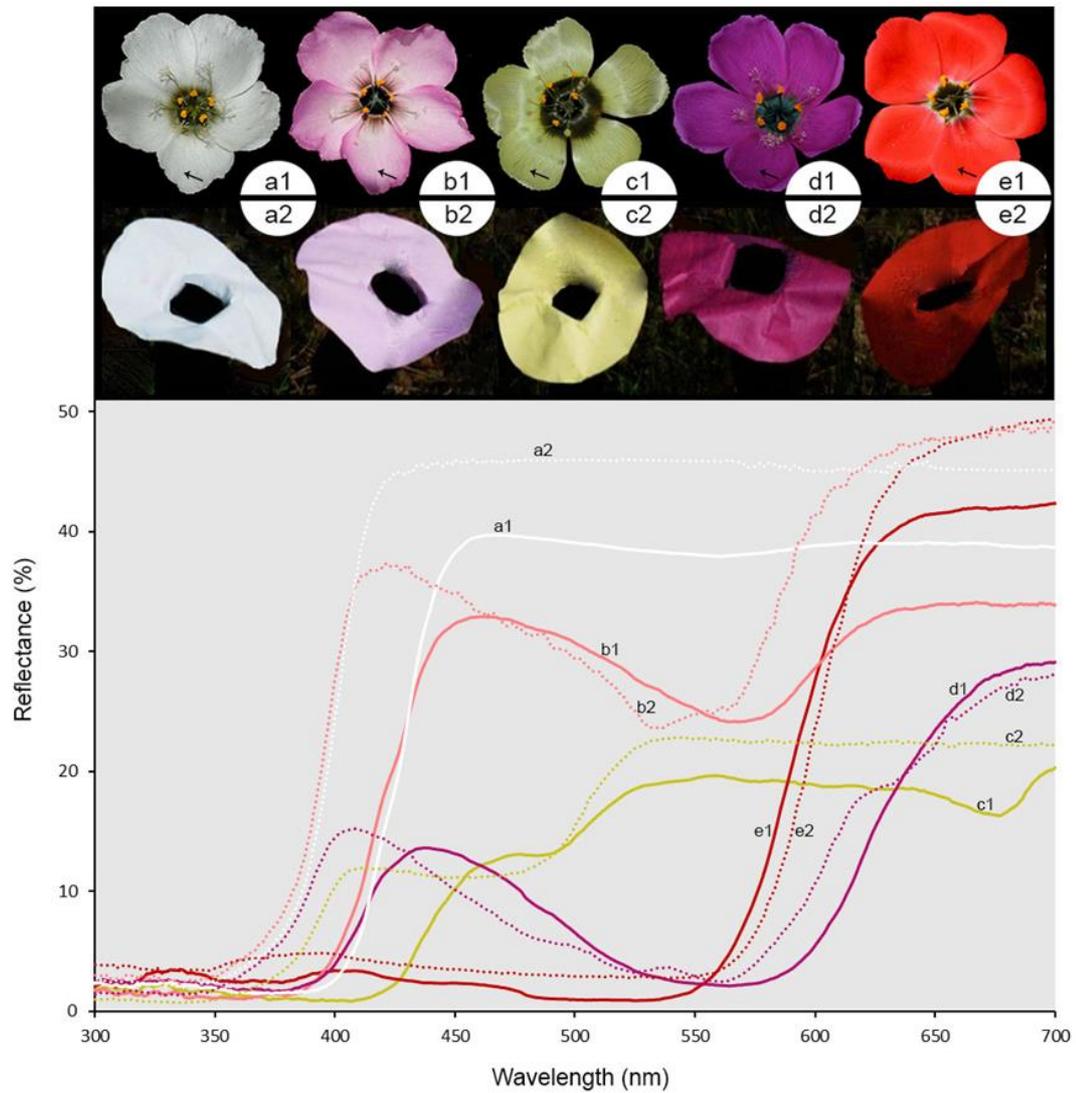


Figure 2. Average spectrophotometer readings in the UV-visible range (300–700 nm) obtained from 5–16 *Drosera cistiflora* s.l. petal readings representing 2–4 populations of white (a1), pink (b1), yellow (c1), purple (d1) and red (e1) floral colour forms (solid curve) and from 1–2 readings for colour paints used in white (a2), pink (b2), yellow (c2), purple (d2) and red (e2) model flowers representing *D. cistiflora* s.l. flower colours (broken curve). *D. cistiflora* s.l. readings were taken from the outer region of petals from populations at Darling 1 and 2 (pink-flowered form); Darling 3 and Durbanville (purple-flowered form); Darling 3, 4 and 5, and Darling-Yzerfontein (red-flowered form); Betty’s Bay and Darling 6 (white-flowered form), and Piketberg 1, 2 and 3 (yellow-flowered form).

A single array of model flowers consisted of a representative of each of the five *D. cistiflora* s.l. flower colours (Figures 1 & 2). The eight randomly placed arrays were checked for trapped insects every 20 minutes and moved to new locations within the population every hour, with each move treated as a replicate (Table 1). Insects were collected and frozen for identification purposes, or recorded and released when their identity had been previously determined. Only insects that were trapped in the black film canister were counted as visitors.

Reciprocal translocations

On different days to the model flower experiments, experiments using translocated real flowers were carried out, where arrays of potted live plants representing each flower colour were moved to new positions within the population every hour. Since the pollinators could not be trapped in unattended flowers, arrays required constant observation and recording. For this reason, only one array was observed per site at a time and each move thereof was treated as a replicate (Table 1). Visitors were determined to be pollinators if they came into contact with the reproductive parts of the flowers. Pollinators were collected both for identification purposes and to prevent genetic contamination of local flowers with introduced pollen.

Table 1. The number of arrays of model and live flowers (representing all five *Drosera cistiflora* s.l. flower colours) distributed in populations of each *D. cistiflora* s.l. floral colour form

Array type	Floral colour form of experimental <i>Drosera cistiflora</i> s.l. populations				
	Pink	Purple	Red	White	Yellow
Model	200	111	183	119	199
Live	8	8	8	16	8

Pollinator abundance

During colour choice experiments, the abundance and kind of visitor trapped in each model flower, and observed and collected in each live flower, were recorded. Trapped insects that were not also observed visiting *D. cistiflora* s.l. flowers were excluded

from the study. Insects were identified to family, and genus and species where possible. Insect collections are housed at Stellenbosch University.

Pollinator importance

Pollen loads may provide an indication of the importance of *D. cistiflora* s.l. pollinators associated with each colour trap. Pollinator importance was determined as the product of relative abundance on *D. cistiflora* s.l. flowers and average *D. cistiflora* s.l. pollen loads. Pollen grains on all insect visitors observed in 2009 and 2010 were counted under a dissecting microscope and classified as *D. cistiflora* s.l. pollen or ‘other’. A sample size of 1–12 (median = 5) insects was used, depending on their abundance in experimental populations of each *D. cistiflora* s.l. floral colour form. *D. cistiflora* s.l. pollen grains were identified by comparison with a reference set of microscope slide preparations of pollen grains made from *D. cistiflora* s.l. and all other plants in flower at the study sites. Relative pollinator importance (RPI) was calculated as the percentage contribution of each pollinator to the overall pollinator importance in each *D. cistiflora* s.l. colour form.

Statistical analyses

Data were analysed using generalised linear mixed models (GLMMs) implemented in R (R Core Team 2018). Models that accounted for whether a visit was received or not incorporated a binomial distribution, whilst models that accounted for the number of visits incorporated a conditional Poisson distribution.

The probability of visitation was modelled as being dependent on the flower colour in the experimental array, whether or not the array colour was the same as the local *D. cistiflora* s.l. flower colour, and site. The explanatory variables thus comprise categorical fixed effects for colour in array, a binary fixed effect indicating whether or not the array colour matched the local *D. cistiflora* s.l. flower colour, and a random effect for site. An interaction term between colour in array and matching colour status was included, under the assumption that: a) there may be a ‘positive matching effect’, i.e. traps are visited more often if they match the local flower colour at the site, but b)

this effect may be different for different colours. Note that, in contrast to the study of genetic factors conducted in Chapter 2, in the current analysis site characteristics such as pollinator availability are important and so physical site (rather than population) is the appropriate level for the random effect. As usual for binomial GLMMs, the linear dependency between the probability of visitation and explanatory variables was specified using a logit link function. The number of visitors received was modelled using a negative binomial distribution to account for overdispersion in the counts, with the mean modelled as dependent on colour in array, matching status, and site. The explanatory variables thus include a categorical fixed effect for colour in array, a binary fixed effect for matching colour status as before, and a random effect for site. The linear dependency was specified using a log link function. Following model fitting, the Tukey method was used to adjust for multiple comparisons in post hoc tests. Marginal means were obtained by back-transformation of values from the linear scale, resulting in asymmetrical standard errors. In cases in which GLMMs for number of visits did not run because of lack of variance (e.g. no visits for certain combinations of floral colour form and array colour), a single value of one visit was substituted (sensu Zuur et al. 2009), which also makes the test more conservative (Johnson et al. 2019). Interaction effects between matching colour status and colour were included wherever justified by model selection, but for all datasets involving reciprocally translocated arrays (with the exception of data on the number of visits by all pollinators) these resulted in non-convergence of the fitted models, and could thus not be included. As a result, models fitted to reciprocally translocated data include main effects only (with one exception). GLMMs were fitted in this manner for *all* pollinator visits and subsequently for visits by the most important group of pollinators, as determined by the relative importance values of the pollinators in each *D. cistiflora* s.l. floral colour form.

Results

Colour choice experiments

Pollinator abundance

Where pollinator colour preferences in model flower and reciprocally translocated arrays matched the flower colour of the local *Drosera cistiflora* s.l. population, this is termed the ‘positive matching effect’.

Overall, positive matching effects were found for visits by all insects recorded in model flowers, both when the probability of any visit ($\chi^2 = 57.08$, $p < 0.001$) and number of visits ($\chi^2 = 33.23$, $p < 0.001$) were considered, and these effects varied between *D. cistiflora* s.l. floral colour forms (any visit: $\chi^2 = 14.61$, $p = 0.006$; number of visits: 14.66, $p = 0.005$), with a significant interaction effect between matching effect and floral colour form (any visit: $\chi^2 = 72.57$, $p < 0.001$; number of visits: $\chi^2 = 65.52$, $p < 0.001$) [Figure 3, Supplementary Tables 12 & 14]. Specifically, for all insect visits, white [matched marginal mean = 0.52, 95% confidence interval (0.35, 0.68) vs non-matched marginal mean = 0.20, 95% confidence interval (0.12, 0.32)]; pink [0.35, (0.23, 0.49) vs 0.15, (0.09, 0.23)]; red [0.44, (0.31, 0.57) vs 0.31, (0.22, 0.42)], and yellow [0.18, (0.10, 0.31) vs 0.11, (0.06, 0.18)] model flowers had a significantly ($p \leq 0.003$) greater chance of being visited when models matched the local *D. cistiflora* s.l. floral colour phenotype (Figure 3a, Supplementary Tables 10 & 12). Purple models had significantly ($p < 0.001$) reduced odds of receiving a visit among purple *D. cistiflora* s.l. flowers than other model colours [matched marginal mean = 0.08, 95% confidence interval (0.03, 0.16) vs non-matched marginal mean = 0.39, 95% confidence interval (0.27, 0.52)] (Figure 3a, Supplementary Tables 10 & 12). Notably, purple-flowered populations always occurred in the presence of other *D. cistiflora* s.l. flower colours, namely pink or red. Although purple-flowered forms were locally dominant on a microspatial scale, pink or red was the dominant flower colour on the whole at the respective sites investigated, and these received more visits than purple flowers. Pink [matched marginal mean = 0.68, 95% confidence interval (0.40, 1.18)

vs non-matched marginal mean = 0.28, 95% confidence interval (0.17, 0.47)] and white [0.71, (0.37, 1.35) vs 0.31, (0.17, 0.57)] model flowers showed highly significant ($p < 0.001$) positive matching effects when numbers of visits of all insects were considered (Figure 3b, Supplementary Tables 10 & 12). Significant ($p < 0.03$) positive matching effects were also found for the number of insect visits to red [0.67, (0.40, 1.10) vs 0.50, (0.31, 0.79)] and yellow flowers [0.20, (0.10, 0.38) vs 0.12, (0.07, 0.22)]. Purple models had significantly ($p < 0.001$) fewer visits among purple *D. cistiflora* s.l. flowers than other model colours [0.07, (0.03, 0.17) vs 0.62, (0.38, 1.01)] (Figure 3b, Supplementary Tables 10 & 12).

Similarly, for considerations of all insect visits to reciprocally translocated flowers, positive matching effects were found overall, both for the probability of any visit ($\chi^2 = 38.40$, $p < 0.001$) and number of visits ($\chi^2 = 32.85$, $p < 0.001$), and these effects varied between *D. cistiflora* s.l. floral colour forms (any visit: $\chi^2 = 12.33$, $p = 0.015$; number of visits: 10.32, $p = 0.035$), with a significant interaction effect between matching effect and floral colour form for number of visits: $\chi^2 = 16.80$, $p = 0.002$] [Figure 4, Supplementary Tables 13 & 15]. Thus, where reciprocally translocated arrays matched the local *D. cistiflora* s.l. flower colour, there was a significantly ($p < 0.001$) greater chance of receiving a visit than in non-matching array colours (Figure 4a). As no interaction effect between array colour and matching effect could be fitted for this model, this can be interpreted as true for all flower colours, in a decreasing order of magnitude as follows: purple [matched marginal mean = 0.91, 95% confidence interval (0.73, 0.98) vs non-matched marginal mean = 0.22, 95% confidence interval (0.11, 0.41)]; pink [0.91, (0.75, 0.97) vs 0.23, (0.11, 0.42)]; yellow [0.72, (0.43, 0.90) vs 0.07, (0.02, 0.18)]; red [0.94, (0.80, 0.98) vs 0.30, (0.16, 0.49)], and white [0.95, (0.84, 0.99) vs 0.36, (0.19, 0.57)] (Supplementary Tables 11 & 13). The number of visits was significantly ($p < 0.02$) greater when the translocated array flower colour matched that of *D. cistiflora* s.l. flowers in all except purple floral colour forms, in a decreasing order of magnitude from yellow [matched marginal mean = 2.16, 95% confidence interval (0.67, 6.96) vs non-matched marginal mean = 0.06, 95% confidence interval (0.02, 0.22), $p < 0.001$]; to pink [2.09, (0.64, 6.80) vs 0.27, (0.11, 0.63), $p = 0.001$]; to white [1.83, (0.75, 4.45) vs 0.59, (0.26, 1.35), $p = 0.017$], to red [1.54, (0.55, 4.25) vs 0.53, (0.24, 1.15), $p = 0.018$] (Figure 4b, Supplementary Tables 11 & 13).

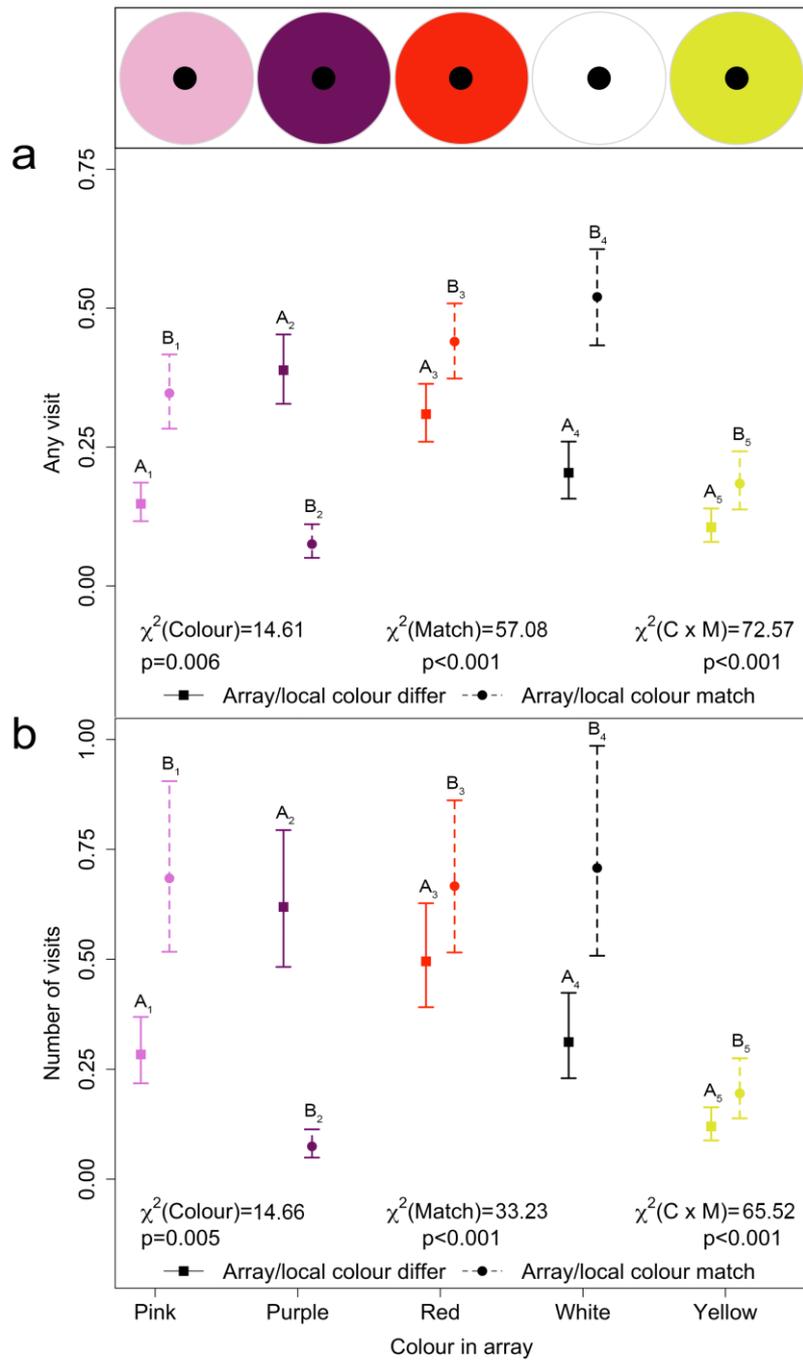


Figure 3. Comparison of matching effects for all insect visitors to model flower arrays in each *Drosera cistiflora* s.l. floral colour form. Values represent marginal model mean (and asymmetrical standard error) proportions for presence/absence of any visits (a) and number of visits (b). Post hoc tests (represented by A and B) are only used to compare means involving matched and non-matched model flowers of the same colour (matched or non-matched means of different colours are not compared). Subscripts 1–5 are used to differentiate the different model flower colours. Means that do not share the same letter-subscript combination are significantly different (Tukey test) [Supplementary Table 12].

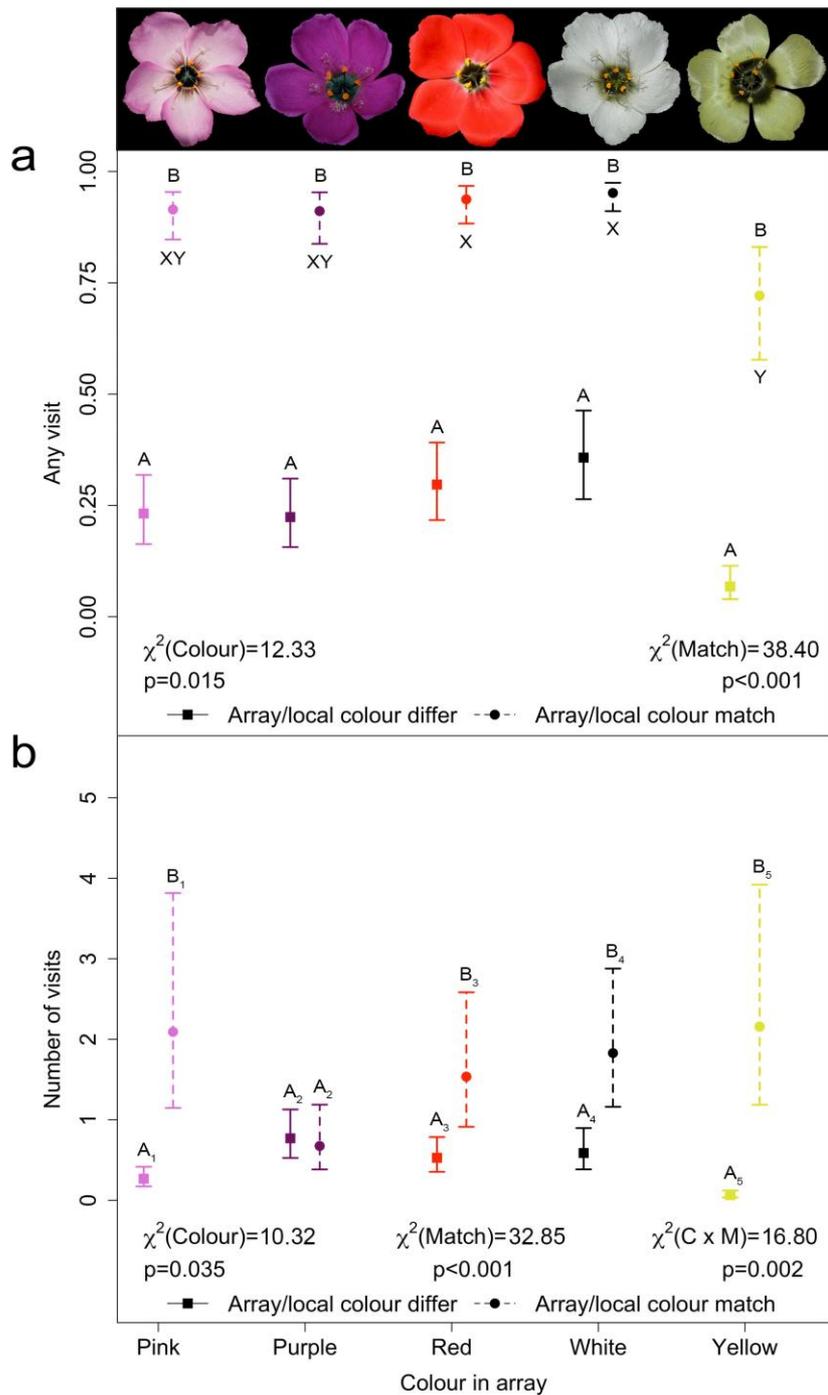


Figure 4. Comparison of matching effects for all insect visitors to reciprocally translocated arrays in each *Drosera cistiflora* s.l. floral colour form. Values represent marginal model mean (and asymmetrical standard error) proportions for presence/absence of any visits (a) and number of visits (b). Means that share the same letter are not significantly different (Tukey test). A and B represent post hoc tests for matching effects and X and Y represent post hoc tests for colour in array (where interactions between matching and colour effects were not significant) [Supplementary Tables 13 & 15].

Pollinator importance

Relative pollinator importance (RPI) values of *D. cistiflora* s.l. pollinators observed during colour choice experiments in 2009 and 2010 indicate that hopliine beetles (Coleoptera: Scarabaeidae: Hopliini) are primary pollinators of all *D. cistiflora* s.l. floral colour forms (Table 2, Supplementary Tables 8 & 9). GLMMs were thus fitted for hopliine beetle visits to model and translocated flowers in the same manner as for all pollinator visits. Total RPI for hopliine assemblages exceeded 90% in purple-, red-, white- and yellow-flowered forms (Tables 2 & 3), with melyrid beetles (Melyridae) also emerging as important pollinators of yellow-flowered forms (RPI: 7.4%). Primary pollinators of pink-flowered forms however comprised hopliine beetles (40.2%) as well as beetles of the families Meloidae (44.1%) and Tenebrionidae (11.6%). Meloid and tenebrionid beetles did not appear very active at the time of trapping and did not visit pink flowers of live experimental arrays in populations of pink-flowered forms. The RPI of hopliine visitors was consequently overestimated for this form in reciprocal translocation experiments (Table 3). Conversely, the RPI of hopliine beetles was underestimated in purple and yellow model experiments, owing largely to a lack of visits by *Chasme* sp. to model flowers representing these flower colours. All *D. cistiflora* s.l. pollinators also bore pollen from other plant species in the local environment.

Table 2. Relative pollinator importance (%), i.e. percentage contribution per *Drosera cistiflora* s.l. floral colour form, of insect pollinators observed visiting *D. cistiflora* s.l. in 2009 and 2010 during model and live flower experiments. Pollinator importance was calculated as the product of abundance in *D. cistiflora* s.l. flowers and average *D. cistiflora* s.l. pollen loads. The pollinator group with the highest RPI value for each floral colour form is indicated in bold type. * RPI of pollinators trapped in model flowers, and † RPI of pollinators trapped in reciprocal translocations. Insects are identified to family, and genus and species where possible. Superscript H denotes hopliine beetles (Coleoptera: Scarabaeidae: Hopliini).

Flower visitor species, genus and/or family	<i>Drosera cistiflora</i> s.l. floral colour form				
	Pink	Purple	Red	White	Yellow
<i>Anisochelus inornatus</i> (Scarabaeidae) ^H					5.6331 ^{*†}
<i>Anisonyx</i> sp. (Scarabaeidae) ^H	8.0123 [†]			3.1954 [†]	
<i>Anisonyx</i> cf. <i>ursus</i> (Scarabaeidae) ^H	2.0057	0.2826		0.1454 [*]	
Apidae	0.3338 [†]			0.1331 [†]	
Ceratopogonidae	0.0292 ^{*†}	0.0129 ^{*†}	0.0017 ^{*†}	0.0291 ^{*†}	0.0183 ^{*†}
<i>Chasme decora</i> (Scarabaeidae) ^H			10.4962 ^{*†}		
<i>Chasme</i> sp. (species 1) [Scarabaeidae] ^H		81.6006[†]		20.5654 [†]	65.9323[†]
<i>Chasme</i> sp. (species 2) [Scarabaeidae] ^H			0.0693 [*]		
Chrysomellidae	0.2734 [*]		0.0070 [*]	0.0140	
Dermestidae (species 1)		0.0129 [†]	0.0017 [†]		
Dermestidae (species 2)				0.0019 [†]	
Empididae (species 2)	0.0348 [*]				
<i>Eristalis crassipes</i> (Syrphidae)	0.0084 [†]				
<i>Heterochelus detritus</i> (Scarabaeidae) ^H					0.0035 [†]
<i>Heterochelus</i> sp. (species 1) [Scarabaeidae] ^H					0.4182
<i>Lepisia rupicola</i> spec. (Scarabaeidae) ^H	25.7909 ^{*†}	2.1042 ^{*†}	87.4243^{*†}	63.8807^{*†}	
<i>Lepithrix</i> sp. (Scarabaeidae) ^H					15.9442 [*]
Lyganidae				0.0888 [*]	
Megachilidae	0.4382 [*]			0.2330	
Meloidae	44.0871[*]			3.3175	
Melyridae (species 1)	0.0434 [*]				
Melyridae (species 2)	0.8904 [*]	1.8403 [†]	0.2367 [†]	0.5918	7.4346 ^{*†}
Muscidae	0.2040 [*]	0.4743 [†]	0.0610 [†]	0.1220 [†]	

<i>Omocrates sp.</i> (species 1)		8.7546 ^{**†}	1.1261 [†]	5.7918 [†]	
[Scarabaeidae] ^H					
<i>Pachycnema murina</i>	3.7558 [†]				
(Scarabaeidae) ^H					
<i>Peritrichia sp.</i> (species 1)					2.1256 [*]
[Scarabaeidae] ^H					
<i>Peritrichia sp.</i> (species 2)					0.3376 [*]
[Scarabaeidae] ^H					
<i>Platychelus lupinus</i>	0.6120 [†]	2.2137 [†]	0.2848 ^{**†}		
(Scarabaeidae) ^H					
Scarabaeidae	1.1685 [*]				0.0976
Tabanidae (species 1)			0.2718 [*]		
Tabanidae (species 2)					1.1499 [*]
Tenebrionidae (species 1)	11.6530 [*]	2.6606 [*]		1.8013 ^{**†}	0.9052 [*]
Tenebrionidae (species 2)			0.0194		
Tenebrionidae (species 3)	0.5759 [*]			0.0166 [*]	
Thomisidae	0.0835 [*]	0.0431			
Tingidae				0.0720 [†]	

Table 3. Relative pollinator importance (%), i.e. percentage contribution per *Drosera cistiflora* s.l. floral colour form, of hopliine beetles (Coleoptera: Scarabaeidae: Hopliini) observed visiting *D. cistiflora* s.l., and of those trapped in model flowers and reciprocal translocations representing each floral colour form, in 2009 and 2010. Pollinator importance was calculated as the product of abundance in *D. cistiflora* s.l. flowers and average *D. cistiflora* s.l. pollen loads.

Hopliine beetle visitors	<i>Drosera cistiflora</i> s.l. floral colour form				
	Pink	Purple	Red	White	Yellow
Observed	40.18	94.96	99.40	93.58	90.39
Trapped (model flowers)	30.24	80.24	99.72	97.06	71.66
Trapped (reciprocal translocations)	99.04	97.59	99.70	97.74	90.57

Overall, when hopliine beetle visits were considered, positive matching effects were found for model flower arrays, both for the probability of any visit ($\chi^2 = 102.65$, $p < 0.001$) and number of visits ($\chi^2 = 65.28$, $p < 0.001$). These effects varied between *D. cistiflora* s.l. floral colour forms (any visit: $\chi^2 = 5.76$, $p = 0.218$; number of visits: 8.03, $p = 0.091$), with a significant interaction between matching effect and floral colour form (any visit: $\chi^2 = 92.07$, $p < 0.001$; number of visits: $\chi^2 = 92.81$, $p < 0.001$) [Figure

5, Supplementary Tables 12 & 14]. Red [matched marginal mean = 0.35, 95% confidence interval (0.17, 0.58) vs non-matched marginal mean = 0.04, 95% confidence interval (0.02, 0.10)]; white [0.37, (0.16, 0.65) vs 0.14, (0.05, 0.33)], and yellow [0.12, (0.04, 0.30) vs 0.05, (0.02, 0.15)] model flowers had a significantly ($p \leq 0.001$) greater chance of being visited by hopliine beetles when models matched the local *D. cistiflora* s.l. floral colour phenotype (Figure 5a, Supplementary Tables 10 & 12). Likewise, the number of visits was significantly ($p \leq 0.005$) greater in matching red [matched marginal mean = 0.42, 95% confidence interval (0.17, 1.07) vs non-matched marginal mean = 0.06, 95% confidence interval (0.02, 0.15), $p < 0.001$]; white [0.44, (0.14, 1.40) vs 0.23, (0.07, 0.68), $p = 0.003$], and yellow [0.12, (0.04, 0.37) vs 0.05, (0.02, 0.17), $p = 0.005$] models (Figure 5b, Supplementary Tables 10 & 12).

In translocated flowers, however, where no interaction between flower colour in array and matching effect could be estimated, a significant ($p < 0.001$) positive matching effect is shown in all flower colours for hopliine visits overall (any visit: $\chi^2 = 26.06$; number of visits: 15.44). These effects differed between flower colours (any visit: $\chi^2 = 12.13$, $p = 0.016$; number of visits: 21.84, $p < 0.001$), both for the chances of array flower colours that matched the local form receiving a visit as well as the number of visits they received (Figure 6, Supplementary Tables 13 & 15). Numbers of visits, reported here in a decreasing order of magnitude, were as follows: purple [matched marginal mean = 3.12, 95% confidence interval (1.12, 8.66) vs non-matched marginal mean = 0.69, 95% confidence interval (0.29, 1.63)]; red [1.91, (0.70, 5.19) vs 0.42, (0.17, 1.03)]; white [1.20, (0.45, 3.17) vs 0.26, (0.11, 0.65)]; yellow [0.48, (0.16, 1.45) vs 0.11, (0.04, 0.30)], and pink [0.38, (0.13, 1.13) vs 0.08, (0.03, 0.25)] (Figure 6b, Supplementary Tables 11 & 13).

The positive matching effects reported for hopliine beetle visits to model flower and translocation experiments are thus akin to those for entire community compositions of pollinators. However, although strong matching effects were found in pink-flowered *D. cistiflora* s.l. when numbers of visits of all insects were considered, these were found to be weak or absent when hopliine beetles were considered in isolation.

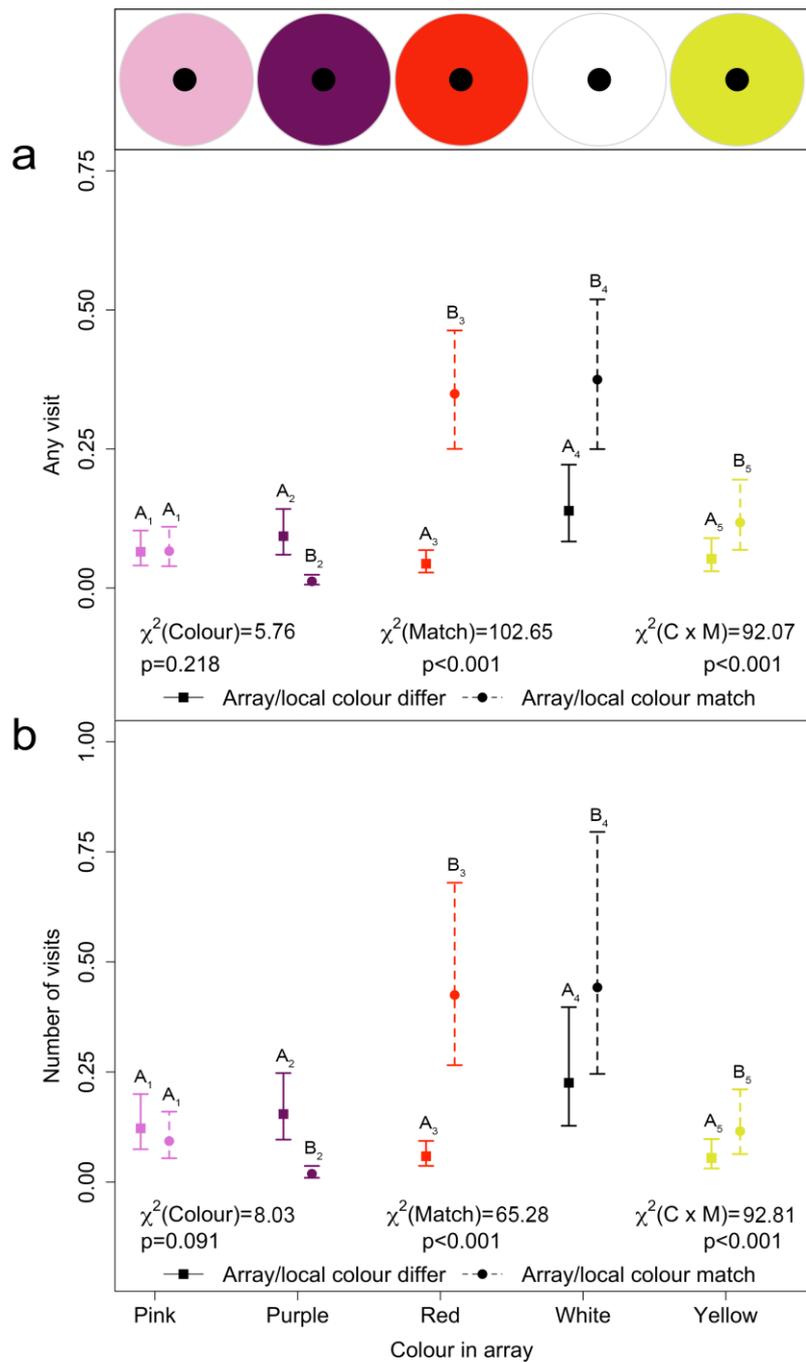


Figure 5. Comparison of matching effects for all hopliine beetle visits to model flower arrays in each *Drosera cistiflora* s.l. floral colour form. Values represent marginal model mean (and asymmetrical standard error) proportions for presence/absence of any visits (a) and number of visits (b). Post hoc tests (represented by A and B) are only used to compare means involving matched and non-matched model flowers of the same colour (matched or non-matched means of different colours are not compared). Subscripts 1–5 are used to differentiate the different model flower colours. Means that share the same letter-subscript combination are not significantly different (Tukey test) [Supplementary Table 12].

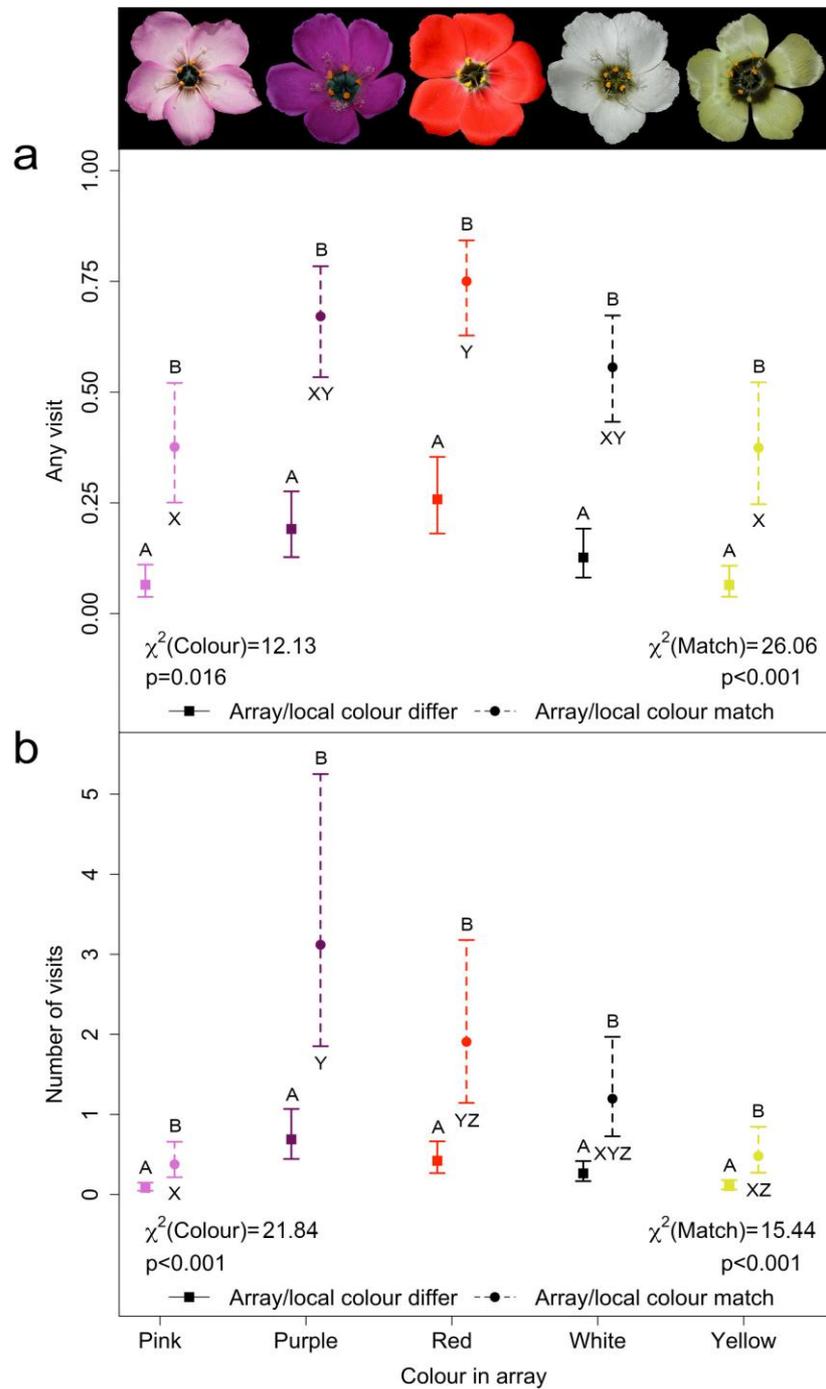


Figure 6. Comparison of matching effects for all hopline beetle visits to reciprocally translocated arrays in each *Drosera cistiflora* s.l. floral colour form. Values represent marginal model mean (and asymmetrical standard error) proportions for presence/absence of any visits (a) and number of visits (b). Means that share one or more of the same letters are not significantly different (Tukey test). A and B represent post hoc tests for matching effects and X, Y and Z represent post hoc tests for colour in array (interactions between matching and colour effects were not significant) [Supplementary Tables 13 & 15].

Discussion

Whereas flower colour selection has scarcely been tested using both model and reciprocally translocated flowers, the results of this study offer reliable evidence for non-random, pollinator-mediated selection for the flower colour of the local population.

The effect of pollinator abundance on flower colour selection

Local *Drosera cistiflora* s.l. flower colours generally had a greater chance of being visited than novel colours when entire community compositions of pollinators were considered. Not only did a match between the model/real flower and the local flower colour improve the chances of receiving a visit, but it also increased the number of visits to all model and reciprocally translocated flower colours except purple. Findings thus accord with the Grant-Stebbins model (q.v. Grant 1949; Grant & Grant 1965; Stebbins 1970; Johnson 2006, 2010; Kay & Sargent 2009; van der Niet et al. 2014; Smith & Kriebel 2018) by demonstrating that divergent floral colour preferences of diverse pollinator assemblages in different populations may have resulted in the evolution of a selection mosaic amid the distribution range of *D. cistiflora* s.l. floral colour forms.

This inferred selection mosaic appears to be congruent with the distribution of *D. cistiflora* s.l. floral colour forms across the landscape. For example, where pollinators preferred white, the *D. cistiflora* s.l. flowers at the site were most likely to conform in colour, and white-flowered *D. cistiflora* s.l. populations are locally adapted to the pollination environment across their area of extent. However, since pink and white floral phenotypes lacked clearly defined pollinator assemblages as well as discrete reflectance spectra, an exception was found in sites where these flower colours occurred together in the Darling region. Here, both pink and white models were selected equally and introgression occurred between floral phenotypes. In contrast, isolated populations of pink-flowered forms at Rawsonville and Riverlands, which

were fixed for the colour pink, showed strong selection by pollinators for pink models over white (Supplementary Figure 4). Similarly, pollinators preferred white models amongst white-flowered forms which were fixed for the colour white. The selective forces of generalist pollinator communities may thus not have been adequate to overcome drift and fix for either end of the pink–white spectrum without geographical isolation of these forms.

The respective absent and weakly positive matching effects of purple and yellow model flowers may be attributed to floral colour dominance of differing sympatric forms in purple-flowered *D. cistiflora* s.l. sites, and/or poor colour matching of model flowers. Floral traits other than petal colour may also be associated with pollinator visits, and these were not considered by model flowers. Petal micromorphology, for instance, may alter visual signalling (Costa et al. 2017). Here, epidermal structural modifications apparent in and around the central region of *D. cistiflora* s.l. flowers may create iridescence (Whitney et al. 2009, van der Kooi et al. 2014), intensify pigment colours (Noda et al. 1994, Glover & Martin 1998, Arnold 2010) to form patterns, or conceivably mimic a nectar reward, thereby potentially altering visitation by some or all insects. Structural colours may also be reorientated by corolla shape, which can at times evolve in response to selection by generalist pollinators (e.g. Gómez et al. 2015). Remarkably, whilst corolla shape was consistent between pink, red and white floral colour forms, viz. shallow bowl-shaped (crateriform) flowers, purple flowers at Durbanville and yellow-flowered forms had unique corolla shapes. The petals of yellow flowers bent perpendicularly approximately halfway along their length, thereby forming cup-shaped flowers with a ring of structural reflectance circumscribing the centre (pers. obs). Purple petals at Durbanville reflexed as anthesis progressed. Although the shape of purple flowers at Darling 3 was similar to pink, red and white floral colour forms, models received very few visits and negative matching effects were found. Other cues (such as metallic-iridescent corolla centres) may thus also play a pivotal role in generating divergent selection by pollinators at this site where two different floral colour forms occur in sympatry (q.v. Liu & Huang 2013). The presence of strongly positive matching effects for any visits in purple and yellow translocated flowers despite their lack in model flowers substantiates the idea that corolla colour may be secondary to shape and/or other floral traits in determining overall pollinator visitation in these populations.

The effect of pollinator importance on flower colour selection

Suites of hopliine beetles, the most important *D. cistiflora* s.l. pollinators, preferred local *D. cistiflora* s.l. flower colours over introduced colours in reciprocally translocated flower arrays placed amongst *all D. cistiflora* s.l. floral colour forms (Figure 6), and chose red, white and yellow model flowers where these matched the local *D. cistiflora* s.l. flower colour (Figure 5). These findings support a pattern-based theory, where floral colour shifts may be adaptations to entire community compositions of pollinators primarily comprising hopliine beetles.

Generally, my outcomes support those of Picker & Midgley (1996), who found matching of model and local flower colour visitation among a variety of hopliine beetle species at three sites (two in the Biedouw Valley and one in Darling). Further evidence of strong hopliine colour discrimination and preference presented by Johnson & Midgley (2001) favours the notion that differential dovetailing of floral colour signals and accompanying pollinator responses may underlie the apparent selection mosaic in *D. cistiflora* s.l. The study by Johnson & Midgley (2001) additionally demonstrated flower colour, rather than dark flower centres or the presence of female or male resting beetles, to be the primary visual signal influencing flower alighting—the latter of these two demonstrations being despite the beetles' frequent use of the flowers as mating sites (Figure 4f&g, Chapter 3; pers. obs). In contrast, van Kleunen et al. (2007) found a significant correlation between dark flower centres and hopliine flower visitation on the Bokkeveld Plateau but no significance for local flower colour preferences by the beetles in any of the three Iridaceae species examined (namely *Hesperantha vaginata*, *Romulea monadelphica* or *Sparaxis elegans*) using colour-controlled models. This inconsistency was ascribed to differences in hopliine visitor species in the areas assessed by Picker & Midgley (1996) and van Kleunen et al. (2007). Likewise, the hopliine species I observed differed largely to those in their studies. The three irid species studied by van Kleunen et al. (2007) however each possess distinct floral beetle marks which appeared to be the primary visual cue to many of their hopliine beetle visitors, thereby potentially obscuring findings relating to colour in isolation.

Pollinators as potential drivers of floral colour divergence in Drosera cistiflora s.l.

The radiation of angiosperms is a recent phenomenon relative to their primary evolution about 140 million years ago (Sun et al. 2002, Willis & McElwain 2002), with conventional wisdom proposing that the evolution of floral display (including petal colour) occurred about 90–100 million years ago (Busch & Zachgo 2009, Crane et. al. 2009). Since insect vision predates flower colour, the evolution of flower colour is certainly pollinator-driven (Arnold 2010). Of the various insect pollen vectors, beetles have been postulated to be one of the earliest drivers of floral evolution (Faegri & van der Pijl 1979). Beetles are also indicated as important pollinators in Mediterranean climatic regions (Bernhardt 2000), such as the Greater Cape Floristic Region where this study was conducted.

This is one of few field-based studies to show that floral colour variation may be chiefly attributed to the local preferences of beetle pollinators, in this case predominantly hopliine beetles of the family Scarabaeidae, and also taxa belonging to the families Meloidae, Tenebrionidae and Melyridae. *D. cistiflora* s.l. floral colour forms were nevertheless additionally pollinated by various other insect families which were also polylectic. The relatively specialised hopliine pollination system (q.v. Goldblatt et al. 1998, Goldblatt & Manning 2011) is thus nested within an overarching generalised floral phenotype/pollinator design. The study is accordingly the first to show that generalist pollinator communities can generate divergent selective pressures on flower colour, and provisionally defines *D. cistiflora* s.l. floral colour forms as geographically divergent generalised pollination ecotypes. In its entirety the pollination system appears to be an example of ‘diffuse coevolution’ (sensu Futuyma & Slatkin 1983, q.v. Jordano 1987), where plant populations may be responding to a ‘pollinator bloc’, i.e. a guild of generalist pollinators that, as a modular unit with a common specialised preference, can generate selective forces on the plants they pollinate.

Although floral attractants and rewards may typically be required to ensure successful pollen export in entomophilous plants (Faegri & van der Pijl 1979), where floral colour signals can heavily influence pollinator visitation (Peter & Johnson 2008), *D. cistiflora*

s.l. flowers are nectarless and odourless and pollen is easily visible. It is thus possible that pollinator colour preferences may be an outcome of local conditioning to the flowers and their readily accessible pollen rewards. This is apparent for the principal hopliine pollinator *Lepisia rupicola* spec., where colour preferences of the beetle varied in accordance with the local *D. cistiflora* s.l. floral colour form in red- and white-flowered populations (Supplementary Figure 6). Whilst those of *Lepisia rupicola* spec. remain to be isolated, at least three photoreceptor types have been identified in certain species of hopliine beetles (Arnold 2010) and spectral colour discrimination could thus also be inborn. The variation in colour preferences of the beetle might then portray geographical differences in photoreception of taxa within the complex, and hence in innately preferred colours. If pollinators are conditioned by the local flower colour at a site, the establishment of novel flower colours through evolution in sympatry, or via immigration, may be opposed, thereby maintaining the local flower colour and potentially restraining floral colour divergence. However, flower colour choice most likely reflects an interaction between both learned and innate preferences (e.g. Weiss 1997) and future investigation into plant community composition as well as the visual systems, mate-searching and foraging strategies of naïve and experienced *D. cistiflora* s.l. pollinators may determine the relative contributions of these modes of behaviour to flower colour selection.

Even if current findings favour pollinator-mediated selection as an explanation for the maintenance of floral colour variation in *D. cistiflora* s.l., pollinators may not be the sole driver behind this phenomenon and some alternatives deserve attention. Pleiotropy or selection by non-pollinator biotic factors may act either for or against selection imposed by pollinators. For example, Irwin & Strauss (2005) found that although the yellow morphotype of *Raphanus sativus* (Brassicaceae) was selected for by pollinators over other colour morphotypes, it was also the most vulnerable to herbivory. Their results suggest that herbivores, or ‘plant antagonists’, exert selection in an opposite direction to that of pollinators (Strauss & Whittall 2006). Whilst antagonistic selection by herbivores reduced the fitness of yellow morphotypes and limited their spread, it did not prevent the spread of yellow alleles altogether. Only one florivore, *Hycleus lunatus* (Coleoptera: Meloidae: Meloinae: Mylabrini) [Supplementary Figure 3], was observed in one *D. cistiflora* s.l. population, hence the effect of florivory was considered negligible in its entirety. Although not appreciable

in my observations, seed predation, by acting through the female component, could influence the relative fitness of floral colour forms. Additionally, if pollinator distributions are characterised through edaphic factors, pollinator-mediated selection for flower colour may in fact be indirectly associated with soils.

Conclusions

The discussed caveats do not detract from the important role of pollinators, whether as specialised individual species (q.v. Newman et al. 2012) or the generalist communities shown here, in local adaptation of flower colour. It remains to be assessed through phylogenetic analyses of each floral colour form whether generalised pollination ecotypes in *D. cistiflora* s.l. embody genetically distinct taxonomic units. If so, and should changes in pollination niche precede changes in corolla colour, it would appear that pollinators have not only maintained but also driven floral colour divergence.

References

- Anderson, B. (2010) Did *Drosera* evolve long scapes to stop their pollinators from being eaten? *Annals of Botany* **106** (4), 653–657.
- Arnold, S. E. J. (2010) *Flowers through insect eyes: The contribution of pollinator vision to the evolution of flower colour*. PhD Thesis. Queen Mary, University of London, UK.
- Bernhardt, P. (2000) Convergent evolution and adaptive radiation of beetle-pollinated angiosperms. *Plant Systematics and Evolution* **222**, 293–320.
- Briscoe, A. D. & Chittka, L. (2001) The evolution of colour vision in insects. *Annual Review of Entomology* **46**, 471–510.
- Busch, A. & Zachgo, S. (2009) Flower symmetry evolution: Towards understanding the abominable mystery of angiosperm radiation. *BioEssays* **31**, 1181–90.
- Campbell, D. R., Bischoff, M., Lord, J. M. & Robertson, A. W. (2010) Flower colour influences insect visitation in alpine New Zealand. *Ecology* **91** (9), 2638–2649.
- Campbell, D. R., Bischoff, M., Lord, J. M. & Robertson, A. W. (2012) Where have all the blue flowers gone: Pollinator responses and selection on flower colour in New Zealand *Wahlenbergia albomarginata*. *Journal of Evolutionary Biology* **25** (2), 352–364.
- Chittka, L. (1992) The colour hexagon: A chromaticity diagram based on photoreceptor excitations as a generalised representation of colour opponency. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural and Behavioural Physiology* **170**, 533–543.

- Chittka, L. (1997) Bee colour vision is optimal for coding flower colour, but flower colours are not optimal for being coded – why? *Israel Journal of Plant Sciences* **45**, 115–127.
- Chittka, L., Beier, W., Hertel, H., Steinmann, E. & Menzel, R. (1992) Opponent colour coding is a universal strategy to evaluate the photoreceptor inputs in Hymenoptera. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural and Behavioural Physiology* **170**, 545–563.
- Chittka, L., Dyer, A. G., Bock, F. & Dornhaus, A. (2003) Bees trade off foraging speed for accuracy. *Nature* **424**, 388–388.
- Chittka, L., Thomson, J. D. & Waser, N. M. (1999). Flower constancy, insect psychology and plant evolution. *Naturwissenschaften* **86**, 361–377.
- Costa, V. B. S., Pimentel, R. M. M., Chagas, M. G. S., Alves, G. D. & Castro, C. C. (2017) Petal micromorphology and its relationship to pollination. *Plant Biology* **19**, 115–122.
- Crane, P., Friis, E. M. & Pedersen, K. J. (2009) The origin and early diversification of the angiosperms. *Nature* **374**, 27–33.
- Dafni, A., Bernhardt, P., Shmida, A., Ivri, Y. Greenbaum, S., O’Toole, Ch. & Losito, L. (1990) Red bowl-shaped flowers: Convergence for beetle pollination in the Mediterranean region, *Israel Journal of Botany* **39** (1–2), 81–92.
- Dyer, A. G. & Chittka, L. (2004) Fine colour discrimination requires differential conditioning in bumblebees. *Naturwissenschaften* **91**, 224–227.
- Dyer, A. G., Whitney, H. M., Arnold, S. E. J. & Glover, B. J. (2007) Mutations perturbing petal cell shape and anthocyanin synthesis influence bumblebee perception of *Antirrhinum majus* flower colour. *Arthropod-Plant Interactions* **1**, 45–55.

- Epperson, B. K. & Clegg, M. T. (1987) Frequency-dependent variation for outcrossing rate among flower-colour morphs of *Ipomoea purpurea*. *Evolution* **41**, 1302–1311.
- Faegri, K. & van der Pijl, L. (1979) *The principles of pollination ecology*. Pergamon Press, Oxford, UK.
- Futuyma, D. J. & Slatkin, M. [Eds] (1983) *Coevolution*. Sinauer Associates, Sunderland, Massachusetts, USA.
- Gigord, L. D. B., Macnair, M. R. & Smithson, A. (2001) Negative frequency-dependent selection maintains a dramatic flower colour polymorphism in the rewardless orchid *Dactylorhiza sambucina* (L.) Soð. *Proceedings of the National Academy of Sciences of the United States of America* **98** (11), 6253–6255.
- Giurfa, M., Núñez, J., Chittka, L. & Menzel, R. (1995) Colour preferences of flower-naïve honeybees. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural and Behavioural Physiology* **177**, 247–259.
- Glover, B. J. & Martin, C. (1998) The role of petal cell shape and pigmentation in pollination success in *Antirrhinum majus*. *Heredity* **80**, 778–784.
- Goldblatt, P., Bernhardt, P. & Manning, J. C. (1998) Pollination of petaloid geophytes by monkey beetles (Scarabaeidae: Rutelinae: Hopliini) in Southern Africa. *Annals of the Missouri Botanical Garden* **85**, 215–230.
- Goldblatt, P. & Manning, J. C. (2011) Hopliine beetles (Scarabaeidae: Rutelinae: Hopliini), specialised pollinators of the southern African flora. *Curtis's Botanical Magazine* **28** (4), 238–259.
- Gómez, J. M., Bosch, J., Perfectti, F., Fernández, J. D., Abdelaziz, M. & Camacho, J. P. M. (2008) Spatial variation in selection on corolla shape in a generalist plant is promoted by the preference patterns of its local pollinators. *Proceedings of the Royal Society B — Biological Sciences* **275**, 2241–2249.

- Gómez, J. M., Muñoz-Pajares, A. J., Abdelaziz, M., Lorite, J. & Perfectti, F. (2014) Evolution of pollination niches and floral divergence in the generalist plant *Erysimum mediohispanicum*. *Annals of Botany* **113** (2), 237–249.
- Gómez, J. M., Perfectti, F. & Lorite, J. (2015) The role of pollinators in floral diversification in a clade of generalist flowers. *Evolution* **69** (4), 863–878.
- Grant, V. (1949) Pollination systems as isolating mechanisms in angiosperms. *Evolution* **3**, 82–97.
- Grant, V. & Grant, K. A. (1965) *Flower pollination in the Phlox family*. Columbia University Press, New York, USA.
- Gumbert, A. (2000) Colour choices by bumblebees (*Bombus terrestris*): Innate preferences and generalisation after learning. *Behavioural Ecology and Sociobiology* **48**, 36–43.
- Hopkins, R. & Rausher, M. D. (2012) Pollinator-mediated selection on flower colour allele drives reinforcement. *Science* **335**, 1090–1092.
- Ilse, D. & Vaidya, V. G. (1956) Spontaneous feeding response to colours. Bibliography 203 in *Papilio demoleus* L. *Proceedings of the Indian Academy of Sciences, Section B* **43**, 23–31.
- Irwin, R. E. & Strauss, S. Y. (2005) Flower colour microevolution in wild radish: Evolutionary response to pollinator-mediated selection. *The American Naturalist* **165**, 225–237.
- Johnson, S. D. (2006) Pollinator-driven speciation in plants. In L. D. Harder & S. C. H. Barrett [Eds], *The ecology and evolution of flowers*, 296–306. Oxford University Press, Oxford, UK.

- Johnson, S. D. (2010) The pollination niche and its role in the diversification and maintenance of the southern African flora. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365** (1539), 499–516.
- Johnson, S. D., Butler, H. C. & Robertson, A. W. (2019) Breeding systems in *Cyrtanthus* (Amaryllidaceae): Variation in self-sterility and potential for ovule discounting. *Plant Biology*. ISSN 1435-8603.
- Johnson, S. D. & Midgley, J. J. (2001) Pollination by monkey beetles (Coleoptera: Scarabaeidae: Hopliini): Do colour and dark centers of flowers influence alighting behaviour? *Environmental Entomology* **30**, 861–868.
- Jones, K. N. & Reithel, J. S. (2001) Pollinator-mediated selection on a flower colour polymorphism in experimental populations of *Antirrhinum* (Scrophulariaceae). *American Journal of Botany* **88** (3), 447–454.
- Jordano, P. (1987) Patterns of mutualistic interactions in pollination and seed dispersal: Connectance, dependence asymmetries, and coevolution. *The American Naturalist* **129** (5), 657–677.
- Kay, Q. O. N. (1978) The pollination of flowers by insects. In A. J. Richards [Ed.], *Linnean Society Symposium Series* **6**, 175–190. Academic, London, UK.
- Kay, K. M. & Sargent, R. D. (2009) The role of animal pollination in plant speciation: Integrating ecology, geography, and genetics. *Annual Review of Ecological Systems* **40**, 637–656.
- Kevan, P. G. & Baker, H. G. (1983) Insects as flower visitors and pollinators. *Annual Review of Entomology* **28**, 407–453.
- Liu, C. Q. & Huang, S. Q. (2013) Floral divergence, pollinator partitioning and the spatiotemporal pattern of plant-pollinator interactions in three sympatric *Adenophora* species. *Oecologia* **173**, 1411–1423.

- Lunau, K. (2014) Visual ecology of flies with particular reference to colour vision and colour preferences. *Journal of Comparative Physiology A: Neuroethology, Sensory Neural, and Behavioural Physiology* **200**, 497–512.
- Lunau, K. & Maier, E. J. (1995) Innate colour preferences of flower visitors. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural and Behavioural Physiology* **177**, 1–19.
- McCall, C. & Primack, R. (1992) Influence of flower characteristics, weather, time of day and season on insect visitation rates in three plant communities. *American Journal of Botany* **79**, 434–442.
- McGimpsey, V. J. & Lord, J. M. (2015) In a world of white, flower colour matters: A white–purple transition signals lack of reward in an alpine *Euphrasia*. *Austral Ecology* **40**, 701–708.
- Menzel, R. & Erber, J. (1978) Learning and memory in bees. *Scientific American* **239**, 102–109.
- Morante, J. & Desplan, C. (2008) The colour-vision circuit in the medulla of *Drosophila*. *Current Biology* **18**, 553–565.
- Newman, E., Anderson, B. & Johnson, S. D. (2012) Flower colour adaptation in a mimetic orchid. *Proceedings of the Royal Society B — Biological Sciences* **279**, 2309–2313.
- Noda, K., Glover, B. J., Linstead, P. & Martin, C. (1994) Flower colour intensity depends on specialised cell shape controlled by a Myb-related transcription factor. *Nature* **369**, 661–664.
- Peter, C. I. & Johnson, S. D. (2008) Mimics and magnets: The importance of colour and ecological facilitation in floral deception. *Ecology* **89** (6), 1583–1595.

- Picker, M. D. & Midgley, J. J. (1996) Pollination by monkey beetles (Coleoptera: Scarabaeidae: Hopliini): Flower and colour preferences. *African Entomology* **4** (1), 7–14.
- R Core Team (2018) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, <http://www.R-project.org/>.
- Rausher, M. D. (2008) Evolutionary transitions in floral colour. *International Journal of Plant Sciences* **169**, 7–21.
- Real, L. A. (1981) Uncertainty and plant-pollinator interactions: The foraging behaviour of bees and wasps on artificial flowers. *Ecology* **62**, 20–26.
- Schemske, D. W. & Bradshaw, H. D., Jr (1999) Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proceedings of the National Academy of Sciences of the United States of America* **96** (21), 11910–11915.
- Smith, S. D. & Kriebel, R. (2018) Convergent evolution of floral shape tied to pollinator shifts in Iochrominae (Solanaceae). *Evolution* **72** (3), 688–697.
- Smithson, A. & McNair, M. R. (1997a) Density-dependent and frequency-dependent selection by bumblebees *Bombus terrestris* (L.) (Hymenoptera: Apidae). *Biological Journal of the Linnean Society* **60**, 401–417.
- Smithson, A. & McNair, M. R. (1997b) Negative frequency-dependent selection by pollinators on artificial flowers without rewards. *Evolution* **51**, 715–723.
- Stebbins, G. L. (1970) Adaptive radiation of reproductive characteristics in angiosperms, I: Pollination mechanisms. *Annual Review of Ecological Systems* **1**, 307–326.

- Strauss, S. Y. & Whittall, J. B. (2006) Non-pollinator agents of selection on floral traits. In L. D. Harder & S. C. H. Barrett [Eds], *Ecology and evolution of flowers*, 120–138. Oxford University Press, Oxford, UK.
- Streinzer, M., Roth, N., Paulus, H. F. & Spaethe, J. (2019) Colour preference and spatial distribution of glaphyrid beetles suggest a key role in the maintenance of the colour polymorphism in the peacock anemone (*Anemone pavonina*, Ranunculaceae) in Northern Greece. *Journal of Comparative Physiology*. doi:10.1007/s00359-019-01360-2.
- Sun, G., Ji, Q., Dilcher, D. L., Zheng, S., Nixon, K. C. & Wang, X. (2002) Archaeofractaceae, a new basal angiosperm family. *Science* **296**, 899–904.
- Tao, Z. B., Ren, Z. X., Bernhardt, P., Liang, H., Li, H. D., Zhao, Y. H., Wang, H. & Li, D. Z. (2018) Does reproductive isolation reflect the segregation of colour forms in *Spiranthes sinensis* (Pers.) Ames complex (Orchidaceae) in the Chinese Himalayas? *Ecology and Evolution* **00**, 1–15. doi:10.1002/ece3.4067.
- Troje, N. (1993) Spectral categories in the learning behaviour of blowflies. *Zeitschrift für Naturforschung* **48** (c), 96–104.
- van der Kooi, C. J., Dyer, A. G., Kevan, P. G. & Lunau, K. (2019) Functional significance of the optical properties of flowers for visual signalling. *Annals of Botany* **123** (2), 263–276.
- van der Kooi, C. J., Dyer, A. G. & Stavenga, D. G. (2014) Is floral iridescence a biologically relevant cue in plant–pollinator signalling? *The New Phytologist* **205** (1), 18–20. doi:10.1111/nph.13066.
- van der Niet, T., Peakall, R. & Johnson, S. D. (2014) Pollinator-driven ecological speciation in plants: New evidence and future perspectives. *Annals of Botany* **113** (2), 199–211.

- van Kleunen, M., Nänni, I., Donaldson, J. S. & Manning, J. C. (2007) The role of beetle marks and flower colour on visitation by monkey beetles (Hopliini) in the Greater Cape Floral Region, South Africa. *Annals of Botany* **100** (7), 1483–1489.
- von Witt, C. G., Anderson, B. A., Durbach, I. N. & Johnson, S. D. (2019) Breeding systems of floral colour forms in the *Drosera cistiflora* species complex. Manuscript under revision.
- Waser, N. M. (1983) The adaptive nature of floral traits: Ideas and evidence. In L. A. Real [Ed.], *Pollination biology*, 241–285. Academic Press, New York, USA.
- Weiss, M. R. (1997) Innate colour preferences and flexible colour learning in the pipevine swallowtail. *Animal Behaviour* **53**, 1043–1052.
- Whitney, H. M., Kolle, M., Andrew, P., Chittka, L., Steiner, U. & Glover, B. J. (2009) Floral iridescence, produced by diffractive optics, acts as a cue for animal pollinators. *Science* **323**, 130–133.
- Willis, K. J. & McElwain, J. C. (2002) Flowering plant origins. In *The evolution of plants*. Oxford University Press, Oxford, UK.
- Wright, S. (1931) Evolution in Mendelian populations. *Genetics* **28**, 114–138.
- Wright, S. (1943a) Isolation by distance. *Genetics* **28**, 114–138.
- Wright, S. (1943b) An analysis of local variability of flower colour in *Linanthus parryae*. *Genetics* **28**, 139–156.
- Wright, S. (1978) *Evolution and the genetics of populations, volume 4: Variability within and among natural populations*. University of Chicago Press, Chicago, USA.
- Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A. & Smith, G. M. (2009) *Mixed effects models and extensions in ecology with R*. Springer-Verlag, New York, USA.

CHAPTER FIVE

REPRODUCTIVE ISOLATION OF SYMPATRIC FLORAL COLOUR FORMS IN THE *DROSERA CISTIFLORA* SPECIES COMPLEX

Abstract

Quantifying reproductively isolating components is critical for determining stage of speciation and alleviating taxonomic obscurity in closely related plant forms. Some of these components assess the degree to which differential foraging behaviour by pollinators may prevent gene flow between plant populations. Variation in floral traits such as corolla colour may impose an ethologically isolating barrier if pollinator colour preferences diverge. However, pollination ecotypes involving plants that feature floral colour gradations seldom present as easily quantifiable systems for study. The *Drosera cistiflora* species complex (Droseraceae) is exceptional; here five discrete floral colour forms show evidence of pollinator-mediated divergence, with some of these forms being maintained in sympatry. I examine potential pre-F₁ reproductive barriers of ecogeography, habitat, flowering phenology, pollinator preferences and siring capacity between two of these *D. cistiflora* s.l. floral colour forms (namely purple and red) in South Africa's Greater Cape Floristic Region. Reproductive isolation (RI) indices were calculated for each barrier and total RI computed as the relative cumulative sequential contribution of each component to total isolation. Strong ecogeographical barriers emerged alongside habitat isolation, which was assessed as an index of non-random pollinator foraging on account of putative fine-scale geographical barriers among sympatric floral colour forms. Overall, RI varied between 0.93 (high RI) and 1.00 (complete isolation), depending on the level of conservativeness of the isolation index chosen between alternatives defined for a single component. Minimum (93%) RI

reflects the conservative strength of isolating barriers at the only known site of floral colour form sympatry. Here, habitat and pollinator isolation posed the greatest barriers to gene transfer between purple- and red-flowered forms, with relative pollinator isolation reaching 34% when pollinator importance was assessed in addition to abundance. Hopliine beetles (Coleoptera: Scarabaeidae: Hopliini) comprised most visitors to each floral colour form, with *Omocrates sp.* and *Lepisia rupicola spec.* as principal pollinators of purple and red respectively. Neither of these pollinator species cross-foraged between *D. cistiflora* s.l. flower colours and each carried form-specific pollen loads (pollen of the two forms can be distinguished by colour). Only pollinators of minimal importance were trapped in both floral colour forms. Pollinator isolation is attributed to ethological isolation by means of asymmetrical pollinator colour preferences. Significantly reduced seed set after inter- vs intra-colour crosses among purple and red flowers yielded relative postpollination barriers of 0–8% total isolation. Morphological observations show that *D. cistiflora* s.l. forms differ in traits besides corolla colour; these findings coupled with strong pre-F₁ isolation indicate that the populations may represent distinct taxa above the level of form. These insights demonstrate how form classification can downgrade real ecological and evolutionary substance, highlighting the need for a pluralistic approach to taxonomy. Such an approach could have significant conservation implications for these rare purple- and red-flowered pollination ecotypes which, although currently red-listed as Least Concern, may both be reclassified as Endangered.

Introduction

Adaptive interplay between plants and their pollinators is conventionally perceived to be a pivotal evolutionary driver in angiosperms (Darwin 1859, Whittall & Hodges 2007). Pollinator shifts have been put forward as a starting point in the putative pollinator-driven speciation process. Such shifts are defined by the capacity of differential pollinator preferences amidst geographically isolating barriers to generate divergent selective pressures among plant populations (q.v. Grant 1949; Grant & Grant 1965; Stebbins 1970; Johnson 2006, 2010; Kay & Sargent 2009; van der Niet et al. 2014). Subsequently, the establishment of pollination ecotypes (e.g. Johnson 1997; Anderson et al. 2010; Newman et al. 2010, 2012; Sun et al. 2014; van der Niet et al. 2014; Newman et al. 2015), reproductive isolation (e.g. Hodges et al. 2002, Ramsey et al. 2003, Kay & Schemske 2003, Kay 2006, Tao et al. 2018, Minnaar et al. 2019) and reinforcement (e.g. Levin & Kerster 1967, Hopkins & Rausher 2012) may ultimately lead to macroevolutionary changes and speciation (Grant 1949, 1994; Schluter 2000; Sargent 2004; Waser & Campbell 2004; Rundle & Nosil 2005; Sobel et al. 2010; van der Niet et al. 2014).

The moulding of regional pollination ecotypes through local floral character adaptation within a ‘pollination climate’ (sensu Grant & Grant 1965) is now a well founded concept (q.v. van der Niet et al. 2014), but the theoretical partitioning and consolidation of singular pollination ecotypes—should they converge in secondary sympatry—remains relatively unexplored. Few studies have reduced the knowledge gap brought by ostensible sympatric reproductive isolation of otherwise allopatric pollination ecotypes. One of these studies (Anderson et al. 2010) showed that the allopatric divergence of corolla tube length in *Gladiolus longicollis* (Iridaceae) is forged by the bimodal distribution of hawkmoth pollinator guilds differing in tongue length, and alluded to potential for such disruptive hawkmoth pollinator-mediated selection to hinder gene flow between short- and long-tubed plants in sympatry (through floral scent cues and short- and long-tongued hawkmoth foraging preferences or capabilities). Here, reproductive isolation involving pollinator behaviour (pollinator isolation) may thus be achieved through ‘floral isolation’ (sensu Grant 1949), which can either occur

through: i) ethological isolation (via the preferential responses of different pollinators to different floral traits) or ii) mechanical isolation (where the response of one or more pollinators is determined by the floral mechanism or structure per se). Kay (2006) revealed that mechanical isolation, in concert with habitat isolation, of *Costus pulverulentus* and *C. scaber* (Costaceae) hummingbird-pollinated ecotypes has largely driven their sympatric reproductive isolation. Although the study by Kay (2006) examined two (very closely related) *formerly speciated* plant taxa, novel evidence by Minnaar et al. (2019) has demonstrated mechanical isolation to be a significant link between floral trait divergence and reproductive isolation of *intraspecific* ecotypes at their contact zone. By tracking pollen movement among sympatric short- and long-tubed flowers of *Lapeirousia anceps* (Iridaceae) using quantum dots, this latter study showed how floral tube length determines the location of pollen placement along the proboscis of the *Moegistorhynchus longirostris* (Diptera: Nemestrinidae) fly pollinator, which in turn contributes substantially to overall reproductive isolation between the two forms of the plant. Similarly, mechanical isolation would likely explain pollinator isolation in sympatric short- and long-tubed *Gladiolus longicollis* ecotypes, where short-tongued moths are unable to access nectar from long-tubed plants (q.v. Anderson et al. 2010). A role for the alternative mode of floral isolation, ethological isolation, in maintaining the integrity of typically allopatric ecotypes in contact zones has yet to be elucidated.

Of all floral traits, flower colour is regarded as an instrumental visual signal influencing visitation by pollinators (Sun et al. 2018). Flower colours can frequently vary among populations (Rausher 2008), thereby altering visual signalling and potentially switching pollinators between different floral colour forms (e.g. Jones & Reithel 2001, Bradshaw & Schemske 2003, Campbell et al. 2012, McGimpsey & Lord 2015). Several studies suggest that floral colour transitions are maintained as the product of natural selection imposed by pollinator shifts (e.g. Levin & Kerster 1967, Waser & Price 1981, Meléndez-Ackerman & Campbell 1998, Schemske & Bradshaw 1999, Gigord et al. 2001, Irwin & Strauss 2005, Campbell et al. 2012, Hopkins & Rausher 2012, Newman et al. 2012, Tao et al. 2018, Streinzer et al. 2019). In this way, floral colour divergence may result in reduced gene flow between floral colour forms following pollinator identity changes (Levin & Kerster 1967, Schemske & Bradshaw 1999, Bradshaw & Schemske 2003), leading to floral isolation and, conceivably, speciation.

Given the paucity of empirical evidence establishing pollinators as the common thread of both allopatric and sympatric plant divergence, there is little consensus on whether ecological speciation can be a direct consequence of pollinator shifts (van der Niet et al. 2014), even in specialist plants (q.v. Armbruster et al. 2014). Since geographical and habitat differences tend to act as barriers to gene flow in most plant species (van der Niet & Johnson 2009), the role of pollinators in speciation is more readily related to diversification of phenotype than reproductive isolation (Johnson 2010). Patterson & Givnish (2003) termed this process ‘consequent radiation’, suggesting that habitat-related divergent selection among species at a site was the primary driver of floral radiation in *Calochortus* (Liliaceae), with ensuing adaptation to the pollinators and abiotic environmental conditions (in lieu of direct selection for plant-pollinator interaction partitions). Similarly, Wright (1943) proposed that local adaptation through natural selection across the range of a species may be preceded by random genetic drift, and geographical and habitat differences facilitate this process. Although pollinator shifts have been raised as a prevailing driving force of speciation in the southern African *Lapeirousia* subgenus *Lapeirousia* (Iridaceae) [q.v. Forest et al. 2014], genetic differentiation between founder and parent populations may in some instances be attributed to edaphic specialisation, resulting in ‘microgeographical speciation’ (sensu Goldblatt & Manning 1996). In these cases, accompanying floral morphological changes and pollinator shifts may have consequently enhanced genetic differentiation.

Evidence for pollinator shifts is provided by Chapter 4 of this thesis, where the maintenance of five discrete floral colour forms in the insectivorous *Drosera cistiflora* species complex (namely pink, purple, red, white and yellow) was shown to be an adaptive response to local pollinator communities across the geographical range of the complex, rather than to local abiotic factors (Chapter 3). In contrast to the conventional self-compatibility and pollinator non-dependence of essentially small-flowered *Drosera* species (q.v. Murza et al. 2006; Sciligo et al. 2007; Sciligo 2009; Cross et al. 2018), the large-flowered *D. cistiflora* species complex is characterised by partial self-incompatibility, pollen limitation and high pollinator dependence (von Witt et al. unpublished). Pollinator communities were found to comprise primarily hopliine beetles (Coleoptera: Scarabaeidae: Hopliini) [Goldblatt et al. 1998, Chapters 3 & 4], although a variety of other pollinator taxa, particularly beetles of the families Meloidae, Melyridae and Tenebrionidae, were also associated with respective floral colour forms

(Chapter 3). In light of these findings, I provisionally defined *D. cistiflora* s.l. floral colour forms as geographically divergent pollination ecotypes.

Some *D. cistiflora* s.l. floral colour ecotypes occur in sympatry, raising questions about whether flower colour at these sites is a polymorphism with typically weak or non-existent barriers to gene flow—or an indication of distinct, reproductively isolated gene pools of different species. Sympatric combinations of discrete floral colour forms include pink and purple, purple and red, purple and white, and red and white, with each occurring at 1–2 respective locations, and no instances of grading observed between colours. In contrast, contact zones of pink- and white-flowered forms visibly appeared to hybridise introgressively, with flower colours expressed as shades of pink and white along a gradient of varying pigment concentrations (q.v. Narbona & Wang et al. 2017, Figure 4, Chapter 1). The occurrence of all these colour combinations is sporadic, whereas floral colour forms are found chiefly in isolation (viz. populations with one flower colour only) across the range of *D. cistiflora* s.l. (Chapter 3).

Here I test the power of the pollinator-shift model and associated potential for shifts in flower colour and pollinator assemblage to achieve ‘incipient speciation’ (sensu Pellmyr 1986) through floral (specifically ethological) isolation in *D. cistiflora* s.l. I do this by assessing the comparative strength of spatio-temporal reproductively isolating parameters and barriers imposed by pollinator assemblages in the absence of macrogeographical isolation between populations. To assess whether these parameters are offset by postpollination barriers, I determine the hybridisation potential among populations and whether the flower colour of the population determines the capacity for hybrid seed set. Coyne and Orr (1989)’s seminal work on rates of genetic divergence in *Drosophila* (Diptera: Drosophilidae) suggests that prezygotic isolation is stronger and evolves more rapidly than postzygotic isolation in sympatry than in allopatry. Thus, by establishing the strength of isolating barriers before, during and after pollination, and further comparing the intensity of these components in sympatry and allopatry, this study addresses the plausibly fundamental mechanisms influencing divergence between *D. cistiflora* s.l. ecotypes and offers insight into their stage of speciation.

Schemske & Bradshaw (1999) define two experimental processes for estimating inferred genetic effects of pollinator isolation: i) field-based evaluation of pollinator responses to the floral traits of closely related plants in sympatry and ii) molecular analysis of DNA sequences to compare the genetic basis of phenotypic variance in floral traits. I apply the first of these in two sympatric populations of *D. cistiflora* s.l. floral colour forms (namely purple and red) which co-occur at a site near Darling, but otherwise occur in allopatry across their distribution ranges. Although the study site is the only locality where the purple-flowered *D. cistiflora* s.l. form with non-reflexed petals has been formally documented, recent evidence reveals the presence of at least four more populations (in sand fynbos, alluvium fynbos and renosterveld) along the West Coast region of the Cape of South Africa. Five additional extant populations of red-flowered *D. cistiflora* s.l. are known to occur, in sand fynbos and renosterveld, in the southwestern Cape. The hue and intensity of the purple and red *D. cistiflora* s.l. flower colours, which are partially governed by anthocyanin pigments (Wilbert et al. 1997), differ markedly in these two forms (Figure 2, Chapter 3) without visible intermediates in any populations. The lack of intermediates does not however preclude hybridisation, and genetic drift (q.v. Futuyma 2009) may be possible between forms at the site of secondary sympatry.

On account of the strong links shown between overall pollinator specificity and flower colour in the apparent absence of abiotic agency (Chapters 3 & 4) in *D. cistiflora* s.l., I hypothesise that the discrete purple- and red-flowered forms may be pollinator-isolated and that they may represent distinct taxa warranting taxonomic recognition above the rank of form. Although I provisionally treat these populations as forms on the basis of striking differences in flower colour, this classification does not hold ecological or evolutionary significance and may underestimate the conservation value of these populations, which are currently red-listed synonymously with *Drosera cistiflora* L. (Table 2, Chapter 1) as Least Concern (Foden & Potter 2005). I test my hypothesis by adapting the reproductive isolation indices outlined by Ramsey et al. (2003) for each pre-F₁ life history stage of purple and red *D. cistiflora* s.l. floral colour forms. These indices include: i) ecogeographical isolation, ii) pollinator fidelity, i.e. isolation through pollinator foraging specificity, and iii) pollen competition, i.e. isolation through intraspecific pollen precedence and/or further interspecific seed set barriers. I further define and consider indices of habitat, i.e. isolation through microgeographical barriers,

and flowering phenological isolation in sympatry. In this way, I demonstrate how the study of reproductive isolation in pollination ecotypes may influence taxonomy and red-listing assessments, which guide conservation of plant taxa and their associated pollinators.

Materials and Methods

Study site

Experimentation took place in 2016 in a disturbed 42m x 120m fragment of privately-owned farmland north of Darling (namely ‘Darling 3’; Table 1, Chapter 2; Supplementary Table 6). The farm is located in an ecotone between Atlantis Sand Fynbos, Swartland Shale Renosterveld and Swartland Alluvium Renosterveld in the winter-rainfall zone of the Fynbos Biome of South Africa. Here purple- (non-reflexed petals) and red-flowered *Drosera cistiflora* s.l. co-exist on a predominantly level plain of stony soils overlying a ferricrete outcrop. Commonly known as ironstone or koffieklip, ferricrete is frequently found as deposits at the ground surface or topsoil level (Cilliers & Withers 2014). The combination of ferricrete deposits and the small size of the clay particles in the topsoil at the site appears to have generated a hardpan basin that impedes drainage. The result is an ephemeral perched water table, providing the seasonally wet soils that support seasonal (springtime) aerial growth and flowering in *D. cistiflora* s.l. from its perennial swollen rootstock.

Invasive alien vegetation (q.v. Downey & Richardson 2016), namely *Acacia saligna*, overgrazing (q.v. Krausman et al. 2009), and habitat fragmentation (q.v. Schlaepfer et al. 2018) appear to pose the greatest threats to long-term native plant survival at the site.

Morphological observations

Plants in each population were randomly selected and checked for phenotypic differences in addition to flower colour. Qualitative and quantitative traits, namely corolla colour; flower quantity; petal quantity, shape and size; sepal quantity and size; plant height; stem diameter; leaf quantity, shape and size; stigma, style, stamen, filament and anther quantity and/or size, and pollen colour and morphology, were assessed and measured using callipers to the nearest 1mm where applicable. A subset

of 3–34 plants of each floral colour form was used. Pollen colour was deemed to have taxonomic significance based on consistent variance between floral colour forms (q.v. Kearns & Inouye 1993). Binary corolla and pollen colour assessments were made personally (by a single observer) and categorised according to colour perception by the human eye.

To compare traits among forms, Levene's tests and applicable independent samples t-tests were performed in Microsoft Excel for Office 365. Accordingly, morphometric mean values for petal length and width, style and style branch length, filament and anther length, flower height, stem diameter, number of cauline leaves, cauline leaf length and width, and number of rosette leaves were compared between purple- and red-flowered forms using the independent samples t-test assuming equal variances, and sepal length was compared using the independent samples t-test assuming unequal variances.

Reproductive isolation experiments

Ecogeography

To test for any detectable elevational segregation, altitude was measured using a GPS at the even horizontal surface of the study site where purple- and red-flowered *D. cistiflora* s.l. floral colour forms co-occurred.

To assess the spatial distribution of the two floral colour forms across the landscape, GPS co-ordinates for all known extant populations were obtained from specimens housed at the Bolus and Compton Herbaria, Custodians of Rare and Endangered Wildflowers (CREW) locality records (Supplementary Table 6), and personal observations. Geographical distances between all populations were measured using Google Earth.

Habitat

Since it is not known exactly how far each individual pollinator travelled, nor the potential effects of imperceptible fine-scale geographical barriers, an appraisal of habitat separation in sympatric *D. cistiflora* s.l. populations accounts for the possibility that pollinators may have focussed their foraging efforts around clusters of plants in microgeographical space rather than moving freely around the site (q.v. Drüsedau 1953, Stephens & Finkner 1953, Fryxell 1956a&b). Non-random foraging on account of fine-scale spatial distributions of different flower colours may thus alter the extent of gene flow between them (cf. Finkner 1954).

I divided the entire study area into 12 quadrats, i.e. equi-sized plots, of 10m x 40m (hemisected by a dirt road approximately 2m wide). This quadrat size catered for a reasonable estimate of the reach of pollinator foraging events in keeping with the approximate flight distance of pollinators observed between plants at the site.

After counting the number of purple- and/or red-flowered *D. cistiflora* s.l. plants in each quadrat, I assessed the population structure of the two floral colour forms by calculating their respective ‘Variance-to-Mean Ratios (VMR)’ (sensu Clapham 1936), also termed ‘Indices of Dispersion (ID)’, q.v. Krebs (1999), as follows:

$$ID = \frac{\sigma^2}{\mu} = \frac{\text{variance}}{\text{mean}}$$

$$\text{where } \sigma^2 = \frac{\sum(x_q - \mu)^2}{n}$$

$$\text{and } \mu = \frac{\sum x_q}{n}$$

x_q is the number of purple or red-flowered plants in each quadrat and n is the number of quadrats sampled.

As per the VMR, the plants are most likely to be randomly distributed if the variance equals the mean, in which case $ID = 1$ (Poisson distribution). If $0 < ID < 1$, they may be underdispersed or uniformly distributed (binomial distribution), and if $ID > 1$, they will tend to have an overdispersed or clumped distribution pattern (negative binomial distribution). In the instance of a constant random variable, $ID = 0$ (not dispersed).

Spatial clustering is often observed in populations of perennial plants that reproduce vegetatively, but also in cases of sexually reproducing populations with short dispersal distances, where offspring tend to aggregate around seed parents (Clapham 1936). Fine-scale geographical barriers may facilitate such short seed dispersal (q.v. Chan et al. 2018).

By determining the mode of distribution of purple- and red-flowered *D. cistiflora* s.l. plants, this analysis laid the groundwork for comparisons to be made between the dispersion patterns of each floral colour form and the potential effect thereof on pollinator foraging behaviour when the two forms occurred in sympatry.

Flowering phenology

Shifts in timing between the flowering phenologies of two taxa may impose important prezygotic reproductive barriers (Martin et al. 2007), both directly, by preventing gene flow during temporal separation, but also indirectly, through their plausible association with the timing of emergence of the respective primary pollinators of each taxon.

To determine the degree of separation achieved by shifts in flowering time, all flowers of each colour were counted at three-day intervals from the start (21 August, no flowers open) to end (5 October, all flowers over) of the flowering season, in 2016. Only open flowers were counted, and one open flower was present on each plant at a time. Buds were thus excluded, but the brief intervals between counts allowed for the inclusion of most of these flowers once they had opened, since each flower lasts for 1–3 days.

Pollinator isolation

Given that flower colour may be maintained through assortative mating in the *D. cistiflora* species complex, I used choice experiments to assess whether pollinator colour choice acts as a prezygotic barrier. Four experiments were carried out during peak pollinator activity (11h30–15h30) on different days, each of which comprised recording insect visitors to five replicates of purple and red flower colour pairs. Buds were removed in the instances where plants had more than one flower so that floral displays were consistent between pairs. Arrays of purple and red flower pairs were

placed one metre apart and arranged in a circle to accommodate 360° observation of visitors. Visitors were considered to be pollinators if they touched the anthers or stigmas of the flower, but were removed after 15 seconds to ensure that subsequent visitors were attracted by flower colour rather than an existing visitor. Consequently, no more than one visitor was present on each flower at a time and each visit thus represented a flower colour choice of purple or red.



Figure 1. Purple- and red-flowered *Drosera cistiflora* s.l. forms (a) arranged in circular arrays of five pairs (b), where each insect visit represented a choice of flower colour between purple and red

Pollen loads were determined for each insect pollinator to give an indication of their effectiveness and hence the degree to which they may influence gene flow between purple and red *D. cistiflora* s.l. floral colour forms. The product of each pollinator's abundance and average purple- and/or red-flowered *D. cistiflora* s.l. pollen load defined its importance. *D. cistiflora* s.l. pollen grains were counted under a dissecting microscope on 1–18 (median = 3.5) individuals of each insect species and differentiated according to purple- or red-flowered *D. cistiflora* s.l. form by way of colour. They were also distinguished from the pollen of other plant species using a reference set of slide preparations of pollen collected at the site.

Genetic compatibility

By determining the capacity for seed set when cross-pollination occurs between purple and red *D. cistiflora* s.l. floral colour forms and assessing whether differences in population flower colour can influence their potential for hybrid seed set, I could infer genetic compatibility among forms. Four treatments were applied to a subset of 17–21 plants per treatment, with one flower treated per plant, as follows: i) hand cross-pollination within the purple-flowered form; ii) hand cross-pollination within the red-flowered form; iii) hand cross-pollination of the purple-flowered form using pollen from the red-flowered form, and iv) hand cross-pollination of the red-flowered form using pollen from the purple-flowered form. Pollen donors were situated at least five metres from recipient plants to deter pollen collection from possible ramets, i.e. clonal members. For all treatments, recipient plants were emasculated while in bud and bagged with fine bridal veil mesh bags to preclude selfing and prevent the import of external pollen by insect pollinators. Bags were fastened around wire frames and firmly tied at the base and pegged to the ground to avoid loss to wind or livestock.

Ripe seed capsules were collected after 20–30 days and seeds counted under a dissecting microscope.

Differences between mean seed set per flower after intra- and inter-colour form hand cross-pollinations were assessed using generalised linear models implemented in IBM SPSS Statistics 26.0 (IBM Corp. 2019). Fixed factors were maternal flower colour, paternal flower colour and the interaction of these factors. Data were modelled using a

negative binomial distribution, and the linear dependency between number of seeds per flower and explanatory variables (flower colour and parent gender) was specified using a log link function. Significance was assessed using likelihood ratios. Back-transformation of marginal means from the linear scale resulted in asymmetrical standard errors.

Empirical mean values for seed set were compared using the independent samples t-test assuming unequal variances performed in Microsoft Excel for Office 365.

Reproductive isolation indices

Following Ramsey et al. (2003) and Coyne & Orr (1989), I computed total reproductive isolation as a multiplicative function of individual reproductive isolation components in chronological order of the *D. cistiflora* s.l. life history. Ramsey et al. (2003) used three pre-F₁ components: ecogeographical isolation, pollinator fidelity and pollen competition. I inserted the indices of habitat (microspatial) and phenological (temporal) isolation between ecogeographical and pollinator stages.

Ecogeographical isolation (EI)

EI provides a measure of the potential for reproductive isolation of populations amidst large-scale geographical barriers.

In their assessment of the differences in elevational range between herbarium specimens of *Mimulus lewisii* and *M. cardinalis* (Phrymaceae), Ramsey et al. (2003) used computer simulations to compare the frequency of natural co-occurrences to random assignment simulations which assumed complete sympatry across the range of both species, thus defining EI as:

$$EI = 1 - \frac{\text{number of co-occurrences in natural distribution simulation}}{\text{number of co-occurrences in random species assignment simulation}}$$

These populations of *Mimulus spp.* were segregated along an altitudinal gradient, usually with a vertical range overlap of approximately 400m and, along a horizontal gradient, seldom occurred at positions less than 10km apart. Here, the assessment of co-ordinates of herbarium collections may have compromised precision, particularly since the two species are known to frequently occur in sympatry (q.v. Ramsey et al. 2003). In contrast, purple- and red-flowered *D. cistiflora* s.l. studied here are only known to co-occur at a single site and GPS co-ordinates are available for all known extant populations of each form, thereby enabling straightforward quantitative comparisons to be made between sympatric and allopatric populations. I thus define EI for the two *D. cistiflora* s.l. colour forms as:

$$EI = 1 - \frac{\text{number of sympatric populations}}{\text{total number of populations}}$$

Habitat isolation (HI)

Here I define two alternative indices that estimate the potential for habitat segregation to give rise to reproductive isolation among *D. cistiflora* s.l. floral colour forms in sympatry. The first of these indices applied the ‘Raunkiaer Frequency’ (sensu Raunkiaer 1909), which involved recording the presence or absence of each form in all 12 quadrats and calculating their percentage co-occurrence, as follows:

$$HI_1 = 1 - \frac{\text{number of quadrats where both floral colour forms co - occurred}}{\text{total number of quadrats}}$$

The HI_1 index measures the frequency of overlap between distributions of purple- and red-flowered forms in the quadrats sampled but does not compare the abundance of each floral colour form among quadrats. On account of this simplism, a second index of habitat isolation was calculated, as follows:

$$HI_2 = 1 - \sum_{q=0}^Q \sqrt{\frac{p_1(q)r_1(q)}{P_1R_1}}$$

where $p_1(q)$ and $r_1(q)$ are, respectively, the number of purple- and red-flowered forms that were observed in quadrat q , and P_1 and R_1 are the respective total numbers of purple- and red-flowered forms in all 12 quadrats, so that $\frac{p_1(q)}{P_1}$ is the proportion of all purple-flowered forms that were observed in quadrat q .

This formula is known as the ‘Bhattacharyya Coefficient’ (sensu Bhattacharyya 1943) and is one potential way of measuring the similarity of two probability mass functions. Whilst the HI_1 index describes the extent of differences between distributions of purple- and red-flowered forms by determining *whether* they coincide, HI_2 describes the extent of differences in *how* they coincide by evaluating the extent of similarity between the abundance of the two forms among the sampled quadrats.

Both HI_1 and HI_2 therefore provide an indication of the potential for non-random foraging of pollinators and consequent assortative pollen movement between the floral colour forms.

Phenological isolation (PhI)

Phenological isolation measured the overlap between flowering periods of purple and red *D. cistiflora* s.l. floral colour forms to determine the temporal potential for inter-form gene transfer. There is no generally accepted way of measuring this overlap. I used the following two possible indices of phenological isolation:

$$PhI_1 = 1 - \frac{\text{number of days when both purple and red flowers observed}}{\text{total number of study days}}$$

$$PhI_2 = 1 - \sum_{t=0}^T \sqrt{\frac{p_2(t)r_2(t)}{P_2R_2}}$$

where $p_2(t)$ and $r_2(t)$ are, respectively, the number of purple and red flowers that were observed on day t , and P_2 and R_2 are the total number of purple and red flowers respectively, so that $\frac{p_2(t)}{P_2}$ is the proportion of all purple flowers that were observed on day t .

PhI_1 is a simple measure of the proportion of study days on which both purple and red flowers were observed, but does not differentiate according to the absolute or relative abundance of each flower colour.

The Bhattacharyya Coefficient in PhI_2 provides a measure of similarity between the two distributions, each of which shows the relative abundance of a particular flower colour over time.

Pollinator isolation (PI)

Here, flower colour choices of pollinator species that were observed visiting purple and/or red flowers in array pairs were used to determine the degree of pollinator isolation between *D. cistiflora* s.l. floral colour forms.

Ramsey et al. (2003) computed pollinator isolation as:

$$PI = 1 - \frac{\text{number of cross - species foraging bouts}}{\text{total number of foraging bouts}}$$

As my study design did not track individual pollinators, it is impossible to calculate pollinator isolation in the same way. My data are counts for colour choices of pollinator species in each of a specific number of flowers, and also, for each pollinator species, an average pollen load. From these data I define the following two possible indices of pollinator isolation:

$$PI_1 = 1 - \frac{\text{number of pollinator species found in both floral colour forms}}{\text{total number of pollinator species found}}$$

$$PI_2 = 1 - \sum_{s=0}^S \sqrt{\frac{p_3(s)r_3(s)}{P_3R_3}}$$

where $p_3(s)$ and $r_3(s)$ are, respectively, the number of pollinators of species s that were observed in purple and red flowers in arrays, and P_3 and R_3 are the total number

of pollinators found in purple and red array flowers respectively, so that $p_3(s)/P_3$ is the proportion of all pollinators of purple flowers that belong to species s .

Since not all pollinators are equally important, I computed a third index that weights each species count by its importance as measured by average pollen load:

$$PI_3 = 1 - \sum_{s=0}^S \sqrt{\frac{w_p(s)p_3(s)w_r(s)r_3(s)}{P_4R_4}}$$

Here, $w_p(s)$ is the average load of purple flower pollen carried by species s and $w_r(s)$ is the average load of red flower pollen carried by the same species. One can therefore think of $w_r(s)r_3(s)$, the product of average pollen load and pollinator abundance, as the total pollen contributed by species s in red flowers. The quantities $P_4 = \sum_{s=1}^S w_p(s)p_3(s)$ and $R_4 = \sum_{s=1}^S w_r(s)r_3(s)$ are the total pollen loads for visitors to purple and red flowers respectively, and scale the weighted counts $w_p(s)p_3(s)$ and $w_r(s)r_3(s)$ to both sum to one across species.

The PI_3 index thus describes the extent of differences in the way *pollen contribution* is distributed over visitor species between the two *D. cistiflora* s.l. floral colour forms. In contrast, the PI_2 index describes the extent of differences in the way *visits from pollinators* are distributed over species between the two floral colour forms.

Genetic incompatibility (GI)

GI is a measure of the extent to which postpollination barriers between populations may influence their fecundity and is applied under the assumption that inter-population pollen transfer can occur.

To determine whether siring capacity is constrained by inter-specific pollen transfer in *Mimulus*, Ramsey et al. (2003) compared inter-specific and conspecific pollen contribution to progeny [pollen competition (PC)] as:

$$PC = 1 - \frac{\text{number of hybrids (mixed pollination)}}{\text{number of parentals (intra - specific cross)}}$$

Since no hybrid plants could be visibly observed among purple- and red-flowered *D. cistiflora* s.l. forms, I calculated a proxy for PC by examining the mean number of seeds set per flower in mixed vs intra-colour conditions and expressed this as a measure of genetic incompatibility between floral colour forms:

$$GI = 1 - \frac{\text{mean number of seeds set (inter - floral colour form cross)}}{\text{mean number of seeds set (intra - floral colour form cross)}}$$

Results

Morphological observations

Apart from conspicuous disparity in peripheral petal colour (namely purple or red), flowers of the two *Drosera cistiflora* s.l. forms were macroscopically alike in terms of the following shared morphological characters: 1–2(–3) large, actinomorphic flowers with dark centres; shallowly bowl-shaped corollas; 5 petals; the 3-locular ovary; tristily; bifid styles with fringed stigmas; 5 anthers; herkogamy, i.e. spatially separated stigmas and anthers; cauline and basal rosette leaves; the presence of leaf and stem anthocyanins; tentacles predominant on leaf margins and ventral surface; the prominent dorsal leaf midvein, and swollen roots.

Characters varied in colour, quantity, shape and/or size (Table 1), with the most distinct apparent phenotypic differences being flower colour; pollen colour (consistently darker orange in purple-flowered forms and lighter yellow-orange in red-flowered forms); calyx, stem and leaf anthocyanin concentration (markedly greater in red than purple floral colour forms) [Figure 2], and the presence of a lateral shoot at the junction of the stem and peduncle in purple-flowered forms (absent in red-flowered forms) [Figure 6b&c, Chapter 1]. Style length, filament length and rosette leaf number were significantly ($p \leq 0.009$) greater in purple-flowered forms than red-flowered forms, whilst style branch length and cauline leaf length were significantly ($p \leq 0.003$) greater in red-flowered forms than purple-flowered forms (Table 1). No intermediate *D. cistiflora* s.l. floral colour forms were apparent at the site.



Figure 2. Habit of purple- (a) and red-flowered (b) *Drosera cistiflora* s.l. forms. Conspicuous phenotypic differences shown here include petal colour, pollen colour, leaf shape and leaf anthocyanin concentration. Purple petals in (a) are in the process of unfolding and, consequently, differences in petal shape between purple and red floral colour forms cannot be discerned from these images.

Table 1. Morphological assessment of quantitative and qualitative characters of purple and red floral colour forms of *Drosera cistiflora* s.l. Means \pm SE in millimetres are provided for morphometric data, with sample sizes shown in parentheses and t-statistics and p-values reported for differences between mean values.

Morphological character	<i>Drosera cistiflora</i> s.l. floral colour form		t	p
Corolla colour	Purple	Red		
	Metallic greenish blue centre	Dark greenish centre often encircled by a ring of white flecks		
Number of flowers per plant	1–2(–3) [699 plants]	1–2(–3) [919 plants]		
Number of petals	5	5		
Petal shape	Oblong-spathulate	Obdeltoid-widely obovate		
Petal length	25.9 \pm 0.99 (15)	23.25 \pm 1.14 (13)	1.76	0.090
Petal width (at widest point)	17.48 \pm 1.36 (13)	17.27 \pm 1.01 (13)	0.13	0.900
Number of sepals	5	5		
Sepal length	9.92 \pm 0.83 (4)	7.76 \pm 0.25 (5)	2.50	0.067
Number of styles	3 (bifid)	3 (bifid)		
Style length	13.01 \pm 0.42 (14)	11.38 \pm 0.30 (13)	3.09	0.005
Style branch length	2.34 \pm 0.14 (34)	3.20 \pm 0.16 (33)	4.01	< 0.001
Number of stigmas	6	6		
Number of stamens	5	5		
Filament length	6.74 \pm 0.27 (13)	4.68 \pm 0.29 (13)	5.17	< 0.001
Anther length	1.91 \pm 0.06 (14)	1.95 \pm 0.09 (13)	0.29	0.772
Pollen colour	Orange	Yellow-orange		
Pollen shape	Tetrahedral tetrad	Tetrahedral tetrad		
Flower height above ground	129.56 \pm 6.39 (9)	110.5 \pm 7.03 (9)	2.01	0.062
Lateral shoot at base of peduncle	Present	Absent		
Stem diameter (mid length)	1.03 \pm 0.09 (3)	1.13 \pm 0.09 (3)	0.80	0.468
Number of cauline leaves	9.5 \pm 0.27 (8)	9.0 \pm 0.38 (7)	1.10	0.291
Cauline leaf shape	Linear-lanceolate	Linear-acuminate		
Cauline leaf length	24.47 \pm 1.19 (3)	41.37 \pm 2.32 (3)	6.49	0.003
Cauline leaf width	1.98 \pm 0.06 (3)	1.93 \pm 0.03 (3)	0.73	0.507
Number of rosette leaves	5.71 \pm 0.92 (7)	1.50 \pm 0.29 (4)	3.34	0.009

Reproductive isolation experiments and indices

Ecogeographical isolation (EI)

At the single site where purple- and red-flowered *D. cistiflora* s.l. populations grow in sympatry, GPS-measured altitude was found to be 61m, with a negligible NW slope across the flat plain. Consequently, altitudinal separation, and hence the elevational diversity gradient, is considered unimportant in terms of pollinator movement at the site. Here, no other large-scale physical geographical reproductive barriers were found between the two floral colour forms and pollinators could thus potentially move freely within the 5040m² area. Where populations of purple- and red-flowered forms occurred in allopatry, they were separated by minimum distances of more than 20km.

Assessment of EI among all populations (in sympatry and allopatry) showed EI between the two floral colour forms to be high, with an EI index of 0.82.

Habitat isolation (HI)

A total of 699 purple-flowered and 919 red-flowered *D. cistiflora* s.l. plants were counted, with variance-to-mean ratios determined to be 41.57 and 769.98 respectively. VMRs thus indicate overdispersed distribution patterns for each form, with particularly high spatial clustering evident in red-flowered forms.

The distributions of the two floral colour forms overlapped in 67% of quadrats, giving an HI_1 index of 0.33. Comparisons of the extent of similarity between the abundance of each form among quadrats, calculated by way of the Bhattacharyya Coefficient, yielded an HI_2 index of 0.40.

Although purple- and red- flowered plants co-occurred in most quadrats, the two forms were generally divided into distinct clusters within these quadrats. Such clusters were only observed to be intermixed (Figure 6a, Chapter 1) in three instances: two where sparse purple-flowered plants were found within essentially red-flowered clusters and one in the reverse configuration. Results of HI indices, which indicate overall isolation between the two forms according to the size of quadrats, should thus be considered

alongside VMRs to make allowance for spatial clustering of plants within quadrats. Considering that clustering within quadrats may have given rise to further segregation of pollinator foraging between purple and red flowers in most instances of their co-occurrence, HI indices may underestimate habitat isolation in reality.

Phenological isolation (PhI)

Purple- and red-flowered *D. cistiflora* s.l. forms started flowering on the same date: 24 August. Red-flowered forms followed a normal distribution over a flowering period extending 12 days longer than purple-flowered forms: until 4 October (Figure 3). The shorter flowering period of purple-flowered forms coincided with an early peak, giving rise to a slight trough between the peak maxima of the two forms (Figure 3).

Despite the distinct temporal isolation between peak flowering times, purple and red floral colour forms flowered contemporaneously on 41% of the study days, thus giving a PhI_1 index of 0.41. Comparisons of relative abundance between purple and red flowers showed similar levels of isolation, giving a PhI_2 index of 0.40.

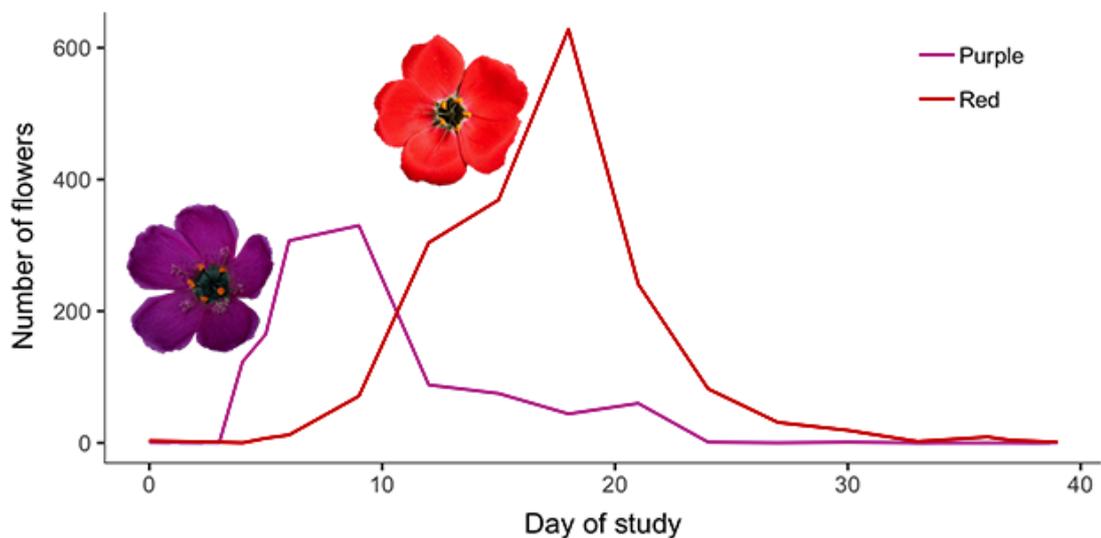


Figure 3. Differences in flowering phenologies of purple and red *Drosera cistiflora* s.l. floral colour forms, shown here as respective total numbers of flowers present per day, from the start to end of each flowering season. Both forms started flowering on the same date (24 August 2016). The final day of study (4 October 2016) is the final day of flowering for the red floral colour form (no purple flowers were observed after 22 September 2016).

Pollinator isolation (PI)

Both *D. cistiflora* s.l. floral colour forms were primarily pollinated by suites of hopliine beetles (Coleoptera: Scarabaeidae: Hopliini). Among these, *Omocestus* sp. and *Lepisia rupicola* spec. were principal pollinators of purple- and red-flowered forms respectively (Figure 5, Supplementary Figure 7). Not only were these two species the most abundant (Figure 4), but they also had a higher pollen load than any other pollinator found in the same floral colour form (Figure 5). They showed strong colour preferences, visiting each form exclusively, viz. *L. rupicola* was not found in purple flowers and *Omocestus* sp. was not found in red flowers. Soft-winged flower beetles (Melyridae) were of sequent importance as pollinators of purple-flowered forms (Table 2). They were observed in abundance in the early stages of peak flowering and appeared to precede the emergence of *Omocestus* sp., which subsequently outcompeted them in abundance and importance (Figures 4 & 5).

Pollinators were polylectic, viz. visits were not restricted to *D. cistiflora* s.l., with all recorded pollinator species also found to carry pollen from other plant species at the site.

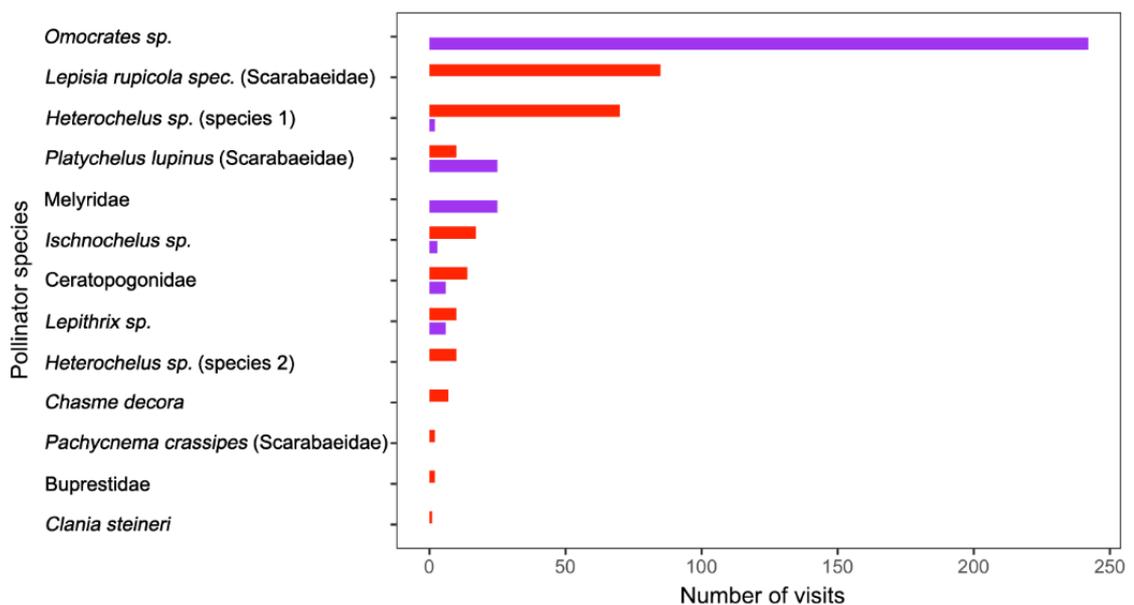


Figure 4. Abundance of each insect pollinator species trapped in purple and red *Drosera cistiflora* s.l. flowers

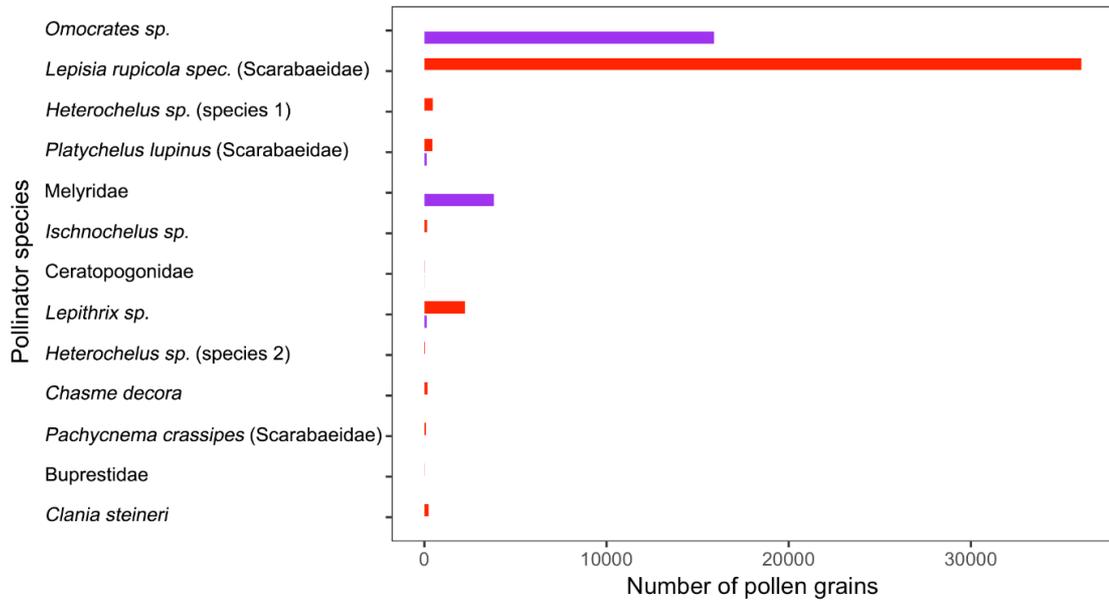


Figure 5. Importance [calculated as the product of abundance and average pollen loads (Table 2)] of each insect pollinator of purple and red *Drosera cistiflora* s.l. floral colour forms

Pollinator isolation indices were high overall, yielding a PI_1 index of 0.62 and PI_2 of 0.81 (Tables 3, 4 & 5). Percentage isolation escalated to 97.4% when pollen loads were accounted for by the PI_3 index (Table 5). These results highlight the strength of prezygotic barriers imposed by the most important pollinators choosing either purple- or red-flowered forms exclusively in array pairs (at 100% purple and red) [Figure 6].

Table 2. Means \pm SE for importance values (abundance*pollen loads) and percentage relative mean importance of insect pollinators observed in purple and red *Drosera cistiflora* s.l. flower colours. Pollen of the two forms can be distinguished by colour. Principal pollinators are indicated by bold type in the colour of the relevant *D. cistiflora* s.l. form with which they were associated. Superscript H denotes hopliine beetles (Coleoptera: Scarabaeidae: Hopliini) and letters in parentheses correspond to the images of each pollinator in Supplementary Figure 7.

Insect pollinator	<i>Drosera cistiflora</i> s.l. flower colour			
	Purple	Relative mean importance (%)	Red	Relative mean importance (%)
	Number of pollen grains \pm SE		Number of pollen grains \pm SE	
Buprestidae (r)			8 \pm 0.00	0.02
Ceratopogonidae (e, q)	4.5 \pm 2.87	0.02	10.5 \pm 3.50	0.03
<i>Chasme decora</i> ^H (k)			182 \pm 81.13	0.46
<i>Clania steineri</i> ^H (j)			228 \pm 0.00	0.57
<i>Heterochelus</i> sp. (species 1) ^H [h]	0 \pm 0.00	0.00	476 \pm 373.96	1.19
<i>Heterochelus</i> sp. (species 2) ^H [p]			23.75 \pm 10.68	0.06
<i>Ischnochelus</i> sp. ^H (m)	0 \pm 0.00	0.00	139.78 \pm 44.69	0.35
<i>Lepisia rupicola spec.</i>^H (f)			36061.25 \pm 18305.13	90.42
<i>Lepithrix</i> sp. ^H (d, g)	108 \pm 0.00	0.54	2225 \pm 2215	5.58
Melyridae (b)	3825 \pm 3275	19.17		
<i>Omocrates</i> sp.^H (a)	15891.33 \pm 5631.26	79.65		
<i>Pachynema crassipes</i> ^H (n)			84 \pm 0.00	0.21
<i>Platycheilus lupinus</i> ^H (c, i)	121.88 \pm 57.95	0.61	446 \pm 317.83	1.12

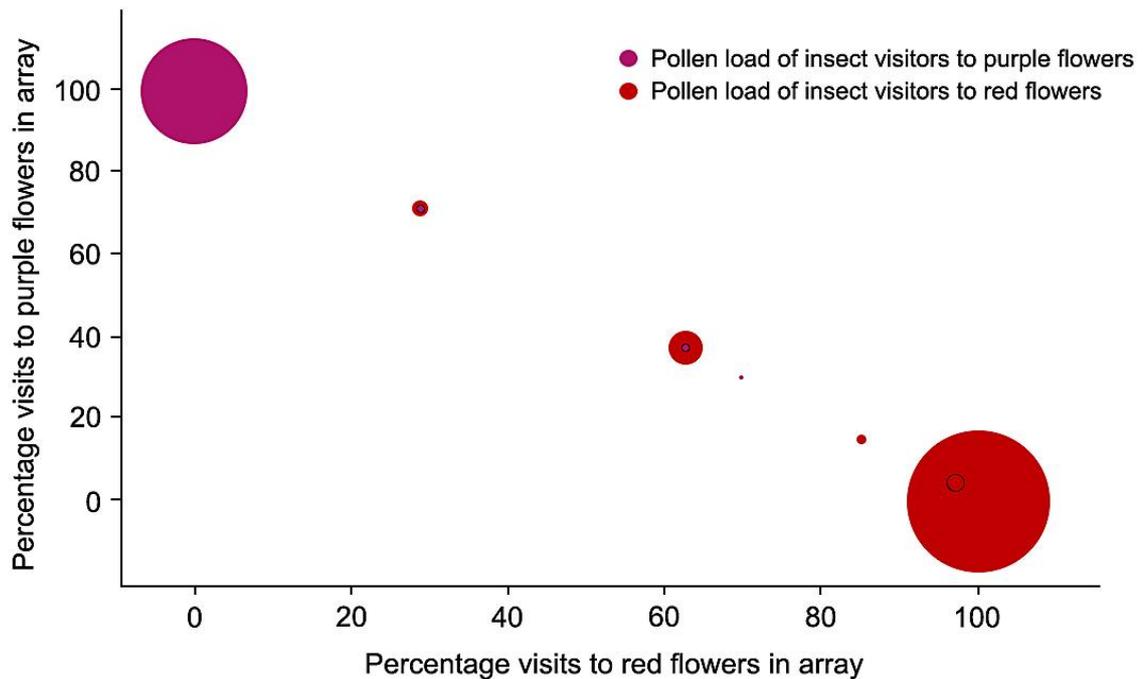


Figure 6. Percentage visits of insect pollinators to purple or red *Drosera cistiflora* s.l. floral colour forms, where each visit represented a choice between a pair of flower colours in an array. Bubble size is proportionate to the number of pollen grains carried by visitors. Where pollinators visited either purple- or red-flowered forms exclusively, these visits are clustered at 100% purple or red. Since more than one insect species visited purple or red flowers exclusively, visits from multiple species are grouped together at each pole. Consequently, bubble sizes for 100% visits to purple or red flowers are proportionate to the respective collective pollen loads of all exclusively purple or red flower visitor species. In some instances overlap appears between pollen load bubbles. These overlapping bubbles are associated with discrete percentage abundances of different pollinator species. Such overlaps are distinguished by circles with black outlines inside larger bubbles, where the circle fill colour represents the flower colour visited.

Genetic incompatibility (GI)

Overall, marginal means for seed production per flower varied between maternal and paternal flower colours [female parent: $\chi^2(1) = 3.281$, $p = 0.070$; male parent: $\chi^2(1) = 0.001$, $p = 0.980$], with a significant interaction effect between comparisons of seed set from parents of the same and different flower colours [female parent*male parent: $\chi^2(1) = 8.439$, $p = 0.004$] (Figure 7).

For red flowers, the mean number of seeds produced per flower after hand-crossing treatments using pollen donors from the same floral colour form (red*red: mean \pm SE = 351.18 \pm 101.43) was significantly ($p < 0.001$) greater than after treatments using pollen donors from the purple floral colour form (red*purple: mean \pm SE = 155.43 \pm 40.44). Seed set per flower after intra-colour crosses was significantly ($p = 0.002$) lower in purple- than red-flowered forms. As per comparisons between mean seed set per flower after intra- and inter-colour crosses in red flowers, seed set for purple flowers using pollen from the same floral colour form (purple*purple: 213.63 \pm 58.41) was significantly ($p = 0.001$) greater than seed set with pollen from the alternative floral colour form (purple*red: 93.26 \pm 22.55).

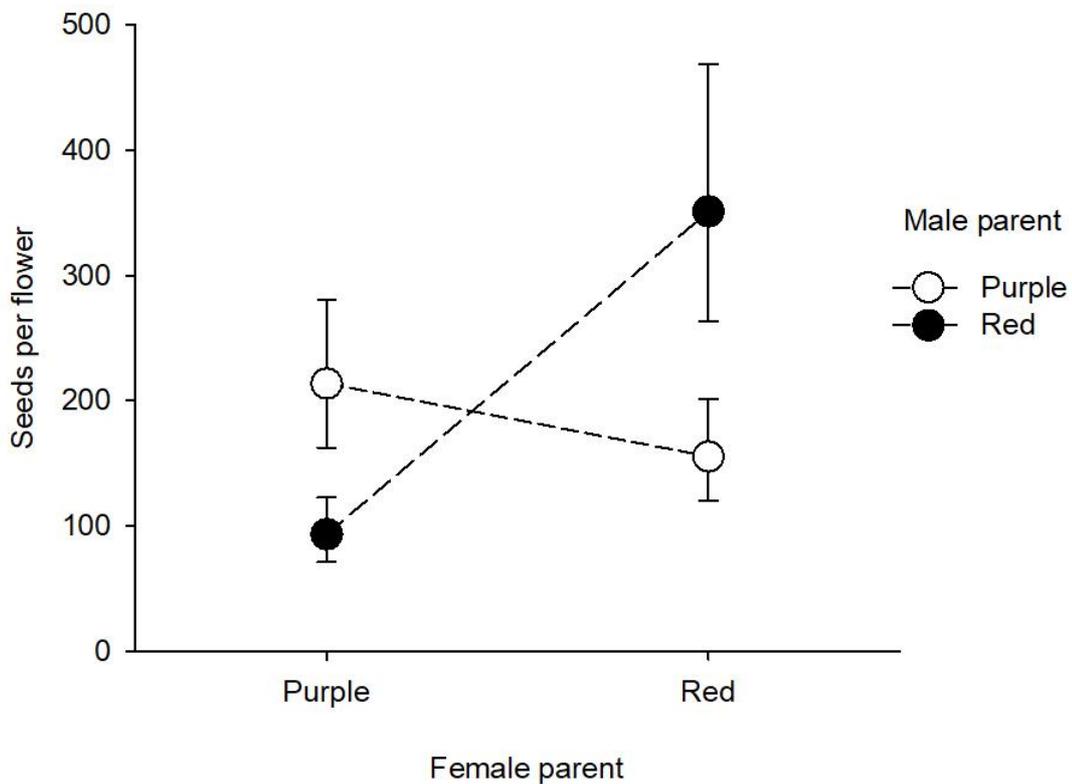


Figure 7. Comparison of the effects of intra- and inter-colour hand cross-pollinations on seed set among purple- and red-flowered *Drosera cistiflora* s.l. Values represent marginal model mean (and asymmetrical standard errors) for number of seeds per flower. Only one flower per plant was treated.

GI indices of 0.56 for both purple- and red-flowered forms indicate the presence of early-acting postzygotic barriers to hybrid seed formation and, when considered as individual isolating components, suggest that these barriers are important contributing factors to reproductive isolation between floral colour forms.

Summary of reproductive isolation indices

Reproductive isolation between purple and red *D. cistiflora* s.l. floral colour forms is high regardless of which of the indices is chosen to represent the individual components. Both definitions of habitat and phenological isolation indices gave respectively similar results, though pollinator isolation indices gave quite different results, with isolation increasing if pollinator abundance was taken into account and increasing further if average pollen load was taken into account.

Results for HI_1 , PhI_2 and PI_2 , as the conservative indices, are shown in Table 4 below.

Tables 4 and 5 consider total reproductive isolation in the presence of ecogeographical barriers (involving both sympatric and allopatric populations of purple- and red-flowered *D. cistiflora* s.l.). As a comparative measure, Table 3 provides an assessment of the strength of reproductive isolation among purple and red floral colour forms in the absence of ecogeographical isolation. Here, EI is reasonably set to zero (complete sympatry).

Table 3. Individual components of reproductive isolation and their relative cumulative contributions to total isolation among purple and red *Drosera cistiflora* s.l. floral colour forms occurring in sympatry only (EI = 0), providing ranges for all defined habitat, phenological and pollinator isolation indices. A value of zero indicates a complete lack of isolating barriers and 1.00 indicates complete isolation. Relative cumulative contributions for each isolating barrier were calculated as percentages of the remainder from the previous barrier level (in order of effect in *D. cistiflora* s.l. life history), so that the sum of all contributions provides a value for total isolation between purple- and red-flowered *D. cistiflora* s.l. (shown in bold type).

	Individual components of reproductive isolation		Relative cumulative contribution to total isolation	
	Purple	Red	Purple	Red
Ecogeographical isolation	0.00	0.00	0.00	0.00
Habitat isolation	0.33–0.40	0.33–0.40	0.33–0.40	0.33–0.40
Phenological isolation	0.40–0.41	0.40–0.41	0.27–0.25	0.27–0.25
Pollinator isolation	0.62–0.97	0.62–0.97	0.25–0.34	0.25–0.34
Genetic incompatibility	0.56	0.56	0.08–0.01	0.08–0.01
Total isolation			0.93–1.00	0.93–1.00

Table 4. Individual components of reproductive isolation and their relative cumulative contributions to total isolation among purple and red *Drosera cistiflora* s.l. floral colour forms, for conservative choices of reproductive isolation indices defined for habitat, phenological and pollinator isolation. Here, ecogeographical isolation refers to isolation amidst the entire geographical range of purple- and red-flowered populations (involving sympatric and allopatric populations). A value of zero indicates a complete lack of isolating barriers and 1.00 indicates complete isolation. Relative cumulative contributions for each isolating barrier were calculated as percentages of the remainder from the previous barrier level (in order of effect in *D. cistiflora* s.l. life history), so that the sum of all contributions provides a value for total isolation between purple- and red-flowered *D. cistiflora* s.l. (shown in bold type).

	Individual components of reproductive isolation		Relative cumulative contribution to total isolation	
	Purple	Red	Purple	Red
Ecogeographical isolation	0.82	0.82	0.82	0.82
Habitat isolation	0.33	0.33	0.06	0.06
Phenological isolation	0.40	0.40	0.05	0.05
Pollinator isolation	0.81	0.81	0.06	0.06
Genetic incompatibility	0.56	0.56	0.01	0.01
Total isolation			1.00	1.00

The results in Table 5 include ranges encompassing all index definitions of HI, PhI and PI. Note that if the very conservative PI_1 index is used, there is greater overlap (less isolation) between the *D. cistiflora* s.l. floral colour forms following the pollinator isolation stage, but that this is compensated for by an increase in the amount of isolation accounted for by the GI stage. As a result, total isolation remains high (although not as high as when PI_2 or PI_3 indices are used).

Table 5. Individual components of reproductive isolation and their relative cumulative contributions to total isolation among purple and red *Drosera cistiflora* s.l. floral colour forms, providing ranges for all defined habitat, phenological and pollinator isolation indices. Ecogeographical isolation refers to isolation amidst the entire geographical range of purple- and red-flowered populations (involving sympatric and allopatric populations). A value of zero indicates a complete lack of isolating barriers and 1.00 indicates complete isolation. Relative cumulative contributions for each isolating barrier were calculated as percentages of the remainder from the previous barrier level (in order of effect in *D. cistiflora* s.l. life history), so that the sum of all contributions provides a value for total isolation between purple- and red-flowered *D. cistiflora* s.l. (shown in bold type).

	Individual components of reproductive isolation		Relative cumulative contribution to total isolation	
	Purple	Red	Purple	Red
Ecogeographical isolation	0.82	0.82	0.82	0.82
Habitat isolation	0.33–0.40	0.33–0.40	0.06–0.07	0.06–0.07
Phenological isolation	0.40–0.41	0.40–0.41	0.05	0.05
Pollinator isolation	0.62–0.97	0.62–0.97	0.04–0.06	0.04–0.06
Genetic incompatibility	0.56	0.56	0.02–0.00	0.02–0.00
Total isolation			0.99–1.00	0.99–1.00

Discussion

Reproductive isolation of floral colour forms

The modification of the reproductive isolation indices of Ramsey et al. (2003) for use in the *Drosera cistiflora* s.l. system, in combination with assessments of habitat and phenological isolation, offer a robust model for delineating barriers to gene flow in sympatry and allopatry. Here, purple- and red-flowered *D. cistiflora* s.l. are shown to possess only a narrow (0–7%) window of opportunity for pre-F₁ gene transfer, with a central barrier being imposed by pollinator preferences. Although ecogeographical isolation emerged as the prime isolating factor (82%) among floral colour forms when the entire geographical range of populations was considered, relative pollinator isolation was notably high (25–34%) when the single site of sympatry was assessed independently (cf. 4–6% in sympatry and allopatry overall). These findings lend support to the theory that pollinator shifts constitute important drivers of reproductive isolation and incipient speciation among closely related plants through ecological niche adaptation and assortative mating (q.v. Smith 1966). They also offer new evidence for ethological isolation (driven largely by pollinator colour preferences) as a strong gene flow barrier between pollination ecotypes in their zone of secondary contact.

In addition to ecogeographical and pollinator isolation, predominant prezygotic barriers impeding gene flow were i) microspatial (habitat isolating through segregative clumping of floral colour forms) and ii) temporal (phenologically isolating through shifts in peak flowering times). The 67% overlap of distributions of purple- and red-flowered plants in quadrats does not however adequately account for overdispersion. Clusters of plants appeared to be segregated within quadrats in reality and these clusters seldom comprise more than one floral colour form. The HI index may consequently underestimate habitat isolation on account of large quadrat size. Such overdispersion may be attributed to dispersal mode in *D. cistiflora* s.l. Because the small seeds (approximately 0.5mm long) do not appear to possess inherent structural characteristics that aid dispersal, this likely occurs through a combination of boleochory, i.e. shaking of dehiscent capsules by wind, and barochory, i.e. dispersal by gravity, both of which

have short dispersal distances, with boleochory dispersal distances recorded to be less than 0.1–1m in plants under 30cm tall (q.v. Vittoz and Engler 2007). Apparent microgeographic isolation of habitat between floral colour forms could also be associated with the seasonally wet substrate requirements of *D. cistiflora* s.l., where colonisation of moist shallow microdepressions may further constrain dispersal and localise recruitment. Ensuing competition may in turn limit immigration of novel forms (or possible hybrids) into pre-existing plant clusters, giving rise to the observed infrequency of floral colour form mixing within clusters and enabling assortative gene transfer through non-random pollinator foraging.

Inferential individual percentage pollinator isolation on account of pollinator importance was particularly high (97.4%), but this measure is not without limitations. Although there is ultimately no ‘correct’ choice of isolation index, consideration of pollinator isolation in terms of the index of abundance (81.0% isolation) may offer a more realistic estimate than importance, since: i) average pollen loads relied on relatively few observations and hence may be uncertain; ii) the taxonomic use of inter-form variance in pollen colour (Kearns & Inouye 1993) as a means of distinguishing between *D. cistiflora* s.l. floral colour forms may be flawed given the potential lability of grain pigments (q.v. Stanley & Linskens 1974), and iii) insect pollen loads corresponding to stigma deposition loads (Howlett et al. 2011) may be inconsistent within and/or between pollinator species.

Early-acting postzygotic isolation was detected by inter-colour hand cross-pollination treatments, which produced significantly fewer seeds than intra-colour hand cross-pollination treatments in both purple- and red-flowered forms; possibly owing to attrition of inter-colour pollen (q.v. Ramsey et al. 2003) after pollen tubes either fail to germinate, or reach or fertilise ovules, or abortion of hybrid zygotes (q.v. Baack et al. 2015). However, such postpollination isolation contributed minimally (0–8%) towards reproductive isolation overall.

Pollinator isolation as a function of hopliine beetle colour preferences

Whilst all *D. cistiflora* s.l. pollinators also carried pollen of other plant species and pollination was thus considered to be generalised (q.v. Waser et al. 1996), purple and red *D. cistiflora* s.l. floral colour forms were specialised for beetle, particularly hopliine, pollination, with beetles comprising 92.3% of all visitors recorded and 83.3% of these being hopliine beetles (Table 2, Supplementary Figure 7). The strong pollinator isolation (97.4%) reported on account of insect pollen loads may be the outcome of phenotypic adaptation to contrasting colour preferences of hopliine beetle assemblages—particularly those of the principal hopliine pollinators *Omocrates* sp. (purple flowers: 79.7% relative mean importance) and *Lepisia rupicola* spec. (red flowers: 90.4% relative mean importance) [Table 2, Supplementary Figure 7], in keeping with the ‘most effective pollinator principle’ (sensu Stebbins 1970). Melyrid beetles, which were most active at the beginning of the flowering season, also showed a strong preference for purple flowers (19.2% relative mean importance, Table 2, Supplementary Figure 7) over red flowers. It is possible that these beetles may have selected for the early flowering peak in purple floral colour forms, facilitating phenological isolation of purple- and red-flowered *D. cistiflora* s.l. populations.

Hopliine beetles are known to distinguish between flower colours (e.g. Picker & Midgley 1996, Johnson & Midgley 2001, Arnold 2010) and Chapter 4 of this thesis demonstrated significant hopliine beetle preferences for local *D. cistiflora* s.l. flower colours over introduced colours in model flower array experiments overall (where model flowers matched the outer petal colour of the local population, precluding selection for other floral traits) [Figure 5, Chapter 4]. However, when considered for individual *D. cistiflora* s.l. flower colours, these findings were significant for red-flowered forms, but not for purple. Given the significant matching effect found for local purple *D. cistiflora* s.l. flowers in reciprocal translocation arrays (Figure 6, Chapter 4), it is possible that visual signals other than outer petal colour may be important factors determining pollinator preferences for the purple-flowered form. Apart from outer petal colour, visible *D. cistiflora* s.l. floral traits that may serve as visual signals for pollinators (e.g. corolla shape, flower size, dark centres, pollen presentation and pollen colour) appeared remarkably similar between the two floral colour forms, with the exception of the metallic-iridescent corolla centre of purple flowers (absent in red

flowers) [Figure 1a; Figure 1, Chapter 6]. Although Whitney et al. (2009) and Vignolini et al. (2014a&b) have intuited iridescence as a signalling cue for pollinators, their findings have been refuted by van der Kooi et al. (2014), who suggested that the iridescence signal disappears under natural light conditions; and this hypothesis remains unresolved. Consequently, in combination with the findings of Chapter 4, this study provides evidence for pollinator isolation of red-flowered *D. cistiflora* s.l. forms driven by asymmetrical colour preferences of local pollinator assemblages (ethological isolation), but the mechanism underlying opposing pollinator preferences for purple-flowered forms is undetermined. My field observations nevertheless found that the two primary pollinating insect taxa of purple *D. cistiflora* s.l. flowers (*Omocrates* sp. and Melyridae) only tended to visit flowers with short-wavelength-reflecting corolla colours at the study site [e.g. *Babiana leipoldtii*, *B. ambigua* (Iridaceae), *Drosera pauciflora* s.l., *Felicia tenella* (Asteraceae) and *Geissorhiza aspera* (Iridaceae)] aside from purple-flowered *D. cistiflora* s.l. These preliminary observations suggest that the outer petal colour of purple-flowered *D. cistiflora* s.l. may indeed be an important visual signal for these beetles. They also suggest that the purple model flower findings in Chapter 4 may reflect poor colour matching of purple model flowers to real flowers, rather than actual pollinator colour preferences. Investigation into iridescence and other petal micromorphological traits may thus elucidate integrated visual signalling in purple-flowered *D. cistiflora* s.l.

Substantial concordant selection, viz. synergistic selective forces mediated by pollinators and/or non-pollinator agents, is required to overcome interference by genetic drift and/or opposing selective forces (Wright 1951, Armbruster et al. 2014). Floral evolution may thus be inhibited should large pollinator assemblages impose conflicting selection on floral traits (Goméz et al. 2014). It follows that reproductive isolation in *D. cistiflora* s.l. may not be achieved unless the selective preferences of pollinating groups associated with each floral colour form are strongly aligned. Here, the hopliine beetle, *Lepithrix* sp., was the only species observed cross-foraging between floral colour forms in direct succession, as seen on two occasions. Although not observed to directly cross-forage, it is possible that some of the other insect taxa that were trapped in both purple and red flowers, namely Ceratopogonidae (Diptera), *Heterochelus* sp. (species 1), *Ischnochelus* sp. and *Platychelus lupinus*, may have represented the same individuals. A particular individual may thus have visited

different array colours on separate visits and essentially cross-foraged. Given the similarities in corolla shape, size and other plausible signalling traits among purple and red flowers at the site, the visitation of both flower colours by the same pollinator may be the result of local conditioning to overall floral form rather than corolla colour in isolation. The red colour preference of the principal hopliine pollinator *Lepisia rupicola* spec. (Supplementary Figure 6) was nevertheless absolute, since individuals visited red flowers exclusively despite the similar shape of purple flowers. Furthermore, non-exclusive pollinators were of minimal importance to one or both floral colour forms. For example, biting midges of the family Ceratopogonidae, which were the only non-beetle pollinators observed, were of negligible importance in purple- and red-flowered forms (0.02% and 0.03% respectively). These small, soft-bodied flower visitors appeared to be the most prevalent species trapped in leaf tentacles, and their importance as prey thus outweighs their consideration as primary pollinators. The hopliine pollinators *Heterochelus* sp. (species 1) and *Ischnochelus* sp., although trapped in both colour forms, did not carry pollen from purple forms. *Lepithrix* sp. and *Platycheilus lupinus* were thus the only dual visitors of importance, howbeit minor, to purple- and red-flowered *D. cistiflora* s.l. (Table 2). The strong mating discrimination on account of minimal cross-foraging of polylectic pollinator assemblages between floral colour forms suggests that floral isolation (Grant 1949, Schiestl & Schlüter 2009), viz. ethological isolation through differences in floral morphology and divergent pollinator preferences, may be attributed to assortative mating by generalist pollinator groups, thereby counteracting drift (Mayr 1965). The direction of gene flow may be more precisely resolved using genetic (microsatellite) markers (Sork et al. 1999, Darvill et al. 2004, Selkoe & Toonen 2006, Moe & Weiblen 2012) or pollen-tracking techniques such as labelling with dye (q.v. Stephens & Finkner 1953, Kay 2006) or quantum dots (q.v. Minnaar et al. 2019), but large-scale experimentation would not be possible in this environmentally sensitive area.

Postpollination isolation and potential for hybridisation of floral colour forms

Even if the capacity for hybridisation is strongly limited by both pre- and postzygotic barriers, and no intermediate flower colours were observed at the study site over four generations, this does not preclude introgression through fertile F₁ hybrids. The potential for quantification of postzygotic barriers through ex situ experimentation is however constrained in the *D. cistiflora* s.l. system, given the plants' highly specific habitat requirements. My attempts to test the viability of seed generated from hand-crossing experiments were accordingly unsuccessful in greenhouse conditions, where no germination was achieved from seed produced by either intra- or inter-colour crosses. Since this outcome is more likely to reflect the difficulty of ex situ simulation of the plants' specialised growth requirements, rather than seed viability or fertility, these findings were excluded from the study and the mechanisms for postzygotic isolation remain undetermined.

Although the contribution of prezygotic barriers towards total isolation typically outweighs that of postzygotic barriers in the early stages of speciation (q.v. Husband & Sabara 2004, Rieseberg & Willis 2007, Tao et al. 2018), postzygotic barriers have been shown to be important contributors towards total reproductive isolation of closely related taxa in the absence of ecogeographical, phenological and pollination barriers (q.v. Liao et al. 2019). Likewise, orchids with weak pollinator isolation have strong intrinsic postmating isolation, and vice versa (Cozzolino & Scopece 2008). Similarly, my findings show particularly high ecogeographical, phenological and pollinator isolation among *D. cistiflora* s.l. floral colour forms with low relative importance of postmating isolation, indicating their somewhat recent divergence. In view of speciation being a process, rather than an event, such comparison of pre- and postzygotic isolation offers a mere snapshot of the present stage of divergence of purple- and red-flowered *D. cistiflora* s.l., which is subject to change until speciation may be achieved, if at all. Coyne & Orr (1989) articulated that although hybrid sterility and inviability may increase at similar rates over time in allopatry, the evolution of prezygotic isolation precedes postzygotic isolation in instances where populations arise in sympatry. A more balanced perspective on stage of speciation among these *D. cistiflora* s.l. forms could thus be reached by comparing pre- and postzygotic isolation

intensity between sympatric and allopatric populations. According to ‘Haldane’s Rule’ (sensu Haldane 1922), hybrid sterility would likely be evident through the male component of fitness. Sex chromosome analyses (Charlesworth 2002), ongoing assessment of siring potential among *D. cistiflora* s.l. populations and comparisons between sympatric and allopatric populations may therefore shed light on the stage of speciation of purple and red floral colour forms.

Can floral isolation be explained by reinforcement?

Reinforcing selection acts to reduce the cost of hybridisation by favouring prezygotic reproductive isolation of incipient species in instances of secondary contact (Hopkins 2013). Flower colour may respond to reinforcing selection, with shifts in colour presenting a means to curtail hybridisation (Levin & Kerster 1967, Hopkins & Rausher 2012, Hopkins 2013). Since many *D. cistiflora* s.l. floral colour forms occur both in sympatry and allopatry, it is reasonable to question whether the floral colour divergence in sympatry examined in this study demonstrates reinforcement. However, considering the current lack of experimental evidence of hybridisation in sympatry, and that the same striking differences in flower colour of both purple and red floral colour forms are also retained in allopatry, I cannot yet assess whether their presence in sympatry at Darling 3 may be attributed to reinforcing selection of a novel trait [c.f. Levin & Kerster (1967), who found that corolla colour displacement in *Phlox pilosa* (Polemoniaceae) only occurred in zones of *P. pilosa*-*P. glaberrima* sympatry with flowering phenological overlap, thereby facilitating pollinator isolation and strengthening ethological barriers true to the ‘Wallace effect’ (sensu Grant 1966)]. All *D. cistiflora* s.l. floral colour forms have been shown through reciprocal translocation experiments to be the product of local adaptation to pollinator communities across the range of the species complex (Figure 4, Chapter 4). The strong prezygotic isolation by means of floral isolation of pollen-limited sympatric purple- and red-flowered *D. cistiflora* s.l. at Darling 3 is thus provisionally most parsimoniously explained by local adaptation.

Other mechanisms of reproductive isolation such as shifts in flowering times may also result from reinforcing selection (McNeilly & Antonovics 1968, Silvertown et al. 2005, Hopkins 2013), where greater phenological divergence may be observed in sympatric

populations than in allopatric populations. Further investigation of flowering times in purple and red *D. cistiflora* s.l. floral colour forms and comparisons thereof in sympatry and allopatry, coupled with comparisons of timing of emergence of primary pollinators and siring potential among forms, would help to establish the role of reinforcement in floral colour divergence among *D. cistiflora* s.l. forms.

Taxonomic and red-listing implications of reproductive isolation

Given that pollination ecotype formation may, by way of pollinator isolation, precede speciation (Gervasi & Schiestl 2017), it is essential that natural pollination processes are conserved to sustain biological diversity through adaptive radiation. This is particularly relevant in ‘biodiversity hotspots’ (sensu Mittermeier et al. 1998) such as the Cape Floristic Region, which includes the terrain of the taxa in this study. Lying at the root of red-listing accuracy and informed conservation action, is taxonomy. In this study, quantitative evaluation of reproductively isolating components (Tables 4 & 5) combined with quali-quantitative morphological assessment showed high levels of pre-F₁ isolation and significant differences between morphological characters (Table 1) of *D. cistiflora* s.l. floral colour ecotypes. These findings provide strong evidence for incipient speciation and offer cause for taxonomic classification of purple- and red-flowered *D. cistiflora* s.l. above the level of form. Thus, by assessing speciation potential using a standard ecological technique, the study shows how the rank of form can fail to appraise evolutionary substance among plant populations. Conservation assessments typically apply IUCN Red List Categories and Criteria (IUCN 2012) to species, subspecies, varieties and “biologically isolated subpopulations of species” (SANBI 2017). In recognising that forms may not constitute biologically isolated subpopulations (q.v. Hamilton & Reichard 1992), along with the current consideration of *D. cistiflora* s.l. as a single species, namely *Drosera cistiflora* L. (Seine & Barthlott 1994, Schlauer 1996), the present red-list assessment of *Drosera cistiflora* L. as Least Concern (Foden & Potter 2005) may be defended. However, such an assessment does not make allowance for the high extinction threats and evolutionary significance of these *D. cistiflora* s.l. ecotypes (Supplementary Table 16) and consequently minimises their perceived conservation value and prospects for conservation action.

Arguably the most conventional of the numerous species concepts (q.v. Aldhebiani 2018) that have been proposed is the ‘Biological Species Concept’ (sensu Mayr 1942), which defines species as “groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups”. In order to delineate species, the following criteria (sensu Stace 1989) are commonly used by taxonomists: i) morphological similarities; ii) presence of gaps in variation between two closely related species; iii) occupation of a specific geographical range and adaptation to local environmental conditions, and iv) interbreeding with minimal to no loss of fertility between conspecifics in sexual taxa. Stace additionally suggests that interbreeding may occur between different species but with reduced success; conversely, subspecies can interbreed without a fitness cost, should prohibitive geographical barriers be removed. It is thus conceivable that purple- and red-flowered *D. cistiflora* s.l. may represent different species, thereby suggesting that the purple-flowered taxon at Darling 3 may be an undescribed species, and lending support to the proposed species-level split of the red-flowered taxon, namely *Drosera rubripetala* Debbert (Table 2, Chapter 1). Purple-flowered forms have historically been described as *Drosera violacea* Willd. (presently synonymous with *D. cistiflora* L.), which has a remarkably similar flower colour to the purple-flowered taxon studied here. However, *D. violacea* describes plants with reflexed petals which do not occur north of Malmesbury, whilst the purple-flowered taxon at Darling 3 has consistently crateriform corollas and a broader distribution range: along the West Coast. I have provisionally named this taxon *Drosera cistiflora* ‘Purple West Coast’ (Table 2, Chapter 1). Systematics methodologies such as DNA sequencing, in combination with the morphological and ecological findings of this study, will help delineate relatedness among purple-and red-flowered populations.

In the interim, precautionary measures that involve: i) formally recognising these phenotypically distinct populations as different taxa or ecotypes [to which IUCN Red List Categories and Criteria (IUCN 2012) may be applied (SANBI 2017)] and ii) suitably considering their individual extinction threats, would likely elevate the Red List statuses of both purple- (West Coast) and red-flowered *D. cistiflora* s.l. from Least Concern (Foden & Potter 2005) to Endangered B2ab(i,ii,iii,iv,v) [Supplementary Table 16].

Conclusions

Conclusive evidence is provided for strong prezygotic (ecogeographical, habitat, flowering phenological and pollinator) isolation of purple- and red-flowered *D. cistiflora* s.l. Pollinator isolation manifests as floral, specifically ethological, isolation, with positive assortative pairing among ecotypes being driven mainly by the respective discrimination of purple or red flower traits by hopliine beetle assemblages, particularly the colour preference of *Lepisia rupicola* spec. for red-flowered ecotypes, but also by a variety of other pollinating species (Table 2, Figures 4 & 5, Supplementary Figures 6 & 7). These *D. cistiflora* s.l. floral colour forms thus appear to represent incipient species which are reproductively isolated largely through local adaptations to ‘pollinator blocs’ (sensu Chapter 4). Although taxonomic ranks may only be accurately assigned once DNA sequences have been analysed, findings support the need for revised red-listing of *D. cistiflora* s.l. ecotypes to reflect their high risk of extinction. A better understanding of reproductive isolation and, in turn, taxonomic relationships, in *D. cistiflora* s.l. can therefore enable the appropriate conservation of its remarkable floral colour diversity and correlative pollinators.

References

- Aldhebiani, A. Y. (2018) Species concept and speciation. *Saudi Journal of Biological Sciences* **25** (3), 437–440.
- Anderson, B., Alexandersson, R. & Johnson, S. D. (2010) Evolution and co-existence of pollination ecotypes in an African *Gladiolus* (Iridaceae). *Evolution* **64** (4), 960–972.
- Armbruster, W. S., Pélabon, C., Bolstad, G. H. & Hansen, T. F. (2014) Integrated phenotypes: Understanding trait covariation in plants and animals. *Philosophical Transactions of the Royal Society B: Biological Sciences* **369** (1649), 20130245.
- Armbruster, W. S., Shi, X. Q. & Huang, S. Q. (2014) Do specialised flowers promote reproductive isolation? Realised pollination accuracy of three sympatric *Pedicularis* species. *Annals of Botany* **113** (2), 331–340.
- Arnold, S. E. J. (2010) *Flowers through insect eyes: The contribution of pollinator vision to the evolution of flower colour*. PhD Thesis. Queen Mary, University of London, UK.
- Baack, E., Melo, M. C., Rieseberg, L. H. & Ortiz-Barrientos, D. (2015) The origins of reproductive isolation in plants. *New Phytologist* **207** (4), 968–984.
- Bhattacharyya, A. (1943) On a measure of divergence between two statistical populations defined by their probability distributions. *Bulletin of the Calcutta Mathematical Society* **35**, 99–109.
- Bradshaw, H. D. & Schemske, D. W. (2003) Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature* **426**, 176–178.

- Campbell, D. R., Bischoff, M., Lord, J. M. & Robertson, A. W. (2012) Where have all the blue flowers gone: Pollinator responses and selection on flower colour in New Zealand *Wahlenbergia albomarginata*. *Journal of Evolutionary Biology* **25** (2), 352–364.
- Chan, Y. M., Tnah, L. H., Lee, S. L., Bhassu, S., Lee, C. T. & Chua, L. S. L. (2018) Limited dispersal and geographic barriers cause population differentiation and structuring in *Begonia maxwelliana* at both large and small scales. *Plant Ecology & Diversity* **11** (1), 69–83.
- Charlesworth, D. (2002) Plant sex determination and sex chromosomes. *Heredity* **88** (2), 94–101.
- Cilliers, C. & Withers, A. (2014) Overstrand IDF: Towards 2050, Environmental Management Framework. <https://www.overstrand.gov.za/en/documents/town-planning/strategic-documents-1/idf-emf/emf/1291-2-overstrand-emf/file>. Accessed on 30 November 2018.
- Clapham, A. R. (1936) Over-dispersion in grassland communities and the use of statistical methods in plant ecology. *Journal of Ecology* **24** (1), 232–251.
- Coyne, J. A. & Orr, H. A. (1989) Patterns of speciation in *Drosophila*. *Evolution* **43** (2), 362–381.
- Cozzolino, S. & Scopece, G. (2008) Specificity in pollination and consequences for postmating reproductive isolation in deceptive Mediterranean orchids. *Philosophical Transactions of the Royal Society B: Biological Sciences* **363** (1506), 3037–3046.
- Cross, A. T., Davis, A. R., Fleischmann, A., Horner, J. D., Jürgens, A., Merritt, D. J., Murza, G. L. & Turner, S. R. (2018) Reproductive biology and pollinator-prey conflicts. In A. M. Ellison and L. Adamec [Eds], *Carnivorous plants: Physiology, ecology, and evolution*, 294–313. Oxford University Press, New York, USA.

Darvill, B., Knight, M. E. & Goulson, D. (2004) Use of genetic markers to quantify bumblebee foraging range and nest density. *Oikos* **107** (3), 471–478.

Darwin, C. (1859) *On the origin of species*. London: J. Murray, UK.

Downey, P. O. & Richardson, D. M. (2016) Alien plant invasions and native plant extinctions: A six-threshold framework. *AoB PLANTS* **8**, plw047. doi:10.1093/aobpla/plw047.

Drüsedau, E. (1953) Untersuchungen über die bestäubungsverhältnisse innerhalb frei abblühender populationen einer fremdbefruchtenden pflanze. *Zeitschrift für Pflanzenzüchtung* **32**, 421–444.

Finkner, M. D. (1954) Random activity of pollen vectors in isolated plots of Upland cotton. *Agronomy Journal* **46**, 68–70.

Foden, W. & Potter, L. (2005) *Drosera cistiflora* L. National Assessment: Red List of South African Plants version 2017.1. Accessed on 4 August 2019.

Forest, F., Goldblatt, P., Manning, J. C., Baker, D., Colville, J. F., Devey, D. S., Jose, S., Kaye, M. & Buerki, S. (2014) Pollinator shifts as triggers of speciation in painted petal irises (*Lapeirousia*: Iridaceae). *Annals of Botany* **113** (2), 357–371.

Fryxell, P. A. (1956a) The breeding structure of an outcrossed population. *Wichita State University Stud. Ser. No. 33*. **31** (1), 1–6.

Fryxell, P. A. (1956b) Effect of varietal mass on percentage of outcrossing in *Gossypium hirsutum* in New Mexico. *Journal of Heredity* **47**, 299–301.

Futuyma, D. J. (2009) Genetic drift: Evolution at random. In D. J. Futuyma, *Evolution*. Sinauer Associates, Sunderland, Massachusetts, USA.

Gervasi, D. D. L. & Schiestl, F. P. (2017) Real-time divergent evolution in plants driven by pollinators. *Nature Communications* **8**, 14691.

- Gigord, L. D. B., Macnair, M. R. & Smithson, A. (2001) Negative frequency-dependent selection maintains a dramatic flower colour polymorphism in the rewardless orchid *Dactylorhiza sambucina* (L.) Soò. *Proceedings of the National Academy of Sciences of the United States of America* **98** (11), 6253–6255.
- Goldblatt, P., Bernhardt, P. & Manning, J. C. (1998) Pollination of petaloid geophytes by monkey beetles (Scarabaeidae: Rutelinae: Hopliini) in Southern Africa. *Annals of the Missouri Botanic Garden* **85**, 215–230.
- Goldblatt, P. & Manning, J. C. (1996) Phylogeny and speciation in *Lapeirousia* subgenus *Lapeirousia* (Iridaceae: Ixioideae). *Annals of the Missouri Botanical Garden* **83** (3), 346–361.
- Gómez, J. M., Muñoz-Pajares, A. J., Abdelaziz, M., Lorite, J. & Perfectti, F. (2014) Evolution of pollination niches and floral divergence in the generalist plant *Erysimum mediohispanicum*. *Annals of Botany* **113** (2), 237–249.
- Grant, V. (1949) Pollination systems as isolating mechanisms in angiosperms. *Evolution* **3**, 82–97.
- Grant, V. (1966) The selective origin of incompatibility barriers in the plant genus *Gilia*. *American Naturalist* **100**, 99–118.
- Grant, V. (1994) Modes and origins of mechanical and ethological isolation in angiosperms. *Proceedings of the National Academy of Sciences of the United States of America* **91** (1), 3–10. doi:10.1073/pnas.91.1.3.
- Grant, V. & Grant, K. A. (1965) *Flower pollination in the Phlox family*. Columbia University Press, New York, USA.
- Haldane, J. B. S. (1922) Sex ratio and unisexual sterility in hybrid animals. *Journal of Genetics* **12** (2), 101–109.

- Hamilton, C. W. & Reichard, S. H. (1992) Current practice in the use of subspecies, variety, and forma in the classification of wild plants. *Taxon* **41** (3), 485–498.
- Hodges, S. A., Whittall, J. B., Fulton, M. & Yang, J. Y. (2002) Genetics of floral traits influencing reproductive isolation between *Aquilegia formosa* and *Aquilegia pubescens*. *The American Naturalist* **159**, S51–S60.
- Hopkins, R. (2013) Reinforcement in plants. *New Phytologist* **197** (4), 1095–1103.
- Hopkins, R. & Rausher, M. D. (2012) Pollinator-mediated selection on flower colour allele drives reinforcement. *Science* **335**, 1090–1092.
- Howlett, B., Walker, M. K., Rader, R. & Butler, R. C. (2011) Can insect body pollen counts be used to estimate pollen deposition on pak choi stigmas? *New Zealand Plant Protection* **64**, 25–31.
- Husband, B. C. & Sabara, H. A. (2004) Reproductive isolation between autotetraploids and their diploid progenitors in fireweed, *Chamerion angustifolium* (Onagraceae). *New Phytologist* **161** (3), 703–713.
- IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.
- Irwin, R. E. & Strauss, S. Y. (2005) Flower colour microevolution in wild radish: Evolutionary response to pollinator-mediated selection. *The American Naturalist* **165**, 225–237.
- IUCN (2012) IUCN Red List Categories and Criteria: Version 3.1. Second edition. Gland, Switzerland and Cambridge, UK: IUCN. iv + 32pp.
- Johnson, S. D. (1997) Pollination ecotypes of *Satyrium hallackii* (Orchidaceae) in South Africa. *Botanical Journal of the Linnean Society* **123**, 225–235.

Johnson, S. D. (2006) Pollinator-driven speciation in plants. *In* L. D. Harder & S. C. H. Barrett [Eds], *The ecology and evolution of flowers*, 296–306. Oxford University Press, Oxford, UK.

Johnson, S. D. (2010) The pollination niche and its role in the diversification and maintenance of the southern African flora. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365** (1539), 499–516.

Johnson, S. D. & Midgley, J. J. (2001) Pollination by monkey beetles (Coleoptera: Scarabaeidae: Hopliini): Do colour and dark centers of flowers influence alighting behaviour? *Environmental Entomology* **30**, 861–868.

Jones, K. N. & Reithel, J. S. (2001) Pollinator-mediated selection on a flower colour polymorphism in experimental populations of *Antirrhinum* (Scrophulariaceae). *American Journal of Botany* **88** (3), 447–454.

Kay, K. M. (2006) Reproductive isolation between two closely related hummingbird-pollinated neotropical gingers. *Evolution* **60** (3), 538–552.

Kay, K. M. & Sargent, R. D. (2009) The role of animal pollination in plant speciation: Integrating ecology, geography, and genetics. *Annual Review of Ecological Systems* **40**, 637–656.

Kay, K. M. & Schemske, D. W. (2003). Pollinator assemblages and visitation rates for 11 species of Neotropical *Costus* (Costaceae). *Biotropica* **35**, 198–207. doi:10.1111/j.1744-7429.2003.tb00279.x.

Kearns, C. A. & Inouye, D. W. (1993) *Techniques for pollination biologists*. University Press of Colorado, Boulder, Colorado, USA.

Krausman, P. R., Naugle, D. E., Frisina, M. R., Northrup, R., Bleich, V. C., Block, W. M., Wallace, M. C. & Wright, J. D. (2009) Livestock grazing, wildlife habitat, and rangeland values. *Rangelands* **31** (5), 15–19.

Krebs, C. J. (1999) *Ecological methodology*. Addison Wesley Longman, Menlo Park, California, USA.

Levin, D. A. & Kerster, H. W. (1967) Natural selection for reproductive isolation in *Phlox*. *Evolution* **21** (4), 679–687.

Liao, W-J., Zhu, B-R., Li, Y-F., Li, X-M., Zeng, Y-F. & Zhang, D-Y. (2019) A comparison of reproductive isolation between two closely related oak species in zones of recent and ancient secondary contact. *BMC Evolutionary Biology* **19** (1), 70. doi: 10.1186/s12862-019-1399-y.

Martin, N. H., Bouck, A. C. & Arnold, M. L. (2007) The genetic architecture of reproductive isolation in Louisiana irises: Flowering phenology. *Genetics* **175** (4), 1803–1812.

Mayr, E. (1942) *Systematics and the origin of species*. Columbia University Press, New York, USA.

Mayr, E. (1965) *Animal species and evolution*. Belknap Press of Harvard University Press, Cambridge, Massachusetts, USA.

McGimpsey, V. J. & Lord, J. M. (2015) In a world of white, flower colour matters: A white–purple transition signals lack of reward in an alpine *Euphrasia*. *Austral Ecology* **40**, 701–708.

McNeilly, T. & Antonovics, J. (1968) Evolution in closely adjacent plant populations IV. Barriers to gene flow. *Heredity* **23**, 205–218.

Meléndez-Ackerman, E. J. & Campbell, D. R. (1998) Adaptive significance of flower colour and inter-trait correlations in an *Ipomopsis* hybrid zone. *Evolution* **52** (5), 1293–1303.

- Minnaar, C., de Jager, M. L. & Anderson, B. (2019) Intraspecific divergence in floral tube length promotes asymmetric pollen movement and reproductive isolation. *New Phytologist*. doi:10.1111/nph.15971.
- Mittermeier, R. A., Myers, N., Thomsen, J. B., da Fonseca, G. A. B. & Olivieri, S. (1998) Biodiversity hotspots and major tropical wilderness areas: Approaches to setting conservation priorities. *Conservation Biology* **12**, 516–520.
- Moe, A. M. & Weiblen, G. D. (2012) Pollinator-mediated reproductive isolation among dioecious fig species (*Ficus*, Moraceae). *Evolution* **66** (12), 3710–3721.
- Murza, G. L., Heaver, J. R. & Davis, A. R. (2006) Minor pollinator-prey conflict in the carnivorous plant, *Drosera anglica*. *Plant Ecology* **184**, 43–52.
- Narbona, E. & Wang, H., Ortiz, P., Arista, M. & Imbert, E. (2017). Flower colour polymorphism in the Mediterranean Basin: Occurrence, maintenance and implications for speciation. *Plant Biology* **20**, Suppl 1. doi:10.1111/plb.12575.
- Newman, E., Anderson, B. & Johnson, S. D. (2012) Flower colour adaptation in a mimetic orchid. *Proceedings of the Royal Society B — Biological Sciences* **279**, 2309–2313.
- Newman, E., Manning, J. C. & Anderson, B. (2015) Local adaptation: Mechanical fit between floral ecotypes of *Nerine humilis* (Amaryllidaceae) and pollinator communities. *Evolution* **69** (9), 2262–2275.
- Patterson, T. B. & Givnish, T. J. (2003) Geographic cohesion, chromosomal evolution, parallel adaptive radiations, and consequent floral adaptations in *Calochortus* (Calochortaceae): Evidence from a cpDNA phylogeny. *New Phytologist* **161** (1), 253–264.
- Pellmyr, O. (1986) Three pollination morphs in *Cimicifuga simplex*: Incipient speciation due to inferiority in competition. *Oecologia* **68**, 304–307.

- Picker, M. D. & Midgley, J. J. (1996) Pollination by monkey beetles (Coleoptera: Scarabaeidae: Hopliini): Flower and colour preferences. *African Entomology* **4** (1), 7–14.
- Ramsey, J., Bradshaw, H. D. & Schemske, D. W. (2003) Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution* **57** (7), 1520–1534.
- Raunkiaer, C. (1909) Formationsundersøgelse og formationsstatistik. *Botanisk Tidsskrift* **30** (1).
- Rausher, M. D. (2008) Evolutionary transitions in floral colour. *International Journal of Plant Sciences* **169**, 7–21.
- Rieseberg, L. H. & Willis, J. H. (2007) Plant speciation. *Science* **317** (5840), 910–914.
- Rundle, H. D. & Nosil, P. (2005) Ecological speciation. *Ecology Letters* **8**, 336–352.
- SANBI 2017. Assessment methodology: Red List of South African Plants version 2017.1. Downloaded from Redlist.sanbi.org on 2019/08/23.
- Sargent, R. D. (2004) Floral symmetry affects speciation rates in angiosperms. *Proceedings of the Royal Society B — Biological Sciences* **271**, 603–608.
- Schemske, D. W. & Bradshaw, H. D., Jr (1999) Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proceedings of the National Academy of Sciences of the United States of America* **96** (21), 11910–11915.
- Schiestl, F. P. & Schlüter, P. M. (2009) Floral isolation, specialised pollination, and pollinator behaviour in orchids. *Annual Review of Entomology* **54** (1), 425–426.
- Schlaepfer, D. R., Braschler, B., Rusterholz, H. P. & Baur, B. (2018) Genetic effects of anthropogenic habitat fragmentation on remnant animal and plant populations: A meta-analysis. *Ecosphere* **9** (10). doi:10.1002/ecs2.2488.

- Schlauer, J. (1996) A dichotomous key to the genus *Drosera* L. (Droseraceae). *Carnivorous Plant Newsletter* **25**, 67–88.
- Schluter, D. (2000) *The ecology of adaptive radiation*. Oxford University Press, Oxford, UK.
- Sciligo, A. R. (2009) *Food or sex: which would you choose? Pollinator-prey conflict and reproductive assurance in New Zealand Drosera*. PhD Thesis. Lincoln University, New Zealand.
- Sciligo, A. R., Sullivan, J. J., Jesson, L. K., Witt, T., Jürgens, A., Hale, R. J. & Newstrom, L. (2007) *Is selfing more advantageous than sex in Drosera due to the pollinator-prey conflict?* ESA/SER Joint Meeting, San Jose McEnery Convention Centre, California.
- Seine, R. & Barthlott, W. (1994) Some proposals on the infrageneric classification of *Drosera* L. *Taxon* **43**, 583–589.
- Selkoe, K. A. & Toonen, R. J. (2006) Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. *Ecology Letters* **9**, 615–629.
- Silvertown, J., Servaes, C., Biss, P. & Macleod, D. (2005) Reinforcement of reproductive isolation between adjacent populations in the Park Grass Experiment. *Heredity* **95** (3), 198–205.
- Smith, J. M. (1966) Sympatric speciation. *The American Naturalist* **100** (916), 637–650.
- Sobel, J. M., Chen, G. F., Watt, L. R. & Schemske, D. W. (2010) The biology of speciation. *Evolution* **64** (2), 295–315.
- Sork, V. L., Nason, J., Campbell, D. R. & Fernandez, J. F. (1999) Landscape approaches to historical and contemporary gene flow in plants. *Trends in Ecology and Evolution* **14** (6), 219–223.

- Stace, C. A. (1989) *Plant taxonomy and biosystematics*. Edward Arnold, London, UK.
- Stanley, R. G. & Linskens, H. F. (1974) *Pollen: Biology, biochemistry, management*. Springer-Verlag, Berlin-Heidelberg, Germany.
- Stebbins, G. L. (1970) Adaptive radiation of reproductive characteristics in angiosperms, I: Pollination mechanisms. *Annual Review of Ecological Systems* **1**, 307–326.
- Stephens, S. G. & Finkner, M. D. (1953) Natural crossing in cotton. *Economic Botany* **7**, 257–269.
- Streinzer, M., Roth, N., Paulus, H. F. & Spaethe, J. (2019) Colour preference and spatial distribution of glaphyrid beetles suggest a key role in the maintenance of the colour polymorphism in the peacock anemone (*Anemone pavonina*, Ranunculaceae) in Northern Greece. *Journal of Comparative Physiology*. doi:10.1007/s00359-019-01360-2.
- Sun, M., Gross, K. & Schiestl, F. P. (2014) Floral adaptation to local pollinator guilds in a terrestrial orchid. *Annals of Botany* **113** (2), 289–300.
- Sun, S., Leshowitz, M. I. & Rychtář, J. The signalling game between plants and pollinators. *Scientific Reports* **8**, 6686. doi:10.1038/s41598-018-24779-0.
- Tao, Z. B., Ren, Z. X., Bernhardt, P., Liang, H., Li, H. D., Zhao, Y. H., Wang, H. & Li, D. Z. (2018) Does reproductive isolation reflect the segregation of colour forms in *Spiranthes sinensis* (Pers.) Ames complex (Orchidaceae) in the Chinese Himalayas? *Ecology and Evolution* **00**, 1–15. doi:10.1002/ece3.4067.
- van der Kooi, C. J., Dyer, A. G. & Stavenga, D. G. (2014) Is floral iridescence a biologically relevant cue in plant–pollinator signalling? *The New Phytologist* **205** (1), 18–20. doi:10.1111/nph.13066.

van der Niet, T. & Johnson, S. D. (2009) Patterns of plant speciation in the Cape Floristic Region. *Molecular Phylogenetics and Evolution* **51**, 85–93.

van der Niet, T., Peakall, R. & Johnson, S. D. (2014) Pollinator-driven ecological speciation in plants: New evidence and future perspectives. *Annals of Botany* **113** (2), 199–211.

van der Niet, T., Pirie, M. D., Shuttleworth, A., Johnson, S. D. & Midgley, J. J. (2014) Do pollinator distributions underlie the evolution of pollination ecotypes in the Cape shrub *Erica plukenetii*? *Annals of Botany* **113** (2), 301–316.

Vignolini, S., Moyroud, E., Hingant, T., Banks, H., Rudall, P. J., Steiner, U. & Glover, B. J. (2014a) The flower of *Hibiscus trionum* is both visibly and measurably iridescent. *New Phytologist* **205** (1), 97–101. doi:10.1111/nph.12958.

Vignolini, S., Moyroud, E., Hingant, T., Banks, H., Rudall, P. J., Steiner, U. & Glover, B. J. (2014b) Is floral iridescence a biologically relevant cue in plant–pollinator signalling? A response to van der Kooi et al. (2014b). *The New Phytologist* **205**, 21–22. doi:10.1111/nph.13178.

Vittoz, P. & Engler, R. (2007) Seed dispersal distances: A typology based on dispersal modes and plant traits. *Botanica Helvetica* **117**, 109–124.

von Witt, C. G., Anderson, B. A., Durbach, I. N. & Johnson, S. D. (2019) Breeding systems of floral colour forms in the *Drosera cistiflora* species complex. Manuscript under revision.

Waser, N. M. & Campbell, D. R. (2004) Ecological speciation in flowering plants. In U. Dieckmann, M. Doebeli, J. A. J. Metz & D. Tautz [Eds], *Adaptive speciation*, 264–277. Cambridge University Press, Cambridge, UK.

Waser, N. M., Chittka, L., Price, M. V., Williams, N. M. & Ollerton, J. (1996) Generalisation in pollination systems and why it matters. *Ecology* **77**, 1043–1060.

Waser, N. M. & Price, M. V. (1981) Pollinator choice and stabilising selection for flower colour in *Delphinium nelsonii*. *Evolution* **35** (2), 376–390.

Whitney, H. M., Kalle, M., Andrew, P., Chittka, L., Steiner, U. & Glover, B. J. (2009) Floral iridescence, produced by diffractive optics, acts as a cue for animal pollinators. *Science* **323**, 130–133.

Whittall, J. B. & Hodges, S. A. (2007) Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature* **447**, 706–709.

Wilbert, S. M., Schemske, D. W. & Bradshaw, H. D. (1997) Floral anthocyanins from two monkeyflower species with different pollinators. *Biochemical Systematics and Ecology* **25** (5), 437–443.

Wright, S. (1943) Isolation by distance. *Genetics* **28**, 114–138.

Wright, S. S. (1951) The genetical structure of populations. *Annals of Eugenics* **15** (4), 323–354.

CHAPTER SIX

GENERAL CONCLUSIONS

Key findings

The key findings of this study are: i) *Drosera cistiflora* s.l. is a partially self-compatible, highly pollinator-dependent species complex with individuals that appear to have strongly pollen-limited seed production; ii) soil chemistry does not seem to play a significant role in determining flower colour, since no colours are specific to a single vegetation or soil type and floral colour pigmentation was maintained in common-garden and soil switching experiments; iii) there is an overall association between flower colour and pollinator assemblages; iv) populations that are close together generally have similar pollinating fauna whilst those that are geographically distant have pollinating fauna that are more dissimilar; v) local *D. cistiflora* s.l. flower colours usually have a greater chance of being visited by pollinators than novel flower colours; vi) floral colour shifts across the distribution range of *D. cistiflora* s.l. typically appear to be adaptations to entire community compositions of pollinators mainly comprising hopliine beetles, and vii) robust pre-F₁ reproductive isolation of purple and red floral colour forms is largely attributed to geographical and pollinator isolation.

Information on pollinator assemblages is fundamental to unravelling the ecological evolution of plant reproductive traits (Waser et al. 1996, Johnson & Steiner 2000, Huber et al. 2005). To this end, findings linking the various *D. cistiflora* s.l. floral colour forms with pollinators of differing species, abundance and importance (Chapter 3), considered in the context of predominantly pollinator-reliant reproductive strategies of floral colour forms (Chapter 2), established potential for pollinator-mediated evolutionary shifts in flower colour. The subsequent use of reciprocal translocations in Chapter 4 provides strong evidence for local adaptation of *D. cistiflora* s.l. floral traits to the geographically framed pollinator environment. Since floral forms differ in traits other than colour (Figure 1), the additional use of model flowers was important for

establishing that colour itself formed the basis for insect choices. Whilst pink-, white-, red- and yellow-flowered forms are adapted to diverse assemblages of generalist pollinators, the more specialised hopliine beetle communities emerge as core selective agents in red-, white- and yellow-flowered forms overall.

Flower colour selection likely represents direct, combined innate and conditioned responses of insect pollinators to corolla colour (q.v. Weiss 1997) and visible pollen rewards of *D. cistiflora* s.l. flowers (Figure 1, Chapter 1; Chapter 4). Investigation into such pollinator response modes, which is beyond the scope of this thesis, should however consider whether corolla colour might be the product of convergence or advergence (such as Batesian floral mimicry) in floral signalling (q.v. Schiestl & Johnson 2013) rather than a direct selective response of pollinators to *D. cistiflora* s.l. flower colour per se. Here, although *D. cistiflora* s.l. flowers were locally abundant and dominant amid flowers of co-occurring species in all study populations, the colour preferences of a shared polylectic pollinator may be conditioned by the flower colour of rewarding, abundant co-flowering species in the pollinator's foraging range (e.g. Brown & Kodric-Brown 1979, Johnson 1994, Jersáková et al. 2012). For example, the presence of 500-1000 yellow-flowered *Sparaxis grandiflora* subsp. *fimbriata* (Iridaceae) plants adjacent one site of yellow-flowered *D. cistiflora* s.l. might exert influence on pollinator visits to *D. cistiflora* s.l. through their abundance, nectar rewards and apparent similarity in corolla colour to the yellow *D. cistiflora* s.l. floral colour form. Alternative floral traits including corolla shape and epidermal structural modifications may play a central role in local adaptation of yellow- and (also) purple-flowered *D. cistiflora* s.l. forms (Figure 1), possibly serving to facilitate pollinator visitation by enhancing ostensible food deception.

Whereas geographical floral colour shifts have previously been shown to be adaptive to specialist pollinator preferences (e.g. Newman et al. 2012), this is the first study to reveal that floral colour variation may be attributed to the local colour preferences of suites of essentially generalist pollinators. Considering the resulting selection mosaic corresponds to the broad distribution of *D. cistiflora* s.l. floral colour forms across the landscape, I define the forms as geographically divergent generalised pollination ecotypes, viz. where populations are frequently locally adapted to pollinator communities within their geographical area of extent. Coupled with those of Gómez et

al. (2008, 2014), my findings support the previously contested concept that variation in floral phenotype may represent a response to spatially divergent selection between partially different generalist pollinator assemblages.

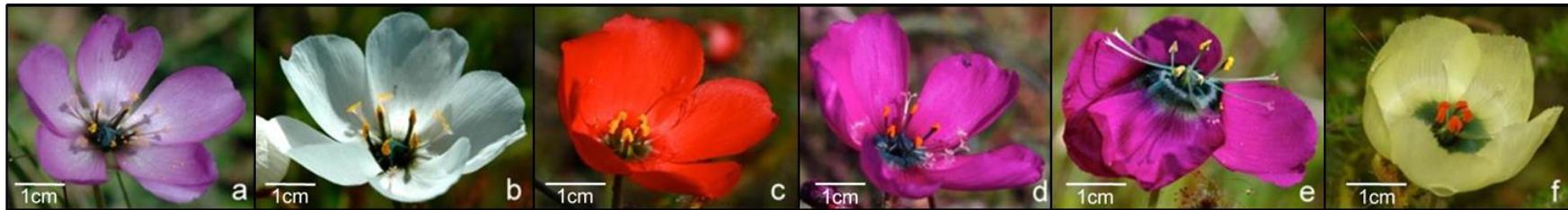


Figure 1. Visible differences in corolla traits between *Drosera cistiflora* s.l. floral colour forms, arranged from left to right in descending order of extant population number. A crateriform corolla shape is consistent between pink- (a), white- (b) and red-flowered (c) forms, as well as purple flowers at Darling 3 (d). Purple flowers at Durbanville (e) and all yellow-flowered forms (f) have reflexed petals and deep cup-shaped flowers, respectively. Corolla centres are distinctly metallic-iridescent in purple-flowered forms, and centres are circumscribed by patterns of structural reflectance in all forms. Corolla size does not appear to vary substantially among forms.

Evolutionary significance

***Drosera cistiflora* s.l.: a case for pollinator-driven speciation?**

The selection mosaic imposed by functional pollination groups appears to underlie the evolution and diversification of floral colour forms in *D. cistiflora* s.l., and to have moderately induced prezygotic reproductive isolation in the sympatric purple- and red-flowered forms examined (Chapter 5). Compelling evidence is thus provided by the *D. cistiflora* s.l. system for generalised pollination ecotypes undergoing incipient speciation, and this study accordingly sets the scene for further evolutionary research.

The apparent speciation potential of purple- and red-flowered ecotypes in secondary sympatry (Chapter 5) is measurably ascribable to well-defined pollinator isolation in the form of ethological isolation (q.v. Grant 1949). This is consistent with the notion of modularity, where flower colour responds to the divergent selective pressures of generalist yet coacting pollinator groups and their respective antagonistic colour preferences. On account of small population sizes that limit study potential, it is not, however, possible to determine whether sympatric pink and purple, purple and white, and red and white *D. cistiflora* s.l. ecotypes are ethologically isolated. Analyses for phylogenetic signal in *D. cistiflora* s.l. floral colour ecotypes and their association with pollination niches may nevertheless establish whether generalist pollinators are primary drivers of divergence among ecotypes.

Even though *D. cistiflora* s.l. flower colour did not appear to be physiologically influenced by soils (Chapter 3), it is conceivable that incidental mutations in flower colour arose in microgeographical niches across the range of the species complex, with putative genetic differentiation being maintained and/or enhanced thereafter by local pollinator preferences. Mutations in *D. cistiflora* s.l. flower colour may have emerged as floral pigment-gain events from a basal colour, plausibly controlled by a pink or white allele, since these colours occur across the entire geographical range of the species complex. White flowers may alternatively be mutants caused by an enzyme deficiency in the anthocyanin biosynthetic pathway (Coberly & Rausher 2003, Strauss & Whittall 2006) of certain pink- and red-flowered populations. The work of McCarthy

et al. (2015) suggests that sympatric floral colour mutations in *Nicotiana* consequent to polyploid and homoploid hybrid divergence may theoretically be driven or retained by pollinator shifts. Polyploidy, as inferred by the initial phylogenetic analyses of Rivadavia et al. (2003), combined with divergent selection by pollinators, may hypothetically explain floral colour variation in *D. cistiflora* s.l., in particular the evolution of purple-flowered forms in sympatry with red, pink and white, respectively. In whichever manner flower colours may have arisen, this manuscript highlights the evolutionary significance of generalised pollination systems as potential drivers of phenotypic divergence both in sympatry and allopatry, and suggests a role for pollinator colour preferences in incipient speciation of *D. cistiflora* s.l.

The prevalence of divergent pollinator-mediated selection does not preclude the potential for subsidiary drivers to either counter or bolster the impact thereof on flower colour. Thus, floral colour expression may instead be the outcome of integrated conflicting and/or congruent mechanistic effects. Meriting particular consideration are pleiotropy or non-pollinator biotic selective agents (e.g. Levin & Brack 1995; Irwin et al. 2003; Strauss & Whittall 2006; Coberly & Rausher 2008; Carlson & Holsinger 2010, 2012), and pollinator habitat associations, all of which can indirectly influence flower colour selection by pollinators (q.v. Patterson & Givnish 2003).

Taxonomic implications

Generalised pollination ecotypes with floral colour divergence substantiate the need for a multidisciplinary approach to taxonomy encompassing molecular systematics, pollination ecology, biogeography, physiology, cytology, biochemistry and morphology. This approach may establish whether *D. cistiflora* s.l. represents a monotypic species [according to the ‘Biological Species Concept’ (sensu Mayr 1942)] with high intraspecific variability, or rather a polytypic species complex where population groups should be ranked as subspecies and/or varieties, or whether it may even constitute several distinct species.

Molecular study of chloroplast and nuclear DNA sequences of floral colour ecotypes may help to identify genetic differences. If monophyly is not supported, reconstruction of ancestral character states may determine whether lineages of different ecotypes (if any) correspond to generalist pollinator shifts, resulting in patterns of convergent evolution.

Hand cross-pollinations between all floral colour forms would shed light on whether barriers to gene flow exist among *D. cistiflora* s.l. ecotypes. Such experimentation would however be unethical if carried out in allopatric populations in situ unless seeds were harvested prior to dispersal, and the difficulty of seed germination and rearing of plants to the flowering stage in greenhouse conditions may render ex situ efforts ineffectual. Apparent introgressive hybridisation between pink- and white-flowered ecotypes growing in proximity in the Darling region indicates that some forms may interbreed and produce viable seed in sites where they are not isolated by geographical or pollination barriers. Hand-crossing between emasculated, discrete purple- and red-flowered ecotypes in sympatry resulted in poor seed set (Chapter 5). Here, the strong influence of pollinator isolation would be asserted should seed be determined to be viable. Pink-flowered ecotypes were not compatible with sympatric purple-flowered ecotypes, nor were white-flowered ecotypes compatible with pollen from sympatric red-flowered ecotypes (Chapter 2). Anther and stigma formation is abnormal in these particular white flowers, and since they did not set seed with other treatments, the plants may be functionally female sterile. Their pollen was however compatible with red-flowered ecotypes, although such seed set was low in comparison to that from intra-ecotypic hand-cross, hand-self and natural pollination treatments in these red flowers. These preliminary findings indicate that pink- and white-flowered ecotypes may represent varieties subject to incipient speciation, whilst the strong reproductive isolation of purple- (crateriform corolla) and red-flowered ecotypes (Chapter 5) suggests that this purple-flowered taxon is genetically novel and defends the classification of red-flowered forms as *Drosera rubripetala* Debbert (Chapter 1, Table 2). It remains to be tested whether the yellow-flowered ecotype is reproductively isolated, although my findings of pollinator-mediated adaptation and geographical isolation suggest that it may at the very least be a candidate for description as a subspecies of *D. cistiflora* s.l. Evidence thus refutes the notion that *D. cistiflora* s.l. may be a monotypic taxon. Microsatellite markers may be used to confirm the absence

or validate the presence of interbreeding (Sork et al. 1999, Selkoe & Toonen 2006, Malan 2013), thereby allowing more accurate taxonomic ranks to be assigned to *D. cistiflora* s.l. ecotypes.

Further examination of morphological traits of each ecotype (for which I have collected floral trait data) and study of pigment biosynthetic pathways will also aid the requisite revision of the species complex.

Conservation implications

Partial self-incompatibility, high pollinator dependence and inference of pollen quantity and quality limitation, and disparities thereof between *D. cistiflora* s.l. floral colour forms (Chapter 2) emphasise the importance of recognising pollination ecotypes in conservation assessments of taxonomically unresolved species. Additionally, since diverse generalist pollinators may ensure reproductive success of pollinator-dependent plants by offsetting the risks of pollinator failure (Bond 1994, Knight et al. 2005, Anderson et al. 2014), and may even have the potential to drive floral evolution, environmental impact assessments and ecological management plans must not underestimate the value of all-inclusive pollinator conservation. Protected areas should therefore always incorporate large buffer zones (that minimise edge effects) as well as biodiversity corridors to uphold pollinator diversity and mitigate pollination failure. Future study of *D. cistiflora* s.l. habitat fragmentation combined with knowledge of breeding systems (Chapter 2) may inform best practice for conservation of generalised pollination ecotypes.

A number of *Drosera* populations are under threat from carnivorous plant collectors and “so-called plant lovers” (Debbert 1991), at times under the guise of ex situ conservation. Of all the sundews, *D. cistiflora* s.l. ecotypes are amongst the most desirable for cultivation. In light of natural pollination requirements, ex situ conservation in the absence of pollinators is usually in vain. Secondly, considering all floral colour forms are already kept in ex situ collections, there are no grounds for propagation material to be collected from the wild. Seed collection is only advised for

the purposes of Kew's Millennium Seed Bank Partnership. Any further collection should henceforth be strictly prohibited and only in situ conservation executed. Collection exceptions may be granted by the relevant local conservation authority for: i) ornamental horticulturists to sustainably harvest leaf cuttings, since these can potentially be grown successfully using the water strike method, and ii) whole plants; leaf, root and/or stem cuttings, and seeds (in addition to those intended for banking) to be collected by authorised plant search and rescue operation teams prior to construction activities that would otherwise destroy the plants.

A more appropriate taxonomic treatment may produce significant positive conservation implications for *D. cistiflora* s.l., its associated pollinator and plant communities, and overall habitats. The species complex is currently evaluated as Least Concern in the Red List of South African Plants (Foden & Potter 2005, Raimondo et al. 2009) as it was not selected in any one of four screening processes highlighting potential taxa of conservation concern for detailed assessment by the Threatened Species Programme of the South African National Biodiversity Institute. Yet when red-listed individually using IUCN Categories and Criteria (IUCN 2012), all of the *D. cistiflora* s.l. floral colour ecotypes examined in this study were found to be of conservation concern, with purple (reflexed petals) proposed to be Critically Endangered; purple (crateriform corolla), red and yellow Endangered; white Vulnerable, and pink Declining (Table 1). Purple, red and yellow floral colour ecotypes persist by means of few, threatened populations. Purple-flowered forms with reflexed petals are highly threatened by invasive alien vegetation; overcollection; severe habitat fragmentation; urban sprawl, as well as a proposed pipeline (pers. obs and Western Cape Wetlands Forum, pers. comm.). Habitat fragmentation may constrain entomophily (Pauw 2004, 2007) through pollinator loss, and absence of pollinators places pollinator-reliant plants at risk of ultimate extinction (Bond 1994, Pauw & Bond 2011, Pauw & Hawkins 2011, Anderson et al. 2014). Likely fragmentation-induced pollinator declines, plant infertility (Chapter 2) and apparent survival through clonality thus present the small urban population of purple-flowered forms at Durbanville with a significant risk of extinction in the imminent future. Two of the three localities of yellow-flowered forms are threatened by impending tungsten mining north of Piketberg (Verlorenvlei Coalition members and local farmers, pers. comm. and pers. obs) and the third population is severely habitat-fragmented. *D. cistiflora* s.l. is thus in dire need of a revised conservation assessment

for the future of these ecotypes and their associated ecosystems to be secured through effective conservation management strategies.

Table 1. Draft Red List assessments of *Drosera cistiflora* s.l. ecotypes examined in this study with proposed Red List statuses as per criteria of the International Union for Conservation of Nature (2012). Purple-flowered forms are separated into two morphological groups to accommodate their unique extinction threats and recognise the conservation urgency of the purple-flowered populations with reflexed petals.

	<i>Drosera cistiflora</i> s.l. ecotype					
	Pink	Purple (crateriform corolla)	Purple (reflexed petals)	Red	White	Yellow
Number of extant populations	< 100	≈ 5 (-10)	3–4	≈ 6	< 40	3
Observed or estimated reduction in population number	≥ 20%	≥ 30%	≥ 50%	≥ 30%	≥ 20%	≥ 50%
Total number of plants	> 10000	< 3000	< 500 (< 250 mature individuals)	< 3000	≈ 10000 (< 10 000 mature individuals)	≈ 1000
Extent of occurrence (km ²)	> 20000	< 10000	< 1000	< 6000	> 20000	< 500
Area of occupancy (km ²)	< 10	< 1	< 0.2	< 0.5	< 10	< 0.2
Endemism	N., W. & E. Cape	W. Cape	W. Cape	W. Cape	N. & W. Cape	Piketberg
Rarity	Widespread	Rare	Critically rare	Rare	Widespread	Critically rare
Major habitat	Fynbos	Fynbos	Fynbos	Fynbos	Fynbos	Fynbos
Major system	Seasonal terrestrial wetlands	Seasonal terrestrial wetlands	Seasonal terrestrial wetlands	Seasonal terrestrial wetlands	Seasonal terrestrial wetlands	Seasonal terrestrial wetlands
Habitat description	Sandy loam to clay soils on flats and slopes in fynbos and renosterveld	Sandy, clayey or loam soils on flats in sand fynbos, alluvium fynbos and renosterveld	Sand to loam soils on flats in sand fynbos and granite and shale renosterveld	Sand to loam soils on flats in sand fynbos and granite renosterveld	Sandy loam to clay soils on flats and slopes in sand fynbos, sandstone fynbos and granite renosterveld	Sandy loam to clay soils on flats and slopes in sandstone fynbos and shale renosterveld

Breeding system and inferred pollen limitation	Partially self-incompatible, highly pollinator-dependent, pollen-limited	Partially self-incompatible, completely pollinator-dependent and pollen-limited	Indeterminable; extremely low natural seed set	Partially self-incompatible, highly pollinator-dependent, pollen-limited	Partially self-incompatible, highly pollinator-dependent, pollen-limited	Partially to fully self-incompatible, highly pollinator-dependent and pollen-limited
Pollination system	Generalised, primarily hopliine beetles and beetles of families Meloidae and Tenebrionidae	Generalised, primarily hopliine beetles and beetles of family Melyridae	Generalised, primarily hopliine beetles and beetles of family Melyridae	Generalised, primarily hopliine beetles	Generalised, primarily hopliine beetles	Generalised, primarily hopliine beetles and beetles of family Melyridae
Threats	Urban expansion, infrastructure development, agricultural expansion, overgrazing, overcollection, invasive alien vegetation, habitat fragmentation, pollution, drought, groundwater extraction	Urban expansion, infrastructure development, agricultural expansion, overgrazing, overcollection, invasive alien vegetation, severe habitat fragmentation, population size, pollution, drought, groundwater extraction	Urban expansion, infrastructure development, agricultural expansion, overgrazing, overcollection, invasive alien vegetation, severe habitat fragmentation, population size, infertility, pollution, drought, groundwater extraction	Urban expansion, infrastructure development, agricultural expansion, overgrazing, overcollection, invasive alien vegetation, severe habitat fragmentation, pollution, drought, groundwater extraction	Urban expansion, infrastructure development, agricultural expansion, overgrazing, overcollection, invasive alien vegetation, habitat fragmentation, pollution, drought, groundwater extraction	Tungsten mining, infrastructure development, agricultural expansion, too frequent fire, overcollection, invasive alien vegetation, habitat fragmentation, predisposition to inbreeding, depression, pollution, drought, groundwater extraction
Population trend	Decreasing	Decreasing	Decreasing	Decreasing	Decreasing	Decreasing
Proposed IUCN Red List status and criteria	Least Concern	Endangered B2ab(i,ii,iii,iv,v)	Critically Endangered B2ab(i,ii,iii,iv,v); C2a(i)	Endangered B2ab(i,ii,iii,iv,v)	Vulnerable C2a(i); D2	Endangered A4cde; B1ab(i,ii,iii,iv,v)+2ab(i,ii,iii,iv,v)

Proposed South African Red List status and criteria	Declining	Endangered B2ab(i,ii,iii,iv, v)	Critically Endangered B2ab(i,ii,iii,iv, v); C2a(i)	Endangered B2ab(i,ii,iii,iv, v)	Vulnerable C2a(i); D2	Endangered A4cde; B1ab(i,ii,iii,iv, v)+2ab(i,ii,iii,iv,v)
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An improved understanding of the patterns and processes influencing floral colour divergence in *D. cistiflora* s.l. draws further attention to the often-undervalued role of pollinators, particularly generalist pollinators, in plant species diversity and habitat integrity maintenance in the Greater Cape Floristic Region. A sound comprehension of plant-pollinator relationships is thus crucial for successful long-term conservation of biodiversity and the ensuing sustenance of ecosystem goods and services.

Further research areas

In summary, the main areas for further work are as follows: i) construction of a phylogenetic tree for the *D. cistiflora* species complex to determine if ecotypes are different lineages, and to establish the number and direction of corolla colour shifts and whether these correspond to pollinator shifts (q.v. Valente et al. 2012); ii) ex situ crossing experiments to help assess patterns of gene flow between sympatric and allopatric ecotypes, particularly yellow-flowered ecotypes which have not yet been tested for compatibility with other ecotypes; iii) microsatellite markers to confirm the absence or establish the presence of interbreeding (q.v. Selkoe & Toonen 2006); iv) thorough examination of morphological differences between all floral colour forms combined with the results of i, ii and iii to establish the accurate taxonomic rank of each ecotype, and v) interim revision of the *D. cistiflora* s.l. Red List assessment with provision for ecotypes pending the outcome of taxonomic assessments.

References

Anderson, B., Allsopp, N., Ellis, A. G., Johnson, S. D., Midgley, J. J., Pauw, A. & Rodger, J. (2014) Biotic interactions. *In* N. Allsopp, J. F. Colville, A. G. Verboom & R. M. Cowling [Eds], *Ecology and evolution of fynbos: Understanding megadiversity*. Oxford University Press, Oxford, UK.

Bond, W. J. (1994) Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical Transactions of the Royal Society B: Biological Sciences* **344**, 83–90.

Brown, J. H. & Kodric-Brown, A. (1979) Convergence, competition, and mimicry in a temperate community of hummingbird-pollinated flowers. *Ecology* **60** (5), 1022–1035.

Carlson, J. E. & Holsinger, K. E. (2010) Natural selection on inflorescence colour polymorphisms in wild *Protea* populations: The role of pollinators, seed predators and inter-trait correlations. *American Journal of Botany* **97**, 934–944.

Carlson, J. E. & Holsinger, K. E. (2012) Direct and indirect selection on floral pigmentation by pollinators and seed predators in a colour polymorphic South African shrub. *Oecologia* **171**, 905–919.

Coberly, L. C. & Rausher, M. D. (2003) Analysis of a chalcone synthase mutant in *Ipomoea purpurea* reveals a novel function for flavonoids: amelioration of heat stress. *Molecular Ecology* **12**, 1113–1124.

Coberly, L. C. & Rausher, M. D. (2008) Pleiotropic effects of an allele producing white flowers in *Ipomoea purpurea*. *Evolution* **62**, 1076–1085.

Debbert, P. (1991) Einige neue arten der gattungen *Drosera* (Droseraceae) und *Pinguicula* (Lentibulariaceae). *Mitteilungen der Botanischen Staatssammlung München* **30**, 373–380.

Foden, W. & Potter, L. (2005) *Drosera cistiflora* L. National Assessment: Red List of South African Plants version 2014.1. Accessed on 16 June 2014.

Gómez, J. M., Bosch, J., Perfectti, F., Fernández, J. D., Abdelaziz, M. & Camacho, J. P. M. (2008) Spatial variation in selection on corolla shape in a generalist plant is promoted by the preference patterns of its local pollinators. *Proceedings of the Royal Society B — Biological Sciences* **275**, 2241–2249.

Gómez, J. M., Muñoz-Pajares, A. J., Abdelaziz, M., Lorite, J. & Perfectti, F. (2014) Evolution of pollination niches and floral divergence in the generalist plant *Erysimum mediohispanicum*. *Annals of Botany* **113** (2), 237–249.

Grant, V. (1949) Pollination systems as isolating mechanisms in angiosperms. *Evolution* **3**, 82–97.

Huber, F. K., Kaiser, R., Sauter, W. & Schiestl, F. P. (2005) Floral scent emission and pollinator attraction in two species of *Gymnadenia* (Orchidaceae). *Oecologia* **142** (4), 564–575.

Irwin, R. E., Strauss, S. Y., Storz, S., Emerson, A. & Guibert, G. (2003) The role of herbivores in the maintenance of a flower colour polymorphism in wild radish. *Ecology* **84** (7), 1733–1743.

IUCN (2012) *IUCN Red List Categories and Criteria: Version 3.1*. Second Edition. Gland, Switzerland and Cambridge, UK: IUCN. iv + 32pp.

Jersáková, J., Jürgens, A., Šmilauer, P. & Johnson, S. D. (2012) The evolution of floral mimicry: Identifying traits that visually attract pollinators. *Functional Ecology* **26** (6), 1381–1389.

Johnson, S. D. (1994) Evidence for Batesian mimicry in a butterfly-pollinated orchid. *Biological Journal of the Linnean Society* **53** (1), 91–104.

- Johnson, S. D. & Steiner, K. E. (2000) Generalisation versus specialisation in plant pollination systems. *Trends in Ecology and Evolution* **15**, 140–143.
- Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mitchell, R. J. & Ashman, T. L. (2005) Pollen limitation of plant reproduction: Pattern and process. *Annual Review of Ecology Evolution and Systematics* **36**, 467–497.
- Levin, D. A. & Brack, E. T. (1995) Natural selection against white petals in phlox. *Evolution* **49**, 1017–1022.
- Malan, M. (2013) Intraspecific variation in *Erica coccinea*. M.Sc. thesis. University of Cape Town, South Africa.
- Mayr, E. (1942) *Systematics and the origin of species*. Columbia University Press, New York, USA.
- McCarthy, E. W., Arnold, S. E. J., Chittka, L., le Comber, S. C., Verity, R., Dodsworth, S., Knapp, S., Kelly, L. J., Chase, M. W., Baldwin, I. T., Kovařík, A., Mhiri, C., Taylor, L. & Leitch, A. R. (2015) The effect of polyploidy and hybridisation on the evolution of floral colour in *Nicotiana* (Solanaceae). *Annals of Botany* **115**, 1117–1131.
- Newman, E., Anderson, B. & Johnson, S. D. (2012) Flower colour adaptation in a mimetic orchid. *Proceedings of the Royal Society B — Biological Sciences* **279**, 2309–2313.
- Patterson, T. B. & Givnish, T. J. (2003) Geographic cohesion, chromosomal evolution, parallel adaptive radiations, and consequent floral adaptations in *Calochortus* (Calochortaceae): Evidence from a cpDNA phylogeny. *New Phytologist* **161** (1), 253–264.
- Pauw, A. (2004) *Variation in pollination across a fragmented landscape at the Cape of Africa*. PhD thesis. University of Cape Town.

- Pauw, A. (2007) Collapse of a pollination web in small conservation areas. *Ecology* **88** (7), 1759–1769.
- Pauw, A. & Bond, W. J. (2011) Mutualisms matter: Pollination rate limits the distribution of oil-secreting orchids. *Oikos* **120**, 1531–1538.
- Pauw, A. & Hawkins, J. A. (2011) Reconstruction of historical pollination rates reveals linked declines of pollinators and plants. *Oikos* **120**, 344–349.
- Raimondo, D., von Staden, L., Foden, W., Victor, J. E., Helme, N. A., Turner, R. C., Kamundi, D. A. & Manyama, P. A. (2009) Red List of South African plants. *Strelitzia* **25**. South African National Biodiversity Institute, Pretoria, South Africa.
- Rivadavia, F., Kondo, K., Kato, M. & Hasebe, M. (2003) Phylogeny of the sundews, *Drosera* (Droseraceae), based on chloroplast *rbcL* and nuclear 18S ribosomal DNA sequences. *American Journal of Botany* **90** (1), 123–130.
- Schiestl, F. P. & Johnson, S. D. (2013) Pollinator-mediated evolution of floral signals. *Trends in Ecology and Evolution* **28** (5), 307–315.
- Selkoe, K. A. & Toonen, R. J. (2006) Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. *Ecology Letters* **9**, 615–629.
- Sork, V. L., Nason, J., Campbell, D. R. & Fernandez, J. F. (1999) Landscape approaches to historical and contemporary gene flow in plants. *Trends in Ecology and Evolution* **14** (6), 219–223.
- Strauss, S. Y. & Whittall, J. B. (2006) Non-pollinator agents of selection on floral traits. In L. D. Harder and S. C. H. Barrett [Eds], *Ecology and evolution of flowers*, 120–138. Oxford University Press, Oxford, UK.
- Valente, L. M., Manning, J. C., Goldblatt, P. & Vargas, P. (2012) Did pollination shifts drive diversification in southern African *Gladiolus*? Evaluating the model of pollinator-driven speciation. *The American Naturalist* **180** (1), 83–98. doi:10.1086/666003.

Waser, N. M., Chittka, L. Price, M. V., Williams, N. M. & Ollerton, J. (1996) Generalisation in pollination systems and why it matters. *Ecology* **77**, 1043–1060.

Weiss, M. R. (1997) Innate colour preferences and flexible colour learning in the pipevine swallowtail. *Animal Behaviour* **53**, 1043–1052.

APPENDICES

Supplementary Tables

Supplementary Table 1. Marginal model means (and asymmetrical 95% confidence intervals) calculated for proportion of flowers that set fruit and number of seeds set per fruit, proceeding from autonomous self-pollination, open pollination, hand self-pollination and hand cross-pollination in each *Drosera cistiflora* s.l. floral colour form. One flower per plant was treated (sample sizes, followed by the number of experimental populations, are shown in parentheses after 95% confidence intervals).

<i>Drosera cistiflora</i> s.l. flower colour	Fruit set per flower				Seed set per fruit			
	Autonomous self-pollination	Open pollination	Hand self-pollination	Hand cross-pollination	Autonomous self-pollination	Open pollination	Hand self-pollination	Hand cross-pollination
Pink	0.31 (95% confidence interval 0.12, 0.60) [31, 3]	0.91 (0.74, 0.97) [73, 4]	0.93 (0.77, 0.98) [31, 3]	0.99 (0.95, 1.00) [34, 3]	36.84 (18.56, 73.11) [31, 3]	109.79 (62.69, 192.25) [73, 4]	115.59 (62.69, 213.11) [31, 3]	257.50 (140.94, 470.44) [34, 3]
Purple	0.01 (0.00, 0.05) [10, 1]	0.14 (0.03, 0.48) [40, 2]	0.18 (0.04, 0.58) [8, 1]	0.60 (0.19, 0.91) [8, 1]	0.93 (0.06, 14.93) [10, 1]	1.20 (0.29, 5.01) [40, 2]	204.87 (51.75, 811.05) [8, 1]	130.01 (42.13, 401.17) [8, 1]
Red	0.26 (0.08, 0.58) [35, 3]	0.88 (0.66, 0.97) [56, 3]	0.91 (0.70, 0.98) [28, 3]	0.99 (0.93, 1.00) [28, 3]	24.04 (10.00, 57.82) [35, 3]	253.58 (134.91, 476.61) [56, 3]	237.90 (120.08, 471.34) [28, 3]	301.83 (154.07, 591.31) [28, 3]
White	0.28 (0.07, 0.68) [16, 2]	0.90 (0.61, 0.98) [35, 2]	0.92 (0.66, 0.98) [16, 2]	0.99 (0.92, 1.00) [16, 2]	40.22 (12.78, 126.61) [16, 2]	353.21 (161.82, 770.95) [35, 2]	279.92 (121.46, 645.11) [16, 2]	385.53 (167.36, 888.10) [16, 2]
Yellow	0.01 (0.00, 0.03) [26, 3]	0.13 (0.03, 0.38) [51, 3]	0.16 (0.04, 0.46) [24, 3]	0.57 (0.23, 0.85) [24, 3]	23.31 (7.69, 70.66) [26, 3]	22.09 (8.39, 58.18) [51, 3]	32.57 (11.19, 94.83) [24, 3]	174.81 (80.87, 377.88) [24, 3]

Supplementary Table 2. Posthoc (Tukey) tests of differences in proportion of fruit set and numbers of seeds set between treatment conditions (O = Open pollination, AS = Autonomous self-pollination, HS = Hand self-pollination and HC = Hand cross-pollination) in *Drosera cistiflora* s.l.

Treatment comparison	Proportion of fruit set				Number of seeds per fruit			
	Estimate	SE	z	p	Estimate	SE	z	p
O - AS	3.084	0.386	7.995	< 0.001	1.661	0.193	8.624	0.000
HS - AS	3.358	0.445	7.546	< 0.001	1.725	0.196	8.812	0.000
HC - AS	5.28	0.581	9.09	< 0.001	2.209	0.196	11.280	0.000
HS - O	0.273	0.39	0.699	0.894	0.065	0.131	0.492	0.961
HC - O	2.196	0.49	4.484	< 0.001	0.548	0.130	4.207	0.000
HC - HS	1.923	0.545	3.526	0.002	0.484	0.141	3.424	0.003

Supplementary Table 3. Posthoc (Tukey) tests of differences in proportion fruit set and seed set per fruit between *Drosera cistiflora* s.l. floral colour forms

<i>Drosera cistiflora</i> s.l. floral colour form comparison	Proportion of fruit set				Number of seeds per fruit			
	Estimate	SE	z	p	Estimate	SE	z	p
Pink - Purple	4.049	1.071	3.781	0.001	1.301	0.575	2.261	0.158
Red - Purple	3.821	1.112	3.437	0.005	1.922	0.595	3.232	0.011
White - Purple	3.928	1.222	3.215	0.011	2.226	0.634	3.509	0.004
Yellow - Purple	-0.138	1.117	-0.123	1.000	0.478	0.629	0.760	0.942
Red - Pink	-0.228	0.904	-0.252	0.999	0.621	0.411	1.511	0.555
White - Pink	-0.121	1.037	-0.117	1.000	0.925	0.466	1.983	0.274
Yellow - Pink	-4.187	0.953	-4.394	< 0.001	-0.823	0.462	-1.780	0.385
White - Red	0.107	1.084	0.099	1.000	0.305	0.490	0.622	0.972
Yellow - Red	-3.959	0.997	-3.973	0.001	-1.444	0.487	-2.967	0.025
Yellow - White	-4.066	1.119	-3.635	0.003	-1.748	0.534	-3.271	0.009

Supplementary Table 4. Posthoc (Tukey) tests of differences in seed set per fruit between treatments in each *Drosera cistiflora* s.l. floral colour form

Treatment comparison	Seed set per fruit				
	<i>Drosera cistiflora</i> s.l. floral colour form	Estimate	SE	z	p
O - AS	Pink	1.092	0.270	4.041	0.000
HS - AS	Pink	1.143	0.277	4.134	0.000
HC - AS	Pink	1.944	0.275	7.063	< 0.000
HS - O	Pink	0.051	0.223	0.230	0.996
HC - O	Pink	0.852	0.216	3.955	0.000
HC - HS	Pink	0.801	0.231	3.465	0.003
O - AS	Purple	0.254	1.498	0.170	0.998
HS - AS	Purple	5.391	1.424	3.786	0.001
HC - AS	Purple	4.936	1.365	3.615	0.002
HS - O	Purple	5.137	0.858	5.987	< 0.000
HC - O	Purple	4.682	0.757	6.181	< 0.000
HC - HS	Purple	-0.455	0.598	-0.760	0.872
O - AS	Red	2.356	0.362	6.502	< 0.000
HS - AS	Red	2.292	0.379	6.043	< 0.000
HC - AS	Red	2.530	0.376	6.728	< 0.000
HS - O	Red	-0.064	0.222	-0.287	0.992
HC - O	Red	0.174	0.212	0.821	0.845
HC - HS	Red	0.238	0.248	0.960	0.772
O - AS	White	2.173	0.484	4.490	< 0.000
HS - AS	White	1.940	0.511	3.798	0.001
HC - AS	White	2.260	0.509	4.441	< 0.000
HS - O	White	-0.233	0.274	-0.849	0.831
HC - O	White	0.088	0.273	0.320	0.989
HC - HS	White	0.320	0.312	1.025	0.735
O - AS	Yellow	-0.054	0.498	-0.108	1.000
HS - AS	Yellow	0.335	0.539	0.620	0.926
HC - AS	Yellow	2.015	0.546	3.691	0.001
HS - O	Yellow	0.388	0.470	0.826	0.842
HC - O	Yellow	2.069	0.470	4.398	< 0.000
HC - HS	Yellow	1.680	0.525	3.204	0.007

Supplementary Table 5. Posthoc (Tukey) tests of differences in seed set per fruit between *Drosera cistiflora* s.l. floral colour forms for each treatment

		Seed set per fruit			
<i>Drosera cistiflora</i> s.l.					
floral colour form					
comparison	Treatment	Estimate	SE	z	p
Pink - Purple	AS	3.675	1.456	2.523	0.086
Red - Purple	AS	3.248	1.483	2.190	0.183
White - Purple	AS	3.763	1.530	2.459	0.100
Yellow - Purple	AS	3.217	1.521	2.115	0.214
Red - Pink	AS	-0.427	0.568	-0.751	0.944
White - Pink	AS	0.088	0.681	0.129	1.000
Yellow - Pink	AS	-0.458	0.663	-0.691	0.958
White - Red	AS	0.515	0.737	0.698	0.957
Yellow - Red	AS	-0.031	0.724	-0.043	1.000
Yellow - White	AS	-0.546	0.812	-0.672	0.962
Pink - Purple	O	4.513	0.781	5.775	0.000
Red - Purple	O	5.350	0.795	6.725	0.000
White - Purple	O	5.681	0.829	6.850	0.000
Yellow - Purple	O	2.909	0.878	3.315	0.008
Red - Pink	O	0.837	0.430	1.945	0.294
White - Pink	O	1.169	0.490	2.384	0.120
Yellow - Pink	O	-1.603	0.569	-2.818	0.039
White - Red	O	0.331	0.512	0.647	0.967
Yellow - Red	O	-2.441	0.589	-4.143	0.000
Yellow - White	O	-2.772	0.634	-4.369	0.000
Pink - Purple	HS	-0.572	0.768	-0.745	0.946
Red - Purple	HS	0.149	0.784	0.191	1.000
White - Purple	HS	0.312	0.821	0.380	0.996
Yellow - Purple	HS	-1.839	0.885	-2.078	0.230
Red - Pink	HS	0.722	0.468	1.542	0.535
White - Pink	HS	0.884	0.528	1.675	0.450
Yellow - Pink	HS	-1.267	0.629	-2.012	0.260
White - Red	HS	0.163	0.551	0.295	0.998
Yellow - Red	HS	-1.989	0.648	-3.069	0.018
Yellow - White	HS	-2.151	0.692	-3.108	0.016
Pink - Purple	HC	0.683	0.652	1.048	0.833
Red - Purple	HC	0.842	0.670	1.258	0.717
White - Purple	HC	1.087	0.715	1.519	0.550
Yellow - Purple	HC	0.296	0.697	0.425	0.993

Red - Pink	HC	0.159	0.461	0.345	0.997
White - Pink	HC	0.404	0.525	0.768	0.940
Yellow - Pink	HC	-0.387	0.499	-0.776	0.938
White - Red	HC	0.245	0.547	0.448	0.992
Yellow - Red	HC	-0.546	0.522	-1.046	0.834
Yellow - White	HC	-0.791	0.580	-1.365	0.650

Supplementary Table 6. Detailed geology and vegetation attributes of GPS-georeferenced populations of *Drosera cistiflora* s.l. floral colour forms. GPS points and other precise locality information have been omitted owing to the sensitive nature of these populations and their vulnerability to overcollection.

<i>Drosera cistiflora</i> s.l. flower colour	Locality	Geology code and description	Vegetation type
Pink	Abbotsdale	NCa (Granite, mainly coarse-grained porphyritic with fine-grained leucocratic, fine-to-medium-grained porphyritic and medium-grained biotitic variants)	Swartland Granite Renosterveld
Pink	Bain's Kloof	Qt (Gritty sand; scree)	Hawequas Sandstone Fynbos
Pink	Between Worcester and Villiersdorp	Qs (Light-grey to pale-red sandy soil)	Brede Quartzite Fynbos
Pink	Caledon	Dr (Light-grey feldspathic sandstone, siltstone and micaceous shale bands)	Rûens Silcrete Renosterveld
Pink	Ceres 1	Dv (Shale, siltstone and subordinate sandstone; fossiliferous)	Ceres Shale Renosterveld

Pink	Ceres 2	Qt (Gritty sand; scree)	Breede Alluvium Fynbos
Pink	Darling 1 (i)	NCd (Granite, mainly coarse-grained porphyritic with porphyritic biotitic, leucocratic, oven-grained biotitic and tourmaline-bearing variants; granodiorite)	Swartland Granite Renosterveld
Pink	Darling 1 (ii)	NCd (Granite: mainly coarse-grained porphyritic with porphyritic biotitic, leucocratic, oven-grained biotitic and tourmaline-bearing variants; granodiorite)	Swartland Granite Renosterveld
Pink	Darling 2 (i)	NCd (Granite, mainly coarse-grained porphyritic with porphyritic biotitic, leucocratic, oven-grained biotitic and tourmaline-bearing variants; granodiorite)	Swartland Granite Renosterveld
Pink	Darling 2 (ii)	NCd (Granite, mainly coarse-grained porphyritic with porphyritic biotitic, leucocratic, oven-grained biotitic and tourmaline-bearing variants; granodiorite)	Swartland Granite Renosterveld
Pink	Darling 8	Qgg (Gravelly clay/loam soil)	Swartland Granite Renosterveld

Pink	Durbanville 1 (i)	Qg (Loam and sandy loam)	Swartland Shale Renosterveld
Pink	Durbanville 1 (ii)	Nt (Greywacke, phyllite and quartzitic sandstone; interbedded lava and tuff)	Swartland Shale Renosterveld
Pink	Durbanville 2	Qs (Light-grey to pale-red sandy soil)	Swartland Shale Renosterveld
Pink	Joostenberg	NCs (Granite, mainly coarse-grained porphyritic with medium-to-coarse-grained, fine-grained porphyritic, fine-grained leucocratic, hybridic, fine-to-medium-grained tourmaline-bearing and coarse-grained biotitic variants)	Swartland Granite Renosterveld
Pink	Karwyderskraal Road	Dv (Shale, siltstone and subordinate sandstone; fossiliferous)	Kogelberg Sandstone Fynbos
Pink	Napier	Dv (Shale, siltstone and subordinate sandstone; fossiliferous)	Western Rûens Shale Renosterveld
Pink	Paardeberg	Qs (Light-grey to pale-red sandy soil)	Swartland Granite Renosterveld
Pink	Philadelphia	Qf (Ferricrete)	Swartland Shale Renosterveld
Pink	Rawsonville	Sg (Red-brown-weathering, thin-bedded quartzitic sandstone, thin shale beds in places)	Hawequas Sandstone Fynbos

	Riverlands		
Pink	Nature Reserve (i)	Qs (Light-grey to pale-red sandy soil)	Atlantis Sand Fynbos
	Riverlands		
Pink	Nature Reserve (ii)	Qs (Light-grey to pale-red sandy soil)	Atlantis Sand Fynbos
	Riverlands		
Pink	Nature Reserve (iii)	Qs (Light-grey to pale-red sandy soil)	Atlantis Sand Fynbos
	Riverlands		
Pink	Nature Reserve (iv)	Qg (Loam and sandy loam)	Atlantis Sand Fynbos
		NCs (Granite, mainly coarse-grained porphyritic with medium-to-coarse-grained, fine-grained porphyritic, fine-grained leucocratic, hybridic, fine-to-medium-grained tourmaline-bearing and coarse-grained biotitic variants)	
Pink	Stellenbosch		Boland Granite Fynbos
			Eastern Rûens Shale
Pink	Swellendam	Qg (Loam and sandy loam)	Renosterveld
		Dw (Micaceous siltstone, shale and subordinate sandstone)	Eastern Rûens Shale
Pink	Swellendam		Renosterveld
		Npo (Phyllite shale, schist and greywacke with dark-grey limestone, sporadic quartzitic sandstone beds and conglomerate beds)	
Pink	Tulbagh 1		Breede Shale Fynbos

Pink	Tulbagh 2	Npo (Phyllite shale, schist and greywacke with dark-grey limestone, sporadic quartzitic sandstone beds and conglomerate beds)	Breede Shale Renosterveld
Pink	Tulbagh 3	Npo (Phyllite shale, schist and greywacke with dark-grey limestone, sporadic quartzitic sandstone beds and conglomerate beds)	Breede Shale Fynbos
Pink	Tulbagh 4	Npo (Phyllite shale, schist and greywacke with dark-grey limestone, sporadic quartzitic sandstone beds and conglomerate beds)	Breede Shale Renosterveld
Pink	Tulbagh 5	Npo (Phyllite shale, schist and greywacke with dark-grey limestone, sporadic quartzitic sandstone beds and conglomerate beds)	Breede Shale Renosterveld
Pink	Tulbagh 6	Npo (Phyllite shale, schist and greywacke with dark-grey limestone, sporadic quartzitic sandstone beds and conglomerate beds)	Breede Shale Renosterveld
Pink	Villiersdorp	Dr (Light-grey feldspathic sandstone, siltstone and micaceous shale bands)	Breede Shale Renosterveld

Pink	Wellington 1	Nn (Phyllite, medium-grained to gritty greywacke, feldspathic and sericitic quartzite, limestone, dolomite and gritstone; greenstone, highly sheared and partly replaced by calcite and chert)	Swartland Shale Renosterveld
Pink	Wellington 2	Qs (Light-grey to pale-red sandy soil)	Swartland Alluvium Fynbos
Pink	Wolseley (i)	Npo (Phyllite shale, schist and greywacke with dark-grey limestone, sporadic quartzitic sandstone beds and conglomerate beds)	Breede Alluvium Fynbos
Pink	Wolseley (ii)	Npo (Phyllite shale, schist and greywacke with dark-grey limestone, sporadic quartzitic sandstone beds and conglomerate beds)	Breede Alluvium Fynbos
Pink	Wolseley (iii)	Npo (Phyllite shale, schist and greywacke with dark-grey limestone, sporadic quartzitic sandstone beds and conglomerate beds)	Breede Shale Fynbos
Purple	Darling 3	Qg (Loam and sandy loam)	Atlantis Sand Fynbos
Purple	Durbanville 2	Qs (Light-grey to pale-red sandy soil)	Swartland Shale Renosterveld

Purple	Malmesbury District	Nm (Greywacke and phyllite with beds and lenses of quartz schist, limestone and grit; quartz-sericite schist with occasional limestone lenses)	Atlantis Sand Fynbos
Purple	Paarl	Nm (Greywacke and phyllite with beds and lenses of quartz schist, limestone and grit; quartz-sericite schist with occasional limestone lenses)	Swartland Granite Renosterveld
Red	Darling 3	Qg (Loam and sandy loam)	Atlantis Sand Fynbos
Red	Darling 4 (i)	Qg (Loam and sandy loam)	Hopefield Sand Fynbos
Red	Darling 5 (ii)	Qg (Loam and sandy loam)	Hopefield Sand Fynbos
Red	Darling-Yzerfontein	NCd (Granite, mainly coarse-grained porphyritic with porphyritic biotitic, leucocratic, oven-grained biotitic and tourmaline-bearing variants; granodiorite)	Hopefield Sand Fynbos
Red	Hopefield	Qg (Loam and sandy loam)	Hopefield Sand Fynbos
Red	Paarl	Nm (Greywacke and phyllite with beds and lenses of quartz schist, limestone and grit; quartz-sericite schist with occasional limestone lenses)	Swartland Granite Renosterveld

White	Abbotsdale	Nca (Granite, mainly coarse-grained porphyritic with fine-grained leucocratic, fine-to-medium-grained porphyritic and medium-grained biotitic variants)	Swartland Granite Renosterveld
White	Betty's Bay	Os (Light-grey quartzitic sandstone with thin siltstone, shale and polymictic conglomerate beds)	Kogelberg Sandstone Fynbos
White	Darling 4	Qg (Loam and sandy loam)	Hopefield Sand Fynbos
White	Darling 6	NCd (Granite, mainly coarse-grained porphyritic with porphyritic biotitic, leucocratic, oven-grained biotitic and tourmaline-bearing variants; granodiorite)	Swartland Granite Renosterveld
White	Darling 7	Qg (Loam and sandy loam)	Hopefield Sand Fynbos
White	Darling 9	NCd (Granite, mainly coarse-grained porphyritic with porphyritic biotitic, leucocratic, oven-grained biotitic and tourmaline-bearing variants; granodiorite)	Atlantis Sand Fynbos
White	Hermanus	Ss (Light-grey, massively bedded, quartzitic sandstone; thin lenticular conglomerate and grit beds)	Kogelberg Sandstone Fynbos

White	Malmesbury District	Nm (Greywacke and phyllite with beds and lenses of quartz schist, limestone and grit; quartz-sericite schist with occasional limestone lenses)	Atlantis Sand Fynbos
White	Nieuwoudtville	Ss (Light-grey, massively bedded, quartzitic sandstone; thin lenticular conglomerate and grit beds)	Bokkeveld Sandstone Fynbos
White	Stanford	Qs (Light-grey to pale-red sandy soil)	Overberg Sandstone Fynbos
White	Yzerfontein	QI (Limestone and calcrete, partially cross-bedded, calcified parabolic dune sand)	Atlantis Sand Fynbos
Yellow	Piketberg 1	Np (Grit and greywacke)	Swartland Shale Renosterveld
Yellow	Piketberg 2	Og (Thinly-bedded sandstone, siltstone and mudstone, mainly reddish)	Piketberg Sandstone Fynbos
Yellow	Piketberg 3	Np (Grit and greywacke)	Piketberg Sandstone Fynbos

Supplementary Table 7. Relative abundance of insect pollinators observed visiting purple and red sympatric *Drosera cistiflora* s.l. floral colour forms at Darling 3 in 2009 and 2010. Superscript H denotes hopliine beetles (Coleoptera: Scarabaeidae: Hopliini).

Flower visitor species, genus and/or family	Relative abundance in each <i>Drosera cistiflora</i> s.l. floral colour form at Darling 3	
	Purple	Red
<i>Anisonyx cf. ursus</i> (Scarabaeidae) ^H	1	0
Ceratopogonidae	4	4
<i>Chasme decora</i> (Scarabaeidae) ^H	0	9
<i>Lepisia rupicola</i> spec. (Scarabaeidae) ^H	1	202
<i>Omocrates sp.</i> (species 1) [Scarabaeidae] ^H	14	0
<i>Platychelus lupinus</i> (Scarabaeidae) ^H	0	4
Tabanidae (species 1)	0	3
Tenebrionidae (species 1)	19	0
Thomisidae	1	0

Supplementary Table 8. Average pollen loads, abundance and importance of each insect pollinator found in *Drosera cistiflora* s.l. flowers during observations in 2009 and 2010 while conducting model flower choice experiments, and the total number trapped in model flower arrays. Superscript H denotes hopliine beetles (Coleoptera: Scarabaeidae: Hopliini).

<i>Drosera cistiflora</i> s.l. floral colour form	Flower visitor species, genus and/or family	Average pollen load	Abundance	Number trapped	Pollinator importance per floral colour form (average pollen load *abundance)
Pink	<i>Anisonyx cf. ursus</i> (Scarabaeidae) ^H	65.54	11	0	720.94
	Ceratopogonidae	0.75	14	107	10.5
	Chrysomellidae	2.52	39	5	98.28
	Empididae (species 2)	2.5	5	2	12.5
	<i>Lepisia rupicola</i> spec. (Scarabaeidae) ^H	487.92	19	22	9270.48
	Megachilidae	52.5	3	17	157.5
	Meloidae	299	53	4	15847
	Melyridae (species 1)	2.6	6	7	15.6
	Melyridae (species 2)	106.68	3	3	320.04
	Muscidae	18.33	4	1	73.32
	Scarabaeidae	28	15	29	420
	Tenebrionidae (species 1)	32.47	129	11	4188.63
	Tenebrionidae (species 3)	3	69	5	207
	Thomisidae	10	3	4	30
Purple	<i>Anisonyx cf. ursus</i> (Scarabaeidae) ^H	65.54	1	0	65.54
	Ceratopogonidae	0.75	4	126	3
	<i>Lepisia rupicola</i> spec. (Scarabaeidae) ^H	487.92	1	128	487.92
	<i>Omocrates</i> sp. (species 1) [Scarabaeidae] ^H	145	14	4	2030
	Tenebrionidae (species 1)	32.47	19	11	616.93
	Thomisidae	10	1	0	10
	Ceratopogonidae	0.75	4	395	3
Red	<i>Chasme decora</i> (Scarabaeidae) ^H	402.58	47	58	18921.26

	<i>Chasme sp.</i> (Scarabaeidae) ^H	31.25	4	2	125
	Chrysomellidae	2.52	5	2	12.6
	<i>Lepisia rupicola</i> spec. (Scarabaeidae) ^H	487.92	323	168	157598.16
	<i>Platychelus lupinus</i> (Scarabaeidae) ^H	73.33	7	1	513.31
	Tabanidae (species 1)	163.33	3	18	489.99
	Tenebrionidae (species 2)	1.75	20	0	35
White	<i>Anisonyx sp.</i> (Scarabaeidae) ^H	180	16	0	2880
	<i>Anisonyx cf. ursus</i> (Scarabaeidae) ^H	65.54	2	2	131.08
	Ceratopogonidae	0.75	35	9	26.25
	Chrysomellidae	2.52	5	0	12.6
	<i>Lepisia rupicola</i> spec. (Scarabaeidae) ^H	487.92	118	66	57574.56
	Lyganidae	20	4	2	80
	Megachilidae	52.5	4	0	210
	Meloidae	299	10	0	2990
	Melyridae (species 2)	106.68	5	0	533.4
	Tenebrionidae (species 1)	32.47	50	5	1623.5
	Tenebrionidae (species 3)	3	5	3	15
	Yellow	<i>Anisochelus inornatus</i> (Scarabaeidae) ^H	80.83	20	16
Ceratopogonidae		0.75	7	18	5.25
<i>Heterochelus sp.</i> (species 1) [Scarabaeidae] ^H		120	1	0	120
<i>Lepithrix sp.</i> (Scarabaeidae) ^H		169.47	27	17	4575.69
Melyridae (species 2)		106.68	20	21	2133.6
<i>Peritrichia sp.</i> (species 1) [Scarabaeidae] ^H		122	5	11	610
<i>Peritrichia sp.</i> (species 2) [Scarabaeidae] ^H		96.88	1	22	96.88
Scarabaeidae		28	1	0	28
Tabanidae (species 2)		110	3	4	330
Tenebrionidae (species 1)		32.47	8	9	259.76

Supplementary Table 9. Average pollen loads, abundance and importance of each insect pollinator found in *Drosera cistiflora* s.l. flowers during observations in 2009 and 2010 while conducting model flower and reciprocally translocated choice experiments, and the total number trapped in reciprocally translocated flower arrays. Superscript H denotes hopline beetles (Coleoptera: Scarabaeidae: Hopliini).

<i>Drosera</i>					Pollinator
<i>cistiflora</i>	Flower visitor species,	Average	Abundance	Number	importance
s.l. floral	genus and/or family	pollen load		trapped	(average
colour form					pollen load
					*abundance)
Pink	<i>Anisonyx. sp.</i> (Scarabaeidae) ^H	180	16	1	2880
	<i>Anisonyx cf. ursus</i> (Scarabaeidae) ^H	65.54	11	0	720.94
	Apidae	120	1	1	120
	Ceratopogonidae	0.75	14	8	10.5
	Chrysomellidae	2.52	39	0	98.28
	Empididae (species 2)	2.5	5	0	12.5
	<i>Eristalis crassipes</i> (Syrphidae)	3	1	1	3
	<i>Lepisia rupicola spec.</i> (Scarabaeidae) ^H	487.92	19	3	9270.48
	Megachilidae	52.5	3	0	157.5
	Meloidae	299	53	0	15847
	Melyridae (species 1)	2.6	6	0	15.6
	Melyridae (species 2)	106.68	3	0	320.04
	Muscidae	18.33	4	0	73.32
	<i>Pachycnema murina</i> (Scarabaeidae) ^H	450	3	3	1350
	<i>Platychelus lupinus</i> (Scarabaeidae) ^H	73.33	3	3	219.99
	Scarabaeidae	28	15	0	420
	Tenebrionidae (species 1)	32.47	129	0	4188.63
	Tenebrionidae (species 3)	3	69	0	207
Thomisidae	10	3	0	30	
Purple	<i>Anisonyx cf. ursus</i> (Scarabaeidae) ^H	65.54	1	0	65.54
	Ceratopogonidae	0.75	4	3	3

	<i>Chasme sp.</i> (species 1) [Scarabaeidae] ^H	386.15	49	2	18921.35
	Dermestidae (species 1)	1	3	3	3
	<i>Lepisia rupicola spec.</i> (Scarabaeidae) ^H	487.92	1	24	487.92
	Melyridae (species 2)	106.68	4	1	426.72
	Muscidae	18.33	6	2	109.98
	<i>Omocrates sp.</i> (species 1) [Scarabaeidae] ^H	145	14	10	2030
	<i>Platychelus lupinus</i> (Scarabaeidae) ^H	73.33	7	3	513.31
	Tenebrionidae (species 1)	32.47	19	0	616.93
	Thomisidae	10	1	0	10
Red	Ceratopogonidae	0.75	4	3	3
	<i>Chasme decora</i> (Scarabaeidae) ^H	402.58	47	2	18921.26
	<i>Chasme sp.</i> (species 2) [Scarabaeidae] ^H	31.25	4	0	125
	Chrysomellidae	2.52	5	0	12.6
	Dermestidae (species 1)	1	3	3	3
	<i>Lepisia rupicola spec.</i> (Scarabaeidae) ^H	487.92	323	24	157598.16
	Melyridae (species 2)	106.68	4	1	426.72
	Muscidae	18.33	6	2	109.98
	<i>Omocrates sp.</i> (species 1) [Scarabaeidae] ^H	145	14	10	2030
	<i>Platychelus lupinus</i> (Scarabaeidae) ^H	73.33	7	3	513.31
	Tabanidae (species 1)	163.33	3	0	489.99
	Tenebrionidae (species 2)	1.75	20	0	35
White	<i>Anisonyx sp.</i> (Scarabaeidae) ^H	180	16	1	2880
	<i>Anisonyx cf. ursus</i> (Scarabaeidae) ^H	65.54	2	0	131.08
	Apidae	120	1	1	120
	Ceratopogonidae	0.75	35	34	26.25
	<i>Chasme sp.</i> (species 1) [Scarabaeidae] ^H	386.15	48	1	18535.2
	Chrysomellidae	2.52	5	0	12.6

	Dermestidae (species 2)	1.75	1	1	1.75
	<i>Lepisia rupicola</i> spec. (Scarabaeidae) ^H	487.92	118	7	57574.56
	Lyganidae	20	4	0	80
	Megachilidae	52.5	4	0	210
	Meloidae	299	10	0	2990
	Melyridae (species 2)	106.68	5	0	533.4
	Muscidae	18.33	6	2	109.98
	<i>Omocrates</i> sp. (species 1) [Scarabaeidae] ^H	145	36	32	5220
	Tenebrionidae (species 1)	32.47	50	2	1623.5
	Tenebrionidae (species 3)	3	5	0	15
	Tingidae	32.47	2	2	64.94
Yellow	<i>Anisochelus inornatus</i> (Scarabaeidae) ^H	80.83	20	2	1616.6
	Ceratopogonidae	0.75	7	6	5.25
	<i>Chasme</i> sp. (species 1) [Scarabaeidae] ^H	386.15	49	2	18921.35
	<i>Heterochelus detritus</i> (Scarabaeidae) ^H	1	1	1	1
	<i>Heterochelus</i> sp. (species 1) [Scarabaeidae] ^H	120	1	0	120
	<i>Lepithrix</i> sp. (Scarabaeidae) ^H	169.47	27	0	4575.69
	Melyridae (species 2)	106.68	20	10	2133.6
	<i>Peritrichia</i> sp. (species 1) [Scarabaeidae] ^H	122	5	0	610
	<i>Peritrichia</i> sp. (species 2) [Scarabaeidae] ^H	96.88	1	0	96.88
	Scarabaeidae	28	1	0	28
	Tabanidae (species 2)	110	3	0	330
	Tenebrionidae (species 1)	32.47	8	0	259.76

Supplementary Table 10. Marginal model means (and asymmetrical 95% confidence intervals) of matching effects between model flower array colour and local *Drosera cistiflora* s.l. flower colour for: i) presence/absence of visits and ii) number of visits, of all insects, all hopliine beetles (Coleoptera: Scarabaeidae: Hopliini) [primary pollinators] only, and the hopliine beetle *Lepisia rupicola* spec. (principal pollinator) only. Number of populations sampled, followed by number of array replicates, are shown in parentheses after flower colour, for each *D. cistiflora* s.l. floral colour form.

		Any visit			Number of visits		
<i>Drosera cistiflora</i> s.l. flower colour	Array/local colour match	All insects	Hopliine beetles	<i>Lepisia rupicola</i> spec.	All insects	Hopliine beetles	<i>Lepisia rupicola</i> spec.
Pink (4, 200)	Yes	0.35 (95% confidence interval 0.23, 0.49)	0.07 (0.02, 0.17)	0.00 (0.00, 0.05)	0.68 (0.40, 1.18)	0.09 (0.03, 0.27)	0.00 (0.00, 0.06)
	No	0.15 (0.09, 0.23)	0.07 (0.03, 0.16)	0.00 (0.00, 0.04)	0.28 (0.17, 0.47)	0.12 (0.05, 0.32)	0.00 (0.00, 0.05)
Purple (2, 112)	Yes	0.08 (0.03, 0.16)	0.01 (0.00, 0.05)	0.00 (0.00, 0.03)	0.07 (0.03, 0.17)	0.02 (0.01, 0.07)	0.00 (0.00, 0.03)
	No	0.39 (0.27, 0.52)	0.09 (0.04, 0.21)	0.03 (0.00, 0.20)	0.62 (0.38, 1.01)	0.15 (0.06, 0.39)	0.05 (0.01, 0.33)
Red (4, 184)	Yes	0.44 (0.31, 0.57)	0.35 (0.17, 0.58)	0.16 (0.02, 0.57)	0.67 (0.40, 1.10)	0.42 (0.17, 1.07)	0.14 (0.02, 1.00)
	No	0.31 (0.22, 0.42)	0.04 (0.02, 0.10)	0.01 (0.00, 0.08)	0.50 (0.31, 0.79)	0.06 (0.02, 0.15)	0.01 (0.00, 0.09)
White (3, 120)	Yes	0.52 (0.35, 0.68)	0.37 (0.16, 0.65)	0.07 (0.01, 0.53)	0.71 (0.37, 1.35)	0.44 (0.14, 1.40)	0.07 (0.00, 0.97)
	No	0.20 (0.12, 0.32)	0.14 (0.05, 0.33)	0.01 (0.00, 0.14)	0.31 (0.17, 0.57)	0.23 (0.07, 0.68)	0.02 (0.00, 0.24)
Yellow (3, 200)	Yes	0.18 (0.10, 0.31)	0.12 (0.04, 0.30)	0.00 (0.00, 0.05)	0.20 (0.10, 0.38)	0.12 (0.04, 0.37)	0.00 (0.00, 0.05)
	No	0.11 (0.06, 0.18)	0.05 (0.02, 0.15)	0.00 (0.00, 0.01)	0.12 (0.07, 0.22)	0.05 (0.02, 0.17)	0.00 (0.00, 0.01)

Supplementary Table 11. Marginal model means (and asymmetrical 95% confidence intervals) of matching effects between reciprocal translocation array colour and local *Drosera cistiflora* s.l. flower colour for: i) presence/absence of visits and ii) number of visits, of all insects, all hopliine beetles (Coleoptera: Scarabaeidae: Hopliini) [primary pollinators] only, and the hopliine beetle *Lepisia rupicola* spec. (principal pollinator) only. Number of populations sampled, followed by number of array replicates, are shown in parentheses after flower colour, for each *D. cistiflora* s.l. floral colour form.

<i>Drosera cistiflora</i> s.l. flower colour	Array/ local colour match	Any visit			Number of visits		
		All insects	Hopliine beetles	<i>Lepisia rupicola</i> spec.	All insects	Hopliine beetles	<i>Lepisia rupicola</i> spec.
Pink (1, 8)	Yes	0.91 (95% confidence interval 0.75, 0.97)	0.38 (0.16, 0.66)	0.08 (0.01, 0.36)	2.09 (0.64, 6.80)	0.38 (0.13, 1.13)	0.04 (0.01, 0.25)
	No	0.23 (0.11, 0.42)	0.07 (0.02, 0.18)	0.01 (0.00, 0.08)	0.27 (0.11, 0.63)	0.08 (0.03, 0.25)	0.02 (0.00, 0.09)
Purple (1, 8)	Yes	0.91 (0.73, 0.98)	0.67 (0.40, 0.86)	0.05 (0.01, 0.27)	0.67 (0.22, 2.05)	3.12 (1.12, 8.66)	0.03 (0.00, 0.18)
	No	0.22 (0.11, 0.41)	0.19 (0.08, 0.38)	0.01 (0.00, 0.06)	0.77 (0.36, 1.63)	0.69 (0.29, 1.63)	0.01 (0.00, 0.07)
Red (1, 8)	Yes	0.94 (0.80, 0.98)	0.75 (0.49, 0.90)	0.58 (0.22, 0.86)	1.54 (0.55, 4.25)	1.91 (0.70, 5.19)	0.56 (0.17, 1.85)
	No	0.30 (0.16, 0.49)	0.26 (0.12, 0.46)	0.15 (0.04, 0.41)	0.53 (0.24, 1.15)	0.42 (0.17, 1.03)	0.22 (0.07, 0.72)
White (2, 16)	Yes	0.95 (0.84, 0.99)	0.56 (0.32, 0.77)	0.46 (0.17, 0.78)	1.83 (0.75, 4.45)	1.20 (0.45, 3.17)	0.34 (0.10, 1.18)
	No	0.36 (0.19, 0.57)	0.13 (0.05, 0.28)	0.10 (0.03, 0.31)	0.59 (0.26, 1.35)	0.26 (0.11, 0.65)	0.13 (0.04, 0.43)
Yellow (1, 8)	Yes	0.72 (0.43, 0.90)	0.37 (0.16, 0.66)	0.16 (0.03, 0.52)	2.16 (0.67, 6.96)	0.48 (0.16, 1.45)	0.11 (0.03, 0.50)
	No	0.07 (0.02, 0.18)	0.06 (0.02, 0.17)	0.02 (0.00, 0.12)	0.06 (0.02, 0.22)	0.11 (0.04, 0.30)	0.04 (0.01, 0.17)

Supplementary Table 12. Posthoc (Tukey) tests of differences in matching effects (match vs non-match) between model flower array colour and local *Drosera cistiflora* s.l. flower colour for: i) presence/absence of visits and ii) number of visits, for all insect visits, hopliine beetle (Coleoptera: Scarabaeidae: Hopliini) visits only, and hopliine beetle *Lepisia rupicola* spec. visits only, per *D. cistiflora* s.l. floral colour form.

	<i>Drosera cistiflora</i> s.l. flower colour	Array/local colour match comparison	Any visit				Number of visits			
			Estimate	SE	z	p	Estimate	SE	z	p
All insects	Pink	No – Yes	-1.12	0.18	-6.14	< 0.001	-0.88	0.15	-5.97	< 0.001
	Purple	No – Yes	2.05	0.37	5.57	< 0.001	2.12	0.36	5.95	< 0.001
	Red	No – Yes	-0.56	0.17	-3.34	< 0.001	-0.30	0.14	-2.18	0.029
	White	No – Yes	-1.44	0.23	-6.42	< 0.001	-0.82	0.19	-4.24	< 0.001
	Yellow	No – Yes	-0.65	0.22	-2.97	0.003	-0.49	0.22	-2.23	0.026
Hopliine beetles	Pink	No – Yes	-0.02	0.30	-0.06	0.950	0.27	0.27	0.99	0.322
	Purple	No – Yes	2.13	0.53	4.01	< 0.001	2.10	0.49	4.25	< 0.001
	Red	No – Yes	-2.46	0.21	-11.70	< 0.001	-1.98	0.18	-11.07	< 0.001
	White	No – Yes	-1.31	0.24	-5.49	< 0.001	-0.67	0.23	-2.95	0.003
	Yellow	No – Yes	-0.88	0.26	-3.41	0.001	-0.75	0.27	-2.78	0.005
<i>Lepisia rupicola</i> spec.	Pink	No – Yes	-0.15	0.59	-0.25	0.806	-0.15	0.54	-0.28	0.781
	Purple	No – Yes	2.55	0.73	3.49	< 0.001	2.75	0.73	3.78	< 0.001
	Red	No – Yes	-2.72	0.26	-10.56	< 0.001	-2.37	0.21	-11.28	< 0.001
	White	No – Yes	-1.90	0.36	-5.33	< 0.001	-1.39	0.30	-4.69	< 0.001
	Yellow	No – Yes	-1.40	1.42	-0.99	0.324	-1.39	1.42	-0.98	0.329

Supplementary Table 13. Posthoc (Tukey) tests of differences in matching effects (match vs non-match) between reciprocally translocated array flower colour and local *Drosera cistiflora* s.l. flower colour for: i) presence/absence of visits and ii) number of visits, for all insect visits, hopliine beetle (Coleoptera: Scarabaeidae: Hopliini) visits only, and hopliine beetle *Lepisia rupicola* spec. visits only, per *D. cistiflora* s.l. floral colour form.

	<i>Drosera cistiflora</i> s.l. flower colour	Array/local colour match comparison	Any visit				Number of visits			
			Estimate	SE	z	p	Estimate	SE	z	p
All insects	Pink	No – Yes	-3.57	0.58	-6.20	< 0.001	-2.06	0.64	-3.23	0.001
	Purple	No – Yes	-3.57	0.58	-6.20	< 0.001	0.13	0.52	0.26	0.797
	Red	No – Yes	-3.57	0.58	-6.20	< 0.001	-1.07	0.45	-2.36	0.018
	White	No – Yes	-3.57	0.58	-6.20	< 0.001	-1.14	0.48	-2.38	0.017
	Yellow	No – Yes	-3.57	0.58	-6.20	< 0.001	-3.51	0.76	-4.59	< 0.001
Hopliine beetles	Pink	No – Yes	-2.16	0.42	-5.11	< 0.001	-1.51	0.38	-3.93	< 0.001
	Purple	No – Yes	-2.16	0.42	-5.11	< 0.001	-1.51	0.38	-3.93	< 0.001
	Red	No – Yes	-2.16	0.42	-5.11	< 0.001	-1.51	0.38	-3.93	< 0.001
	White	No – Yes	-2.16	0.42	-5.11	< 0.001	-1.51	0.38	-3.93	< 0.001
	Yellow	No – Yes	-2.16	0.42	-5.11	< 0.001	-1.51	0.38	-3.93	< 0.001
<i>Lepisia rupicola</i> spec.	Pink	No – Yes	-2.04	0.55	-3.70	< 0.001	-0.94	0.33	-2.87	0.004
	Purple	No – Yes	-2.04	0.55	-3.70	< 0.001	-0.94	0.33	-2.87	0.004
	Red	No – Yes	-2.04	0.55	-3.70	< 0.001	-0.94	0.33	-2.87	0.004
	White	No – Yes	-2.04	0.55	-3.70	< 0.001	-0.94	0.33	-2.87	0.004
	Yellow	No – Yes	-2.04	0.55	-3.70	< 0.001	-0.94	0.33	-2.87	0.004

Supplementary Table 14. Posthoc (Tukey) tests of differences between *Drosera cistiflora* s.l. floral colour forms within matched or unmatched model flowers for: i) presence/absence of visits and ii) number of visits, for all insect visits, hopliine beetle (Coleoptera: Scarabaeidae: Hopliini) visits only, and hopliine beetle *Lepisia rupicola* spec. visits only.

	Flower colour comparison	Array/local colour match	Any visit				Number of visits			
			Estimate	SE	z	p	Estimate	SE	z	p
All insects	Pink - Purple	No	-1.30	0.38	-3.41	0.006	-0.78	0.36	-2.16	0.197
	Pink - Red	No	-0.95	0.37	-2.58	0.074	-0.56	0.35	-1.58	0.512
	Pink - White	No	-0.39	0.42	-0.93	0.887	-0.09	0.40	-0.23	0.999
	Pink - Yellow	No	0.39	0.42	0.92	0.889	0.86	0.41	2.12	0.212
	Purple - Red	No	0.35	0.17	2.08	0.230	0.22	0.14	1.65	0.466
	Purple - White	No	0.91	0.41	2.20	0.178	0.69	0.39	1.74	0.412
	Purple - Yellow	No	1.68	0.41	4.08	< 0.001	1.64	0.40	4.13	< 0.001
	Red - White	No	0.56	0.40	1.40	0.627	0.46	0.39	1.20	0.754
	Red - Yellow	No	1.33	0.40	3.34	0.008	1.42	0.39	3.64	0.003
	White - Yellow	No	0.77	0.45	1.73	0.417	0.96	0.44	2.19	0.182
	Pink - Purple	Yes	1.88	0.52	3.60	0.003	2.22	0.50	4.41	< 0.001
	Pink - Red	Yes	-0.39	0.41	-0.97	0.871	0.03	0.38	0.07	1.000
	Pink - White	Yes	-0.71	0.46	-1.55	0.527	-0.03	0.43	-0.08	1.000
	Pink - Yellow	Yes	0.86	0.46	1.87	0.333	1.26	0.44	2.83	0.037
	Purple - Red	Yes	-2.27	0.39	-5.75	< 0.001	-2.19	0.37	-5.92	< 0.001
	Purple - White	Yes	-2.59	0.55	-4.68	< 0.001	-2.25	0.53	-4.23	< 0.001
	Purple - Yellow	Yes	-1.02	0.55	-1.85	0.344	-0.96	0.54	-1.78	0.385
	Red - White	Yes	-0.32	0.45	-0.72	0.951	-0.06	0.42	-0.14	1.000
	Red - Yellow	Yes	1.25	0.44	2.81	0.040	1.23	0.43	2.87	0.034
	White - Yellow	Yes	1.57	0.49	3.18	0.013	1.29	0.48	2.70	0.054
Hopliine beetles	Pink - Purple	No	-0.39	0.69	-0.56	0.980	-0.24	0.68	-0.35	0.997
	Pink - Red	No	0.42	0.68	0.62	0.973	0.73	0.68	1.08	0.817
	Pink - White	No	-0.84	0.76	-1.10	0.805	-0.61	0.75	-0.82	0.926
	Pink - Yellow	No	0.23	0.76	0.30	0.998	0.80	0.76	1.05	0.830
	Purple - Red	No	0.81	0.19	4.15	< 0.001	0.97	0.18	5.28	< 0.001
	Purple - White	No	-0.45	0.74	-0.61	0.974	-0.38	0.74	-0.51	0.986
	Purple - Yellow	No	0.62	0.75	0.83	0.922	1.04	0.74	1.39	0.633
	Red - White	No	-1.26	0.74	-1.71	0.428	-1.34	0.73	-1.84	0.353
	Red - Yellow	No	-0.19	0.74	-0.26	0.999	0.07	0.74	0.09	1.000
	White - Yellow	No	1.07	0.81	1.32	0.679	1.41	0.81	1.75	0.405
	Pink - Purple	Yes	1.76	0.89	1.99	0.273	1.59	0.86	1.86	0.340
	Pink - Red	Yes	-2.02	0.73	-2.77	0.044	-1.52	0.72	-2.12	0.212

	Pink - White	Yes	-2.13	0.81	-2.64	0.064	-1.56	0.80	-1.95	0.289
	Pink - Yellow	Yes	-0.63	0.81	-0.78	0.938	-0.22	0.81	-0.27	0.999
	Purple - Red	Yes	-3.78	0.56	-6.80	< 0.001	-3.11	0.51	-6.13	< 0.001
	Purple - White	Yes	-3.89	0.91	-4.29	< 0.001	-3.15	0.89	-3.56	0.003
	Purple - Yellow	Yes	-2.39	0.91	-2.62	0.067	-1.81	0.89	-2.03	0.254
	Red - White	Yes	-0.11	0.76	-0.15	1.000	-0.04	0.75	-0.05	1.000
	Red - Yellow	Yes	1.39	0.76	1.83	0.357	1.30	0.76	1.71	0.428
	White - Yellow	Yes	1.50	0.84	1.80	0.376	1.34	0.84	1.60	0.498
<i>Lepista ruficicola</i> spec.	Pink - Purple	No	-2.46	1.63	-1.51	0.558	-2.48	1.62	-1.53	0.540
	Pink - Red	No	-1.44	1.63	-0.88	0.903	-1.22	1.62	-0.76	0.943
	Pink - White	No	-1.44	1.83	-0.79	0.935	-1.51	1.82	-0.82	0.923
	Pink - Yellow	No	2.10	2.15	0.97	0.868	2.40	2.15	1.11	0.800
	Purple - Red	No	1.02	0.22	4.59	< 0.001	1.26	0.20	6.25	< 0.001
	Purple - White	No	1.03	1.66	0.62	0.972	0.97	1.66	0.59	0.977
	Purple - Yellow	No	4.56	2.05	2.22	0.171	4.87	2.06	2.37	0.125
	Red - White	No	0.00	1.66	0.00	1.000	-0.28	1.66	-0.17	1.000
	Red - Yellow	No	3.54	2.05	1.73	0.418	3.62	2.06	1.76	0.399
	White - Yellow	No	3.53	2.21	1.60	0.497	3.90	2.22	1.76	0.398
	Pink - Purple	Yes	0.23	1.83	0.13	1.000	0.43	1.81	0.24	0.999
	Pink - Red	Yes	-4.01	1.69	-2.38	0.122	-3.44	1.66	-2.07	0.232
	Pink - White	Yes	-3.19	1.88	-1.69	0.439	-2.74	1.87	-1.47	0.585
	Pink - Yellow	Yes	0.84	2.20	0.38	0.996	1.16	2.19	0.53	0.985
	Purple - Red	Yes	-4.25	0.75	-5.63	< 0.001	-3.87	0.74	-5.24	< 0.001
	Purple - White	Yes	-3.42	1.80	-1.90	0.319	-3.17	1.81	-1.75	0.403
	Purple - Yellow	Yes	0.61	2.17	0.28	0.999	0.73	2.18	0.34	0.997
	Red - White	Yes	0.83	1.66	0.50	0.987	0.70	1.66	0.42	0.993
	Red - Yellow	Yes	4.85	2.05	2.37	0.124	4.60	2.06	2.23	0.168
	White - Yellow	Yes	4.03	2.21	1.82	0.360	3.90	2.22	1.75	0.401

Supplementary Table 15. Posthoc (Tukey) tests of differences between *Drosera cistiflora* s.l. floral colour forms within matched or unmatched reciprocally translocated flowers for: i) presence/absence of visits and ii) number of visits, for all insect visits, hopliine beetle (Coleoptera: Scarabaeidae: Hopliini) visits only, and hopliine beetle *Lepisia rupicola* spec. visits only.

			Any visit				Number of visits			
Flower colour comparison	Array/local colour match	Estimate	SE	z	p	Estimate	SE	z	p	
All insects	Pink - Purple	No	0.05	0.51	0.09	1.000	-1.06	0.37	-2.84	0.036
	Pink - Red	No	-0.34	0.49	-0.68	0.961	-0.68	0.39	-1.75	0.405
	Pink - White	No	-0.61	0.50	-1.23	0.733	-0.78	0.42	-1.87	0.336
	Pink - Yellow	No	1.42	0.60	2.38	0.122	1.42	0.61	2.35	0.131
	Purple - Red	No	-0.38	0.51	-0.75	0.944	0.38	0.33	1.15	0.779
	Purple - White	No	-0.66	0.51	-1.28	0.705	0.27	0.37	0.73	0.950
	Purple - Yellow	No	1.38	0.62	2.22	0.171	2.48	0.57	4.35	<0.001
	Red - White	No	-0.28	0.50	-0.55	0.982	-0.11	0.37	-0.29	0.998
	Red - Yellow	No	1.76	0.61	2.88	0.033	2.10	0.58	3.64	0.003
	White - Yellow	No	2.03	0.60	3.39	0.006	2.21	0.60	3.70	0.002
	Pink - Purple	Yes	0.05	0.51	0.09	1.000	1.13	0.72	1.57	0.517
	Pink - Red	Yes	-0.34	0.49	-0.68	0.961	0.31	0.69	0.45	0.991
	Pink - White	Yes	-0.61	0.50	-1.23	0.733	0.13	0.66	0.21	1.000
	Pink - Yellow	Yes	1.42	0.60	2.38	0.122	-0.03	0.76	-0.04	1.000
	Purple - Red	Yes	-0.38	0.51	-0.75	0.944	-0.82	0.56	-1.46	0.588
	Purple - White	Yes	-0.66	0.51	-1.28	0.705	-1.00	0.60	-1.67	0.452
	Purple - Yellow	Yes	1.38	0.62	2.22	0.171	-1.16	0.72	-1.62	0.486
	Red - White	Yes	-0.28	0.50	-0.55	0.982	-0.17	0.55	-0.32	0.998
	Red - Yellow	Yes	1.76	0.61	2.88	0.033	-0.34	0.68	-0.50	0.988
	White - Yellow	Yes	2.03	0.60	3.39	0.006	-0.17	0.65	-0.25	0.999
Hopliine beetles	Pink - Purple	No	-1.22	0.59	-2.06	0.238	-2.11	0.55	-3.81	0.001
	Pink - Red	No	-1.61	0.59	-2.74	0.049	-1.62	0.54	-2.98	0.024
	Pink - White	No	-0.73	0.57	-1.28	0.705	-1.16	0.55	-2.11	0.218
	Pink - Yellow	No	0.01	0.65	0.01	1.000	-0.24	0.62	-0.39	0.995
	Purple - Red	No	-0.39	0.51	-0.76	0.942	0.49	0.42	1.16	0.772
	Purple - White	No	0.49	0.52	0.94	0.882	0.96	0.47	2.06	0.238
	Purple - Yellow	No	1.23	0.59	2.08	0.231	1.87	0.53	3.55	0.004
	Red - White	No	0.87	0.51	1.71	0.427	0.47	0.45	1.03	0.840
	Red - Yellow	No	1.61	0.58	2.78	0.044	1.38	0.52	2.65	0.062
	White - Yellow	No	0.74	0.58	1.28	0.705	0.91	0.54	1.70	0.432
	Pink - Purple	Yes	-1.22	0.59	-2.06	0.238	-2.11	0.55	-3.81	0.001
	Pink - Red	Yes	-1.61	0.59	-2.74	0.049	-1.62	0.54	-2.98	0.024

	Pink - White	Yes	-0.73	0.57	-1.28	0.705	-1.16	0.55	-2.11	0.218
	Pink - Yellow	Yes	0.01	0.65	0.01	1.000	-0.24	0.62	-0.39	0.995
	Purple - Red	Yes	-0.39	0.51	-0.76	0.942	0.49	0.42	1.16	0.772
	Purple - White	Yes	0.49	0.52	0.94	0.882	0.96	0.47	2.06	0.238
	Purple - Yellow	Yes	1.23	0.59	2.08	0.231	1.87	0.53	3.55	0.004
	Red - White	Yes	0.87	0.51	1.71	0.427	0.47	0.45	1.03	0.840
	Red - Yellow	Yes	1.61	0.58	2.78	0.044	1.38	0.52	2.65	0.062
	White - Yellow	Yes	0.74	0.58	1.28	0.705	0.91	0.54	1.70	0.432
<i>Lepista ruficicola</i> spec.	Pink - Purple	No	0.48	1.10	0.43	0.993	0.31	1.03	0.30	0.998
	Pink - Red	No	-2.80	0.88	-3.19	0.012	-2.60	0.75	-3.47	0.005
	Pink - White	No	-2.33	0.85	-2.73	0.050	-2.11	0.77	-2.74	0.048
	Pink - Yellow	No	-0.83	0.99	-0.84	0.919	-1.00	0.86	-1.16	0.772
	Purple - Red	No	-3.27	0.96	-3.41	0.006	-2.91	0.75	-3.87	0.001
	Purple - White	No	-2.80	0.92	-3.06	0.019	-2.41	0.78	-3.09	0.017
	Purple - Yellow	No	-1.31	1.04	-1.25	0.721	-1.30	0.87	-1.49	0.568
	Red - White	No	0.47	0.54	0.87	0.908	0.50	0.35	1.42	0.617
	Red - Yellow	No	1.97	0.72	2.73	0.050	1.61	0.52	3.09	0.017
	White - Yellow	No	1.50	0.71	2.10	0.218	1.11	0.54	2.06	0.239
	Pink - Purple	Yes	0.48	1.10	0.43	0.993	0.31	1.03	0.30	0.998
	Pink - Red	Yes	-2.80	0.88	-3.19	0.012	-2.60	0.75	-3.47	0.005
	Pink - White	Yes	-2.33	0.85	-2.73	0.050	-2.11	0.77	-2.74	0.048
	Pink - Yellow	Yes	-0.83	0.99	-0.84	0.919	-1.00	0.86	-1.16	0.772
	Purple - Red	Yes	-3.27	0.96	-3.41	0.006	-2.91	0.75	-3.87	0.001
	Purple - White	Yes	-2.80	0.92	-3.06	0.019	-2.41	0.78	-3.09	0.017
	Purple - Yellow	Yes	-1.31	1.04	-1.25	0.721	-1.30	0.87	-1.49	0.568
	Red - White	Yes	0.47	0.54	0.87	0.908	0.50	0.35	1.42	0.617
	Red - Yellow	Yes	1.97	0.72	2.73	0.050	1.61	0.52	3.09	0.017
	White - Yellow	Yes	1.50	0.71	2.10	0.218	1.11	0.54	2.06	0.239

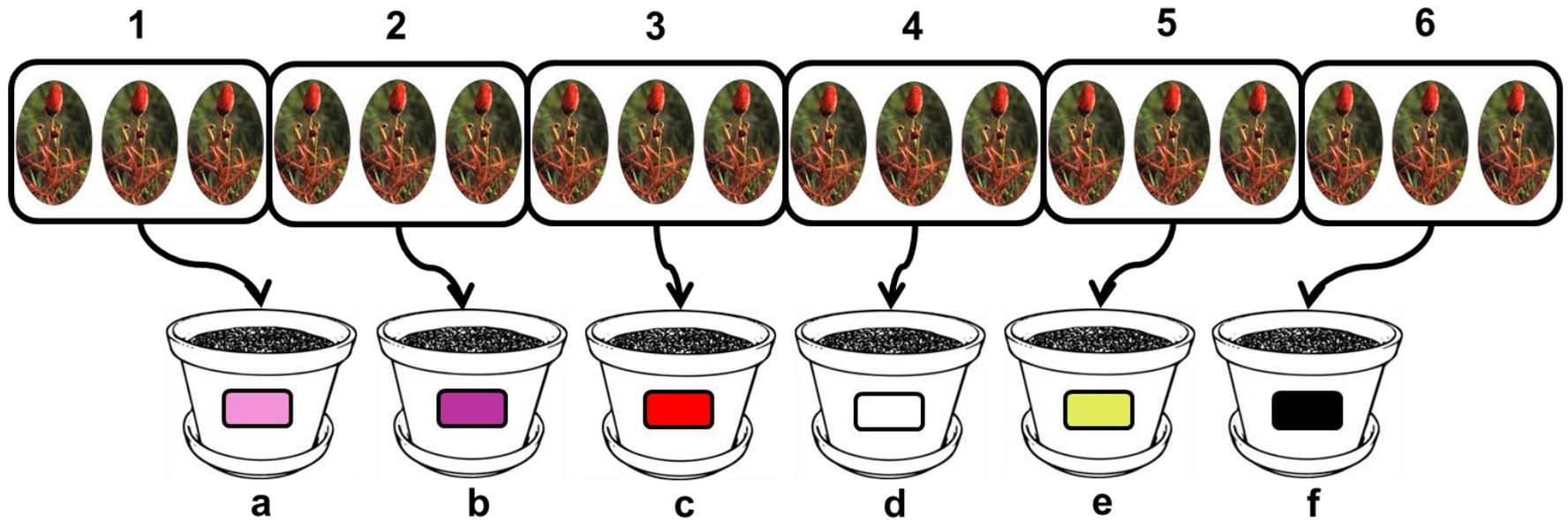
Supplementary Table 16. Draft Red List assessment of purple and red *Drosera cistiflora* s.l. ecotypes with proposed Red List statuses as per criteria of the International Union for Conservation of Nature (2012). ‘Purple’ refers to the provisionally named *D. cistiflora* ‘Purple West Coast’ taxon with crateriform corollas. Considering the *D. cistiflora* species complex is endemic to South Africa, proposed global (IUCN) and regional (South African) Red List statuses and criteria are identical.

	<i>Drosera cistiflora</i> s.l. ecotype	
	Purple	Red
Number of extant populations	≈ 5 (–10)	≈ 6
Observed or estimated reduction in population number	≥ 30%	≥ 30%
Total number of plants	< 3000	< 3000
Extent of occurrence (km ²)	< 10000	< 6000
Area of occupancy (km ²)	< 1	< 0.5
Endemism	W. Cape	W. Cape
Rarity	Rare	Rare
Major habitat	Fynbos	Fynbos
Major system	Seasonal terrestrial wetlands	Seasonal terrestrial wetlands
Habitat description	Sandy, clayey or loam soils on flats in sand fynbos, alluvium fynbos and renosterveld	Sand to loam soils on flats in sand fynbos and granite renosterveld
Breeding system and inferred pollen limitation	Partially self-incompatible, completely pollinator-dependent and pollen-limited	Partially self-incompatible, highly pollinator-dependent, pollen-limited
Pollination system	Generalised, primarily hopliine beetles and beetles of family Melyridae	Generalised, primarily hopliine beetles
Threats	Urban expansion, infrastructure development, agricultural expansion, overgrazing, overcollection, invasive alien vegetation, severe habitat fragmentation, population size, pollution, drought, groundwater extraction	Urban expansion, infrastructure development, agricultural expansion, overgrazing, overcollection, invasive alien vegetation, severe habitat fragmentation, pollution, drought, groundwater extraction
Population trend	Decreasing	Decreasing
Proposed IUCN Red List status and criteria	Endangered B2ab(i,ii,iii,iv,v)	Endangered B2ab(i,ii,iii,iv,v)
Proposed South African Red List status and criteria	Endangered B2ab(i,ii,iii,iv,v)	Endangered B2ab(i,ii,iii,iv,v)

Supplementary Figures



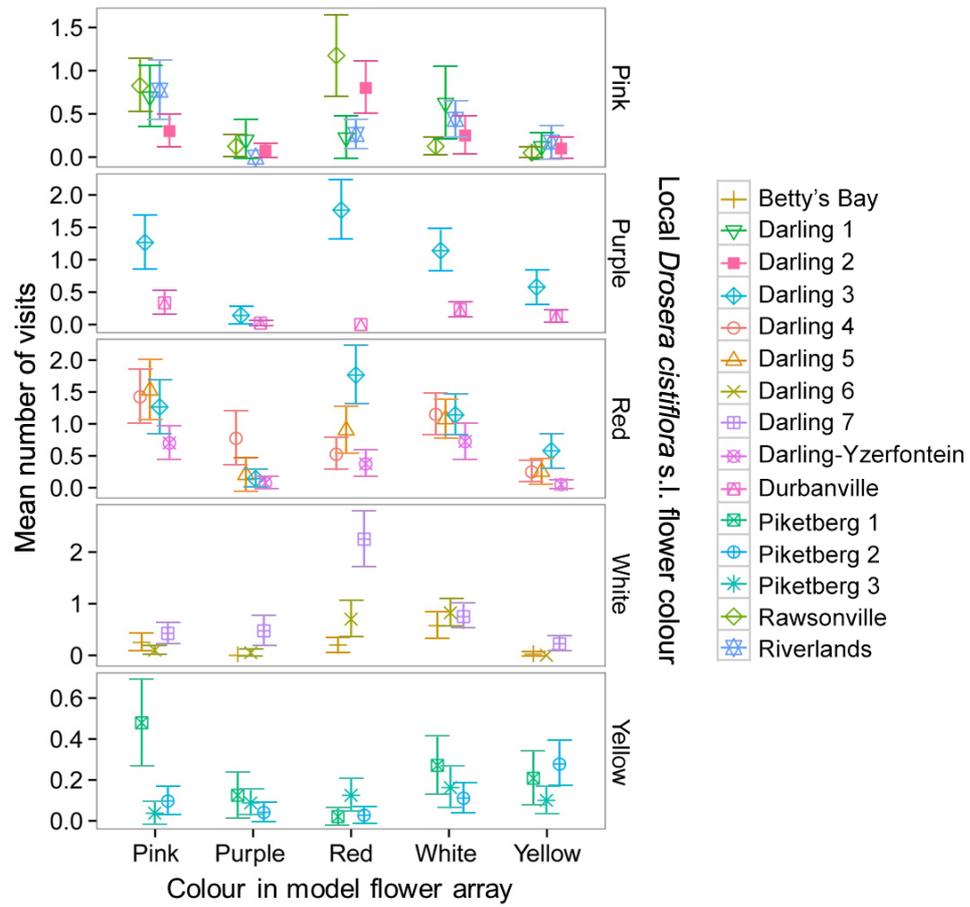
Supplementary Figure 1. A comparison of purple-flowered forms of *Drosera cistiflora* s.l. at Durbanville (a and b) and Darling 3 (c), showing partial (a) and complete (b) lack of pollen formation, and reflexed petals, at Durbanville, versus normal pollen production (c) and crateriform corollas at Darling 3



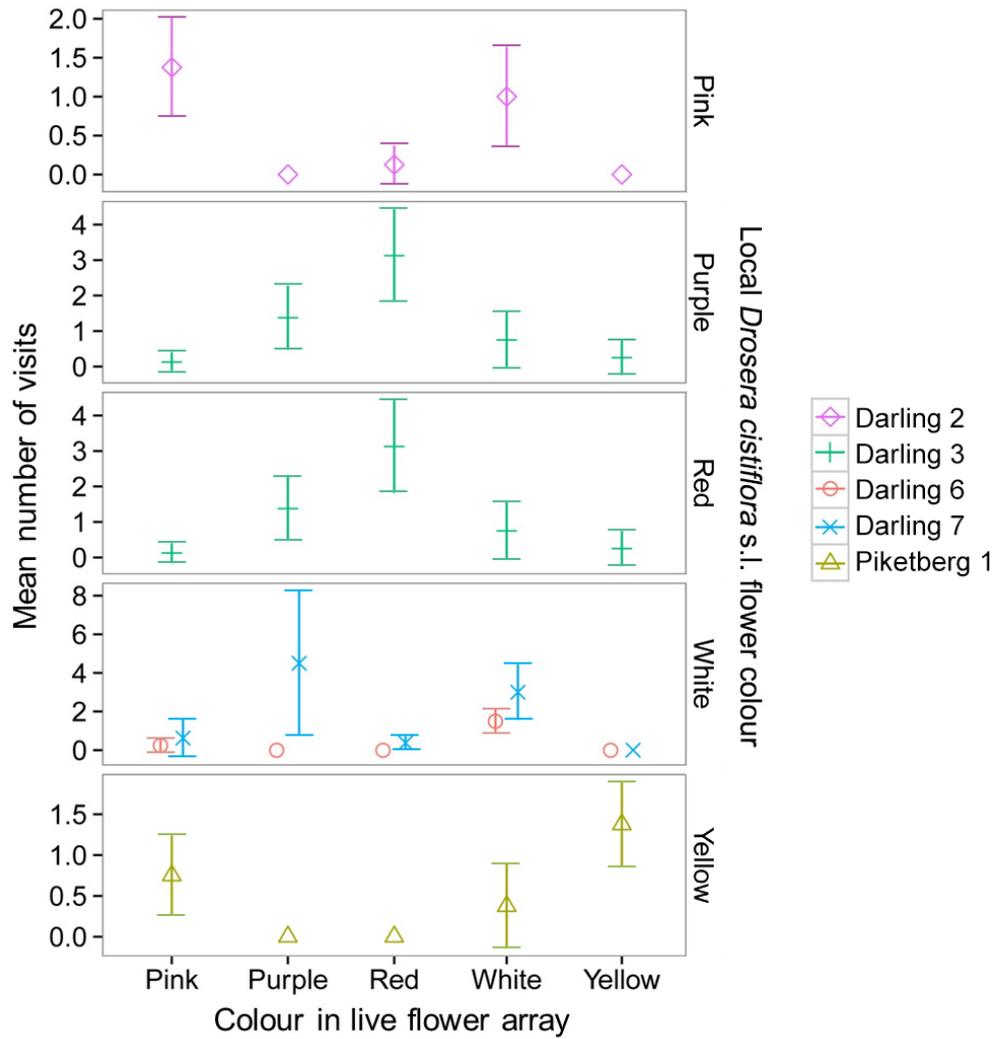
Supplementary Figure 2. Experimental design of the common-garden and soil switching experiment, using the red *Drosera cistiflora* s.l. floral colour form from Darling 5 as an example. Separate samples of three plants in bud (1–6) were grown in soil from each *D. cistiflora* s.l. floral colour form (a–e) as well as from a site where the plants did not occur (f). Control plants (c) were potted in their native soils. The experiment was carried out using plants from one population of each of the pink-, purple-, red-, white- and yellow-flowered *D. cistiflora* s.l. forms. All experimental plants were kept in common environmental conditions and experienced common changes in temperature, light and moisture availability. Plants and soils were obtained from Darling 2 (granite and granodiorite soils supporting the pink-flowered form); Darling 3 (loam soils; purple-flowered form); Darling 5 (loam soils; red-flowered form); Darling 6 (granite and granodiorite soils; white-flowered form), and Piketberg 1 (grit and greywacke soils; yellow-flowered form). Clay soils collected from The Towers Farm, Darling were used for potting plants in soil from a site where *D. cistiflora* s.l. did not occur.



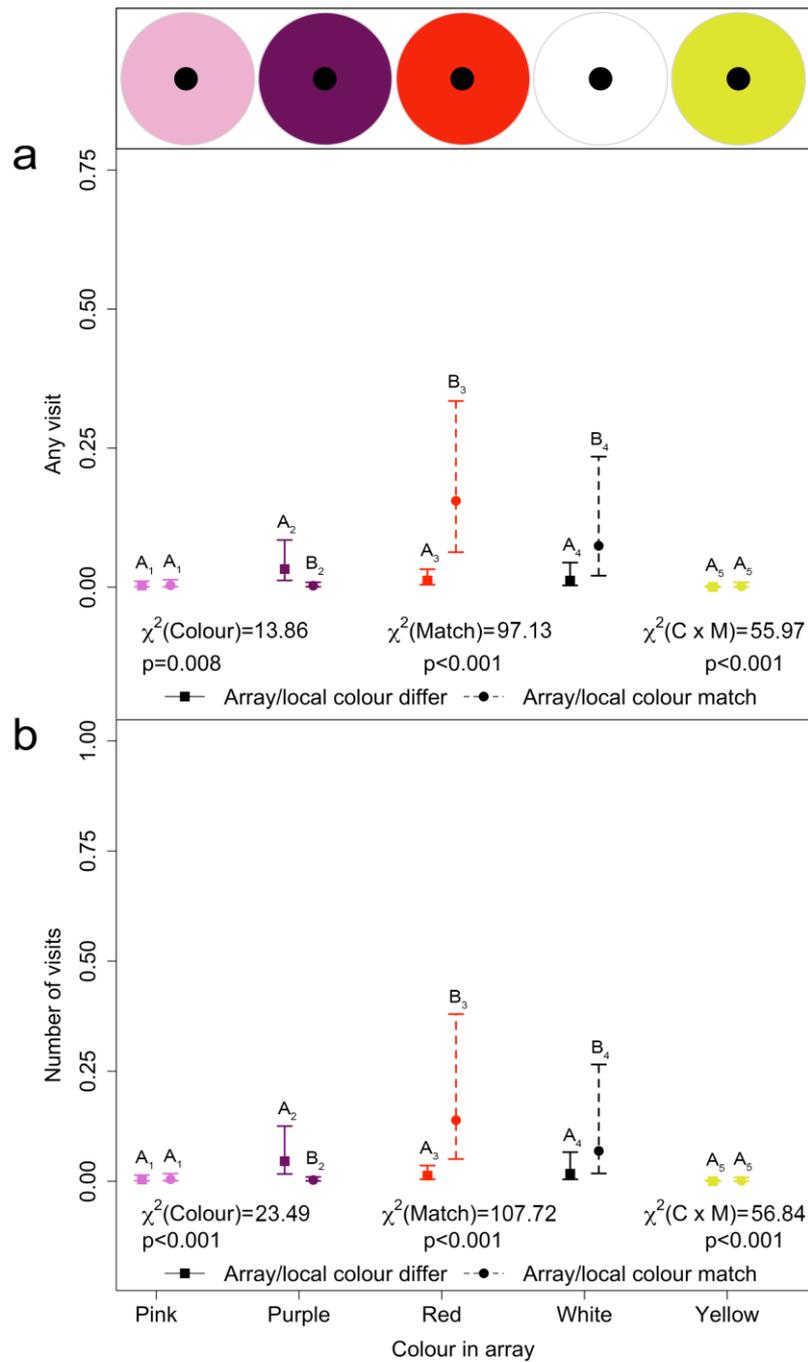
Supplementary Figure 3. Floral destruction by the lunate blister beetle *Hycleus lunatus* (Coleoptera: Meloidae: Meloinae: Mylabrini), the only florivore observed visiting *Drosera cistiflora* s.l.



Supplementary Figure 4. Number of visits of all pollinators to different model flower trap colours at individual populations of pink, purple, red, white and yellow *Drosera cistiflora* s.l. floral colour forms. Values represent mean number of visits and 95% confidence intervals around the mean.



Supplementary Figure 5. Number of visits of all pollinators to different live flower array colours at individual populations of pink, purple, red, white and yellow *Drosera cistiflora* s.l. floral colour forms. Values represent mean number of visits and 95% confidence intervals around the mean.



Supplementary Figure 6. Matching effects for visitation by the hopliine beetle *Lepisia rupicola* spec. (Coleoptera: Scarabaeidae: Hopliini) to model flower arrays in each *Drosera cistiflora* s.l. floral colour form. Values represent marginal model mean (and asymmetrical standard error) proportions for presence/absence of any visits (a) and number of visits (b). Post hoc tests (represented by A and B) are only used to compare means involving matched and non-matched model flowers of the same colour (matched or non-matched means of different colours are not compared). Subscripts 1–5 are used to differentiate the different model flower colours. Means that share the same letter-subscript combination are not significantly different (Tukey test).



Supplementary Figure 7. All insect pollinators observed in sympatric purple and red *Drosera cistiflora* s.l. flowers at Darling 3. Insects are arranged in order of importance from a–e (purple-flowered forms) and f–r (red-flowered forms). Larger circles distinguish principal pollinators, viz. hopliine beetles (Coleoptera: Scarabaeidae: Hopliini), namely *Omocrates* sp. (a) [purple-flowered forms] and *Lepisia rupicola* spec. (f) [red-flowered forms]. Insect names are listed in Table 2, Chapter 5.