Pollination and the evolution of floral traits: selected studies in the Cape flora

by

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Statement

The conception, planning, execution and writing of this study was entirely my own except in the specific instances mentioned below.

Some of the chapters are adapted from published papers which were coauthored with either one of my supervisors, William Bond and Kim Steiner. Their contributions were mainly through discussions and suggestions on how to improve the manuscripts. The cladistic analysis in Chapter 4 was done in collaboration with Peter Linder who is an authority in this field.

Appendix B is a paper written by Kim Steiner, with Vin Whitehead and myself as coauthors. We independently discovered sexual deception in Cape orchids and the paper contains some of my data and observations.

Acknowledgements

I have thoroughly enjoyed working with my supervisors, Professor William Bond and Dr Kim Steiner. As biologists from the ecological and systematic traditions, respectively, their roles were complementary. I particularly appreciated William's broad knowledge and uncanny ability to see the crux of a problem, and Kim's experience and expertise in the field of evolutionary pollination ecology. I wish to thank them wholeheartedly for their support and guidance.

I am also thankful to the many people who took an interest in my work and provided ideas and encouragement; deserving of special mention are Amots Dafni; Louis Vogelpoel, Hubert Kurzweil, John Donaldson, John Manning, Peter Goldblatt, Dave McDonald and Richard Cowling. Peter Linder helped with the cladistic analysis and Ben-Erik van Wyk analyzed nectar sugar ratios for several species. In addition, I am grateful to Vin Whitehead, Connel Eardley, John Manning and David Barraclough for help with insect identification. Bill Liltved played an important role as a companion on countless field trips and was always willing to walk the extra mile or ten. Other friends who helped in the field were Stefaan Steyn, Ian Jennings, Andrew Marquard and Bruno Schostar. Conservation officials Jan Vlok ("the fynbos encyclopedia"), Chris Martens, Rory Allerdice and Frans Krige went out of their way to assist me. I am also grateful to the many farmers who allowed me access to their land, offered coffee and gave me a place to sleep.

Financially, the project was made possible through grants by the Foundation for Research Development, National Botanical Institute and Charles Dixon bequest.

Finally, I am privileged to have a stimulating and supportive family: my parents, whose own love of nature helped inspire me to become a biologist, David, Lindy and most importantly my wife Kathy, without whom I would probably be a quivering wreck.

Frontispiece

Top left. The author in his natural habitat **Top right**. The remarkable Mountain Pride butterfly *Meneris tulbaghia* has an exclusive preference for red flowers; here it is seen visiting the orchid *Disa ferruginea*. **Middle left.** Pollinaria attached to the proboscis of this horsefly betray the fact that it pollinates an orchid. **Bottom left**. Long-proboscid flies are a unique and spectacular component of the Cape fauna. Here the fly *Moegistorynchus longisrostris* (Nemestrinidae) is attempting to insert its 80 mm proboscis into the splendidly adapted flower of *Lapeirousia anceps* (Iridaceae). **Bottom right**. It took a century of observation by naturalists to discover that carpenter bees pollinate the showy blue flowers of the 'blue disa', *Herschelianthe graminifolia.*





ABSTRACT

The study of plant pollination mechanisms has produced some of the most convincing examples of natural selection and adaptation. The aims of this thesis were to determine the role of pollinators in the evolution of floral traits in selected Cape plants, and to reach a better understanding of the relationship between floral adaptation and speciation. To establish a set of testable hypotheses, I asked specifically how adaptation to pollinators can explain three striking patterns in the Cape flora, namely (1) *convergence* in floral form between unrelated lineages (2) floral *mimicry* and (3) *adaptive radiation* within genera.

The convergent evolution of very large red flowers in unrelated Cape plants is addressed in Chapter 1. These species are shown to be pollinated almost exclusively by the Mountain Pride butterfly *Meneris tulbaghia* (Nymphalidae: Satyrinae). The convergent floral features in the guild were found to reflect the peculiar foraging behaviour of the butterfly, which includes a strong preference for large, red flowers over other sizes and colours.

Chapter 2 deals with the evolution of floral similarity through mimicry, rather than convergence. Extensive evidence is provided in support of the hypothesis that *Disa ferruginea*, a non-rewarding orchid, secures pollinator visits through mimicry of the floral traits of two nectar-producing species.

Patterns of radiation in pollination systems are examined in chapters 3-4. I carried out a detailed study of pollination in the large orchid genus, *Disa*, which has radiated extensively in the Cape flora. This first involved intensive field observations, since there was very little pre-existing information on pollination in this genus. Case histories based on these observations are given in Chapter 3. Pollination systems were then mapped onto a phylogeny in order to derive pathways of radiation within the group (Chapter 4). This showed that the cladogenesis of *Disa* is replete with examples of floral shifts between different pollinators.

In chapters 5-7, I deal with the process of radiation, specifically the roles of spatial heterogeneity in pollinator availability and pollen-limitation of plant reproductive success. In Chapter 5, I show that divergence in spur length in *Disa* species complex is attributable to a shift between a short-tongued fly in the mountains and a long-tongued fly on the sandplain. In Chapter 6, I establish that reproductive success in populations of *Disa uniflora* (Orchidaceae) is affected by the habitat preferences of its pollinator, the Mountain Pride butterfly. In Chapter 7, I show that fecundity was limited by pollinator availability, rather than resources, in eleven out of twelve herbaceous Cape wildflower species. Strong selection for traits which improve the effectiveness of pollination may, therefore, explain the numerous pollinator shifts shown by Cape plants.

In Chapter 8, which concludes the thesis, I explore the link between floral adaptation and speciation. I argue from the Darwinian position that speciation is often an incidental consequence of adaptive divergence in floral traits. Since most cases of floral divergence occur in allopatry, selection for isolation between congeners may play only a minor role in floral evolution - contrary to the claims of many evolutionists. To support the idea that pollinator-driven speciation has occurred frequently in Cape plants, I cite the large number of genera whose taxonomy is based almost entirely on floral characters.

Several published papers which are ancillary to the main themes of the thesis have been included as appendices.

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"*Fieldwork [in the post-victorian era] became the domain of amateurs and bumbling old men......It is only relatively recently that field study and the natural history approach have emerged from being considered outmoded and useless.*" Scourse (1983)

"*The pollination component of any ecosystem is in a sense a major evolutionary steering wheel.*" Stirton (1981)

INTRODUCTION

For centuries Cape plants have fascinated botanists, horticulturists and the general public alike. All would agree that there is something unique about the Cape flora: not only its richness (there are 8500 species in an area only 1000 km long by 200 km wide), but also in the showiness and brilliant colours of the flowers. One might have expected that whole books would have been written on the subject of pollination mechanisms in this flora; spectacular flowers, after all, suggest something spectacular about pollination. Yet, until recently, pollination of the Cape flora was a virtually unexplored subject.

Pollination biology in the Cape

The first Cape plant to arouse curiosity as to its method of pollination was the orchid *Disa uniflora* with its dramatic large red flowers. Early botanists and naturalists struggled for years to solve its pollination biology. Roland Trimen (1864), for example, guessed that a "day-flying" insect was responsible for its pollination, but also noted that few flowers showed any evidence of pollination. Later, Bolus (1888), frustrated at his inability to find fruits of the orchid, speculated that frequent fires on the Cape Peninsula may have led to the extinction of the pollinator. But finally, in 1895, Rudolf Marloth reported that he had managed to capture a large satyrid butterfly with a pollinarium of *D. uniflora* attached to its legs.

Marloth continued to make scattered observations on Cape plants, possibly inspired by Darwin's work on orchid pollination (Darwin, 1862). After Marloth there was a dearth of publications, with only a few original observations (eg Garside, 1922). The most important work during this period was Vogel's (1954) volume on floral morphology in which he classifies flowers as belonging to one of several "pollination syndromes"; this paper was important in drawing attention to floral radiation in Cape genera, but it contains very few original field observations. Although there has been a resurgence of interest in the pollination of Cape plants (Wiens *et al*, 1983; Rebelo, 1987a; Johnson, 1992c and references therein), our knowledge is still extremely limited.

Natural history (the study of animals and plants in the field) is basic to any study of pollination: the definitive moment often results from a fleeting encounter between insect and flower under circumstances which cannot be recreated in the laboratory. However, one of the possible reasons why pollination biology made so few advances in the first half of this century, not only in the Cape but also elsewhere, is that natural history became (wrongly) associated with fuzzy thinking and an aesthetic rather than scientific approach to nature study.

This prejudice was essentially a post-victorian phenomenon that coincided with the rise of the physical sciences and physiology (Scourse, 1983). A popular notion persists that scientific endeavour is restricted to the use of expensive laboratory equipment or computers. In practice, however, evolutionary research at the organism level cannot usually proceed without natural history (cf. Mayr, 1992). There is no better place to stimulate the biologist's mind than in the field, where carefully developed theories often crumble in the face of observational evidence.

Special problems of research in the Cape flora

Field work is never as romantic as some would assume and the vagaries of weather can hamper observations or experiments in any region. I am convinced, however, that pollination biologists in the Cape flora face a special set of problems which, in addition to the sociological reasons outlined above, may have contributed to the paucity of our knowledge at present. The first of these is the dependence of many Cape plants on fire to stimulate flowering. Flowering populations of these species can only be found if there has been a recent wildfire. Secondly, populations often occur in almost inaccessible mountain localities. Thirdly, the abundance of pollinators appears to be very low (cf. Marloth, 1908), though this is difficult to quantify directly.

Trends in pollination biology

The study of pollination became one of the fastest growing sub-disciplines of biology during the 1970s and 1980s, as evidenced by the exponential increase in pollination-related papers during this period (Kearns & Inouye, 1993). Most of the research is confined to the well-known floras of the northern hemisphere, though there is also a rapidly developing interest in pollination biology in Australia, as well as in less explored regions such as Madagascar.

Pollination biologists address diverse questions. Some, for instance, are concerned chiefly with the description of the process itself, *viz a vis* natural history, while others are far more interested in using their observations to test hypotheses about ecology and evolution. Most pollination biologists work within either the comparative systematics tradition or the more experimental discipline of evolutionary ecology. Of course, no research category is exclusive and many biologists, including myself, have interests which overlap both traditions. Indeed, there are healthy signs of increasing collaboration between evolutionary ecologists and systematists; for example, the current interest in using phylogenies to reveal pathways of evolution of ecological traits (cf. Manning & Linder, 1992; Armbruster, 1992, 1994; McDade, 1992; Stein, 1992).

Why study orchids?

A large portion of this thesis is devoted to a study of adaptive radiation in the terrestrial orchid genus *Disa*. Orchids have several features which make them ideal for studies of pollination. The most important of these is the presence of pollen in discrete packages (pollinaria), rather than loose pollen as in most other plants. These pollinaria are usually distinctive and can be observed without a microscope on the bodies of insects, making the field identification of pollinators a far simpler task than is the case with other plant families.

On the negative side, orchids are often viewed as "special cases", freaks of the plant world which follow a different set of rules. To some extent this may be true: the precision of pollinaria transfer in most Orchidaceae demands a closer morphological correspondence between flower and pollinator than in other plant families. Adaptation to different pollinators, therefore, is nearly always associated with changes in flower structure. While orchids might be more predisposed to floral radiation than other plants, this does not mean that the insights gained from a study of orchid pollination systems are not applicable to other plants. Indeed, orchids may closely reflect the properties of the pollination environment. For example, a subtle turnover in pollinators from one region to another may be evident from a study of ecotypes within an orchid species (cf. Robertson & Wyatt, 1990).

Aims and rationale of this study

The evolutionary biologist, according to Perrin and Travis (1992), is one who is "given patterns, and must ask whether these patterns are solutions to some problems, and if so, which problems?" Indeed, it was the striking patterns of floral radiation and convergence which stimulated questions in my mind about the role of pollinators in the evolution of Cape plants.

The most obvious pattern to result from adaptive evolution is radiation of forms within taxa (cf. Grant & Grant, 1965) (Fig 1A). A secondary pattern which may emerge is convergence, the evolution of similar features in unrelated lineages (cf. Faegri & van der Pijl, 1979; Brown & Kodric-Brown, 1979) (Fig 1C). Convergence is strong evidence for adaptation (this is because non-adaptive evolution - eg. through drift - is unlikely to produce convergence in suites of characters). A third and much more unusual pattern is advergence or mimicry, where one species evolves an adaptive resemblance to another species (cf. Wiens, 1978; Dafni, 1984) (Fig 1B).

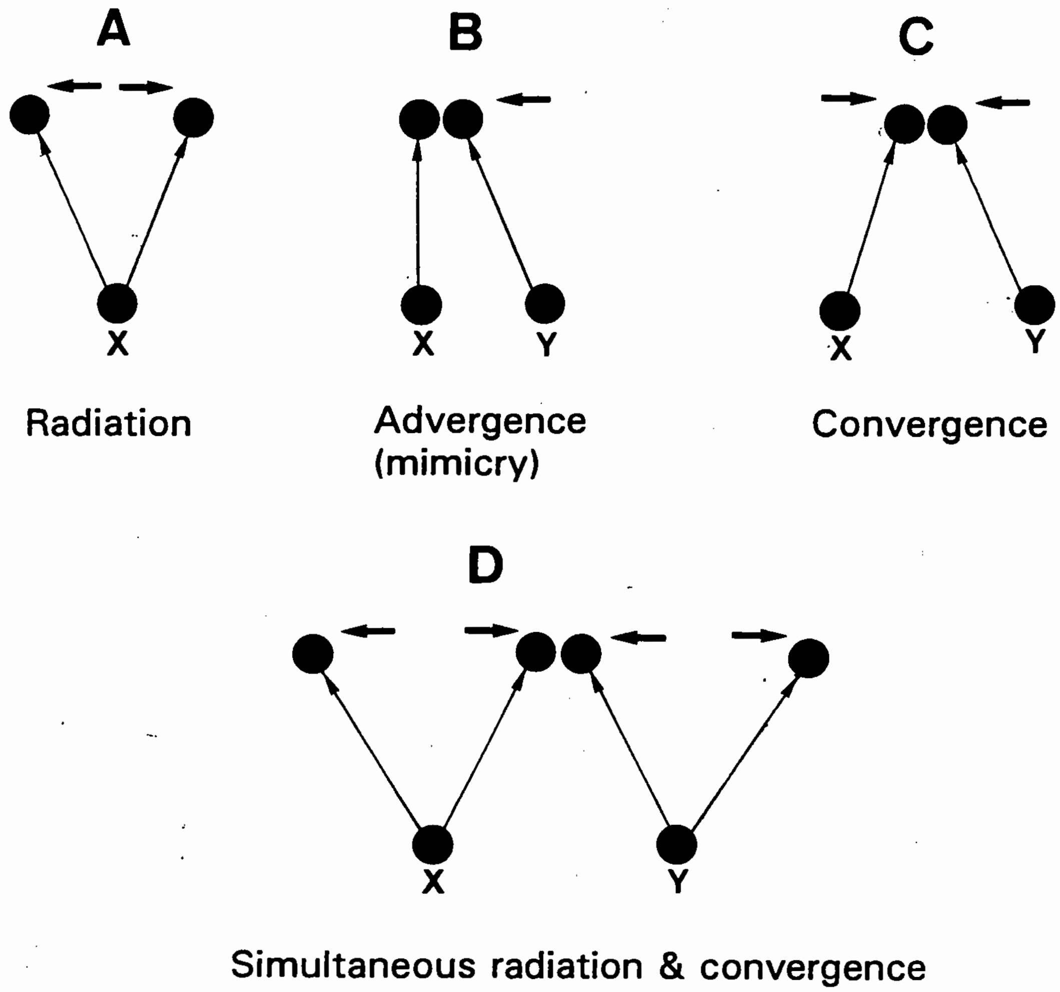

Figure 1. Patterns resulting from adaptive evolution. **A,** Adaptive radiation: taxon X diverges into two taxa. **B,** Advergence (mimicry): taxon Y evolves a mimetic resemblance to taxon X. **C,** Convergence: taxa X and Y evolve similar features under a common selective pressure. **D,** Patterns of radiation and convergence can result from the same evolutionary event; the adaptive radiation of taxon X into two daughter taxa results in a pattern of convergence with one of the daughter taxa of taxon Y.

It is important to note that a single evolutionary event can simultaneously result in more than one pattern. For example, if red flowers evolve independently in two lineages under a common selective pressure, this adaptation results in both convergence between lineages and radiation within each lineage (Fig 1D). This was clearly expressed by Proctor (1978):

> "*Within any evolutionary line we have a complex of selection pressures towards diversity operating in relation to a limited number of groups of specialised pollinators. The consequence is seen in an interplay of adaptive radiation within particular groups of plants and convergent evolution of members of diverse plant families into a limited number of available syndromes of adaptation to particular pollinators.*"

My aim throughout this study has been to demonstrate the importance of pollination biology for an understanding of the floral patterns in the Cape flora. Specific examples were chosen to test the hypothesis that radiation, convergence and mimicry in floral traits are epiphenomena which reflect adaptation to pollinators at a population level. Alternative hypotheses considered were that these floral patterns are pleiotropic or allometric consequences of adaptation in other characters, or the result of non-adaptive processes such as drift. Ultimately, I was interested in arriving at a clearer understanding of how species may be generated by adaptation to pollinators, as this may help explain some of the remarkable species richness of the Cape flora.

PART 1

Convergent evolution

1 Red flowers and butterfly pollination in the Cape flora[1]

A guild of Cape species with red flowers is pollinated almost exclusively by the butterfly *Meneris tulbaghia* (Nymphalidae: Satyrinae). Such dependence on a single species of pollinator is rarely found in plants. Species pollinated by *M. tulbaghia* share several convergent characteristics including large, red flowers with straight, narrow nectar tubes and a flowering period in late summer. The butterfly appears to be attracted primarily to the red colour of the flowers as shown by experiments in which butterflies chose red coloured model flowers over other colours. The straight, narrow nectar tubes of flowers in the guild discourage visits by sunbirds which otherwise visit red flowers. Nectar properties of plants in the guild vary considerably, but most species have nectar of between 15 and 25% sugar concentration. Similar low sugar concentrations were found in a sample of bird-pollinated Cape species, many of which are regularly 'robbed' of nectar by the butterfly. Perception of red colour by *M. tulbaghia* might have evolved because it facilitated exploitation of the copious amounts of dilute nectar available in most red bird-pollinated flowers. The nectar in many of its host plants is more dilute than the optimal predicted by experiments in which the relationship between nectar concentration and sugar uptake by the butterfly was determined. Therefore, the need to maintain a favourable water balance may play as important a role as energetics in nectar selection during the hot, dry summer months. Plants pollinated by other insects might have shifted to *M. tulbaghia* because of the efficiency of a pollinator which visits flowers of only one colour.

"Independent evolution of similar traits in different lineages indicates convergence, one of the strongest types of evidence for adaptation." Wanntorp *et al* (1990)

INTRODUCTION

The association between red flowers and pollination by nectarivorous birds is well known (Grant, 1966; Raven, 1973). Insect-pollinated plants, on the other hand, rarely have red flowers. For example, the near absence of bird pollination in Europe is generally considered the reason for the paucity of red flowers in the European flora. Perception of red flowers, however, is not entirely restricted to birds. In Israel a small guild of red-flowered plants is

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[1] Adapted from Johnson & Bond (1994)

pollinated by *Amphicoma* beetles (Dafni *et al*, 1990). Some flowers in tropical America with contrasting red and yellow patterns (eg. *Lantana camara*, *Caesalpinia pulcherrima*) are visited by butterflies (Cruden & Hermann-Parker, 1979; Boyden, 1980). Butterflies may have the widest visual spectrum of all animals, with perception of both ultraviolet and red light in some species (Bernard, 1979; Eguchi *et al*, 1982).

In South Africa, a number of red-flowered species are pollinated by the butterfly *Meneris tulbaghia* (hereafter simply termed *Meneris* as the genus is monotypic). It is outstanding among the Satyrinae for its large size (wingspan = 80 mm), swift flight and flower-visiting habits. *Meneris* is found throughout the moister mountain ranges of southern Africa and favours rocky outcrops and gorges. It is fairly common in the Cape, a region relatively depauperate in butterflies (Cottrell, 1985).

The attraction of the butterfly to red flowers was noted by early lepidopterists (Trimen, 1887) and botanists (Trimen, 1887; Marloth, 1895, 1915). Marloth (1895) showed that the spectacular red flowers of the orchid *Disa uniflora* are pollinated by *Meneris*, which carries the pollinaria on its legs. The butterfly is also well known to mountaineers for its habit of swooping down on red hats and socks! Despite popular interest in this unusual butterfly (Johnson, 1992a), no proper study of its pollination relationships had been undertaken. This study was based on the following questions:

1. Which species are pollinated by *Meneris*? Although Marloth had recorded visits to many species with red flowers, many other inaccessible species had not been examined in the field.
2. Do these species pollinated by *Meneris* share convergent characters, eg. colour, morphology, flowering time and nectar properties?
3. Can these convergent characters (if any) be explained by the peculiarities in the foraging behaviour, phenology and morphology of the butterfly?

METHODS

Field observations

Field work took place at several sites in the western, southern and eastern Cape Province during 1990-1992 (Table 1.1). Plants known, or suspected, to be visited by *M. tulbaghia* were observed in the field. Careful attention was paid to whether the butterfly contacted the anthers and stigma of flowers and whether pollen was present on the body of the insect.

Colour analysis

The spectral reflectances of flowers of each species were measured using an ACS 550M spectrophotometer. This method avoids the problem of subjectively assessing colour. Only the attractive parts of the flower were measured. The reflectance curve from 400-700 nm gives an objective representation of colour in the wavelengths visible to most butterflies. In addition the spectra were analyzed using the new method of segment classification (Endler, 1990). This involves dividing the spectrum into four equal segments, finding the differences between the areas under the curve at alternating segments and plotting the two "differences" on a two-dimensional colour space. The method allows comparison between a number of reflectance curves at once and compensates for differences in brightness. It also approximates signal processing in animal colour vision which is thought to be based on opponency between signals from photoreceptors with different spectral sensitivities (Endler, 1990). Some butterflies are thought to have up to four visual pigments (Bernard, 1979), as opposed to humans and bees which have only three. Spectral reflectances from a sample of bird pollinated plants were also analyzed.

The colour preference of *Meneris* was examined experimentally by recording the relative proportions of visits to various coloured paper discs (model flowers). These were displayed randomly either in arrays in the field (1990) or in outdoor flight cages containing captive butterflies (1991). A reward of 20% honey solution was offered in glass funnels mounted in the centre of the paper discs in 1991. The proportion of visits to red paper discs of different sizes displayed in the field during 1990 was also recorded.

Nectar analysis

Nectar volumes of flowers in the field were measured using microcapillaries. A Bellingham and Stanley 0-50% refractometer was used to determine nectar sugar concentration. Nectar was extracted from flowers as close to 1200 h as possible in order to standardise results and because butterfly activity is high at this time. Nectar was also spotted onto Whatman no.1 filter paper and kept refrigerated until later analysis of sugar constituents using high pressure liquid chromatography (HPLC) could be performed.

The rate of sugar flux into butterflies at various sugar concentrations was measured for eight captive male butterflies. A range of honey solutions from 10 to 50% sugar by weight was prepared and a 5μl drop of each was placed onto a red plastic disc. The proboscis was unrolled with a pin and feeding usually commenced immediately once the tip of the proboscis was inserted into the drop of honey solution. The time taken to consume the drop was recorded. The sequence of concentrations offered to the butterfly was randomized.

Table 1.1. Species conforming to the syndrome of pollination by *Meneris tulbaghia.*

Family/species	Study site	Flower type	Flowering time
CRASSULACEAE			
Crassula coccinea	Table Mountain	Classic	Dec-Feb
AMARYLLIDACEAE			
Cyrtanthus guthrieae	Bredasdorp mountain	Brush	Mar
C. elatus	Robinson Pass	Brush	Dec-Feb
C. montanus *		Brush	Jan-Feb
Nerine sarniensis	Bettys Bay	Brush	Mar-Apr
Brunsvigia marginata	Du Toits Kloof, Bains Kloof	Brush	Feb-Mar
IRIDACEAE			
Gladiolus cardinalis	Du Toits Kloof, Bains Kloof	Flag	Dec-Jan
G. stefaniae	Montagu	Flag	Mar-Apr
G. nerinoides !	Jonkershoek	Classic	Jan-Feb
G. sempervirens	George Peak	Flag	Mar
G. cruentus # $		Flag	Jan
G. saundersoniae # $		Flag	Feb-Mar
G. stokei *		Flag/classic	Mar
Tritoniopsis leslei *		Classic	Feb-Mar
T. longituba *		Horizontal	Dec-Mar
Schizostylis coccinea $	Maclear district	Brush	Jan-Mar
ORCHIDACEAE			
Disa uniflora	Table Mountain	Flag	Dec-Feb
Disa ferruginea	Table Mountain	Horizontal	Feb-Mar
D. porrecta * $		Horizontal	Feb

* Species which conform to the *Meneris* syndrome but which have not been observed in the field
# Visitation by *Meneris* observed by J. Vlok (pers. comm.)
! Visitation observed by C.L.Wicht (letter housed in Bolus Herbarium)
$ Species occurring outside the Cape region

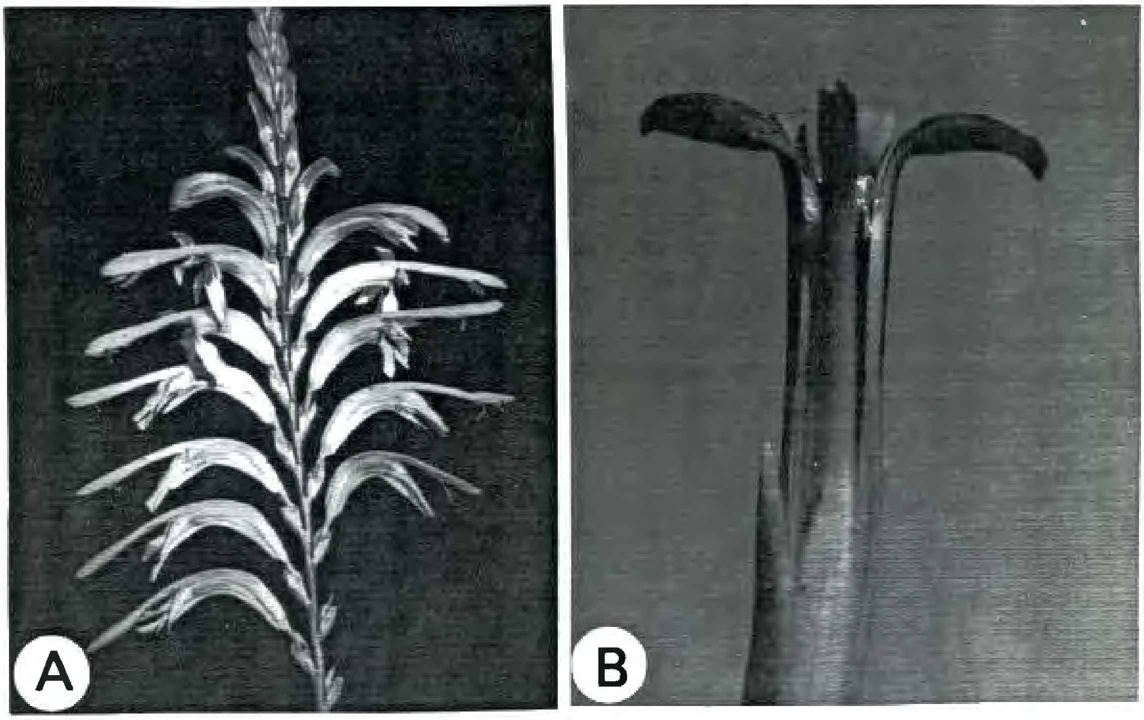


Figure 1.1. A, *Chasmanthe floribunda*, an example of a typical sunbird-pollinated plant. Note the curved perianth tube and the overarching anthers positioned to place pollen on the head of the sunbird. **B,** Cross-section of a flower of *Crassula coccinea*, a classic butterfly-type flower pollinated by *Meneris*, showing the straight perianth tube and inserted anthers positioned to place pollen on the proboscis.

RESULTS

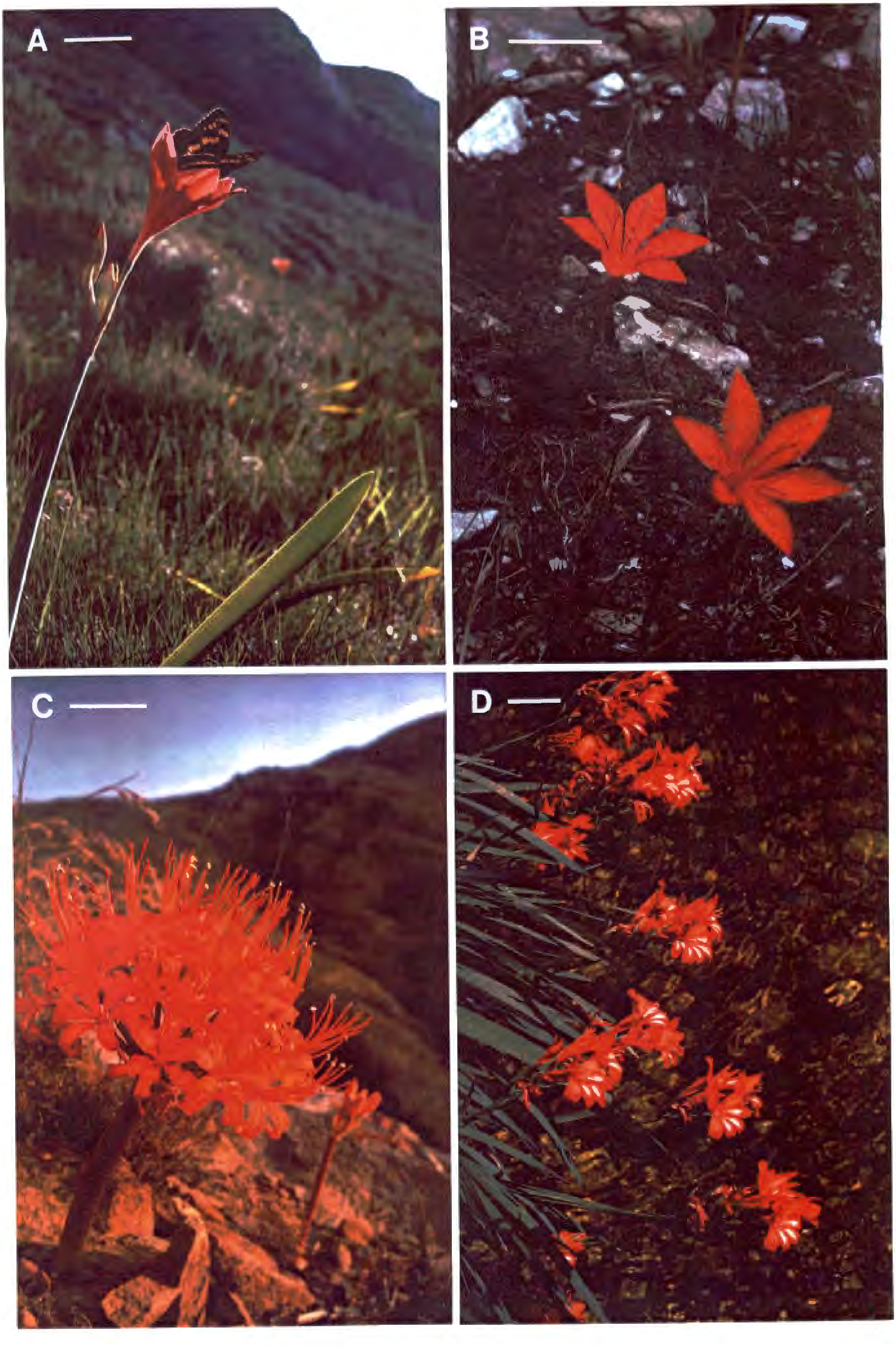
Field observations

Through field observations and examining butterflies for pollen it became clear that Cape species with red flowers which have nectar tubes which are narrow, straight and vertical were invariably pollinated by *Meneris*. These species all flower between December and April, corresponding to the flight period of the butterfly (Johnson, 1992c). The narrow nectar tubes of these species appear to prevent sunbirds from gaining access to the nectar. Sunbirds can obtain nectar from *Crassula coccinea*, for example, only by piercing the nectar tube. I observed a sunbird which apparently managed to extract nectar from *Nerine sarniensis*, but the bird did not contact the anthers due to the brush-type morphology of this flower which is adapted to *Meneris* (see Discussion). By contrast, the narrow proboscis of *Meneris* allows it frequently to "rob" nectar from bird-pollinated flowers. Pollination by *Meneris* occurs in at least four families and eight genera (Table 1.1). Nineteen South African species, of which fifteen are endemic to the Cape region, appear to rely solely on *Meneris* for pollination. All these species, with the exception of *Crassula coccinea* (Crassulaceae), are petaloid monocotyledons.

Figure 1.2. Flowers pollinated by the Mountain Pride butterfly *Meneris tulbaghia*. **A**, The butterfly settled to feed on nectar from *Cyrtanthus elatus*. **B.** Brilliant red brush-type flowers of *Cyrtanthus guthrieae*. **C**, *Brunsvigia marginata*, a brush-type flower. **D**, Flag-type flowers of *Gladiolus cardinalis*.

5 cm

5 cm



5cm

The floral morphology varies greatly within the guild. Four floral types can be identified: *Classic-type* flowers (see Faegri & van der Pijl, 1979) have a narrow upright tube with inserted stamens and a flat rim (Fig 1.1 B). *Brush-type* flowers have tall extended stamens projecting from a funnel-shaped flower (Fig 1.2 A-C). *Flag-type* flowers are large and showy with a broad landing surface (Fig 1.2 D, Fig 3.1A). *Horizontal-type* flowers resemble bird-pollinated flowers closely, except that in some cases the tube is too narrow to allow access of a bird's bill (Fig 2.1C).

Colour analysis

Without exception, species pollinated by *Meneris* have spectral reflectances with a maximum positive slope between 570 and 650 nm, a curve shape perceived as red by humans (Fig 1.3). If these curves, as well as curves for bird-adapted flowers, are analyzed by segment classification, it can be seen that no substantial difference exists in the colours of *Meneris* and many bird-adapted flowers (Fig 1.4). The strong preference shown for red-coloured model flowers (Fig 1.5) is a striking feature of the behaviour of *Meneris*. Large model flowers were also strongly preferred over smaller models (Fig 1.5).

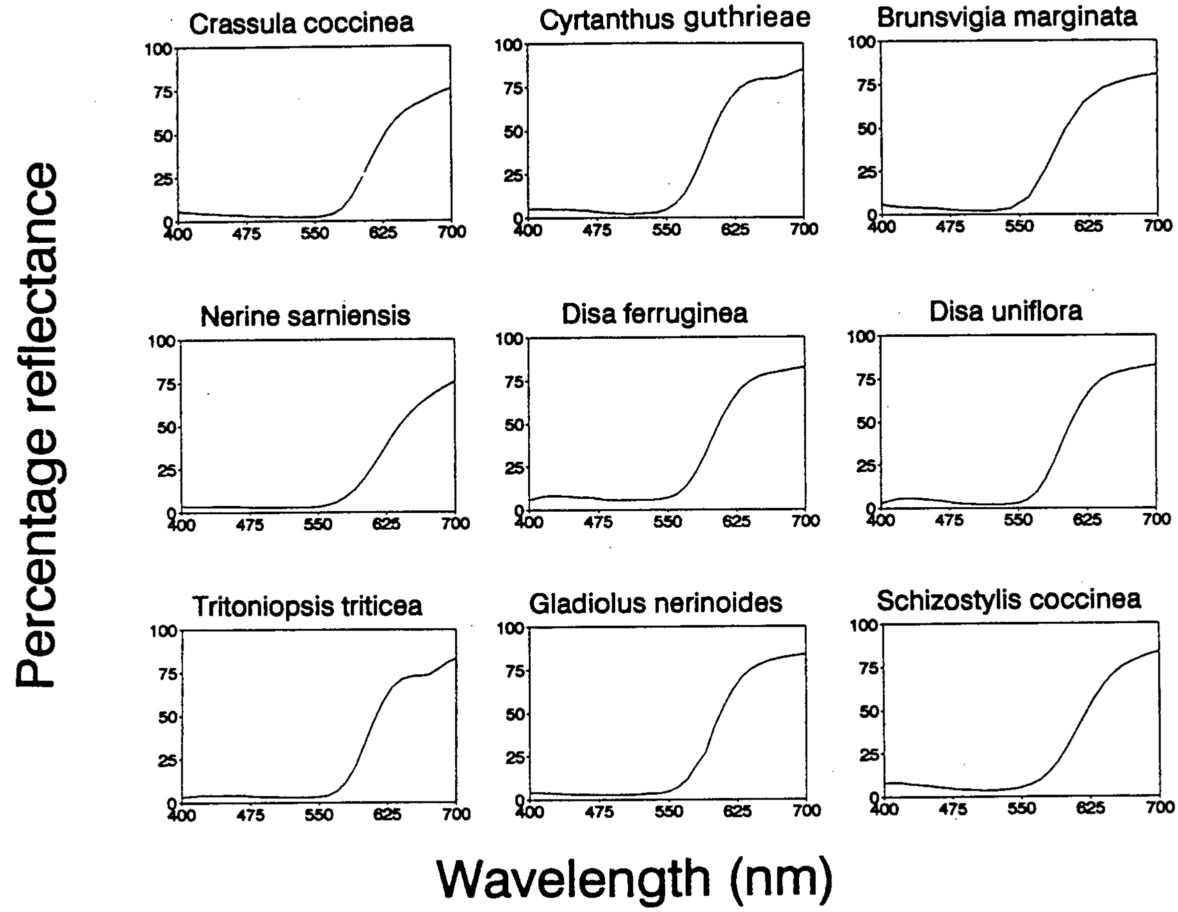


Figure 1.3. Spectral reflectances of the flowers of various species pollinated by *Meneris tulbaghia*.

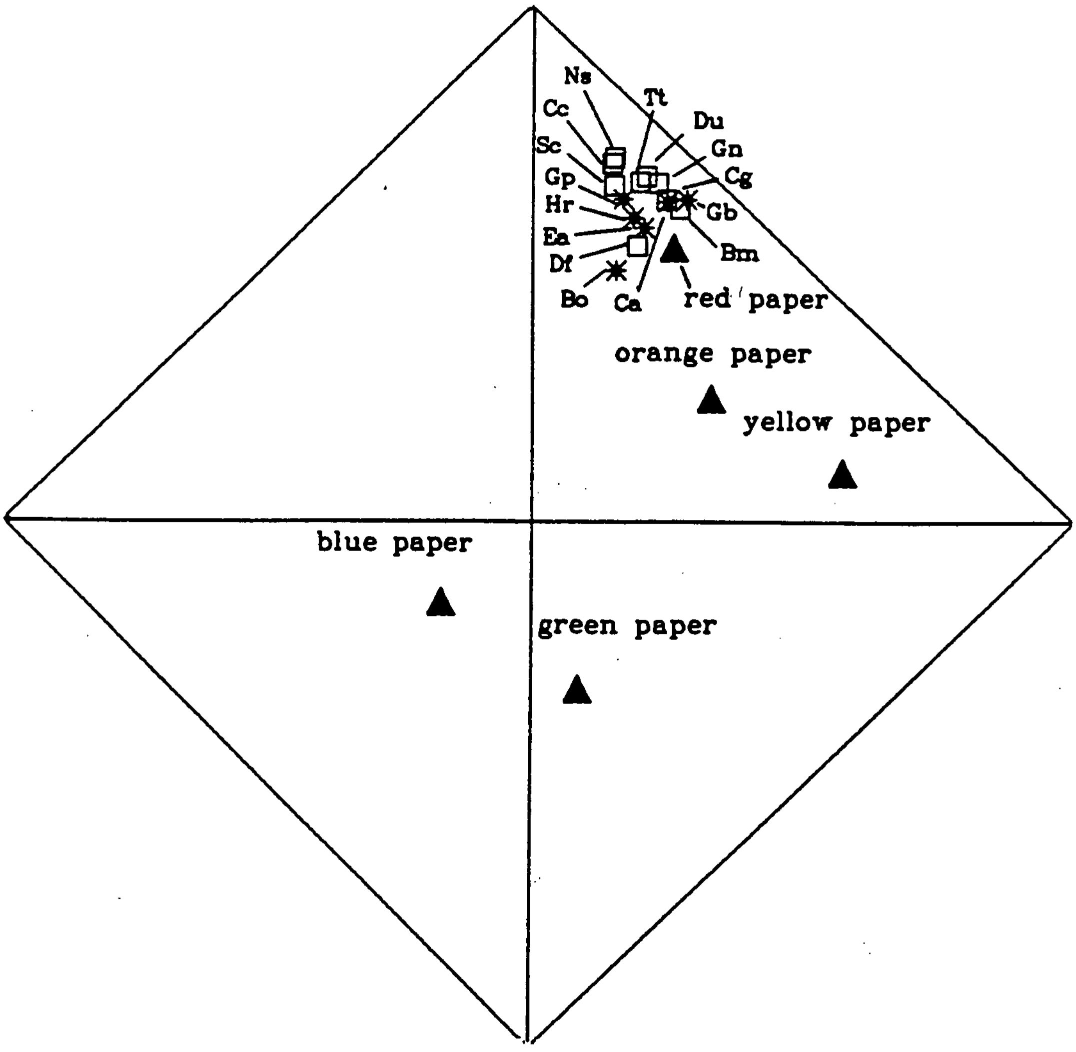


Figure 1.4. Segment classification (Endler, 1990) of reflectance spectra of *Meneris*-pollinated plants (□), bird-pollinated plants (*) and papers used in the discrimination experiments in 1991 (▲). The position of colours in the colour space is independent of human perception. Spectra closer to the origin are less saturated (paler, less chroma) and the angle with respect to the origin is a measure of the shape of the spectrum or hue. Species abbreviations are as follows: Ns - *Nerine sarniensis*, Tt - *Tritoniopsis triticea*, Du - *Disa uniflora*, Gn - *Gladiolus nerinoides*, Cg - *Cyrtanthus guthrieae*, Gb - *Gladiolus bonaespei*, Bm - *Brunsvigia marginata*, Ca - *Chasmanthe aethiopica*, Bo - *Brunsvigia orientalis*, Df - *Disa ferruginea*, Ea - *Erica abietina*, Gp - *Gladiolus priorii*, Hr - *Haemanthus rotundifolius*, Sc - *Schizostylis coccinea*, Cc - *Crassula coccinea*.

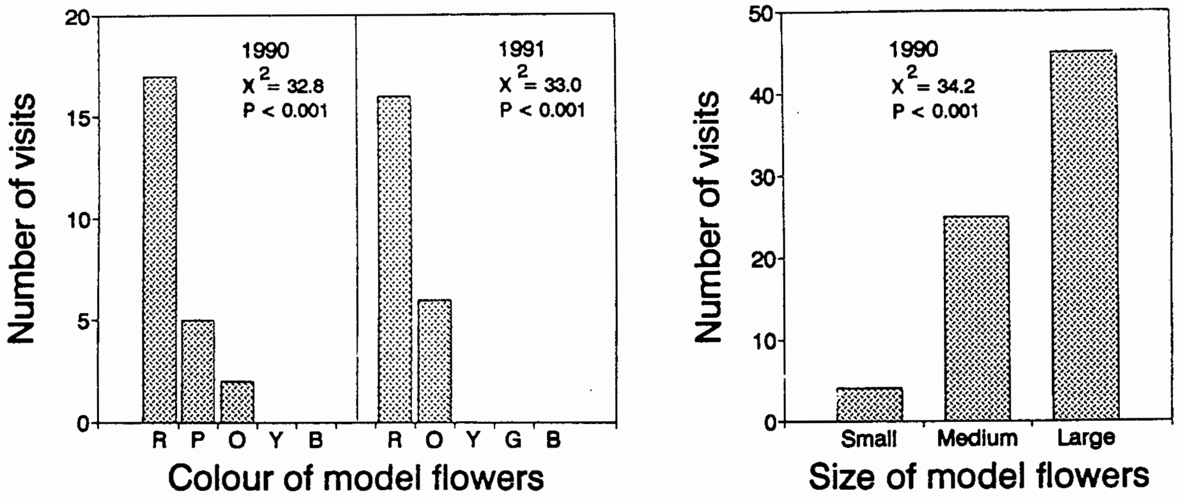


Figure 1.5. Discrimination by *Meneris tulbaghia* between flower models differing in colour and size. R = red, P = pink, O = orange, Y = yellow, G = green, B = blue. The diameters of the paper discs were as follows: small = 5 cm, medium = 10 cm, large = 15cm.

Nectar analysis

The nectar properties of plants adapted to *Meneris* showed great variation in volume, concentration and sugar proportions (Table 1.2). Comparison with a sample of bird-adapted nectars shows that the average nectar volumes and concentrations are almost identical (Table 1.2, 1.3). The orchid *Disa ferruginea* does not produce nectar and appears to mimic the nectar-producing species *Tritoniopsis triticea* and *Kniphofia uvaria* (see Chapter 2).

The feeding experiments showed that the rate of sugar uptake by butterflies is optimal for nectars in a broad range between 20 and 40% sugar concentration (Fig 1.6). Nectar above 40% is rich in sugar, but too viscous for optimal uptake.

Analysis of the constituent nectar sugars did not reveal the trend towards sucrose dominated nectar in butterfly-pollinated plants that has been reported elsewhere (Cruden & Hermann-Parker, 1979; Baker & Baker, 1983). Four species in the sample of *Meneris*-pollinated plants have sucrose dominated nectar and four species have nectar dominated by hexose sugars (glucose and fructose). Among the sample of species adapted for bird pollination, seven have sucrose dominated nectar and four have hexose dominated nectar.

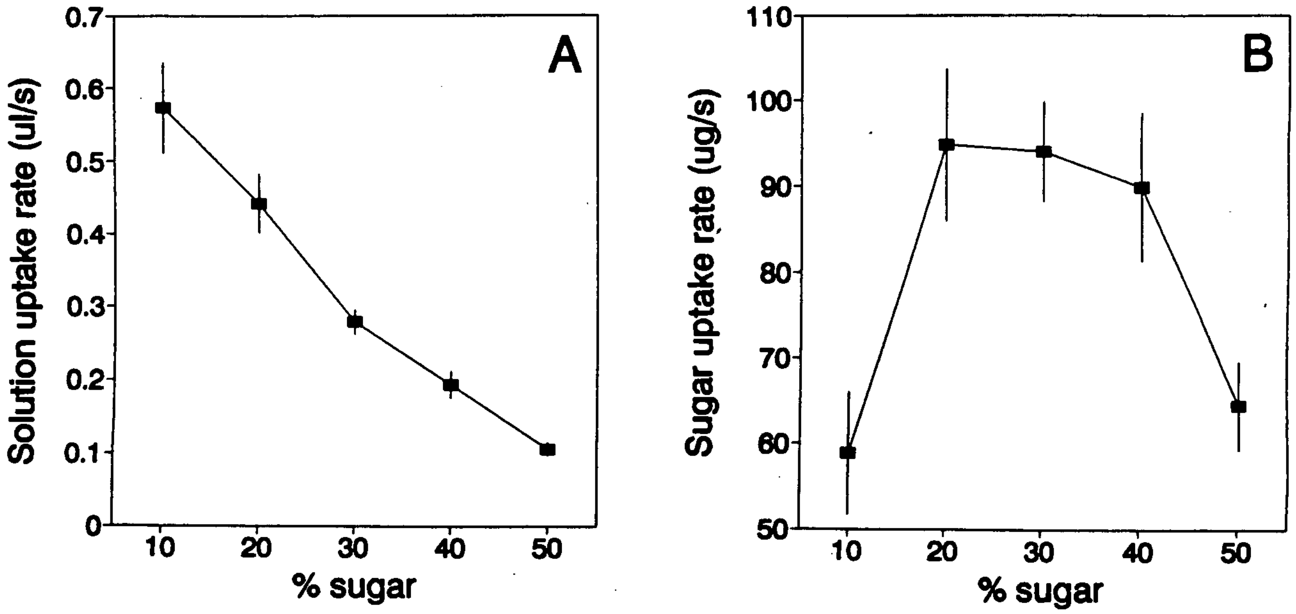


Figure 1.6. The relationship between uptake rates by *Meneris tulbaghia* and the sugar concentration of a honey-based artificial nectar. **A**, solution uptake. **B**, sugar uptake. The vertical bars indicate s.e.

Table 1.2 Nectar properties of species adapted to pollination by *Meneris tulbaghia*.

Family/species	Vol		Conc		Sugars (%)		
	µl	n	g/100g	n	Fru	Glu	Suc
CRASSULACEAE							
Crassula coccinea	2.4	(8)	16.4	(8)	54	39	7
AMARYLLLIDACEAE							
Brunsvigia marginata	3.1	(26)	26.5	(15)	34	44	22
Cyrtanthus elatus	-		-		20	26	54
C. guthrieae	9.8	(11)	17.7	(9)	14	19	67
Nerine sarniensis	4.3	(10)	36.0	(8)	50	42	8
IRIDACEAE							
Gladiolus cardinalis	9.4	(12)	24.8	(12)	9	16	75
G. stefaniae	-		-		10	12	78
Schizostylous coccinea	1.9	(19)	17.4	(11)	42	43	15
ORCHIDACEAE							
Disa uniflora	40.9	(5)	8.0	(5)	0	0	100
D. ferruginea	No nectar produced						
Average	10.2		21.0		29	30	41

Table 1.3. Nectar properties of species adapted to pollination by birds.

Family/species	Vol		Conc		Sugars (%)		
	µl	n	g/100g	n	Fru	Glu	Suc
AMARYLLIDACEAE							
*Cyrtanthus ventricosus**	**1.1**	(10)	**15.2**	(9)	-	-	-
*Haemanthus coccinea**	**2.0**	(23)	**33.0**	(7)	-	-	-
H. rotundifolius	**5.5**	(16)	**23.8**	(14)	**43**	**54**	**3**
Brunsvigia orientalis	**13.2**	(3)	**34.8**	(3)	**18**	**25**	**58**
IRIDACEAE							
*Tritoniopsis triticea**	**2.3**	(7)	**24.2**	(6)	**9**	**10**	**81**
*T. burchelli**	**6.2**	(9)	**20.8**	(8)	**16**	**16**	**69**
T. nervosa	**17.1**	(7)	**33.7**	(10)	**6**	**8**	**86**
*Watsonia tabularis**	**25.6**	(5)	**14.5**	(4)	**17**	**21**	**62**
Witsenia maura	**27.8**	(10)	**13.5**	(10	**45**	**55**	**0**
Gladiolus priorii	**13.4**	(4)	**26.3**	(3)	-	-	-
G. watsonius	**15.6**	(12)	**27.9**	(12)	-	-	-
Chasmanthe aeithiopicum	**10.1**	(6)	**13.9**	(6)	**46**	**51**	**3**
C. floribunda	**15.7**	(8)	**16.8**	(8)	-	-	-
Average	**12.0**		**23.0**		**23**	**27**	**50**

* Species which are important sources of nectar to *Meneris tulbaghia* and which may be partially pollinated by the butterfly.

DISCUSSION

A unique pollination syndrome?

A pollination syndrome is a set of convergent characteristics that appears in unrelated plant groups and is associated with the peculiar selective pressures imposed by shared pollinators. The following convergent characteristics can be identified in *Meneris*-pollinated plants: red flowers, large advertising area, dilute nectar, narrow and straight perianth tubes often with an upright orientation, and a flowering season from December to April. This "*Meneris* syndrome" combines elements of both the classical bird and butterfly syndromes. The red flowers are typical of bird-pollinated flowers, but birds are excluded by the floral morphology. The floral morphology in some species is typical of other butterfly-pollinated plants, but the coloration appears to exclude other butterflies. For example, *Princeps demodocus* (Papilionidae) is a large butterfly which, while often sympatric with *Meneris*, appears to ignore the flowers chosen by *Meneris* and instead tends to visit blue flowers. Thus by virtue of excluding birds (through morphology) and other insects (through colour), *Meneris*-pollinated plants rely almost exclusively on this single insect for pollination.

The various floral morphologies found in *Meneris*-pollinated plants represent distinct strategies for placing pollen on the butterfly. Classic-type flowers, with a narrow, upright nectar tube and flat rim, have inserted or partially inserted anthers which place pollen on the head and proboscis of the butterfly, eg. *Crassula coccinea* (Fig 1.1 B). Brush-type flowers (Fig 1.3 A-C) are most interesting as they represent the biggest deviation from the general butterfly-pollination syndrome (but see Cruden & Hermann-Parker, 1979). These flowers have straight, narrow, upright nectar tubes, but the anthers and stigma are highly exserted and function to brush pollen onto the wings and bodies of flying butterflies. Brush-type flowers are effective as they exploit a habit of *Meneris* of swooping down on flowers without landing. During these "inspection" visits, which usually outnumber feeding visits, pollen is brushed onto the butterfly. In *Brunsvigia marginata* and *Nerine sarniensis* the butterfly does not even contact the anthers during a feeding visit and pollination takes place almost exclusively during inspection visits. Inspection visits appear to form a higher proportion of the butterfly's daily activity later in the season and may be associated with territoriality and mate location. The wings and body are used for pollen placement as opposed to the proboscis which is considered a poor pollen-transporting surface (Wiklund *et al*, 1979).

Flag-type flowers, eg. *Disa uniflora*, have a large advertising area and a broad landing surface (Fig 1.2D, 3.1A). The site of pollen placement varies from the butterfly's legs, as in *Disa uniflora*, to its wings and body, as in *Gladiolus cardinalis*. Finally, horizontal-type flowers are closely allied to bird pollination and in species such as *Tritoniopsis triticea*, pollination by *Meneris* appears to be facultative to bird pollination. Examination of the stigmas of *Tritoniopsis triticea* showed that the majority were plastered with wing scales of *Meneris*, suggesting that pollen transfer may take place when the wings of a feeding butterfly contact the protruding anthers and stigmas of surrounding flowers on the inflorescence. However, birds are the primary pollinators of *T. triticea* (Chapter 2). In *Disa ferruginea* which has narrow horizontal spurs, pollination is exclusively by *Meneris* which carries pollinaria attached to the proboscis (Chapter 2). *Disa ferruginea* has often been considered, on the basis of morphological evidence only, to be bird-pollinated (eg. Vogel, 1954).

In terms of nectar properties, there is no substantial difference between species adapted to *Meneris* and species adapted to birds (Table 1.2, 1.3). Much of the nectar requirements of *Meneris* are met by bird-pollinated plants. In the summer rainfall area of South Africa, *Kniphofia* (Asphodelaceae), an ornithophilous genus, appears to be the primary nectar source for *Meneris*. In the Cape region, bird-pollinated members of *Tritoniopsis*, *Watsonia* (both Iridaceae), *Kniphofia* (Asphodelaceae) and *Erica* (Ericaceae) are all important nectar sources for the butterfly. The dilute nectar of bird-adapted flowers is ideal for butterflies which have to suck nectar through a narrow proboscis. However, measurements of sugar uptake in *Meneris* indicate that many of the nectars it feeds on may be too dilute in the sense that sugar uptake rates are not maximised (Fig 1.6). At least three species adapted to the butterfly have nectar below 20% sugar concentration with *Disa uniflora* at 8% the most dilute.

Predicted optimal sugar concentrations for the nectar of butterfly-pollinated plants, based on theoretical models of uptake through a narrow proboscis, range from 20 to 40% (Kingsolver & Daniel, 1979; Heyneman, 1983; Pivnick & McNeil, 1985). Laboratory experiments, similar to those reported here, show somewhat higher optimal nectar concentrations with the highest rates of sugar uptake by butterflies occuring for nectars varying from 35-40% (May, 1985) to 40% (Pivnick & McNeil, 1985). Field observations by Pivnick and McNeil (1985) indicated that the European Skipper *Thymelicus lineola* fed on nectars up to 65% sugar concentration. The mean nectar concentration for the *Meneris* guild (21%) probably reflects a compromise between optimization of sugar and water uptake by butterflies (see Watt *et al*, 1976). Nectar consisting of 30% sugar by weight is ingested by *Meneris* at half the rate of nectar consisting of 10% sugar by weight (Fig 1.6). However, the rate of sugar uptake is nearly twofold higher in 20% nectar relative to 10% nectar. Water balance and not energetic considerations may be a critical factor in *Meneris* selecting for dilute nectars below 20%. Field observations indicated that the butterfly tends frequently to rest on the shady side of boulders or stream banks during hot days. This behaviour can be interpreted as a strategy to minimize dehydration.

The widely differing proportions of sugars making up the nectar of both *Meneris*- and sunbird-pollinated plants (Table 1.2, 1.3) suggest that sugar ratios play little role in nectar selection by these pollinators. Sugar ratios in the nectar appear to reflect the phylogenetic affinities of taxa (cf. Percival, 1961; Van Wyk *et al*, 1993), rather than adaptation to *Meneris* or sunbirds.

Evolution of the guild

Although red coloration is known in butterfly-pollinated flowers elsewhere (Cruden & Herman-Parker, 1979; Boyden, 1980), the existence of a guild with entirely red flowers dependent on a single butterfly for pollination must be unique. Two questions are immediately raised. Firstly, what advantage could *Meneris* have in possessing such a distinct preference for red? Secondly, why have unrelated plant species converged on this butterfly for pollination?

A spontaneous colour preference has been reported for many butterflies. Swihart and Swihart (1970), for instance, found that *Heliconius charitonius* had a spontaneous preference for blue-green and orange-red model flowers, but that they could be trained to feed on other colours. Preference by butterflies for blue and yellow flowers was noted by Ilse (1928, in Swihart & Swihart, 1970). In *Pieris brassicae* (Pieridae) uncoiling of the proboscis in preparation for feeding occurs in response to blue and orange-red light (Scherer & Kolb, 1987). Distinct colour preferences of various butterfly species feeding on the pink and orange flowers of *Lantana camara* was reported by Dronamraju (1960).

What advantage is red perception to a butterfly? In some butterflies with red wing patterns, discrimination of red assists in mating behaviour. However, there is no red in the wing patterns of *Meneris* and preference for the colour seems to be associated purely with feeding. In most plant communities red flowers signal the presence of the copious nectar found in bird-adapted flowers. It has been suggested that this nectar resource is disguised from most insects by virtue of their poor perception of red (at least in some bees) (Raven, 1973). However, *Meneris* obtains a large percentage of its nectar requirements from bird-adapted flowers, most of which have red coloured flowers. The evolution or inheritance of red perception in *Meneris* must have therefore facilitated exploitation of this abundant nectar resource. Butterflies are particularly capable of "robbing" nectar from flowers adapted to other pollinators and it has been suggested that butterflies evolved long proboscides to enable them to exploit a wide range of flower morphologies (Wiklund *et al*, 1979). The dilute nectar found in bird-pollinated plants is especially suitable for butterflies - nectar which is too viscous cannot be sucked by butterflies (Kingsolver & Daniel, 1979). I suggest, therefore, that through its ability to perceive red colours, *Meneris* evolved a parasitic lifestyle of robbing bird-pollinated flowers.

The effectiveness of *Meneris* as a pollinator is probably directly related to its narrow colour preference. A specialised pollinator holds many advantages. Since very few flowers in any community have red flowers, plants adapted to *Meneris* are assured that a minimum of pollen is wasted on foreign stigmas and conversely that a minimum of foreign pollen clogs its own stigmas. Perhaps more important, however, is the increased probability of visitation because of the "magnetic" attraction of the butterfly to red flowers. This may be of particular benefit to the rare plants in the guild which are quickly "noticed" by the butterfly, despite being a small minority of the nectar-producing plants in a community. A feature of the guild is the large number of rare species. Four species (*Gladiolus nerinoides*, *G. sempervirens*, *G. stefaniae* and *Cyrtanthus guthrieae*) are in the Red Data Book for the Cape flora (Hall & Veldhuis, 1985) and many other have very restricted distributions.

A wide range of unrelated plants have converged to the syndrome of *Meneris* pollination. Many species pollinated by other butterflies and long proboscid flies are morphologically pre-adapted to *Meneris*; all that is required for a shift to *Meneris* pollination is a mutation for red coloration. Dronamraju (1960) has already pointed out that the strict colour preferences of some butterflies might even allow a sympatric speciation event through a single colour mutation.

Part 2

Floral mimicry

2 Evidence for Batesian mimicry in a butterfly-pollinated orchid[1]

Observations in the Cape Province, South Africa, showed that *Disa ferruginea* (Orchidaceae) is dependent on a single butterfly species - *Meneris tulbaghia* (Nymphalidae: Satyrinae) - for pollination. The flowers of *D. ferruginea* contain no food reward and, instead, appear to secure pollinator visits by imitating flowers which are nectar sources for the butterfly. A red-flowered form of *D. ferruginea* appears to mimic the red nectar-producing flowers of *Tritoniopsis triticea* (Iridaceae) in the south western Cape, while an orange-flowered form of *D. ferruginea* appears to mimic the orange nectar-producing flowers of *Kniphofia uvaria* (Asphodelaceae) in the Langeberg Mountains. Reflectance spectra of the orchid's flowers closely match those of its putative models. Analysis of foraging movements of the butterfly in a mixed stand of *D. ferruginea* and *T. triticea* indicated that it does not discriminate between the nectarless orchid and the nectar-producing model. Populations of *D. ferruginea* which are sympatric with *T. triticea* have relatively high levels of pollination and fruit production, compared with populations where the orchid grows alone. Although other studies have reported relatively low fecundity in deceptive orchids, pollination and fruiting success in *D. ferruginea* compare favourably with a nectar-producing congener, *Disa uniflora*, which is also pollinated solely by *M. tulbaghia*.

INTRODUCTION

While protective mimicry in animals has long fascinated evolutionary biologists, floral mimicry has been treated as little more than a botanical curiosity and, consequently, been largely ignored in textbooks and major symposia on mimicry. In this chapter I present evidence for Batesian mimicry in a South African orchid and suggest that our understanding of the evolution of mimicry can be advanced through the study of plant examples.

Botanists have disagreed whether the concept of Batesian mimicry, developed by zoologists, can be extended to plant pollination systems. Little (1983), for example, rejected the term Batesian mimicry for plants on the grounds that floral mimicry involves attraction, rather

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[1] Adapted from Johnson (1994a)

than repulsion of the signal receiver. However, Batesian mimicry in palatable animals and floral mimicry in plants with non-rewarding flowers is the outcome of a common evolutionary process: selection favours resemblance to an unpalatable animal or rewarding plant model, respectively, because fitness of the mimic is increased when it is perceived by a signal receiver (predator or pollinator) to be an example of the model (*sensu* Vane-Wright, 1980). I therefore agree with Brown and Kodric-Brown (1979), Bierzychudek (1981a) and Dafni (1984, 1986) that the category of Batesian mimicry should include floral examples.

As a consequence of shared evolutionary properties, the predictions of floral Batesian mimicry (see Wiens, 1978; Dafni, 1984; Little, 1983; Ackerman, 1986) are essentially the same as those of protective Batesian mimicry in animals (Vane-Wright, 1980; Endler, 1981). They are:

(1) The mimic and model should occur in the same place at the same time (but see Waldbauer, 1988, for exceptions in the case of signal receivers with long memories)
(2) The mimic should occur at low frequency relative to the model
(3) The mimic should resemble the model such that the signal receiver is unable to discriminate between mimic and model
(4) The fitness of the mimic should be higher in the presence of the model than in its absence

Much of the evidence for Batesian mimicry in plants consists of anecdotal information about floral similarity and pollinator sharing (see reviews by Little, 1983; Dafni, 1984, 1986; Ackerman, 1986). Only a few studies contain experimental evidence based on one or more of the above predictions (Bierzychudek, 1981a; Dafni & Ivri, 1981; Dafni, 1983; Nilsson, 1983a).

While studying the butterfly-pollinated flowers of Table Mountain (Johnson 1992a; Johnson & Bond, 1992a, 1994), I was impressed by the similarity between the flowers of *Disa ferruginea* (Thunb.) Sw. (Orchidaceae) and *Tritoniopsis triticea* (Burm.F.) Goldbl. (Iridaceae). To a human observer, these two species are difficult to distinguish in the field from more than about ten metres away. Both have scentless red flowers which are regularly visited by the butterfly *Meneris tulbaghia* (L.) (Nymphalidae: Satyrinae). The orchid has no floral reward, while *T. triticea* is an important source of nectar for the butterfly during late summer when few other Cape species are in flower (Johnson, 1993b). Mixed populations of *D. ferruginea* and *T. triticea* were found at many other sites in the south western Cape, except in the Langeberg Mountains where an orange-flowered form of *D. ferruginea* occurs with an orange-flowered form of the nectar-producing species *Kniphofia uvaria* (L.)Hook (Asphodelaceae).

The butterfly *M. tulbaghia* is found throughout the mountainous regions of South Africa where it specializes in 'robbing' nectar from red or orange flowers of bird-pollinated plants (Johnson & Bond, 1994). It is also the near-exclusive pollinator of a guild of c. 19 montane plant species with scentless red flowers which are morphologically adapted to this butterfly, rather than birds (Johnson, 1992a; Johnson & Bond, 1994). No other butterfly in the region is known to visit red flowers.

As a result of the preliminary observations it was hypothesised that the orchid *D. ferruginea* attracts *M. tulbaghia* through mimicry of the red flowers of *T. triticea* in the south western Cape and the orange flowers of *K. uvaria* in the Langeberg Mountains. A study was undertaken to test whether *D. ferruginea* fits the four predictions of the Batesian mimicry hypothesis (see above).

METHODS

Study sites - The field work was carried out from 1990 to 1993 at the following sites: Constantia Nek and Maclears Beacon on Table Mountain (33°59'S 18°25'E), Wolfkop and Muizenberg Plateau in the Silvermine Nature Reserve (34°07'S 18°26'E), the Jonkershoek valley (33°57'S 18°58'E), and Grootvadersbosch (33°57'S 22°52'E) in the Langeberg Mountains. The plant communities at the study sites are dominated by small heathlike shrubs (mainly Ericaceae) and reedlike Restionaceae. The altitude of the sites varies from 450 m in the Silvermine Reserve to 1500 m in the Langeberg.

Breeding system of D. ferruginea - A breeding system experiment was performed at Wolfkop in 1993 to determine the dependence of *D. ferruginea* on pollinators. Five inflorescences, bearing a total of 129 flowers, were bagged with fine nylon mesh prior to anthesis of the flowers. When the flowers had opened, each was randomly assigned to one of the following treatments: (1) bagged only, (2) bagged and self-pollinated with pollen from the same plant, (3) bagged and pollinated with pollen from another plant. The fruit production, seed crop mass and percentage of seeds with embryos was determined for each treatment.

Plant attributes - Flower dimensions were measured in the field (in the case of *D. ferruginea* and *T. triticea*) and from herbarium material (in the case of *K. uvaria*). An ACS 550M Spectrophotometer with an internal integrating sphere was used to measure the visible spectral reflectance (400-700 nm) of freshly picked flowers of *D. ferruginea* and *T. triticea* from Table Mountain and *D. ferruginea* and *K. uvaria* from Grootvadersbosch.

Distribution and phenology - To ascertain whether the species co-occur and have overlapping flowering times, data on the distribution and flowering times of *D. ferruginea* and its putative models were obtained from field notes taken over four years, material in the Bolus Herbarium and the literature (Lewis, 1960; Linder, 1981a).

Pollinator observations - Extensive observations (c. 30 days in the field) were made of butterfly visits to *D. ferruginea* and *T. triticea* on Table Mountain and at the Silvermine Nature Reserve. Most of the observations took place between 0900 h to 1500 h. Additional observations were made of butterfly visits to *D. ferruginea* and *K. uvaria* in the Langeberg Mountains on 9-10 March 1993. Butterflies visiting the orchid were caught and examined for pollinaria.

The sequence of visits by butterflies to *D. ferruginea* and *T. triticea* in a mixed stand of the two species on Constantia Nek was recorded on 4-5 March 1992, to determine whether the butterfly discriminated between the species. The foraging bouts were analyzed, firstly in terms of the expected vs observed number of visits to each species, and secondly in terms of the expected vs observed number of movements between the species. If there are ***a*** individuals of species A and ***b*** individuals of species B, and the pollinator moves randomly, then the expected proportion *P* of visits to each species will be

$$P_{SpA} = \frac{a}{a+b} \quad \text{and} \quad P_{SpB} = \frac{b}{a+b}$$

and the expected proportions of random interplant moves will be

$$P_{SpA \rightarrow SpA} = \frac{a}{a+b} \times \frac{a-1}{(a-1)+b} \qquad P_{SpA \rightarrow SpB} = \frac{a}{a+b} \times \frac{b}{(a-1)+b}$$

$$P_{SpB \rightarrow SpB} = \frac{b}{a+b} \times \frac{b-1}{(b-1)+a} \qquad P_{SpB \rightarrow SpA} = \frac{b}{a+b} \times \frac{a}{(b-1)+a}$$

Pollination and fruiting success - To determine whether *D. ferruginea* attracts more pollinator visits when sympatric with *T. triticea*, the frequency of pollen deposition on the stigma, pollinarium removal and fruit production in flowers of *D. ferruginea* was recorded at the four study sites on the Cape Peninsula. At two of these sites (Constantia Nek and Wolfkop), the orchid was sympatric with *T. triticea*, while at Muizenberg Plateau the nearest plants of *T. triticea* were at least 500 m away and at Maclears Beacon they were at least 1 km away.

RESULTS

Breeding system

Both the cross- and self-pollination treatments resulted in 100% fruit set in flowers of *D. ferruginea*, but the seed crop mass of the selfed fruits was only 10.1 mg, compared with 24.3 mg in the outcrossed fruits (Table 2.1), suggesting that *D. ferruginea* is only partially self-compatible. The absence of fruits in the bagged treatment (Table 2.1) indicated that the orchid is not autogamous, and therefore requires pollinator visits for fruit production.

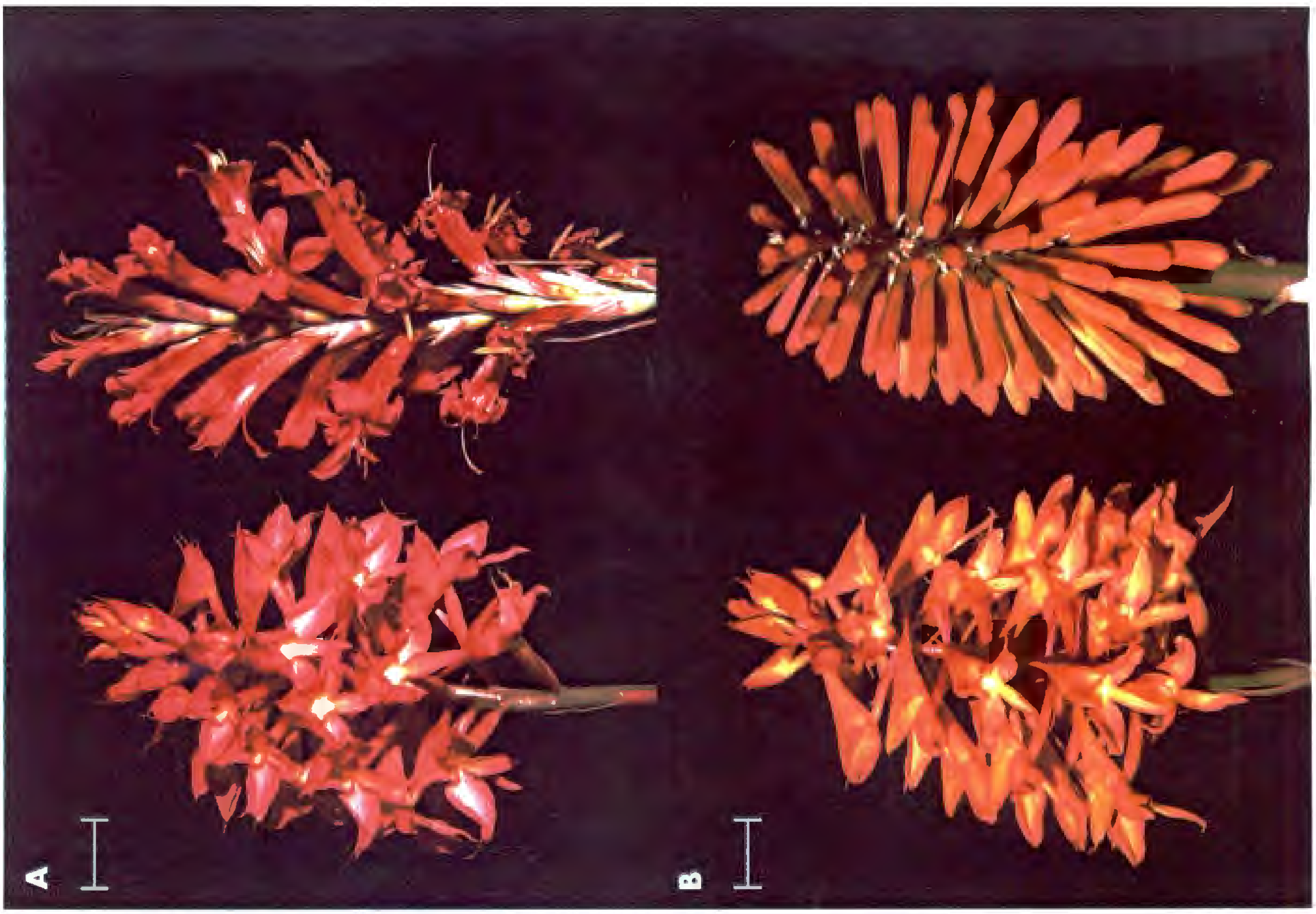
Table 2.1. Results of an experiment to determine the breeding system of *Disa ferruginea*.

Treatment	n	fruit set (%)	Avg. mass of seed crop (mg ±S.D.)	Seeds with embryos (% ±S.D.)
Pollinator- excluded	89	0	0	0
selfed	21	100	10.1 (±5.3)	36.4 (±7.6)
outcrossed	19	100	24.3 (±10.5)	86.4 (±16.4)

Plant attributes

The inflorescence dimensions and morphology of the orchid are similar to those of its putative models (Fig 2.1A,B). The mean spur depth in flowers of *D. ferruginea* from Table Mountain was very similar to the mean corolla depth in flowers of *T. triticea* (Table 2.2). The mean spur depth of flowers of *D. ferruginea* from the Langeberg, however, was shorter than the mean corolla depth of *K. uvaria* (Table 2.2). Both the proboscis length of *M. tulbaghia* and the spur depth of *D. ferruginea* were significantly shorter in the Langeberg than on Table Mountain (Table 2.2). No nectar was found in the flowers of *D. ferruginea* at any of the sites, while *T. triticea* and *K. uvaria* produced copious quantities of nectar.

Figure 2.1. **A**, The red-flowered form of *Disa ferruginea* (left) and *Tritoniopsis triticea* (right) collected on Table Mountain. Scale bar 10 mm. **B**, The orange-flowered form of *Disa ferruginea* (left) and *Kniphofia uvaria* (right) collected in the Langeberg Mountains. Scale bar 10 mm. **C**, The butterfly *Meneris tulbaghia* probing the flowers of *Disa ferruginea*. Note the pollinaria of *D. ferruginea* on the proboscis. Scale bar 10 mm. **D**, *M. tulbaghia* feeding on the flowers of *T. triticea*. The butterfly is carrying a pollinarium which indicates it had previously visited *D. ferruginea*. Scale bar 10 mm.


A
B


C
D

Table 2.2. Floral morphological characteristics and butterfly proboscis measurements at two study sites.

	Cape Peninsula	Langeberg Mountains
Corolla length of *T. triticea*	26.8 ±.22 (n = 8)	-
Corolla length of *K. uvaria*	-	33.6 ±3.1 (n = 22)
Spur length of *Disa ferruginea*	27.2 ±3.5 (n = 34)	21.8 ±2.0* (n = 16)
Proboscis length of *Meneris tulbaghia*	34.0 ±2.9 (n = 11)	30.4 ±2.5* (n = 14)

* $P < 0.01$ (T test)

Analysis of the spectral reflectance of the two colour forms of the orchid and their putative models confirmed their remarkable similarity in the field. The reflectance curve for the red-flowered form of *D. ferruginea* is similar that of *T. triticea* (though the latter has a slightly more saturated red coloration) and the curve for the orange-flowered form closely matches that of *K. uvaria* (Fig 2.2).

Distribution and phenology

D. ferruginea is found between the Cape Peninsula in the west and the Langeberg Mountains in the east (Fig 2.3). Over most of its range the orchid is sympatric with *T. triticea* on lower mountain slopes, except in the Langeberg Mountains where an orange-flowered form is sympatric with *K. uvaria* at high altitudes (Fig 2.3). *T. triticea* has been collected on the lower foothills of the Langeberg, but it does not occur on the moist, high altitude slopes where the orange-flowered form of *D. ferruginea* and *K. uvaria* occur in sympatry. *K. uvaria* is found throughout the Cape region, but in most areas it is not sympatric with *D. ferruginea* as it usually occurs in much wetter habitats than the orchid.

Another exception to the association of *D. ferruginea* with *T. triticea* occurs in the Jonkershoek valley, 50 km east of the Cape Peninsula, where I have found a population of *D. ferruginea* sympatric with *Tritoniopsis burchellii* (Burm. F.) Goldbl., a nectar-producing species which is very similar and closely related to *T. triticea*.

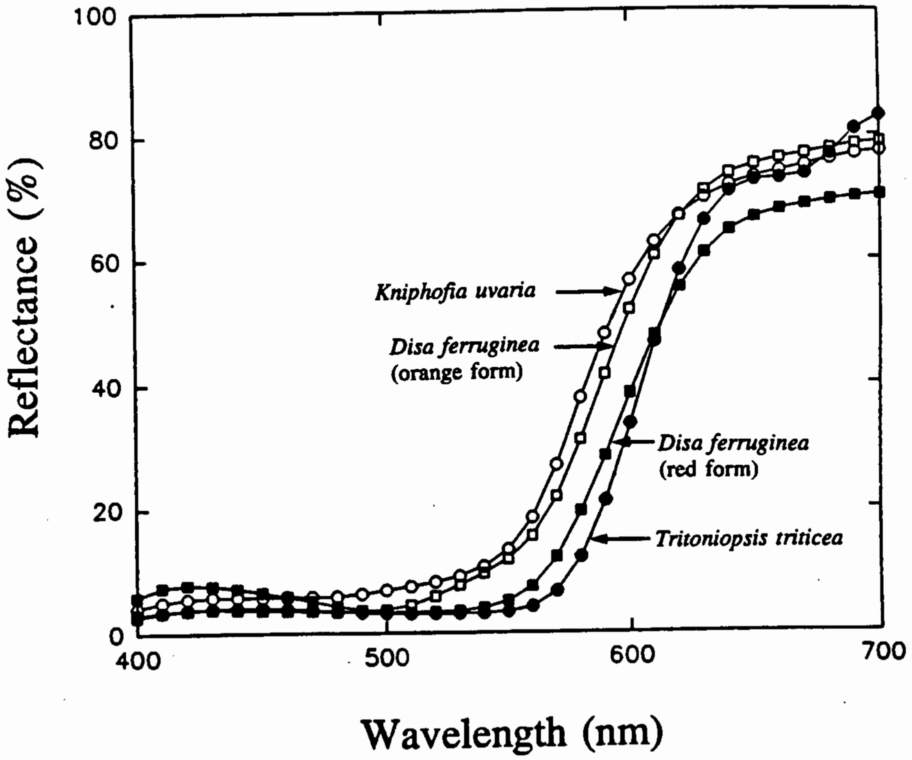


Figure 2.2. Spectral reflectance curves of flowers of the two colour forms of *D. ferruginea* and their putative models.

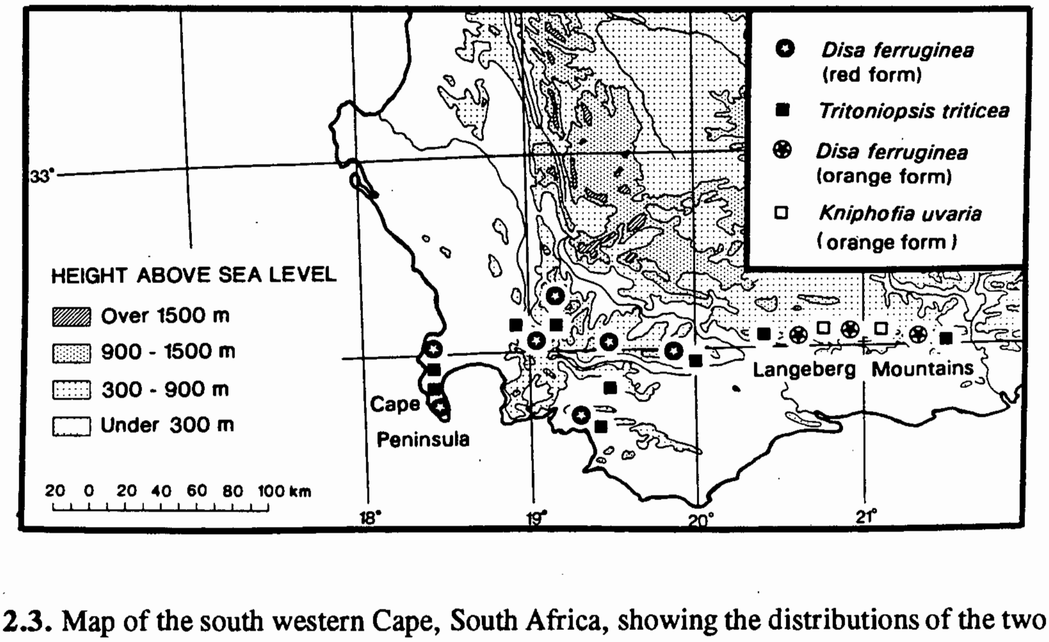


Figure 2.3. Map of the south western Cape, South Africa, showing the distributions of the two colour forms of *D. ferruginea* and their putative models, *T. triticea* and the orange-flowered form of *K. uvaria*.

The flowering times of *D. ferruginea* and *T. triticea* are similar on the Cape Peninsula. *T. triticea* usually begins flowering in early February and peaks in late February, while the orchid begins flowering in mid February and peaks in early March. Both *D. ferruginea* and *K. uvaria* were in full bloom on 10 March 1993 in the Langeberg. The butterfly *M. tulbaghia* has a flight period from December to April (Claassens & Dickson, 1980).

Pollinator observations

Many hundreds of visits by the butterfly *M. tulbaghia* to both the orchid and its models were observed over the four years of this study. The butterflies carried pollinaria of *D. ferruginea* attached to the proboscis (Fig 2.1C). The average number of pollinaria per butterfly in a sample of 22 butterflies carrying pollinaria, collected from Table Mountain and Silvermine, was 2.1 (S.D = 1.2, range = 1-5). No visits to the orchid by birds were observed during the study, despite previous speculation that birds pollinate its red flowers (Vogel, 1954). The very narrow, straight spurs of *D. ferruginea* probably prevent probing by the relatively broad, curved beaks of sunbirds. It was recently shown that many of the red-flowered Cape plants that were previously assumed to be ornithophilous are actually adapted for pollination by the butterfly *M. tulbaghia* (Johnson & Bond, 1994).

Sunbirds (mainly *Nectarinia violacea* and *N. chalybea*) were frequently observed visitors to *T. triticea* and seemed to pollinate the flowers as they contact both the anthers and stigma with their heads. *M. tulbaghia* may play a secondary role in the pollination of *T. triticea*, however, as butterfly scales were found on 14 out of 22 stigmas of *T. triticea* from Table Mountain, suggesting that contact between the body of the butterfly and the exerted anthers and stigmas of *T. triticea* takes place. No other butterflies are known to visit red flowers on Table Mountain. Although it may not be the main pollinator of either *T. triticea* or *K. uvaria*, *M. tulbaghia* nevertheless seemed to rely heavily on the nectar of these and other ornithophilous species. Its unusual ability to perceive red colours gives the butterfly privileged access to the copious nectar of ornithophilous flowers.

I recorded 53 foraging bouts by *M. tulbaghia* within the observation patch on Table Mountain. Of the 34 bouts in which two or more plants were visited, 23 (67%) involved visits to both *D. ferruginea* and *T. triticea* within the same bout. No other plant species were visited at the study site.

M. tulbaghia showed two kinds of flower visiting behaviour which, for convenience, can be termed "inspection visits" (when the butterfly approached a flower without actually settling) and "probing visits" (when the butterfly settled, often in an upside-down position, and systematically probed into open flowers on the inflorescence with its proboscis)(Fig 2.1C,D).

The numbers of inspection and probing visits received by each species were not significantly different from the expectation of random movement (Table 2.3). Similarly, the directions of interplant moves of butterflies in the mixed stand of *D. ferruginea* and *T. triticea* were not statistically distinguishable from the predictions of a random movement model (Table 2.4). The observed butterfly movement patterns strongly suggest that the butterfly cannot distinguish between the non-rewarding orchid and the rewarding *T. triticea*.

Pollination and fruiting success

Populations of *D. ferruginea* found in mixed stands with *T. triticea* on Table Mountain and Silvermine showed significantly higher levels of pollination, pollinarium removal and fruiting success compared to nearby populations where *D. ferruginea* grows alone (Table 2.5).

Table 2.3. Analysis of visits by *Meneris tulbaghia* to *Disa ferruginea* (10 plants) and *Tritoniopsis triticea* (12 plants) in a mixed patch. The expected number of visits is based on the hypothesis that the butterfly does not discriminate between the species (see Methods).

	D. ferruginea	*T. triticea*	G	P
No. of inspection visits observed (no. expected)	59 (51)	52 (61)	0.6	N.S.
No. of probing visits observed (no. expected)	12 (11)	13 (14)	0.2	N.S.
Total visits (no. expected)	71 (62)	65 (74)	2.4	N.S.

Table 2.4. Analysis of interplant movements by *Meneris tulbaghia* in a patch consisting of *Disa ferruginea* (10 plants) and *Tritoniopsis triticea* (12 plants). The expected number of interplant moves are based on the hypothesis that the butterfly does not discriminate between the species.

	Tritoniopsis→ *Tritoniopsis*	*Tritoniopsis*→ *Disa*	*Disa*→ *Tritoniopsis*	*Disa*→ *Disa*
Expected frequency of moves	.285	.259	.260	.195
Expected no. of moves	18.8	17.1	17.2	12.9
Observed no. of moves	22	11	13	20

G test for goodness of fit G = 7.49. df =3, P>0.05

Table 2.5. Comparison of pollination, pollinaria removal and fruit set in flowers of *D. ferruginea* from populations sympatric with *T. triticea* and populations where *T. triticea* is absent.

	Table Mountain		Silvermine Reserve	
	Maclears Beacon (*T. triticea* absent)	Constantia Nek (*T. triticea* present)	Muizenberg Plateau (*T. triticea* absent)	Wolfkop (*T. triticea* present)
	% (n)	% (n)	% (n)	% (n)
Flowers with pollen on stigma	.5 (271)	51*** (150)	3 (306)	34*** (143)
Flowers with pollinaria removed	6 (271)	39*** (150)	4 (306)	28*** (143)
Fruit set	18 (545)	78*** (266)	38 (307)	61*** (334)

*** P < .001 (G test using frequencies prior to transformation to percentages)

DISCUSSION

Is D. ferruginea *a Batesian mimic?*

D. ferruginea satisfies the predictions of the Batesian mimicry hypothesis which were outlined in the Introduction. *D. ferruginea* and its putative models, *T. triticea* and *K. uvaria*, are sympatric and co-blooming and are visited by the same butterfly, *M. tulbaghia*, which depends on the models for nectar. Observations indicated that *D. ferruginea* relies solely on this butterfly for pollination. *D. ferruginea* is a highly dispersed species that does not form dense populations, whereas its models are common species that occur in relatively dense populations. The colour and shape of the flowers of *D. ferruginea* and its models are similar and geographically concordant. A choice experiment in a mixed stand indicated that the butterfly does not discriminate between *D. ferruginea* and *T. triticea*. Finally, the fecundity of *D. ferruginea* is higher when it is sympatric with *T. triticea*.

Alternative hypotheses

Alternative hypotheses which could explain the pollination of *D. ferruginea* are autogamy and nonmodel mimicry. The lack of nectar is often a characteristic of autogamous species, but the results of the breeding system experiment clearly exclude the possibility of autogamy in *D. ferruginea*. Nonmodel mimicry (Dafni, 1986) is a term used to describe non-rewarding species which exploit the instinctive foraging behaviour of naive pollinators - no model is required. This is analogous to innate avoidance by predators of palatable prey with particular aposematic colour patterns. The results show that *D. ferruginea* attracts a limited number of visits where it grows alone (Table 2.5). However, as the fecundity of the orchid is greatly increased by the presence of *T. triticea* (Table 2.5), it appears that Batesian mimicry is the primary mode of pollination attraction in *D. ferruginea*.

While greater fecundity of a mimic where it grows in the presence of its model is a compelling line of evidence for Batesian mimicry, it is not without problems of interpretation; Ackerman (1986) and Nilsson (1992) caution that a non-rewarding species may benefit from growing near a rewarding species simply because pollinators aggregate where food is present (cf. Laverty, 1992).

Finally, an alternative hypothesis for the similarity of *D. ferruginea* and its models is that the species have converged in floral features to attract a common pollinator. But this hypothesis can be rejected out of hand as the models are both primarily bird-pollinated, whereas *D. ferruginea* is pollinated by the butterfly *M. tulbaghia*.

A proposed two-tier mechanism of deception

Deception of the butterfly *M. tulbaghia* can be conveniently divided into two components: (1) long distance attraction and, (2) eliciting the feeding response (probing into flowers).

Long distance attraction of *M. tulbaghia* appears to be based solely on flower colour. In experiments on Table Mountain, the butterfly has been shown to be strongly attracted to red paper discs and, to a lesser extent, orange and pink discs; other colours are ignored (Johnson & Bond, 1994). The butterfly is even attracted to red items of clothing, therefore it can be assumed that flower colour, rather than shape, is important for long distance attraction. The preference for red colour on Table Mountain may be innate or, alternatively, learned through association with the red-flowered ornithophilous species which are important nectar sources for the butterfly. The observation that red and orange forms of *D. ferruginea* closely match the colour of the dominant nectar plants utilized by *M. tulbaghia* in different regions (Fig 2.2), suggests that the colour preference of *M. tulbaghia* may be learned through positive association with nectar plants.

The second stage of deception - eliciting feeding behaviour in the pollinator - probably requires morphological similarity in mimic and model. Colour alone is insufficient to stimulate feeding; the butterfly seldom alights or attempts to feed on red paper discs, even though it is attracted to them. Scent also does not appear to play a role in eliciting a feeding reaction, as none of the flowers of species visited by the butterfly, including *T.triticea* and *D. ferruginea*, are scented (Johnson & Bond, 1994). Lewis (1986) has shown that butterflies take considerable time to learn how to obtain nectar from flowers and consequently tend to specialise on flowers with which they are familiar. Therefore, the morphology of the mimic should not necessitate any further learning by the butterfly, but rather exploit feeding behaviour learned on the rewarding model through positive reinforcement. The butterfly consumes nectar of *T. triticea* by probing into the flowers while in an upside-down position (Fig 2.1D). The butterfly assumes the same position on *D. ferruginea* (Fig 2.1C).

The evolution of Batesian mimicry in plants

Mimicry is essentially an evolutionary concept; usefully termed "adaptive resemblance" by Starrett (1993). To be selected, Batesian mimicry presumably confers advantages over conventional reward-based pollination systems. In the near complete absence of fossil evidence, the evolution of Batesian mimicry has to be deduced from the features and ecology of extant organisms. One approach is simply to compare the reproductive success of deceptive, non-rewarding species and rewarding species.

A great anomaly is that most research indicates low levels of fecundity in non-rewarding, deceptive plants, relative to rewarding plants (Gill, 1989; Nilsson, 1992). It is difficult to understand, therefore, how deceptive pollination systems could have evolved if they confer no obvious advantage to fecundity. One explanation has been that energy saved by the loss of reward outweighs the disadvantage of reduced pollination success (Brown & Kodric-Brown, 1979). This argument is, of course, most applicable to plants in which resources, rather than pollinators, limit fitness. But how does one explain the loss of nectar in the many orchids in which reproduction is pollinator-limited (Nilsson, 1992; Calvo, 1993)? According to theory (Haig & Westoby, 1988), the resources of pollinator-limited plants should be allocated to, and not away from, floral attractants such as nectar.

Comparison of the fecundity of rewarding and non-rewarding plants is usually of little value because of uncontrolled variables, such as locality and the taxon of the pollinator. It is obviously pointless, for example, to compare the fecundity of a non-rewarding species pollinated by bees in one region with that of a rewarding species pollinated by butterflies in another region. Fortunately, for the purposes of comparison, there are both a deceptive and a rewarding *Disa* species pollinated by *M. tulbaghia* on Table Mountain. *D. uniflora* has large red nectar-producing flowers which, like its non-rewarding congener *D. ferruginea*, are pollinated solely by *M. tulbaghia* (Marloth, 1895; Johnson & Bond, 1994). Flowering in *D. uniflora* occurs one month earlier than in *D. ferruginea*.

I have collected four years of pollination and fruiting success data for two adjacent populations of *D. uniflora* along a stream on Table Mountain (Johnson & Bond, 1992a; Chapter 6). This stream is about 1km from the Maclears Beacon and Constantia Nek populations of *D. ferruginea*. Pollination and fruiting success in the first population of *D. uniflora*, which occurs in a habitat not especially favourable to the butterfly, occurred in 9-23% of the flowers over the four years of this study. The second population occurs in a rocky gorge favoured by the butterfly; here pollination and fruiting occurred in 59-87% of the flowers. These data show that pollination and fruiting success in the nectar-producing *D. uniflora* is comparable to the deceptive *D. ferruginea* in which pollination and fruiting occurred in 34-78% of the flowers at sites where it co-occurred with a model (Table 2.5). Although, this comparison is crude as the habitats of the two orchids differ, it does indicate that the loss of a reward, combined with effective mimicry, could result in energy savings without reducing fecundity.

Part 3

Adaptive radiation

3 Pollination in the genus *Disa*: case histories

Flowers in the genus *Disa* are among the most highly modified and variable of all orchids. Despite extensive taxonomic research on this genus in South Africa, almost nothing was known about pollination of the species. In this chapter I describe "case histories" of pollination for 17 species. The main finding to emerge is the extraordinary diversity of specialized pollination systems within this single genus. Pollinators include butterflies, settling-moths, hawkmoths, bees, wasps, short-proboscid flies and long-proboscid flies. In addition, several *Disa* species are capable of auto-pollination. The majority of species do not produce nectar and rely on various forms of deception to attract pollinators. The column of *Disa* species is usually located below the entrance to the flowers; consequently pollinaria are attached to the ventral surface of the insects, either on the mouthparts, thorax or legs.

INTRODUCTION

Disa Berg. (Orchidaceae: Diseae) *sensu stricta* consists of approximately 120 species found mostly in the montane grasslands and "fynbos" shrublands of Africa (Linder, 1981a). In this chapter I have used a concept of the genus which includes *Herschelianthe* Rauschert and *Monadenia* Lindl., but excludes the *Disa* section *Micranthae* Lindl. Phylogenetically, the former genera are embedded in *Disa*, and the taxonomy will soon be changed to accommodate them into a larger monophyletic concept of *Disa* (Linder and Kurzweil, in prep). *Disa sensu lato* then consists of about 80 species in the Cape flora, making it one of the larger genera in the region (Bond & Goldblatt, 1984).

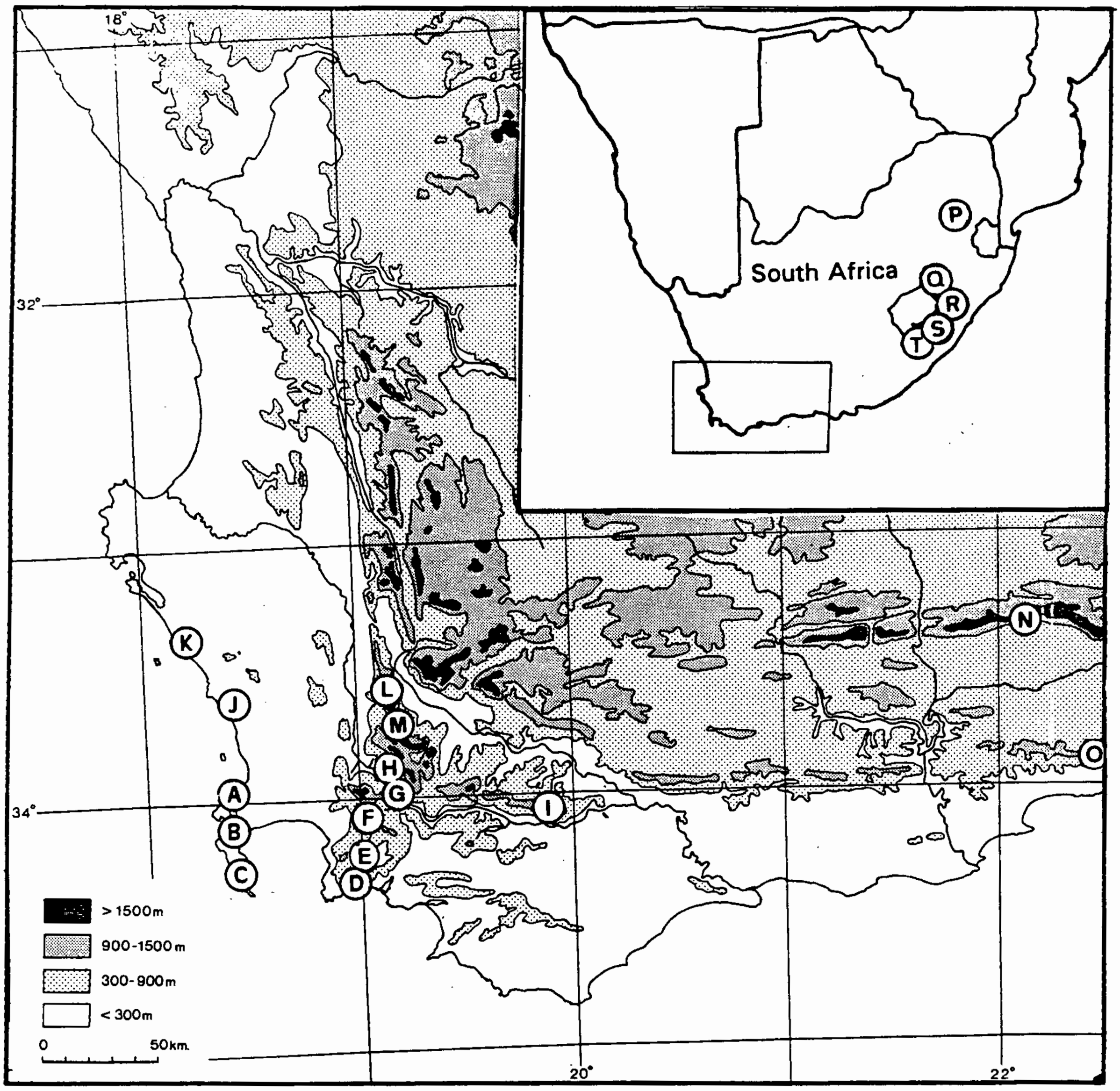
Despite interest in the diverse floral morphology of *Disa* (Vogel, 1954; Dressler, 1993), pollination in the genus has been poorly understood, representing a serious gap in our knowledge of the general biology and evolution of these plants. Until this research commenced, the only existing data were the seminal studies of *Disa uniflora* Berg. and *D. ferruginea* Thunb. (Sw.) by Marloth (1895), a note on possible bee pollination in *Disa rhodantha* Schltr. and *Disa chrysostachya* Sw. (Stewart & Manning, 1982) and descriptions of auto-pollination in *Disa crassicornis* Lindl. (as *D. macrantha*) (Weale, 1871), *Disa erubescens* Rendle ssp. *carsonii* (N. E. Br.) Linder (as *D. stolzii*) (Gassner, 1982) and *Disa ornithantha* Schltr. (Pettersson, 1986).

I managed to find and observe populations of 72 out of the 119 *Disa* species (including *Monadenia* and *Herschelianthe*) in South Africa, and 52 out of the 80 species in the Cape flora during the course of this study. However, reliable pollination data were only obtained for a small proportion of these species. In total we now have pollination data for about 27 species (c. 20 % of the total for South Africa). *Inter alia*, it is known that species in the genus are pollinated by butterflies (Marloth, 1895; Johnson, 1994a), moths (Johnson, 1994b), solitary bees (Johnson, 1992b, 1993a, 1994c; Johnson & Steiner, 1994a), long-proboscid flies (Johnson & Johnson, 1993), nematoceran flies (Johnson & Steiner, 1994b) and wasps (Steiner *et al*, 1994) as well as through auto-pollination (Johnson *et al*, 1994).

This chapter consists of case histories summarized from some of the above published accounts, as well as several unpublished observations. The majority of the species occur in the Cape region, but I have included several species from the nearby Drakensberg Mountains for comparison and to give a sense of the radiation of *Disa* in South Africa as a whole. For greatest clarity, each case history is distinct with its own summary, allowing the reader to focus on a particular species of interest.

Figure 3.1. Representative examples of pollination in *Disa*. **A**, *Disa uniflora* is pollinated by the large butterfly *Meneris tulbaghia*. Scale bar 10 mm. **B**, *Disa versicolor*, a grassland orchid, visited by the anthophorid bee *Amegilla natalensis*. Scale bar 10 mm. **C**, Auto-pollination in *Disa rosea*; the pollinaria have flipped out of the anther and onto the stigma. Scale bar 1 mm. **D**, *Monadenia ophrydea* has dark, evening scented flowers which attract moth pollinators. Scale bar 10 mm. **E**, Hoverfly pollination of *Disa sagitallis*. Scale bar 5 mm. **F**, Tanglewing fly visiting a flower of *Disa rhodantha*. Scale bar 5 mm.


A
B
C
D
E
F



G.P.-S.(L).

Figure 3.2. Localities of study sites referred to in this chapter. A - Table Mountain, B - Silvermine Nature Reserve, C - Cape Point, D - Bettys Bay, E - Kogelberg, F - Viljoens Pass, G - Theewaterskloof Dam, H - Franschhoek Pass, I - Pilaarkop, J - Silverstroomstrand, K - Ysterfontein, L - Bains Kloof, M - DuToits Kloof, N - Swartberg Pass, O - Montagu Pass, P - Dullstroom, Q - Royal Natal Park, R - Giants Castle, S - Himeville Nature Reserve, S - Sehlabathebe, T - Naudes Nek.

POLLINATION OF *DISA TENUIFOLIA* BY LEAFCUTTER BEES[1]

The study species

Disa tenuifolia (Thunb.) Linder (= *Disa patens* (L.f.) Thunb.) is a small, c. 100 mm tall, terrestrial orchid occurring on sandstone-derived soils in the mountains of the western Cape. As in most Cape *Disa* species, flowering in *D. tenuifolia* is most profuse in the first summer following a fire, though flowering sometimes continues for a few seasons in exposed localities such as the edges of paths.

The single inflorescence usually consists of 1 or 2 (sometimes up to 5) bright yellow flowers (Fig 3.3A). There are no ultraviolet patterns in the flowers of *D. tenuifolia* - using the "grey-scale" method of Kevan *et al* (1973) and the photographic apparatus described in Steiner (1990), it was established that the flowers are uniformly absorptive in the ultraviolet component of the bee visual spectrum. The flowers have a compressed appearance as the dorsal sepal is flattened, unlike many other *Disa* species which have a bonnet-shaped or galeate dorsal sepal. The pollinaria, which are characterised by an extremely elongate caudicle, c. 10 mm in length, are presented by long hornlike rostellum arms which project above the flower and come into contact with the ventral surface of the pollinator.

Extensive wild-fires on the Cape Peninsula early in 1992 resulted in a rare opportunity to study pollination in large populations of *Disa tenuifolia* which flowered *en masse* during the following summer.

Study site

Two populations of *D. tenuifolia* on the Cape Peninsula were studied during November and December 1992. The first population consists of about 1000 plants scattered widely over a sandy plain (34°17'S 18°28'E) below Judas Peak in the Cape Point Nature Reserve. The second consists of about 200 plants at the edge of a marsh (34°06'S 18°27'E) on the Muizenberg Plateau in the Silvermine Nature Reserve. Both sites had been burnt the previous summer.

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[1] Adapted from Johnson & Steiner (1994a)

Observations of pollinator behaviour were made between 1000 h and 1500 h on 11, 18 and 27 November and 2 and 12 December at Cape Point, and on 17 November and 7-8 December at Silvermine. Collections were made of insects visiting the flowers of *D. tenuifolia* as well as insects, caught on other flowers, which carried the unmistakable pollinaria of *D. tenuifolia.*

Observations

Pollinators of *D. tenuifolia* were scarce at both study sites; despite constant vigilance I caught fewer than two pollinators, on average, during each of the eight five-hour observation periods. Five males of the carder bee *Immanthidium immaculatum* Smith (Megachilidae: Anthidinii) were caught on *D. tenuifolia* at Cape Point (Fig 3.3B). They carried 4, 12, 12, 14 and 14 pollinaria respectively. Two male leafcutter bees *Megachile albohirsuta* Pasteels (Megachilidae: Megachilini), carrying three pollinaria each, and a single male *Megachile semiflava* Ckll., carrying five pollinaria, were caught on *D. tenuifolia* at Silvermine. In addition I caught four unidentified monkey beetles (Scarabaeidae: Hopliini) carrying 1, 1, 2 and 2 pollinaria respectively.

The pollinaria were precisely attached in two distinct clumps, 3-4 mm apart, on the ventral surface of the thorax of the bees. The spacing between these clumps corresponds to the spacing between the outer lobes of the rostellum, as the bee settles with its thorax in contact with both rostellum lobes. The bees always settled with their heads facing the dorsal sepal. The pollinaria of *D. tenuifolia* are elongate with a flexible caudicle; consequently when a bee settles on the flower, pollinaria already attached to the bee dangle loosely onto the stigma directly below the rostellum. By contrast, pollinaria of *Disa filicornis*, the sister species of *D. tenuifolia*, undergo a 90° curvature once extracted from the anther, thereby positioning the pollinaria correctly to contact the stigma which is tucked beneath the rostellum (Johnson, 1992b).

The carder and leafcutter bees moved rapidly among the flowers and most "visits" were merely close inspections without settling. Only occasionally would a bee settle briefly (1-2 seconds) on the flowers. The short time spent on the flower is probably due to the absence of a nectar reward. I observed a distinct territorial behaviour — individual bees repeatedly completed an identical circuit of approximately 5-20 flowers and returned to the same resting point (usually a small rock). In one case a bee (*Immanthidium immaculatum*) was observed to follow the same circuit of flowers for 30 minutes.

By contrast, the monkey beetles appeared to be rather inefficient pollinators — many visited the flowers without picking up pollinaria and those that did never carried more than two pollinaria. Monkey beetles often spent long periods on the flowers and some were observed eating pollen from pollinaria which were still within the anther sac. Based on these observations, I believe that monkey beetles play only a minor role in the pollination of *D. tenuifolia*.

Discussion

A common explanation for the presence of nectar in flowers is that it promotes loyalty by insects (cf. Larson & Larson, 1987). It was surprising, therefore, that the bees showed a sustained interest in the orchid, even regularly patrolling patches of flowers, when no reward is offered by its flowers. Mimicry by *D. tenuifolia* of sympatric rewarding species (Dafni, 1984; Ackerman, 1986) is unlikely as the flowers of other species in the area were all very different in appearance and colour.

Since all of the bees caught were male, the possibility of sexual deceit by the orchid cannot be excluded. The bright yellow colour of the flowers and lack of discernable scent is, however, inconsistent with this hypothesis. More likely is that the bees have a strong foraging instinct which results in persistent visits to brightly coloured flowers, despite the lack of reinforcement of this behaviour by a reward (cf. Ackerman, 1983; Nilsson, 1983c). *D. tenuifolia* may thus be an effective "food-fraud orchid without a model" (Nilsson, 1992). This form of deception also appears applicable to *Disa filicornis*, the sister species of *D. tenuifolia*, which is visited repeatedly by megachilid bees, despite having no reward in its flowers (Johnson, 1992b).

Although simple food-fraud seems likely in *D. tenuifolia*, the patrolling behaviour of the bees suggests that territorial instincts of the male carder bees may also be involved in their attraction to the orchid. The pollination of the European orchid *Orchis papilionacea*, for example, takes place during the patrolling flights of male *Eucera* bees (Vogel, 1972). Recently Pettersson and Nilsson (1993) found that the pollination system of *Polystachya rosea*, a non-rewarding orchid in Madagascar, was based on the patrolling behaviour of male halictid bees which use the flowers as landmarks for mate-seeking, a similar situation to that observed in *D. tenuifolia.*

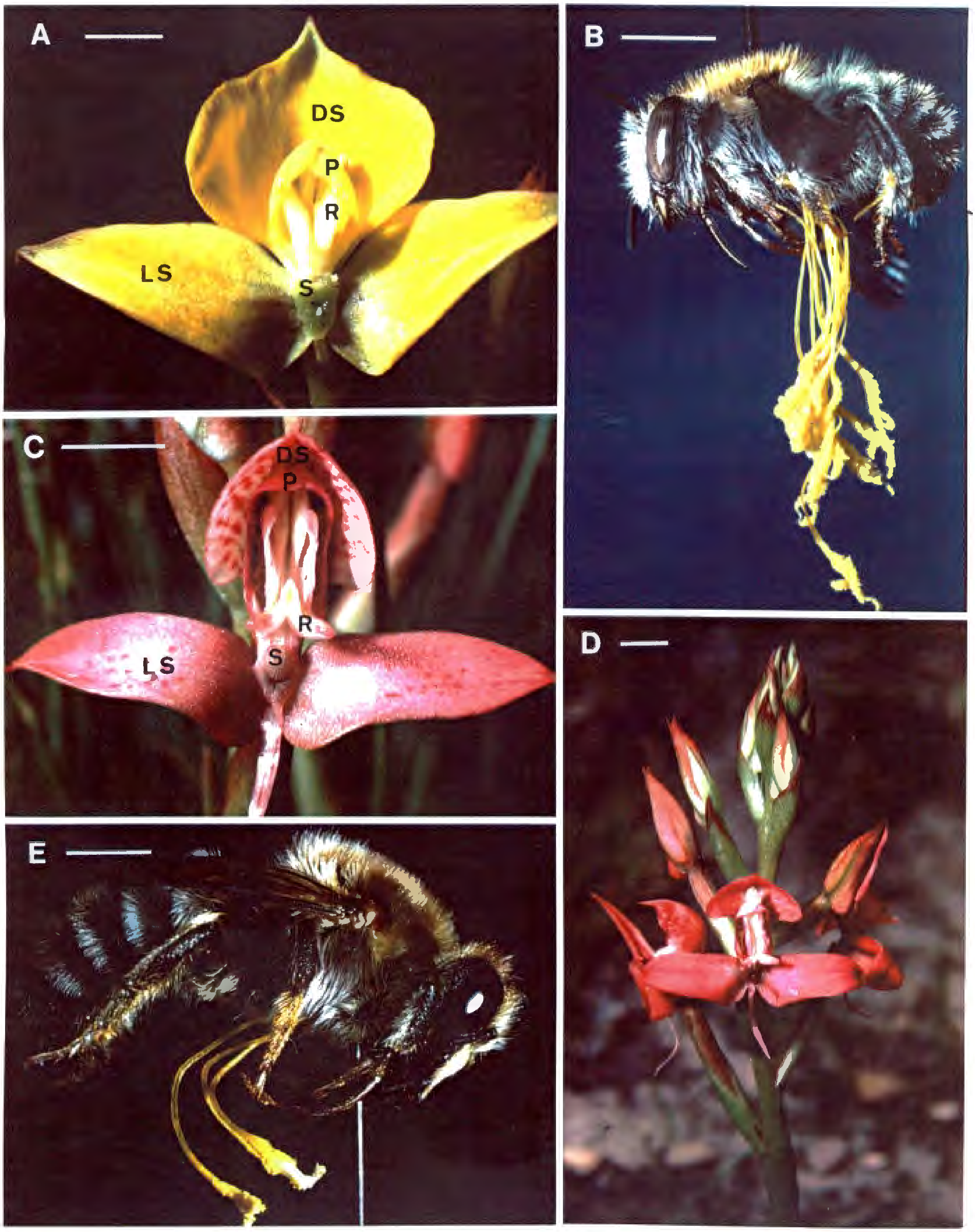
The territorial behaviour of males of the common European carder bee, *Immanthidium manicatum*, as described by O'Toole (1991), is remarkably similar to that observed in *I. immaculatum* in this study. In both bee species, the males constantly patrol a territory consisting of a patch of flowers. According to O'Toole (1991) male carder bees often set up territories at flowers frequented by females. An intriguing possibility then is that *D. tenuifolia* attracts territorial male carder bees because of a resemblance of the orchid to the flowers typically visited by female bees. Female carder bees were not observed at the study sites, but have been observed elsewhere to visit small yellow legume flowers, such as

Aspalathus spp. (V. Whitehead, pers. comm.). From these observations, it seems that either foraging or territorial instincts, or a combination of both, explain the attraction of male carder bees to the flowers of *D. tenuifolia*.

Summary

Studies of *Disa tenuifolia* near Cape Town showed that the bright yellow flowers of the orchid are pollinated by various carder and leafcutter bees (Megachilidae) and occasionally monkey beetles (Scarabaeidae: Hopliini). The highly elongate pollinaria become attached to the underside of the pollinator's thorax. The bees showed strong fidelity towards the orchid, despite the lack of nectar in its flowers. It was concluded that the pollination system of *D. tenuifolia* depends on exploitation of instinctive foraging and territorial behaviour of male megachilid bees.

Figure 3.3. **A,** Flower of *Disa tenuifolia*. Scale bar 10 mm. **B,** Male carder bee *Immanthidium immaculatum* carrying 12 pollinaria of *Disa tenuifolia*. Note the length of the pollinaria in relation to the size of the bee. Scale bar 2 mm. **C,** Flower of *Disa filicornis*. Scale bar 10 mm **D.** Inflorescence of *Disa filicornis*. Scale bar 10 mm. **E,** *Chalicodoma karooensis* (Megachilidae) with pollinaria of *D. filicornis* attached to the thorax. Note the 90° curvature of the pollinaria as well as the irregular surface at the tips of the pollinaria where massulae (pollen clumps) involved in pollination have been torn away. Scale bar 2 mm. Abbreviations: R - rostellum, S - stigma, P - petals, DS - dorsal sepal, LS - lateral sepal.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POLLINATION OF *DISA FILICORNIS* BY MASON BEES[1]

The study species

D. filicornis is the sister species of *D. tenuifolia*. The inflorescence, with between one and five flowers (Fig 3.3C,D), is typically produced in the first two years following fire. The flowers are usually purple-pink, although white forms are not rare (Linder, 1981a).

Study site

The pollination biology of *Disa filicornis* (L.f) Thunb. (Orchidaceae) was investigated on 16 November 1991 at a site in Baviaanskloof, near Wellington. Observation took place between 1100 h and 1500 h. The area had been burnt two years previously and about 100 plants of *D. filicornis* were in flower.

Observations

Four male mason bees carrying pollinaria, positively identified as those of *D. filicornis*, were caught while foraging for nectar on *Moraea tripetala* (L.f.) Ker Gawl. (Iridaceae) which was flowering amongst the orchids at the study site. Three of the bees, identified as *Chalicodoma karooensis* Brauns. (Megachilidae), carried thirteen, nine and six pollinaria respectively, attached to the ventral side of the thorax between the first and second pair of legs (Fig 3.3C,D). The fourth bee, a smaller megachilid which has not been identified, carried a single pollinium of *D. filicornis*. Other bees, including carpenter bees and honey bees were caught at the study site, but they did not carry pollinaria.

Although bees were not observed visiting the orchid, the presence of pollinaria of *D. filicornis* on the ventral side of the thorax indicates that bees straddle the anther when alighting on the flower. Pollinaria undergo a curvature of 90° after being extracted from the anther, an interesting phenomenon which ensures that the ends of the pollinaria project forwards (Fig 3.3E) and thus contact the stigma without being impeded by the rostellum which juts out above the sessile stigma.

No nectar is produced in *D. filicornis*, as is the case with other species of *Disa* which lack a spur (pers. obs.). This lack of a nectar reward does not prevent bees from repeatedly visiting *D. filicornis*, as evidenced by one of the captured bees which had accumulated

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[1] adapted from Johnson (1992b)

thirteen pollinaria on its thorax. This effective deception of bees results in high levels of pollination success for the orchid. I examined one flower from each of 33 plants at the site and found that 27 (82%) had both pollinaria removed and 21 (64%) had pollen deposited on the stigma.

Discussion

There are three possible reasons why bees would visit a nectarless flower. Firstly, the flowers may mimic a sympatric nectar-producing species. Mason bees could have confused the orchid with a similarly shaped and coloured species of *Polygala* (Polygalaceae) which was growing nearby. Secondly, the orchid may exploit the instinctive nectar-foraging strategy of the bees (see Little, 1983; Dafni, 1984) in which case the resemblance to the flowers of *Polygala* is coincidental. Thirdly, bees may be drawn by a female sexual attractant produced by the flower, as all the bees found carrying pollinaria were male. Further detailed experimental studies are needed to distinguish between these hypotheses.

Summary

Disa filicornis (L.f.) Thunb., a Cape orchid, was found to be pollinated by mason bees (Apoidea: Megachilidae). Pollinaria are placed on the ventral side of the thorax when bees straddle the anther. After extraction from the anther, the pollinaria undergo a curvature of 90° which ensures correct orientation relative to the stigma. The flowers do not produce a nectar reward, but the large number of pollinaria carried by bees suggests that bees have a low ability to learn to avoid the flowers. This deception of bees allows the orchid to achieve a high pollination success.

POLLINATION OF *DISA RACEMOSA* BY ANTHOPHORID BEES

The study species

Disa racemosa is one of the most common orchids in the Cape floral region. The inflorescence bears from one to fifteen large (c. 60 mm diameter) pink flowers which lack nectar or any other floral reward. *D. racemosa* is often found flowering together with its sister species *Disa venosa* in recently burnt marshes. The taxa are remarkably similar and sometimes difficult to distinguish; the only character which consistently separates them is the broader sepals, particularly the dorsal sepal, of *D. racemosa*. It was established that the spectral reflectances of flowers of D. racemosa and *D. venosa* are very similar in human-visible wavelengths (see Chapter 2 for methods), supporting the impressions gained in the field; the magenta-pink colour results from strong reflectance of blue and red wavelengths (Fig 3.4). Magenta-pink is due to anthocyanin pigments in *D. racemosa* (Vogelpoel *et al*, 1985) and is a common colour among bee-pollinated flowers in the Cape and elsewhere (cf. Nilsson, 1983c). In addition, photographs of the flowers with and without a Corning 7-60 "black" filter which only transmits ultraviolet light (partially visible to some bees) showed that both species have U.V reflective sepals and relatively, U.V absorbent petals (Fig 3.5) (see Appendix B for more details on this method).

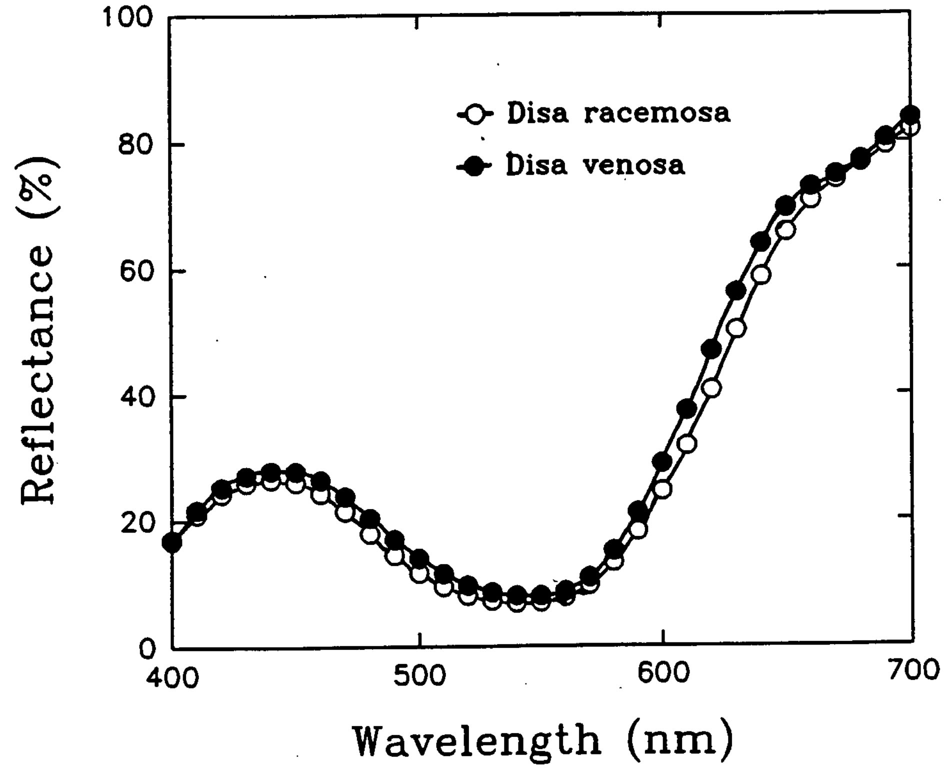

Figure 3.4. Reflectance spectra for *Disa racemosa* and *Disa venosa*.

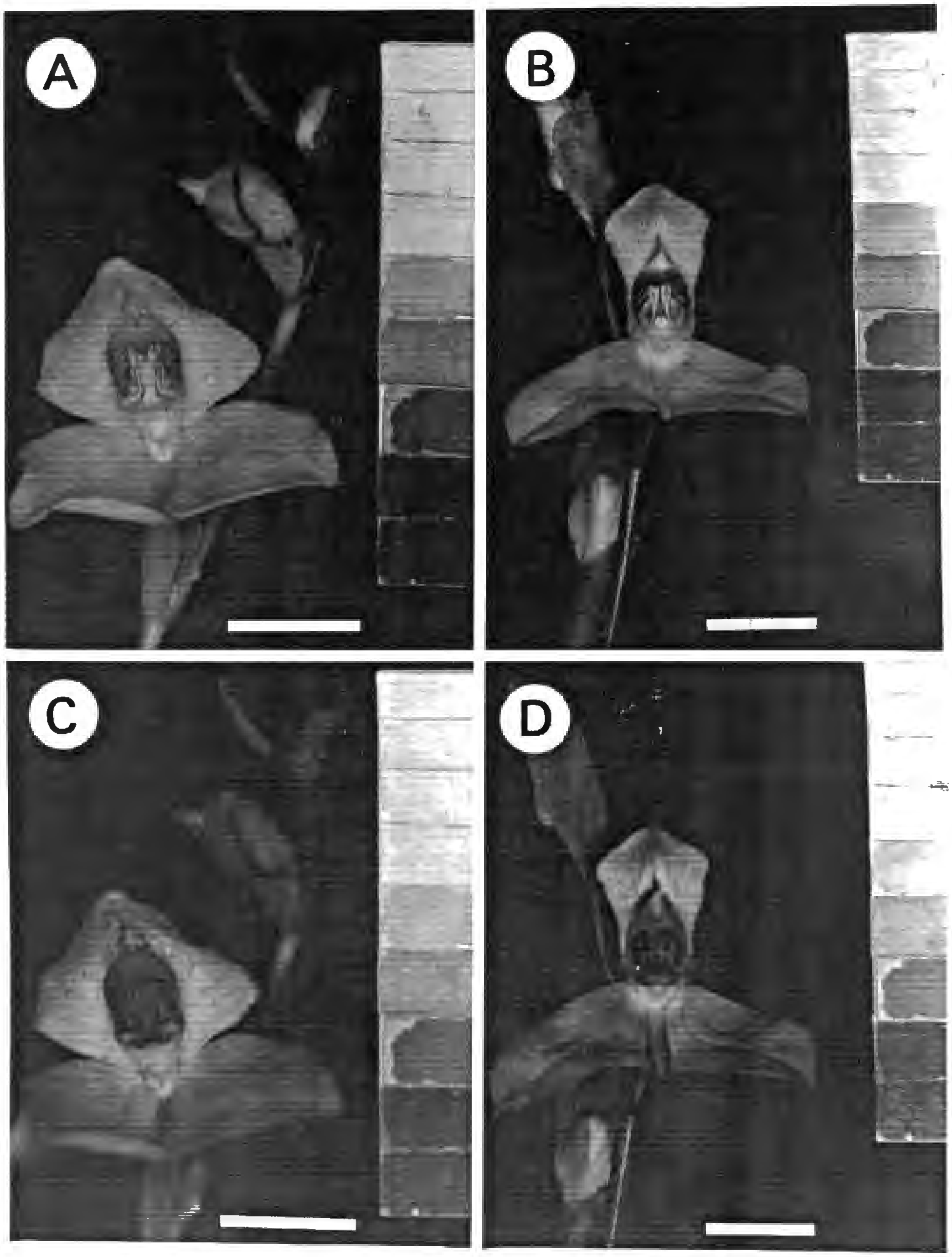

Figure 3.5 Comparison of reflectance of flowers of *Disa racemosa* and *Disa venosa* in both normal and ultraviolet light. The grey scale is used to ensure a comparable range of contrast in each photograph (cf. Kevan *et al*, 1973). **A**, *Disa racemosa* (daylight). **B**, *Disa venosa* (daylight). **C**, *D. racemosa* (U.V.). **D**, *D. venosa* (U.V.) Scale bar 10 mm throughout. Note that both species have ultraviolet absorptive petals and ultraviolet reflective sepals.

Study sites

Observations were made at Olifantsbosch in the Cape Point Nature Reserve (34°16'S 18°33'E), Muizenberg Plateau (34°07'S 18°24'E) in the Silvermine Nature Reserve, Baviaanskloof (33°34'S 19°08'E) in Bains Kloof, Ysterklip (34°16'S 18°59'E) near Botriver, Bettys Bay (34°17'S 18°56'E), Theewaterskloof Dam (33°58'S 19°13'E) near Franschhoek and the Swartberg Pass (32°17'S 22°04'E) near Oudtshoorn. Mixed populations of *D. racemosa* and *D. venosa* occur at the Baviaanskloof and Swartberg sites. The total observation period was c. 30 hours over 10 days.

Observations

Carpenter bees (*Xylocopa rufitarsus* Lepeletier and *X. caffra* L.) were observed to visit *D. racemosa* at five of the sites (Silvermine, Theewaterskloof, Betty's Bay, Bains Kloof and Swartberg Pass). Smaller anthophorid bees (*Amegilla niveata* and *Amegilla spilostoma*) visited *D. racemosa* at the Swartberg site. All of these bees, except the very large *X. caffra*, were found to carry pollinaria attached to the middle legs (Table 3.1; Fig 3.6 D-E).

Since the orchid has no floral rewards, the bees obviously rely on other plants for pollen and nectar. At some sites there were no food plants growing in the same marshes as the orchid. Here, the showy flowers of the orchid appeared to "flag down" bees passing through the population.

After settling on the flower of *D. racemosa*, the bees grasp the protruding petals with their front legs and insert their heads into a small chamber formed by the petals (Fig 3.6A). The bees are presumably guided to this area by the contrast between the strongly UV absorptive petals and the relatively reflective sepals (Fig 3.5), and also by the banded pattern along the insides of the petals. Pollinaria become attached to the basal segment of their middle legs which are placed across each of the rostellum lobes when the bees grasp the petal chamber. Interestingly, pollinaria were found in this position on captured bees of several species, indicating that the floral mechanism is effective for a range of bee sizes. Although bees were sometimes observed to visit several flowers in succession, none of the captured bees carried more than two pollinaria. Thus it appears that the site on each leg can accommodate only a single pollinarium. The bees caught were all female, suggesting that *D. racemosa* primarily attracts pollen-seeking bees.

Table 3.1. Insects found with pollinaria of *D. racemosa*

Study site	Observation time (hrs)	Floral visitors to *D. racemosa*	Number of pollinaria carried	Food plants for the pollinators (P = pollen source, N = nectar source)
Swartberg	15	*Xylocopa rufitarsus*	♂ (1)	-
		Amegilla niveata	♀(2), ♀(2)	*Moreae ramosissima* (Iridaceae) (N)
		Amegilla spilostoma	♀(0), ♀(2), ♀(1), ♀(1), ♀(2)	
Bains Kloof	10	*X. rufitasus*	♀ (2), ♂ (0)	*Drosera regia* (Droseraceae) (P)
		X. caffra	♀ (0)	*Chironia jasminoides* (Gentianaceae) (P)
Silvermine	8	*X. rufitarsus*	♀ (2), ♀ (0)	-
Franschhoek	5	*X. rufitarsus*	♀ (0)	-
Betty's Bay	4	*X. caffra*	♀ (0)	
Cape Point	8	-	-	-

At most sites, visitation by bees was extremely scarce. On average less than one bee was observed per day at each of the sites, except Swartberg Pass where seven bees were caught in two succesive days. Pollination success exceeded 10% of flowers only at Swartberg and Bains Kloof (Table 7.1). At the other sites, the scarcity of pollinators was reflected in very low levels of pollination and fruiting success (Table 7.1).

Observations indicated that *D. racemosa* and *D. venosa* share pollinators. An individual of *X. rufitarsus* was seen to visit the flowers of both *D. venosa* and *D. racemosa* at the Swartberg Pass in January 1993, but could not be captured. Further observations of *D. venosa* were unsuccessful.

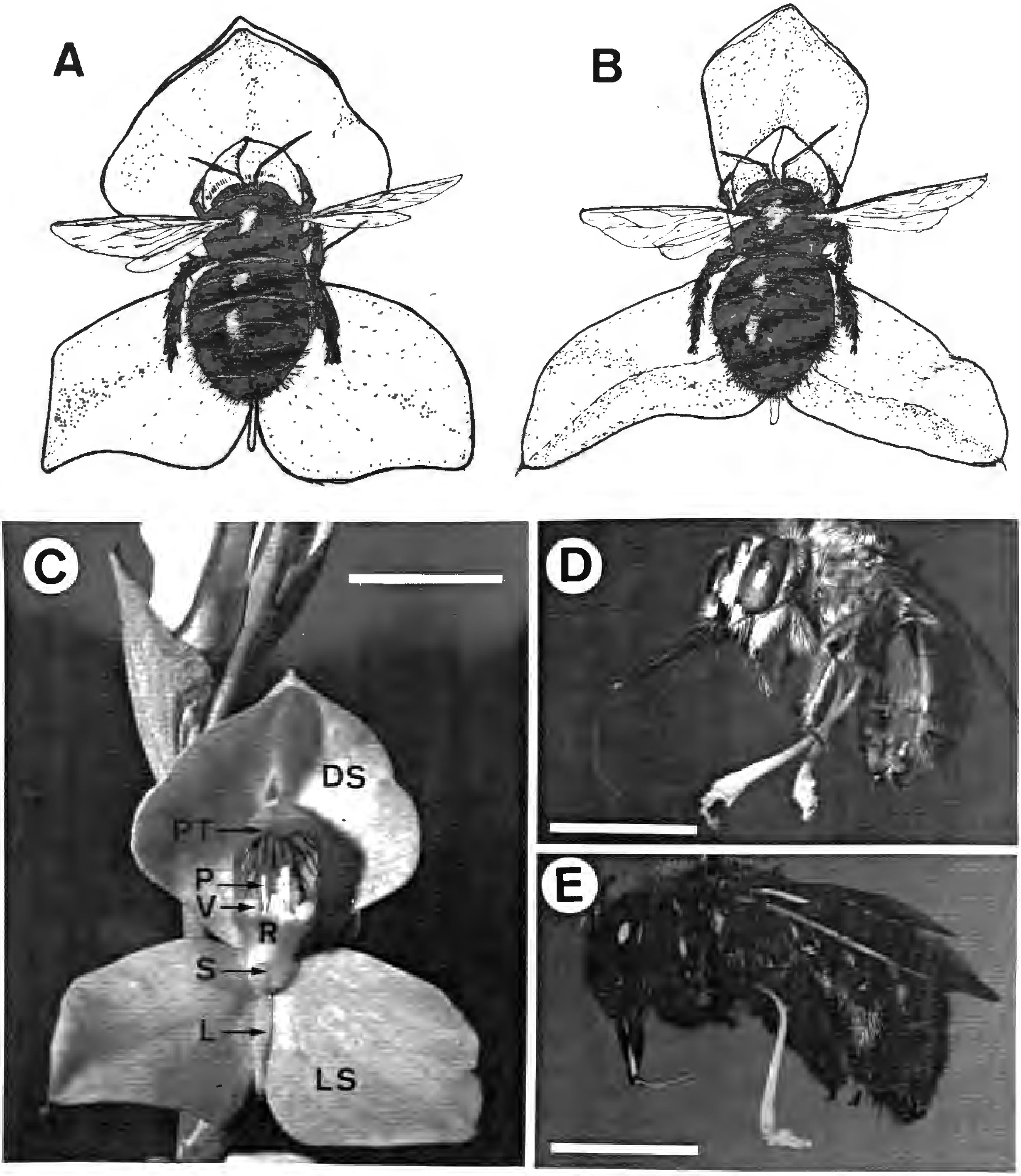

Figure 3.6. **A**, The carpenter bee, *Xylocopa rufitarsus* (Anthophoridae), on a flower of *Disa racemosa*. **B**, *X. rufitarsus* settled on a flower of *D. venosa*. C, Floral morphology of *D. racemosa*. Abbreviations: DS - dorsal sepal, PT -petal, P - pollinarium, V - viscidium, R - rostellum, S - stigma, L - lip, LS - lateral sepal. Scale bar 10 mm. **D**, *Amegilla niveata* (Anthophoridae) carrying two pollinaria of *D. racemosa* attached to the middle legs. Scale bar 5 mm. **E**, *X. rufitarsus* with a single attached pollinarium of *D. racemosa*. Scale bar 5 mm.

Discussion

Disa racemosa appears to be a generalized food mimic of large pink pollen-rewarding flowers. Some of these large pink flowers with prominent yellow anthers, such as *Chironia jasminoides* (which was sympatric with *D. racemosa* at Bains Kloof and Cape Point), are buzz-pollinated by *Xylocopa* females (Johnson, 1992d). Thus the pollination system of *D. racemosa* has several similarities to some northern hemisphere orchids which have large showy deceptive flowers pollinated by bumblebees (Thien & Marcks, 1972; Gill, 1989; Nilsson, 1980, 1983a; Boyden, 1982; Fritz, 1990). Bumblebees are absent from southern Africa, where their ecological role as pollinators of large showy flowers is substituted by *Xylocopa* and other large anthophorid bees.

Coexistence - *Disa* species, like most orchids, can easily be hybridized (Vogelpoel *et al*, 1985; Linder, 1990). How then do *D. racemosa* and *D. venosa,* which are sister species, manage to coexist without apparent hybridization when they have almost identical flowers with closely matched reflectance spectra? Ethological isolation by attraction of different pollinators is well known in other sympatric orchid species pairs (Smith & Snow, 1976; Chase, 1986)., but *D. racemosa* and *D. venosa* apparently share pollinators. Other obvious possibilities, such as mechanical isolation through placing pollinaria on different parts of the body of the pollinator can be ruled out as the species have almost identical column structures. I have tested the possibility that the two species are incompatible, by performing reciprocal crosses between bagged flowers. Fruits were set in flowers from all four treatments (10 crosses per treatment), but germination took longer than expected - over 5 months - but to date, 7 months after the experiment, only seeds from intraspecific crosses have germinated. These preliminary results suggest a genetic barrier between these closely related orchids. The most likely explanation, which will be tested when root tips become available, is that the species differ in chromosome number. Genetic barriers are known between co-flowering orchids of different genera eg. *Dactylorhiza sambucina* and co-flowering *Orchis* species (Nilsson, 1980), but a genetic barrier between sister species is very unusual in the Orchidaceae.

Summary

Observations in the Cape mountains showed that the orchid *D. racemosa* is pollinated by anthophorid bees. Pollinaria are carried on the middle pair of legs of the bees. The orchid lacks floral rewards and appears to rely on pollinators which are instinctively attracted to its large pink flowers. Visits were scarce with less than one bee observed per day at most sites. The puzzling coexistence of *D. racemosa* with its sister species *D. venosa* in the same habitats is discussed. Preliminary experiments suggest that a genetic barrier, rather than ethological or mechanical isolation, prevents hybrids between the species.

AUTO-POLLINATION IN *DISA VAGINATA*, *D. GLANDULOSA* AND *D. ROSEA*[1]

Auto-pollination (automatic self-pollination) is widespread in the Orchidaceae and may occur in up to 20% of the species (Catling, 1990). There have been relatively few reports of auto-pollination in the large African orchid flora (Schelpe, 1970; Gassner, 1982; Williamson, 1984; Pettersson, 1986, 1989; Kurzweil & Johnson, 1993). Here I describe and illustrate the mechanisms and structural modifications which facilitate auto-pollination in three Cape *Disa* species, and suggest possible reasons for the evolution of the trait.

The study species

Disa vaginata Lindl. and *Disa glandulosa* Burch. ex. Lindl. (Fig 3.7) are sister species, currently placed in *Disa* sect. *Coryphaea* Lindl. They share the character combination of cauline leaves and a corymbose inflorescence (Linder, 1981a). They can be distinguished from each other by the longer spur in *D. vaginata* (Fig 3.7) and the conspicuous glandular hairs on the stems and leaves of *D. glandulosa*. Both species are terrestrial herbs up to 20cm tall with small pink flowers c.10 mm in diameter. They tend to occur on moist south-facing slopes which are frequently blanketed in cloud during the flowering season. The two species often occur within close proximity and flower concurrently in December. Both are widely distributed throughout the Cape floral region (Linder, 1981a). *Disa rosea* Lindl., a member of the sect. *Disa* Berg., occurs in similar habitats to *D. glandulosa*. The white or pink flowers of *D. rosea* are c. 20 mm in diameter and do not have a spur.

Methods

Populations of *D. glandulosa* near Muizenberg Cave in the Silvermine Nature Reserve (c.40 plants) and on Newlands Buttress, Table Mountain (c.40 plants), were studied. *D. vaginata* was studied at a site on Newlands Buttress, Table Mountain, where about 100 plants occur. Scattered populations of *D. rosea* were examined on the Constantiaberg and at Newlands Buttress.

The breeding system of *D. glandulosa* was investigated at Silvermine by bagging 53 flower buds on 10 plants in fine nylon mesh on 11 December 1992 and then emasculating 13 of these flowers by removing the pollinaria with forceps. The remaining 40 bagged flowers

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[1] Adapted from Johnson *et al* (1994)

were left with their pollinaria intact in the anthers. A further 87 flowers on 23 plants were marked with thread and left unbagged. Fruit set in these flowers was determined on 7 January 1993. Fruit set in the Table Mountain population of *D. vaginata* was determined on 17 January 1993.

Observations

Mechanism of auto-pollination - Auto-pollination was evident in most of the flowers of *D. vaginata* and *D. glandulosa* which I examined at the study sites. The pollinia had flipped onto the stigma, while the viscidia remained in their original position (Fig 3.8). I found that a gentle tap at the base of a plant with newly opened flowers would result in the pollinia's flipping onto the stigma. Dissection of buds of *D. vaginata* showed that auto-pollination sometimes occurs in this species before the flowers open. Although the viscidia of both *D. vaginata* and *D. glandulosa* are reduced, I found that pollinaria adhered to a needle inserted into the flowers which had not yet self-pollinated, indicating the potential for outcrossing to occur. However, no insects were seen to visit the flowers and no flowers had pollinaria removed or pollen massulae (other than from auto-pollination) deposited on the stigma. Auto-pollination through pollinia flipping onto the stigma was also observed in *D. rosea* on the Constantiaberg and Table Mountain (Fig 3.1C).

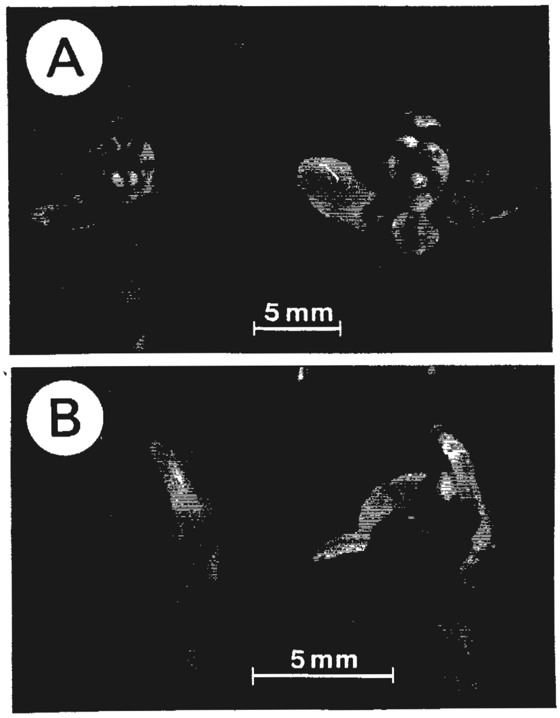

Figure 3.7. **A**, Flower of *Disa vaginata* (left) and *Disa glandulosa* (right). **B**, Lateral view of the flowers of *D. vaginata* (left) and *D. glandulosa* (right) showing the longer spur of D. vaginata. Scale bars 5 mm.

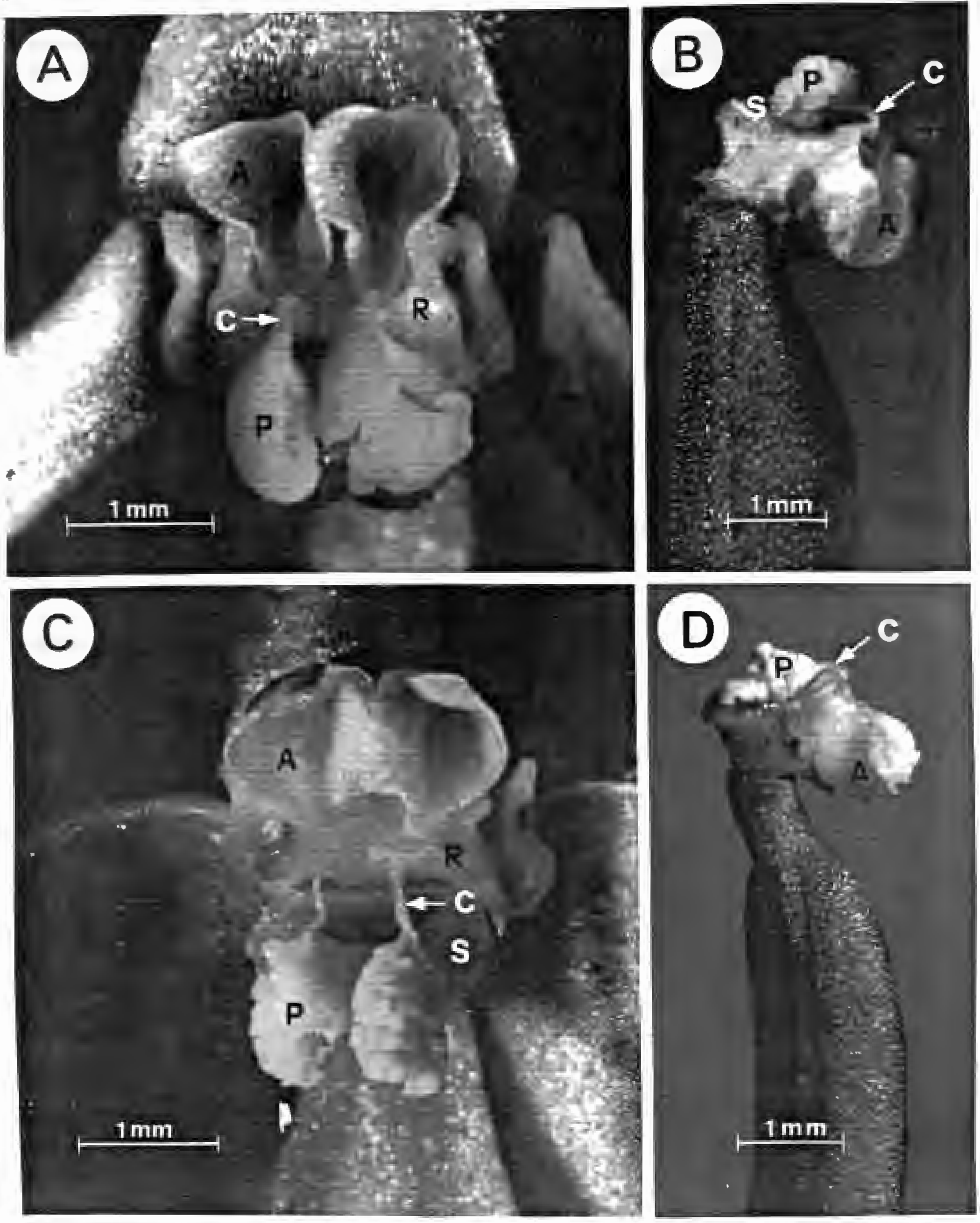

Figure 3.8. **A**, Auto-pollination in *D. glandulosa*. Dorsal view of the column (galea and lateral sepals are removed), showing how both pollinia have flipped out of the anther and onto the stigma. **B**, Lateral view of the column of *D. glandulosa* (petals and sepals are removed) showing one pollinium flipped onto the stigma and one pollinium remaining in the anther. **C**, Dorsal view of the column of *D. vaginata* (galea and lateral sepals are removed) showing how both pollinia have flipped onto the stigma. Note the longer caudicle in *D. vaginata*. **D**, Lateral view of the column, showing auto-pollination. Abbreviations A Anther, C caudicle, P pollinium, R rostellum, S stigma. Scale bars 1 mm.

Table 3.2. Fruit set in open (unmanipulated), bagged (insect excluded) and bagged/emasculated flowers of *D. glandulosa*. Natural levels of fruit set in *D. vaginata* are also indicated.

Species	Treatment	No. of flowers	No. of fruits	Fruit set (%)
D. glandulosa	Open	87	67	78
	Bagged	40	25	63[a]
	Bagged and emasculated	13	2	15[b]
D. vaginata	Open	60	44	74

[a] Fruit set in the bagged and open treatments of *D. glandulosa* are not significantly different ($G = 2.81$, $P > 0.05$)

[b] Fruit set in the bagged/emasculated treatment is significantly lower than fruit set in the bagged treatment ($G = 9.36$, $P < 0.01$)

Experimental manipulations - Natural levels of fruit set were 78% in *D. glandulosa* and 74% in *D. vaginata* (Table 3.2). Fruits developed in 63% of the bagged flowers of *D. glandulosa* (Table 3.2), confirming that fruit production takes place without pollinators. The nylon bags probably reduce the incidence of auto-pollination by sheltering the flowers from disturbances such as wind and rain, which could cause flipping of the pollinia. This may explain why fruit set was lower (though not significantly) in bagged flowers compared with unbagged flowers (Table 3.2). Agamospermy, as a cause of fruit set in the bagged flowers of *D. glandulosa*, is unlikely as only two of the 13 emasculated flowers set fruit (Table 3.2). These two fruits probably resulted from pollen accidentally dropping onto the stigma during the emasculation process.

Adaptations for auto-pollination - *D.vaginata* and *D. glandulosa* have several features, not found in obligate outcrossing relatives, which facilitate auto-pollination. The rostellum lobes are reduced and do not provide a barrier between the stigma and the anther. The pollinia are friable (especially in *D.vaginata*), and break up onto the stigma after "flipping". The caudicles are very slender and flexible and do not provide any resistance to flipping. Catling (1990) called this phenomenon "bending caudicle". The anthers open widely (Fig 3.8A,C), allowing the pollinia to be jerked out when the plant is physically disturbed - in many other disas the anther remains partially closed and the pollinia have to be drawn out by the pollinator (eg. in *Disa uniflora* Berg.). Similarly, González-Díaz and Ackerman (1988) demonstrated that physical disturbance caused by rain accounted for natural fruit set levels in two Puerto Rican populations of *Oecoclades maculata* Lindl.

On the other hand, both *D.vaginata* and *D. glandulosa* have retained several features associated with outcrossing. Both possess spurs, although they do not contain nectar. The fact that spur length differs in these two sister species indicates that pollinators may have played a role in differentiation of the species in the past. Both species have intact pollinia, with a sticky viscidium, which can be extracted from the flower during the "window period" before a disturbance to the plant causes auto-pollination. Although there are no physical reasons why outcrossing should not take place during the window period, it appears that insect visits resulting in outcrossing are rare or non-existent as none of the c.40 flowers examined in each of the study populations had pollinaria removed.

Auto-pollination appears to have evolved independently in several lineages within *Disa* (see Chapter 4). A separate origin of auto-pollination in *D. rosea* (a member of sect. *Disa*) can be assumed as it is not closely related to *D. vaginata* and *D. glandulosa* (members of sect. *Coryphaea*). Auto-pollination through pollinia flipping onto the stigma also occurs in at least two other sections within *Disa*. In sect. *Hircicornes*, auto-pollination has been reported in *Disa crassicornis* Lindl. (as *D. macrantha*) by Weale (1871). According to Weale, a physical disturbance is also required in this species to flip the pollinia onto the stigma. In sect. *Micranthae*, auto-pollination has been reported in the closely related species *Disa erubescens* Rendle ssp. *carsonii* (N. E. Br.) Linder (as *D. stolzii*) (Gassner, 1982) and *Disa ornithantha* Schltr. (Pettersson, 1986). As the anther is erect in sect. *Micranthae*, the flipping of pollinia onto the stigma is aided by gravity and it is likely that auto-pollination is widespread in the section.

Habitat correlates of auto-pollination - It is generally accepted that auto-pollination is a derived trait which evolves in response to extreme pollinator limitation of reproductive success. Thus auto-pollination should be more prevalent in orchids occurring in climates unsuitable for insect activity. Mountainous and boreal regions, for example, tend to have a high proportion of auto-pollinating orchids (Catling, 1990). The disas described here - *D. glandulosa*, *D. vaginata* and *D. rosea* - occur on shady, south-facing rock ledges which are frequently blanketed in cloud and exposed to high wind velocities. While such conditions are unsuitable for insect activity, auto-pollination may actually be promoted by wind and rain if the resultant disturbance causes pollinaria to be jerked out of the anther. The potential for outcrossing during periods of calm, sunny weather has been retained in these orchids as pollinaria can be extracted from the flowers during the "window period" before auto-pollination takes place.

Summary

Auto-pollination (self-pollination without the assistance of pollinators) was observed in three *Disa* species occurring in the cloud-zone of the Cape Peninsula mountains near Cape Town. The mechanism of auto-pollination was studied in detail in *Disa vaginata*, *Disa glandulosa* and *Disa rosea*. The caudicle is very flexible in these species, allowing the pollinia to flip onto the stigma when the plant is jerked by wind or rain. Auto-pollination may occur within unopened buds of *D. vaginata*, but more commonly occurs a few days after the flower has opened. Flowers of *D. glandulosa* which were bagged set almost as many fruits as unbagged flowers, indicating that pollinators are not required for fruit set in this species. Auto-pollination has been previously reported in three other *Disa* species and probably evolved independently in at least four different lineages within *Disa*.

POLLINATION OF *DISA COOPERI* BY HAWKMOTHS

Study species

Disa cooperi Reichb.f., is found in montane grasslands along the eastern escarpment of South Africa (Linder, 1981a). The orchid bears up to 50 flowers on a tall inflorescence (up to 70 cm in height) which stands conspicuously above the surrounding grasses (Fig 3.10B). The flowers are white with a green lip and pinkish tinges on the spur and sepals. A long nectar-producing spur projects from the back of the hoodlike dorsal sepal. The most distinctive feature of this *Disa* species is the broadly spathulate lip.

Study site

Observations were made during the summers of 1993-1994 at the Himeville Nature Reserve (29 45'S 29°31'E) in the foothills of the Natal Drakensberg Mountains. The study population consisted of about 100 plants of *D. cooperi* scattered over an area of 5 hectares of grassland. The flowering period of the orchid at this site is from late December to late January. Observations were made intermittently during daylight hours and continuously from 1800 h to c. 2100 h on each evening of 27, 28 and 29 January 1993 and 1, 2, 24, 25 and 27 January 1994, a total of c. 24 hours of observation. Although the population was extensive, most of the observations were carried out in a fixed observation area of 900 m^2 containing a subpopulation of 20 flowering plants. A flashlight was used to observe the behaviour of moths after dusk. Where possible, moths were caught and examined for pollinaria. A Robinson-type light trap equipped with an 8W UV emitting fluorescent tube was operated at the site, but proved to be completely ineffectual at trapping hawkmoths on both of the nights that it was used, probably because hawkmoth activity peaked at dusk before the onset of the very dark conditions that are optimal for the use of light traps. Nectar volume and nectar sugar concentration were measured with micropipettes and a pocket refractometer, respectively, shortly prior to the start of hawkmoth feeding.

Observations

Hawkmoths were frequent visitors to the flowers of *D. cooperi* at dusk (Fig 3.9). Approximately 10-20 hawkmoth foraging bouts were recorded between 1915 h and 1945 h on each of the eight evenings of the study (the term 'foraging bout' refers to the cumulative activity of a moth while in the observation area). No foraging bouts were observed before 19h15 and very few foraging bouts were observed after 1945 h. The hawkmoths usually probed c. 1-5 flowers on each plant while hovering with their proboscides extended (Fig 3.9, 3.10C-E). Hawkmoths moved directly between plants, indicating that optical cues were primarily used to locate flowers at dusk, even though scent may have attracted the moths from a distance. The moths did not seem to be disturbed by the flashlight, although they were very sensitive to any movement by the observers during dusk and were thus difficult to capture.

The hawkmoths *Basiothia schenki* Möschler and *Agrius convolvuli* L. were the only species observed on the flowers of *D. cooperi*. They are easily distinguished in the field by their distinctive wing coloration patterns (Pinhey, 1962). They also differed greatly in proboscis length. Three captured individuals of *B. schenki* had proboscides of 43 mm (♂), 43 mm (♀) and 42 mm (♂) ($\bar{x}$ = 42.7 mm) and four captured individuals of *A. convolvuli* had proboscides of 80 mm (♀), 88 mm (♂), 104 mm (♀) and 126 mm (♀) ($\bar{x}$ = 99.5 mm). Spur length (measured from the rostellum to the tip of the spur) of the flowers of *D. cooperi* at the site and adjacent localities averaged 41.6 mm (S.D. = 2.5, n = 5 plants).

The relatively short-tongued *B. schenki* was the only hawkmoth species observed to carry pollinaria of *D. cooperi*. During 1994 approximately 50% of the 6-10 moths sighted each evening carried pollinaria attached ventrally to the basal portion of the proboscis (pollinaria of *D. cooperi* are large enough (c. 4 mm) to be seen without capturing the moths). One of the captured moths carried two well-worn pollinaria of *D. cooperi* (Fig 3.10A) and several intact and worn pollinaria can be seen attached to the proboscis of one of the moths photographed while visiting the orchid (Fig 3.10C). None of the individuals of *A. convolvuli* observed during 1993 and 1994 carried pollinaria of *D.cooperi*, even though these moths were very common and probed flowers of the orchid for nectar (Fig 3.10E).

Functional morphology - Features of *D. cooperi* which obviously facilitate hawkmoth pollination are the white flower colour, long spur and evening emission of scent. However, the placement of pollinaria on the proboscis of hawkmoths also requires a sophisticated arrangement of floral parts. The entrance to the flower of *D. cooperi* is mostly blocked by two erect petals which leave only a small aperture directly above the column. These petals are curved inwards so that the proboscides of a hovering hawkmoth is guided into the aperture. This funnel-like arrangement of the petals, which is also found in other long-spurred *Disa* species, is essential for pollinarium attachment as it ensures that the proboscis passes over the rostellum which bears the two closely adjacent viscidia. Funnel-like entrances to the spur are found in many hawkmoth-pollinated orchids; the "funnel" is usually part of the lip, however, eg. *Cynorkis uniflora* (Nilsson *et al*, 1992), *Angraecum arachnites* (Nilsson *et al*, 1985). The deposition of pollinaria onto the base of the proboscis of *B. schenki* indicated that effective proboscis-viscidium contact occurs only when the proboscis of this moth is fully inserted into the flower.

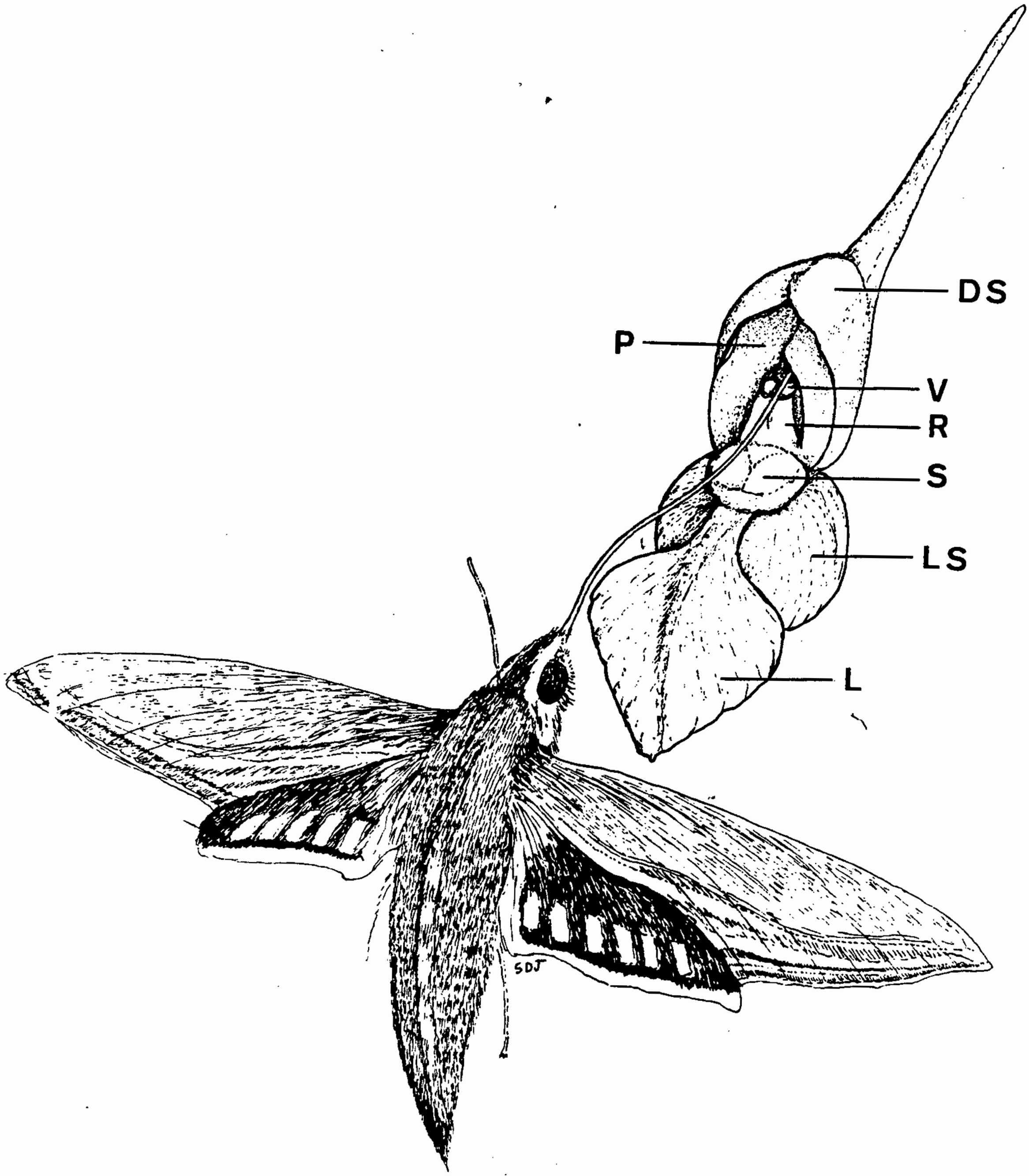

Figure 3.9. Flower of *Disa cooperi* being visited by the hawkmoth *Basiothia schenki*. Note how the proboscis is inserted into a small aperture between the petals and the rostellum. Abbreviations: DS - dorsal sepal. P - petal, V - viscidium, R - rostellum, S - stigma, LS - lateral sepal, L - lip (labellum).

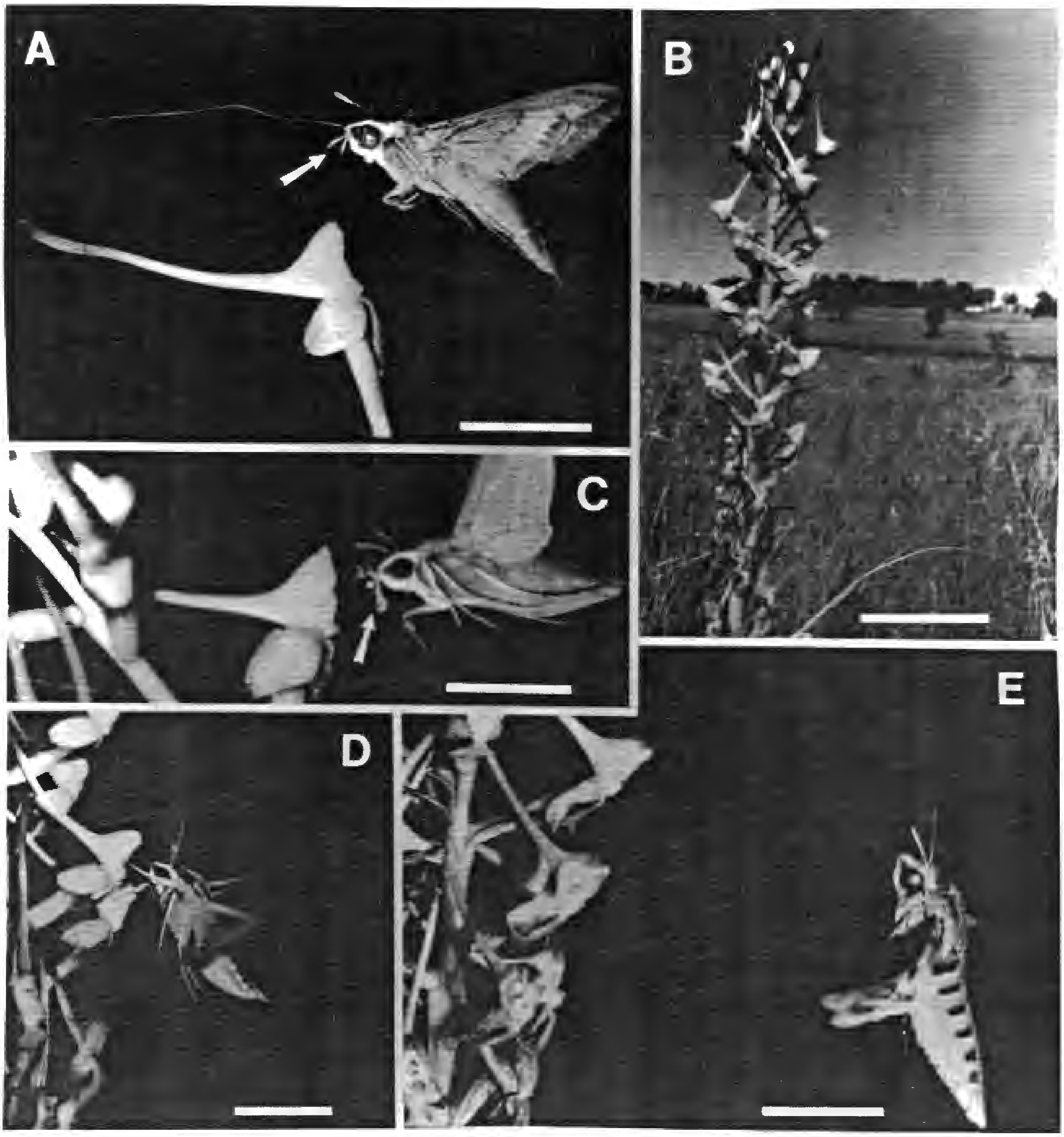

Figure 3.10. A, The captured hawkmoth *Basiothia schenki* positioned next to a flower of *Disa cooperi*, showing the similar proboscis and spur length respectively. Two pollinaria on the ventral base of the proboscis of the moth are indicated by an arrow. Scale bar 20 mm. **B**. Inflorescence of *Disa cooperi* in the Himeville Nature Reserve. Scale bar 50 mm. **C**. Hovering hawkmoth *B. schenki* frozen in flight by an electronic flash, showing three intact pollinaria (arrowed) and the remains of several others attached to the proboscis. Scale bar 20 mm. **D**. *B. schenki* probing flowers of *D. cooperi*. Scale bar 20 mm. **E**. The hawkmoth *Agrius convolvuli*, hovering in front of flowers of *D. cooperi*, with its proboscis extended. Scale bar 20 mm.

Pollination of the orchid occurs when pollinaria dangling from the base of the proboscis contact the large rounded stigma situated directly below the entrance to the flower (Fig 3.10). Pollen massulae which adhere to the sticky mucilage on the surface of the stigma are torn away from the pollinarium as the moth departs from the flower. The pollinaria become progressively worn as massulae are deposited onto the stigmas of consecutive flowers.

Isolation of flower parts of *D. cooperi* in separate glass vials indicated that the broad spathulate lip (Fig 3.9) is the primary source of a strong clove-like scent which is produced in the evening. The mean nectar volume in the spur at 1815 h (shortly before the start of moth activity) on 28 January 1993 was 1.53 μl (S.D. = 0.9, n = 20) and sugar concentration was 34.9 % sugar by weight (S. D. = 3.5, n = 10). The nectar filled about one quarter of the distal part of the spur shortly before moths started foraging.

Discussion

Dodson (1962a) estimated that up to 50% of African orchids are pollinated by lepidoptera. This estimate was presumably based on the high frequency of white, long-spurred flowers among African orchids, especially those of central Africa and Madagascar. A number of studies have recently confirmed that hawkmoth pollination systems are prevalent in the epiphyte-rich orchid flora of Madagascar (Nilsson & Rabokonandrianina, 1988; Nilsson *et al*, 1985, 1986, 1992). The montane grasslands and shrublands of southern Africa, in contrast to Madagascar, have relatively few orchids with white, long-spurred flowers (Stewart *et al*, 1982). These occur mainly in the terrestrial genera *Habenaria* and *Satyrium*. Vogel (1954) was able to observe hawkmoth pollination in *Habenaria epipactidea* Reichb.f. in the Transvaal grasslands, but there have been no subsequent reports of hawkmoth pollination in southern African orchids.

The finding that *D. cooperi* is pollinated only by hawkmoths with proboscides shorter or equal to the length of the flower spur is consistent with several other studies on hawkmoth-orchid interactions (Nilsson, 1988; Nilsson *et al*, 1985, 1992). The positioning of pollinaria of *D. cooperi* on the ventral base of the proboscis of relatively short-tongued hawkmoths is very similar, for example, to that of *Angraecum arachnites* in Madagascar (Nilsson *et al*, 1985). In *A. arachnites*, however, a sophisticated mechanism of flower tilting and spur curvature ensures that contact between the column and the proboscis of hawkmoths is not made until the proboscis is fully inserted into the spur (Nilsson *et al*, 1985). No such mechanism exists in *D. cooperi* and it is therefore more difficult to understand why only the shorter-tongued hawkmoth species should carry pollinaria. Although proboscis-viscidium contact could conceivably be made at any point along the proboscis as it is inserted into the flower, moths were only observed to carry pollinaria attached to the base of the proboscis. It seems, therefore, that firm proboscis-viscidium contact is made only once the proboscis is fully inserted and the head of the moth comes to rest against the petals. Also, pollinaria are more likely to adhere to the thickened basal portion of the proboscis than to the slender distal portion.

Approximately 50% of the visits to *D. cooperi* were by the nectar-thieving hawkmoths *A. convolvuli* with their very long proboscides. Interestingly, this hawkmoth species was also found to be a non-pollinating nectar-thief of *Cynorkis uniflora* in Madagascar (Nilsson *et al*, 1992), although it is the legitimate pollinator of other longer-spurred orchids on Madagascar (Nilsson & Rabakonandrianina, 1988).

Evolution of hawkmoth pollination - The broadly spathulate, scent-producing lip of *D. cooperi* is an autapomorphic trait within *Disa*, and therefore a species-specific adaptation. Closely related species, such as *D. scullyi* H. Bolus and *D. rhodantha* Schltr. share a similar floral structure to *D. cooperi*, but have thin linear lips which do not produce a detectable scent. Both of these species appear to be pollinated by large hovering long-proboscid flies (unpublished data), but additional observations are still needed. I suggest that *D. cooperi* evolved from a long-proboscid fly pollinated ancestor, which was preadapted to hawkmoth pollination as it already possessed a long floral spur. The evolution of a hawkmoth adapted flower from such a long-proboscid fly pollinated ancestor would have required little more than modification of the lip to produce scent. This hypothesis would be consistent with Stebbins' (1970) principle of "lines of least resistance" in the evolution of pollination systems. More data on the pollination of members of *Disa* sect. *hircicornes* is being sought to test this hypothesis.

Summary

Disa cooperi Reichb. f. is a robust grassland orchid with long-spurred white flowers which are strongly scented in the evening. Observations at a site in KwaZulu-Natal Province, South Africa, showed that hawkmoths are frequent visitors to the orchid at dusk. The hawkmoth *Basiothia schenki* was an effective pollinator of *D. cooperi*; this hawk-moth has a medium length proboscis ($\bar{x}$ = 4.3 cm) which can be fully inserted into the nectar-containing spur of the orchid. Pollinaria are attached ventrally to the basal portion of the proboscis where it joins the head. Another hawkmoth, *Agrius convolvuli*, commonly foraged on nectar from *D. cooperi*, but did not carry pollinaria, probably because its proboscis is too long ($\bar{x}$ = 10 cm) to allow contact between the thick basal portion of the proboscis and the orchid column. Lips in *Disa* are typically linear and do not produce scent, thus the autapomorphic spathulate and scent-producing lip of *D. cooperi* indicates that hawkmoth pollination is derived in this species, probably from a long-proboscid fly-pollinated ancestor.

POLLINATION OF *DISA OREOPHILA* BY LONG-PROBOSCID FLIES[1]

The study species

Disa oreophila H. Bolus ssp. *erecta* Linder was considered a candidate for long-proboscid fly pollination on account of its pink, unscented flowers with long (10-25 mm) spurs. It is a member of *Disa* section Stenocarpa Lindl., a clade of long-spurred *Disa* species which do not secrete nectar and are thus pollinated through deceit (unpublished data). *D. oreophila* ssp. *erecta* is restricted to a range of about 100 km along the summits of the Drakensberg Mountains between Lesotho and South Africa (Linder, 1981a).

Study site

The study site was located at the top of Naudesnek Pass (30°44'S 28°08'E), a remote mountain road which crosses the Drakensberg Mountains in the Eastern Cape Province, near the Lesotho border. Populations of *D. oreophila* ssp. *erecta* occur in short montane grassland on the slopes of the summit at c. 2500 m. The population consists of about 100 individuals.

Observations at Naudesnek were made between 1100 h and 1500 h on 24 January 1993. While the summit of Naudesnek is often covered in cloud during summer, the observation days were warm and sunny. Insects visiting the orchids or neighbouring nectar flowers were collected and inspected for pollinaria. The spur length (taken here as the distance between the rostellum and tip of the spur) was measured in a sample of flowers.

Observations

The large tanglewing fly *Prosoeca ganglbaueri* Lichtwardt (Nemestrinidae) was the only species of insect observed to pollinate the flowers of *D. oreophila*. This fly was abundant at Naudesnek; ten individuals were caught in 1993, of which nine carried between one and ten pollinaria ($\bar{x} = 2.8$) of *D. oreophila* attached to the underside of the proboscis (Fig 3.11C). Observations by K. Steiner (pers. comm.) have shown that *P. ganglbauri* also pollinates another long-spurred orchid, *Brownleea macroceras*, at the Naudesnek Pass site. The pollinaria of the two orchids can be readily distinguished by the much longer caudicle of *B. macroceras*. The flies did not confine their activity to the orchids at the study site, but also visited three different nectar plants: *Scabiosa columbaria* L. (Dipsacaceae) and *Cephalaria galpiniana* Szabó (Dipscaceae), both white-flowered, and *Lobelia preslii* A.DC. (Campanulaceae), which has blue flowers.

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[1] Adapted from Johnson & Steiner (1994c)

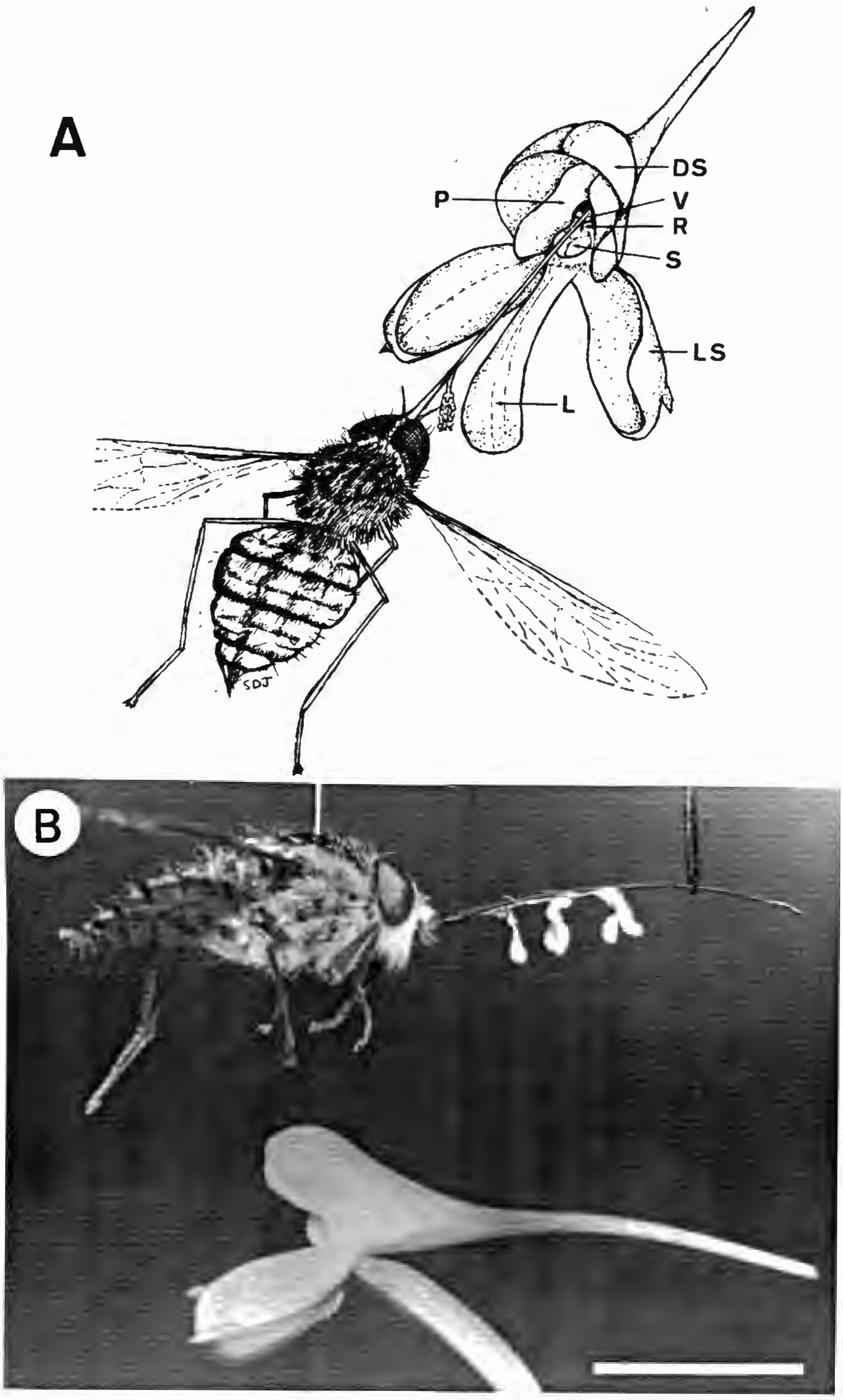

Figure 3.11. **A**, *Prosoeca ganglbaueri* hovering in front of a flower of *Disa oreophila* ssp. erecta. The fly's proboscis enters the spur through a small aperture between the petals and the rostellum. Pollinaria which are already attached to the fly's proboscis are dragged over the ventrally located stigma as the proboscis enters the spur. **B**, Lateral view of the long-proboscid fly Prosoeca ganglbaueri posed next to a flower of *D. oreophila* ssp. *erecta*. Pollinaria of *D. oreophila* are attached to the underside of the fly's proboscis. Scale bar 20 mm.

The proboscides of tanglewing flies are tucked beneath the body during flight, but when a flower is approached the proboscis is brought forwards (Fig 3.11). *Prosoeca ganglbaueri* hovers while inserting its proboscis into the horizontal spurs of *D. oreophila* and then places its front legs on the lateral sepals for support once the proboscis is fully inserted. The proboscis is inserted into a small aperture between the petals and the ventrally located rostellum (Fig 3.11). The rostellum in *D. oreophila* has square lobes which support two closely adjacent viscidia. In *B. macroceras*, the rostellum is a tall triangular structure bearing two small viscidia on the apex. Pollinaria of both orchid species become attached to the fly's proboscis wherever it makes firm contact with the viscidia as it passes through the aperture between the petals and the rostellum. Pollinaria already dangling from the proboscis are dragged over the stigma which is located ventrally at the entrance to the flower (Fig 3.11). Pollen massulae which contact the stigma adhere to the sticky surface and break away from the pollinarium. Since floral morphology and pollinaria placement on the flies is similar in both *D. oreophila* and *B. macroceras*, there is potential for reproductive interference between the species. However, the flowering times of the two orchids differ and no flies were observed to carry mixed loads of pollinaria.

The mean proboscis length of *P. ganglbaueri* at the study site ($\overline{x}$ = 19.6 mm, S.D. = 2.3, n = 10) corresponded well to the spur length of *D. oreophila* ($\overline{x}$ = 20.6 mm, S.D. = 1.1, n = 7). As there is no nectar in the spur of *D. oreophila* the visits are very rapid (1-2 sec).

Discussion

Pollination by long-proboscid flies is one of the most interesting aspects of the South African flora. Although flies with elongated mouthparts up to 10 mm in length are known from many regions of the world, the evolution of nectar-feeding mouthparts has proceeded to extremes in two dipteran families in southern African - Tabanidae and Nemestrinidae - which have mouthparts of up to 40 mm and 80 mm respectively (Bezzi, 1924; Oldroyd, 1957; Bowden, 1978). Their importance as pollinators was first recognised by Vogel (1954) who showed that flowers from a wide variety of families, including Ericaceae, Iridaceae, Geraniaceae and Orchidaceae, are adapted to long-proboscid flies. In general, these flowers have long perianth tubes, bright colours and an absence of scent. Despite the large number of plants with apparent adaptations for long-proboscid fly pollination, few actual observations of long-proboscid flies visiting flowers have been made (Marloth, 1908; Goldblatt & Bernhardt, 1990; Johnson & Johnson, 1993). In the Orchidaceae, a family with many long-spurred flowers, long proboscid fly pollination has been observed in only one species: *Disa draconis* (L.f) Sw., a southwestern Cape endemic (Vogel, 1954; Johnson & Johnson, 1993).

Prosoeca ganglbaueri is one of the most common long-proboscid flies in South Africa, but it is not often collected as it is confined to inaccessible mountainous areas. It has been recorded as a pollinator of a pink-flowered *Satyrium* species (Orchidaceae) in the Transvaal Drakensberg Mountains (Johnson, unpublished), as well as blue-flowered *Nivenia* species (Iridaceae) and pink-flowered *Erica* species (Ericaceae) in the Swartberg Mountains about 400 km east of Cape Town (Goldblatt & Bernhardt, 1990; Johnson, unpublished).

The close correspondence between spur length in *D. oreophila* ssp. *erecta* and proboscis length of *P. ganglbaueri* indicates that spur length in the orchid may have been modified by selection to accommodate the long proboscis of *P. ganglbaueri*. Taxa related to *D. oreophila* ssp. *erecta* have relatively short spurs which could indicate that the long spurs of the orchid are a derived character. It is highly unlikely, however, that proboscis length in the fly has been influenced by the non-rewarding flowers of *D. oreophila*. It is more likely that the proboscis length of *P. ganglbaueri* evolved through a process of diffuse coevolution (*sensu* Futuyama & Slatkin, 1983) in response to a variety of flowers that secrete nectar.

It was interesting to observe how the flies at the study site persistently visited *D. oreophila*, despite the absence of a floral reward in its flowers. None of the nectar-producing species which were also visited by *P. ganglbaueri* at the study site were similar in colour or morphology to *D. oreophila*, thus indicating that the orchid is not a specific mimic of other species. Rather, the flies appeared to be instinctively attracted to the showy pink flowers of *D. oreophila*. The orchid thus fits the category of generalized food-source mimicry (Ackerman, 1986). The Cape orchid *Disa draconis* which is pollinated by long-proboscid horseflies also falls into this category of deception (Johnson & Johnson, 1993).

Comments on the long-proboscid fly pollination syndrome - Flowers visited by long-proboscid flies tend to have flowers with long straight perianth tubes or spurs, bright colours (pink, blue or white) and an absence of scent (Vogel, 1954; Rebelo *et al*, 1985; Goldblatt & Bernhardt, 1990; Johnson, 1992c). However, this combination of characters also occurs in many butterfly-visited flowers. The difficulty in distinguishing between butterfly and long-proboscid fly adapted flowers led Vogel (1954) to incorporate the latter into the traditional "psychophilous" butterfly pollination syndrome.

While it is admittedly difficult to clearly separate flowers visited by butterflies and long-proboscid flies on the basis of floral character syndromes, there is little field evidence to suggest that butterflies and long-proboscid flies have the same flower visiting preferences; in fact they seldom, if ever, visit the same flowers (Johnson & Bond, 1994, unpublished). Long-proboscid flies appear to restrict their visits to blue, white or pink flowers, whereas most butterflies are attracted to a wider range of colours including yellow, orange and red. Another common difference is that long-proboscid flies, like hawkmoths, tend to hover in front of flowers with horizontally orientated perianth tubes, whereas butterflies tend to settle on, and probe flowers with vertically orientated perianth tubes, though exceptions to both these flower visiting patterns occur (Johnson & Bond, 1994). Finally, it should be borne in mind that categorization of flowers into pollination syndromes is an exercise that should never serve as a substitute for field observations as a way of understanding floral function.

Summary

Large hovering flies with elongated nectar-feeding mouthparts play an important role in the pollination of South African plants. I describe and illustrate the pollination *Disa oreophila* H. Bolus ssp. *erecta* Linder by the long-proboscid fly *Prosoeca ganglbaueri* Lichtwardt (Nemestrinidae). This fly was the sole observed visitor to this orchid and 90% of the captured flies carried pollinaria.

POLLINATION OF *DISA VERSICOLOR* (ORCHIDACEAE) BY ANTHOPHORID BEES[1]

The study species

Disa versicolor Reichb.f. is probably the most common orchid in the montane grasslands of South Africa, yet its pollination biology has not previously been investigated. The species is a member of *Disa* sect. *Hircicornes*, a clade of robust, nectar-producing species which occur mainly in grassland habitats (Linder, 1981a). The inflorescence of *D. versicolor* is 10-60 cm tall and bears up to 200 densely packed flowers. The column is ventrally located at the entrance to the flower and partially enclosed in a chamber formed by the galeate dorsal sepal. This chamber narrows posteriorly to form a sharply decurved spur (c. 10 mm in length) containing nectar. The lip is a simple linear structure which hangs below the entrance to the flower. A weak, indistinct scent, described as "vanilla-like" by Linder (1981a) is sometimes detectable in the flowers.

The most distinctive feature of *D. versicolor* is the change in flower colour from pink at the bud stage to brown at anthesis. As a consequence the inflorescence of *D. versicolor* usually has a bicoloured appearance (hence the specific epithet) as the upper portion where flowers are still at the bud stage is pink and the lower portion with opened flowers is brown (see colour plate in Stewart *et al*, 1982).

Study site

Observations were made across the distribution range of *D. versicolor* in South Africa, but it was not possible to visit the outlying populations in Angola and Zimbabwe. Observations were made on 1-2 and 19-21 January 1994 at the Verloren Valei Nature Reserve (25°17'S 33°09'E) near Dullstroom, and 26-27 January 1994 on the Bushmans Nek Pass in Sehlabathebe National Park (29°52'S 29°08'E) in Lesotho. Further observations at the Royal Natal National Park (28°45'S 28°58'E) and Giants Castle Game Reserve (29°13'S 29°33'E) were communicated to me by K. Steiner and J. Manning, respectively.

At both Dullstroom and Sehlabathebe the populations of *D. versicolor* were extensive, consisting of several hundred individuals in an area of about one hectare. Observations were carried out between approximately 0900 h and 1800 h. Insects visiting the orchid were caught and inspected for pollinaria. Bee proboscis length was measured from the face to the tip of the extended tongue. Spur length, defined as the distance from the rostellum to the tip of the

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[1] Adapted from Johnson (1994c)

spur, was measured in a sample of flowers from each site. Nectar volume in the spurs was measured with graduated micropipettes.

Observations

Amegilla natalensis (Friese) (Anthophoridae), a common grassland bee, was a frequent visitor to *Disa versicolor* at both Verloren Valei and Sehlabathebe. This bee has also been caught on *D. versicolor* at the Giants Castle Game Reserve (J. Manning, pers. comm.) and Royal Natal National Park (K. Steiner, pers. comm.) in the central Drakensberg Mountains.

Amegilla natalensis always landed on the lower portion of the inflorescence of *D. versicolor* with its brown opened flowers (Fig 3.1B, 3.12). While the pink colour of the buds on the upper portion of the inflorescence probably acts as a long distance attractant, the bees appear to use the transition zone from pink to brown as a short range orientation cue for locating newly opened flowers on the inflorescence.

The bees visited several flowers on each inflorescence and accumulated large loads of pollinaria on the ventral surface of their mouthparts. Bees sometimes groomed off these pollinaria while hovering in front of the flowers. Samples of seven bees from Verloren Valei and six bees from Sehlabathebe were collected on the flowers of *D. versicolor*. These bees were all female *A. natalensis* and have been deposited in the South African Museum, Cape Town. Each of the bees carried several pollinaria, but exact counts of pollinaria could not be made as they were mostly groomed off by the bees in the killing bottle.

While the orchid appeared to rely solely on *A. natalensis* for pollination, the bees were more generalist in their foraging. At Verloren Valei, *A. natalensis* also visited the flowers of *Gladiolus papilio* Hook.f. (Iridaceae) and *Scabiosa sp*. (Dipsacaceae), but at Sehlabathebe where the choice of alternative nectar sources was more limited, the bees visited *D. versicolor* exclusively.

Functional morphology - The entrance to the flower of *D. versicolor* faces downwards; consequently bees hang from the dorsal sepal by their front legs while feeding (Fig 3.1B, 3.12). The galea, a rigid non-retractible portion of the mouthparts, is inserted into the dorsal sepal chamber and then the true tongue (glossa) is extended into the downwardly curved spur itself. Since the column is located ventrally at the entrance to the dorsal sepal chamber, pollinaria become attached to the underside of the galea (Fig 3.12). There was a good "fit" between the spur length of the orchid ($\bar{x}$ = 9.8 mm, s = 0.8, n = 11 plants) and the length of the bee mouthparts ($\bar{x}$ = 10.0 mm, n = 14). The spur contained 0.3 - 1.0 μl ($\bar{x}$ = 0.7 μl) of nectar in a sample of 10 flowers.

The sharply decurved spur of *D. versicolor* (Fig 3.12) is an unusual trait for a South African orchid. Sharply decurved spurs are found in several European orchids where they accommodate the downwardly curved tongue of bumblebees (Nilsson, 1983c). Since bumblebees are absent from southern Africa, this spur feature appears to be a convergent adaptation to accommodate the long curved tongue of *Amegilla* or similar large long-tongued bees.

Conclusions - *D. versicolor* appears to have a simple reward-based pollination system. The consistency of the field observations at several sites up to 400 km apart in South Africa (Fig 3.2). suggests that *A. natalensis* is the primary pollinator of the orchid. Other studies of bee pollination of orchids show that several bee species may be involved in the pollination of one orchid species (eg Nilsson, 1983c; see also *D. tenuifolia*, and *D. racemosa*, this chapter). Pollination of *D. versicolor* by a single species of bee was therefore a curious and unexpected result. This apparent specificity of the orchid may be a consequence of low diversity of large bees during the flowering period, rather than specific floral attractants. The only other large bees observed at the study sites were *Rediviva* species (Melittidae) which confined their visits to oil-producing flowers (see Steiner & Whitehead, 1988). Both *D. versicolor* and *A. natalensis* are confined to the eastern escarpment of southern Africa. *Amegilla natalensis* has not been collected from the extreme southern end of the orchid's range, however, which could reflect either undercollecting of the bee in remote areas or pollination of the orchid by another vector, probably another *Amegilla* species, in this region.

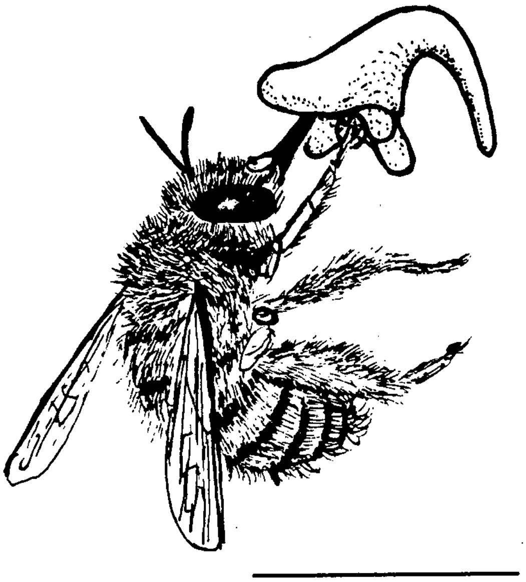

Figure 3.12. The bee *Amegilla natalensis* probing a flower of *Disa versicolor*. Scale bar 10 mm.

Summary

Amegilla natalensis, a large anthophorid bee, was the sole observed pollinator of the orchid *Disa versicolor* at several sites in South Africa and Lesotho. The orchid has a sharply decurved nectar-containing spur which neatly accommodates the long curved tongue of *A. natalensis*. Pollinaria are ventrally attached to the mouthparts of the bee. Flowers of *D. versicolor* undergo a colour change from pink to brown during anthesis. This appears to function as an orientation cue for bees to locate newly opened flowers on the tall, densely packed inflorescence.

POLLINATION OF *DISA OBTUSA* BY BIBIONID FLIES[1]

Small Diptera of the suborder Nematocera have been found to pollinate many plant species possessing small, dull-coloured and pungent-scented flowers. The most important pollinating families are Culicidae, Sciaridae, Mycetophilidae and Bibionidae (Proctor & Yeo, 1972; Kevan & Baker, 1983). Most nematoceran flies are less than 10 mm in length and are generally considered inefficient pollen vectors relative to larger flies and bees (Proctor & Yeo, 1972). A number of studies have shown low levels of fruit and seed set in plants pollinated by nematoceran flies (Thien & Utech, 1970; Bierzychudek, 1981b). The abundance of nematoceran flies in some habitats, however, may compensate for their inefficiency as pollen vectors. Mesler *et al* (1980) attributed high pollination success in the orchid *Listera cordata* (L.) R.Br. to the abundance of Sciaridae and Mycetophilidae which pollinate its flowers in the coastal redwood forests of California.

Very little is known about pollination by nematoceran flies in Africa. The only previous study that I am aware of is Garside's (1922) report that *Satyrium bicallosum* Thunb. (Orchidaceae) is pollinated by Sciaridae. Here I show that *Disa obtusa* Lindl. is pollinated by bibionid flies.

The study species

The inflorescence of *Disa obtusa* Lindl. is 6-40 cm tall and densely packed with up to 150 small (c. 5 mm in diameter) flowers (Fig 3.14A). The flowers are white with some blotchy purple markings and they emit a pungent scent. No nectar was found in the floral spurs, despite careful examination under a stereo microscope. The pollinaria are ventrally located at the entrance to a small chamber formed by the galeate dorsal sepal.

Study site

A population of *D. obtusa* on the Muizenberg Plateau (34°17'S 18°28'E) in the Silvermine Nature Reserve was studied. The population consisted of about 2000 flowering individuals in a marshy area which had been burnt the previous year. Flowering in this species occurs only in the first season following a fire; flowering is thus infrequent as fires in Cape fynbos vegetation occur at five to 40 year intervals. Observations and insect collections were made between 1000 h and 1500 h on 12, 15, 17 and 27 October 1992.

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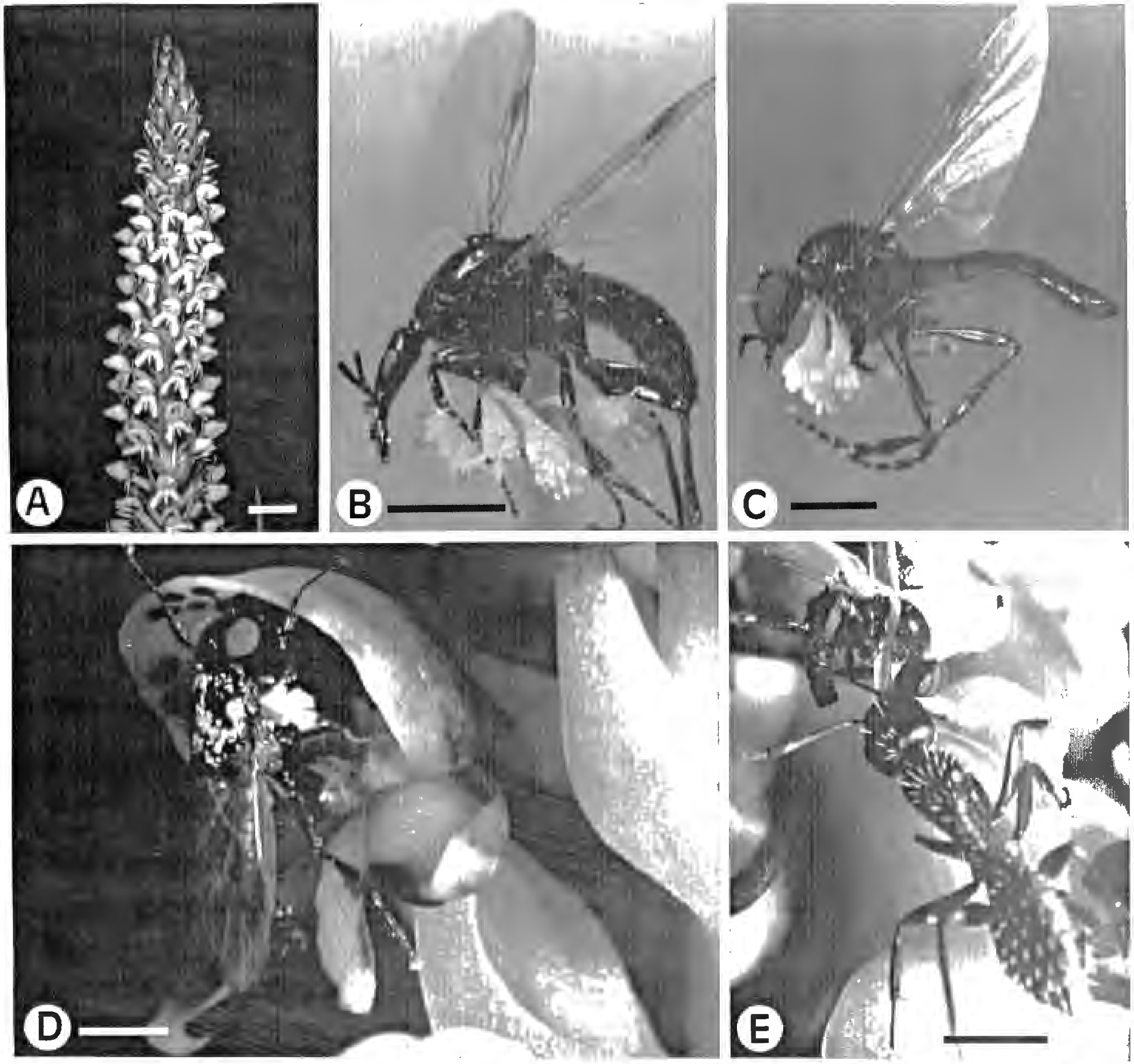
[1] Adapted from Johnson & Steiner (1994b)

Figure 3.14. **A,** Inflorescence of *Disa obtusa*. Scale bar 10 mm. **B,** Female *Bibio turneri* with pollinaria attached to the front legs. Scale bar 1 mm. **C,** Male *B. turneri* with pollinaria attached to the ventral surface of the thorax. Scale bar 1 mm. **D,** Male *B. turneri* carrying pollinaria emerging from the flower of *D. obtusa*. Scale bar 1 mm. **E,** The predatory ant *Camponotus niveosetosus* grasping a male *B. turneri* in its jaws. Scale bar 2 mm.


A
B
C
D
E

Observations

Disa obtusa was pollinated almost exclusively by the small fly *Bibio turneri* Edwards (Bibionidae) at the study site. A sample of 32 flies carrying pollinaria of *D. obtusa* consisted of 31 individuals of *B. turneri* and one unidentified *Bibio* species. Voucher specimens of these flies have been deposited in the South African Museum, Cape Town. Large numbers of flies were active throughout the observation periods. After settling on an inflorescence, the flies crawled in and out of many flowers before departing. Pollinaria became cemented to the ventral surface of the flies as they crawled over the column at the entrance to the flower chamber.

The flowers of *D. obtusa* attracted male and female *Bibio* flies equally and both sexes exhibited similar visitation behaviour. Pollinaria placement differs between the sexes, however, as male and female flies show strong sexual dimorphism in leg and head characters (Hardy, 1950). Pollinaria are generally attached to the swollen front legs of the females, as these are the first body parts to contact the viscidium when the fly crawls into the flower. The males, on the other hand, have slender front legs and pollinaria become attached to the ventral surface of the thorax just behind the head (Table 3.3, Fig 3.14B,C). Females carried slightly more pollinaria (mean = 2.5) than males (mean = 2.0) (Table 3.3), but this difference was not significant (p = 0.25, t = 1.15).

At the height of the flowering season, up to 10 flies were observed on an inflorescence at any one time. Mating regularly took place between flies as they crawled around the inflorescences and male flies engaged in what appeared to be a form of ritual fighting. Heavy predation of the flies by the ant *Camponotus niveosetosus* Mayr (Formicidae) occurred at the site. These ants waited on many of the inflorescences and quickly caught any flies that settled (Fig 3.14E). Ants carrying flies with pollinaria were seen scurrying down inflorescences and over the ground.

Table 3.3. The mean number of pollinaria carried per fly, and placement of pollinaria, on male and female *Bibio turneri* at Muizenberg Plateau.

Sex	n	Mean number of pollinaria (±S.D.)	Percentage of pollinaria	
			on thorax	on legs
Male	13	2.0 (±1.08)	87	13
Female	18	2.5 (±1.38)	40	60

Of 301 flowers examined on 10 randomly chosen inflorescences, 84.0 % had been pollinated and 93.3 % had at least one pollinarium removed. Fruit set was 91.3 % in a sample of 1096 flowers on a further 14 randomly chosen inflorescences. The high pollination and fruiting success appeared to be a consequence of a number of factors including the abundance of *B. turneri* and the dense population structure and mass-flowering in *D. obtusa* which facilitated cross-pollination.

Discussion

Disa obtusa has several features in common with other plants which are reportedly pollinated by Nematocera (Stoutamire, 1968; Kevan & Baker, 1983; Mesler *et al*, 1980; Jones, 1988). These include a pungent odour, small dull-coloured flowers and a moist habitat. The observations indicate that the flowers of *D. obtusa* are attractive to Bibionidae in particular, rather than nematoceran flies in general. Other flies, including Sciaridae, were common at the study site, but were not seen pollinating *D. obtusa*.

The observed dependence of *D. obtusa* on Bibionidae for pollination is probably not an artifact of study at a single site; K. Steiner (pers. comm.) has also collected *B. turneri* on *Disa obtusa hottentotica* at Betty's Bay (34°22'S 18°55'E), as well as a single unidentified bibionid on *D. obtusa hottentotica* at Franschhoek (33°55'S 19°08'E). While the orchid appeared to depend solely on Bibionidae, especially *B. turneri*, for pollination, the flies do not similarly depend on the orchid as they are generalist foragers which visited the flowers of a number of species at the study site. Furthermore, any dependence of Bibionidae on *D. obtusa* is precluded by the infrequent, fire-stimulated flowering pattern of the orchid.

Summary

Observations at a burnt marsh in the Silvermine Nature Reserve near Cape Town showed that the pungent-scented flowers of *Disa obtusa* are pollinated by small (c. 3 mm) bibionid flies. The sexes of these flies are dimorphic, consequently the pollinaria were attached to the underside of the thorax of the male flies and to the front legs of the female flies. The flies were abundant at the study site and pollinated a high percentage of the orchid flowers.

POLLINATION OF *MONADENIA OPHRYDEA* BY SETTLING MOTHS[1]

Apart from a brief description of auto-pollination in *Monadenia bracteata* (Swartz) Dur. & Schinz. (Kurzweil & Johnson, 1993), there have been no studies of pollination in *Monadenia*. The pollination of *Monadenia* has always been enigmatic because of the unusual dark flower coloration in many of the long-spurred species. While these species are usually well-pollinated, I have never observed a daytime visitor.

The study species

Monadenia Lindl. is a small genus of 16 species within the orchid tribe *Diseae* (Dressler, 1993). The most distinctive feature of *Monadenia* is the unlobed, notched rostellum supporting a single viscidium (Linder & Kurzweil, 1990). Thus *Monadenia* - etymologically "single-gland" (viscidium) - differs from the related genus *Disa* Lindl. in which there are two viscidia derived from the lateral lobes of the rostellum, and also *Herschelianthe* (Lindl.) Rauschert in which the single viscidium is a simple fusion of two *Disa*-like viscidia. All *Monadenia* species, except one, are confined to the Cape Floristic Region of South Africa (Linder, 1981b). Flowering is strongly stimulated by fire in most species of *Monadenia* and in at least two species - *M. ophrydea* Lindl. and *M. atrorubens* (Schltr.) Rolfe - flowering occurs exclusively in the first season after fire (Linder, 1981b; Johnson, unpublished).

Monadenia ophrydea is common in the Cape Fold Mountains between Cape Town and Humansdorp, but flowering populations are not often encountered because of the strictly fire-stimulated flowering pattern of the orchid. The inflorescence of *M. ophrydea* is slender, 10-40 cm tall and bears up to 30 dark beetroot-red flowers.

Study site

A flowering population of about 100 plants of *Monadenia ophrydea* on the southern slopes of the "Pilaarkop" mountain (34°04'S 19°51'E) near Riviersonderend was studied on 25-26 October and 1-2 November 1993. The vegetation had been burnt in a wildfire the previous summer. Observations were made during the day and also at night until 2300 h using flashlights to observe moths. Insects visiting the flowers were caught where possible, and their proboscides were unrolled for measurement and inspected for pollinaria. Vouchers of the insects are deposited in the South African Museum, Cape Town. The spur length and

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[1] Adapted from Johnson (1994b)

rates of pollinaria removal and deposition of pollen massulae on stigmas was recorded in a sample of plants.

Observations

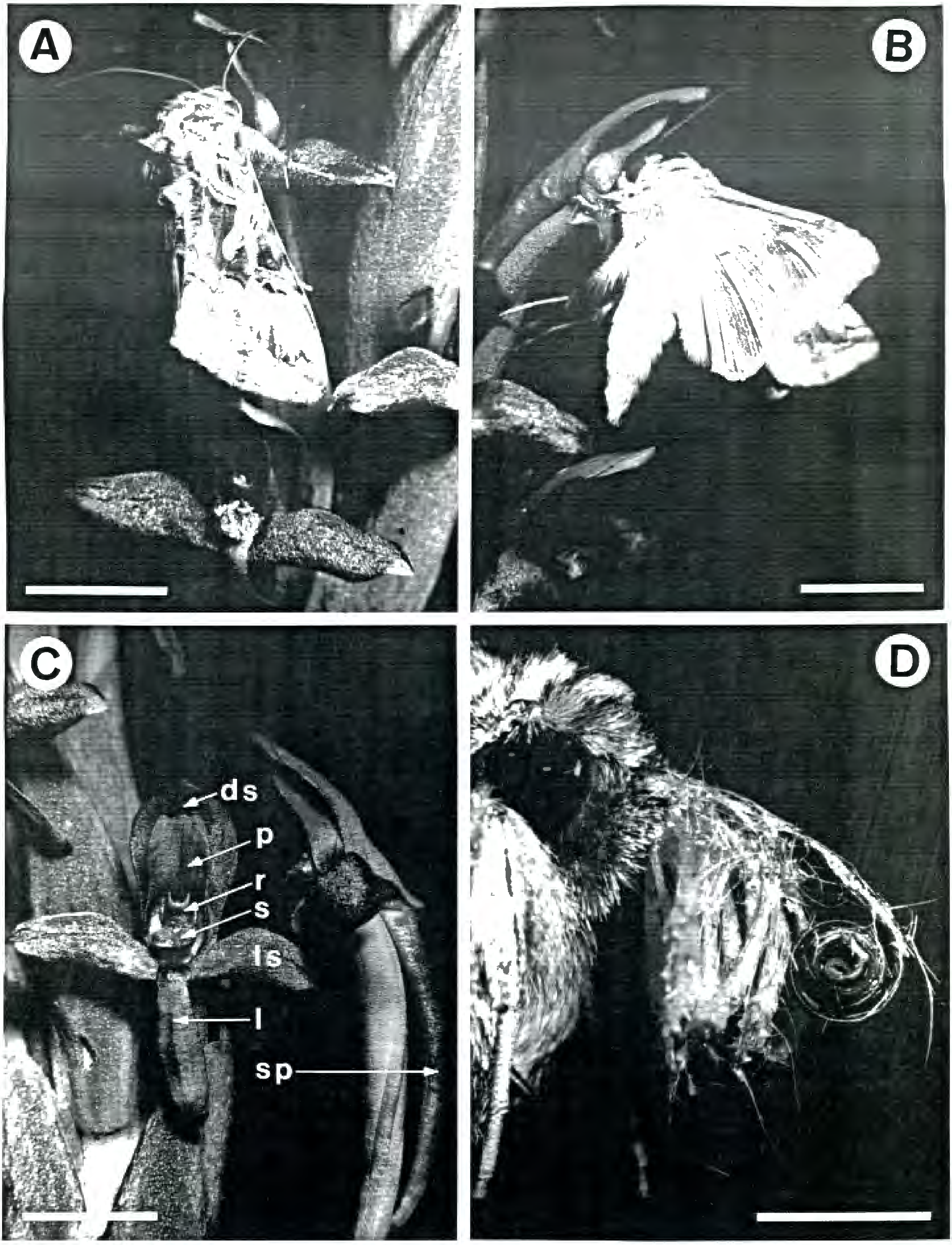
The flowers of *M. ophrydea* were found to emit a strong cinnamon-like scent towards dusk; only a slight scent was detectable during the day. I was unable to locate a specific source of the scent in the flowers of *M. ophrydea* by isolating the floral parts. It seemed as if the sepals and petals, which are fleshy, both produce scent, but a more detailed examination of the flowers would be necessary to confirm this.

Moths were active at the study site from about 1800 h and were frequent visitors to *M. ophrydea.* After landing, the moths crawled rapidly over the inflorescence, stroking the flowers with their antennae and probing open flowers with their proboscides (Fig 3.15A,B)). Moths usually placed their front legs on the lateral sepals for support while feeding (Fig 3.15A). To enter the spur the proboscis is guided by the petals into a groove along the dorsal sepal (Fig 3.15B,C). When the proboscis is fully inserted, the head of the moth comes to rest in a shallow chamber formed by the petals (Fig 3.15B) and the ventral surface of the proboscis contacts the viscidium. After withdrawal from the flowers, the pollinaria hang from the base of the proboscis and contact the stigma of the next flower that is visited.

The most common floral visitor was the noctuid moth *Syngrapha circumflexa* (Linnaeus). Four moths of this species were captured carrying 6, 2, 2 and 1 pairs of pollinaria respectively. The average proboscis length in these moths was 17.6 mm (S.D = 0.47). Another noctuid moth, *Cucullia minuta* Möschler, was caught carrying 10 pairs of pollinaria (Fig 3.15D). The proboscis length of this moth was 19.0 mm. Spur length of *M. ophrydea*, as measured from the rostellum to the tip of the spur, was 26.0 mm (S.D. = 2.1) in a sample of 10 flowers from the study site. It was noted that the nectar filled about half the spur in the evening, so that even moths with proboscides as short as 13 mm would be able to secure some of the nectar.

Flowers of *M. ophrydea* achieved high levels of pollination success at the study site: of the 179 flowers examined on 18 plants, 93% had been pollinated and 94% had pollinaria removed. Auto-pollination does not occur in *M. ophrydea* as the pendant anthers are separated from the stigma by a tall rostellum, unlike *M. bracteata* where the rostellum is reduced and does not prevent pollen from falling onto the stigma (Kurzweil & Johnson, 1993).

Figure 3.15. **A**, *Syngrapha circumflexa* (Noctuidae) visiting the flowers of *M.ophrydea*. Scale bar 10 mm. **B**, Lateral view of *S. circumflexa* with its proboscis fully inserted into the orchid spur and its head resting against the petals. Scale bar 10 mm. **C**, Front and lateral views of the flower of *M. ophrydea*. Abbreviations: ds - dorsal sepal; p - petal; r - rostellum (note the deep notch); s - stigma; ls - lateral sepal: l - lip; sp - spur. Scale bar 10 mm. **D**, Close up of the proboscis of *Cucullia minuta* (Noctuidae) carrying 10 pairs of pollinaria. Scale bar 2 mm.


A
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Discussion

The observations reported here indicate that *M. ophrydea* is adapted to pollination by medium-sized settling moths (Noctuidae, and possibly Geometridae), despite the dark floral coloration of the flowers. Moth pollination is also likely in the other eight *Monadenia* species which have elongate spurs of 10-25 mm long. I have recorded an evening scent in *M. atrorubens* (Schltr.) Rolfe (spur 15-30 mm), *M. comosa* Reichb. F. (spur 17-24 mm), *M. bolusiana* (Schltr.) Rolfe (spur 16-22 mm), *M. reticulata* (Bolus) Dur. & Schinz (spur 10-20 mm) and *M. cernua* (Thunb.) Dur. & Schinz (spur 11-17 mm).

Functional morphology - The rostellum structure in *Monadenia* is well suited to a moth pollination system. The single viscidium, seated in a notch on the rostellum, allows both pollinia to be attached to the narrow proboscis of a moth in a single visit. In *Disa* species, on the other hand, the two pollinia are attached to individual viscidia which are usually separated by the middle lobe of the rostellum. Interestingly, in species such as *Disa cooperi* Reichb. f. (Thunb). Sw. and *Disa draconis* (L.f.) Sw., in which pollinaria are attached to the narrow proboscides of hawkmoths and long-proboscid flies respectively (Johnson unpublished; Johnson & Johnson, 1993), there is a tendency for reduction of the middle rostellum lobe so that the two viscidia are closely adjacent and function as one adhesive unit. Many studies have demonstrated a close relationship between rostellum structure and pollination system in orchids. The taxonomic importance which has been accorded the rostellum in the Disinae (Linder & Kurzweil, 1990) probably reflects the role of adaptation to pollinators in the evolution of this group of orchids.

Floral morphology in the long-spurred species of *Monadenia* can be related to the moth pollination syndrome. The dorsal sepal and petals in these species form a shallow groove which guides the proboscis into the long pendant spur containing nectar. The narrow aperture between this groove and the rostellum ensures contact between the proboscis and the viscidium. Once the viscidium has been removed, the deep notch in the rostellum (Fig 3.15C) serves as a further physical guide for the proboscis. Moths frequently attempted to reach the nectar of *M. ophrydea* while in an upside-down position, but were prevented from doing so by the large dorsal sepal which overarches the entrance to the spur (Fig 3.15B). The overarching dorsal sepal thus forces moths to obtain nectar while in the upright position (Fig 3.15A,B), ensuring that pollinaria are consistently placed on the ventral surface of the proboscis, in the correct position to contact the stigma.

Crypsis - As noted by Linder (1981b), the flowering spikes of *M. ophrydea* are very inconspicuous among the charred and blackened branches of recently burnt fynbos vegetation. It seems likely that the dark coloration of the flowers of *M. ophrydea* and *M. atrorubens* is an adaptation to render the plants cryptic in the post-fire habitat. It was clear that *M.*

ophrydea is palatable as many of inflorescences at the study site had been chewed off, leaving only the base of the flowering stem. K. Steiner (pers. comm.) has shown that grazing by herbivores has a serious demographic effect on orchids, though orchids flowering directly after fire were least affected. Rebelo (1987b) found that most of the inflorescences of *Satyrium odorum* Sond. (Orchidaceae) at his study site were eaten by rock hyraxes, *Procavia capensis*. Since the entire reproductive effort of *M. ophrydea* is devoted to the first flowering season after fire, the need to protect inflorescences is obvious. The cost of crypsis in flowers, however, is reduced conspicuousness to pollinators and *M. ophrydea* relies on scent to attract moths such as Noctuidae which are capable of locating scented flowers without visual cues (cf. Brantjes, 1978).

Summary

Observations at a recently burnt site in the Western Cape Province, South Africa, showed that *Monadenia ophrydea* Lindl. is pollinated by settling moths. The pollinaria become attached to the ventral surface of the moth's proboscis when it is inserted into the nectar-containing spur. Moth pollination was not initially suspected in *M. ophrydea* as the flowers are dark beetroot-red, rather than white as found in classic moth-pollinated flowers. The dark-coloured inflorescences of those *Monadenia* species which flower solely after fire are cryptic among the charred and blackened branches of recently burnt vegetation, possibly to avoid detection by herbivores. The primary attractant to moths in *M. ophrydea* appears to be a strong cinnamon-like scent produced in the evening. Pollination occurred in over 90% of the flowers at the study site.

POLLINATION OF *HERSCHELIANTHE GRAMINIFOLIA* BY CARPENTER BEES[1]

The genus *Herschelianthe* (= *Herschelia* Lindl) is a member of the orchid tribe Diseae (Dressler, 1981), and is closely related to the large genus *Disa* (Linder, 1981c, 1986). The outstanding feature of *Herschelianthe* is the great variety in the shape of the lip, which in some species emits a strong fragrance (Vogel, 1963). The wide variation in floral morphology among the 16 species of *Herschelianthe* suggests a correspondingly wide diversity of pollinators, but there have been no reports of pollination in the genus. Marloth (1908, 1915) commented that in many years of observation he had only twice observed an insect on the flowers of *H. graminifolia*. One was a lycaenid butterfly and the other a small fly, but these appeared to be casual visitors, rather than pollinators, as neither insect carried or removed pollinaria. The chance discovery of a carpenter bee carrying pollinaria of *H. graminifolia* led to further investigations and observations which are reported here.

The study species

H. graminifolia is found throughout the Cape floral region. The orchid grows in sclerophyllous vegetation on mountain slopes. Populations are very sparse with individuals separated by hundreds of metres at most sites. The flowers have brilliant sky-blue sepals, a purple lip and greenish petals. The dorsal sepal is galeate, forming a shallow chamber (Fig 3.16A). A very short spur (c. 2-4 mm), which does not contain nectar, is situated at the base of the dorsal sepal, behind the column. A faint sweet scent is emitted from the flower. The inflorescence may bear up to 10 flowers, but three or four flowers per inflorescence is more typical.

Study site

Observations of *Herschelianthe graminifolia* were made during clear, sunny weather on Table Mountain (33°59'S 18°25'E) on 13, 17, 18, 19 February 1993 and at Silvermine Nature Reserve (34°07'S 18°24'E) on 23, 24, 28 February and 6 and 8 March 1993. In addition to bees caught on the flowers of *H. graminifolia*, bees foraging on other flowers were also caught if they carried pollinaria. The proportion of flowers which had pollen deposited on the stigma and the proportion of flowers which had pollinaria removed was determined at both sites. As the flowers wilt quickly after being pollinated (pers. obs.), wilted flowers were included in the sample so as to avoid underestimating pollination success.

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[1] Adapted from Johnson (1993a)

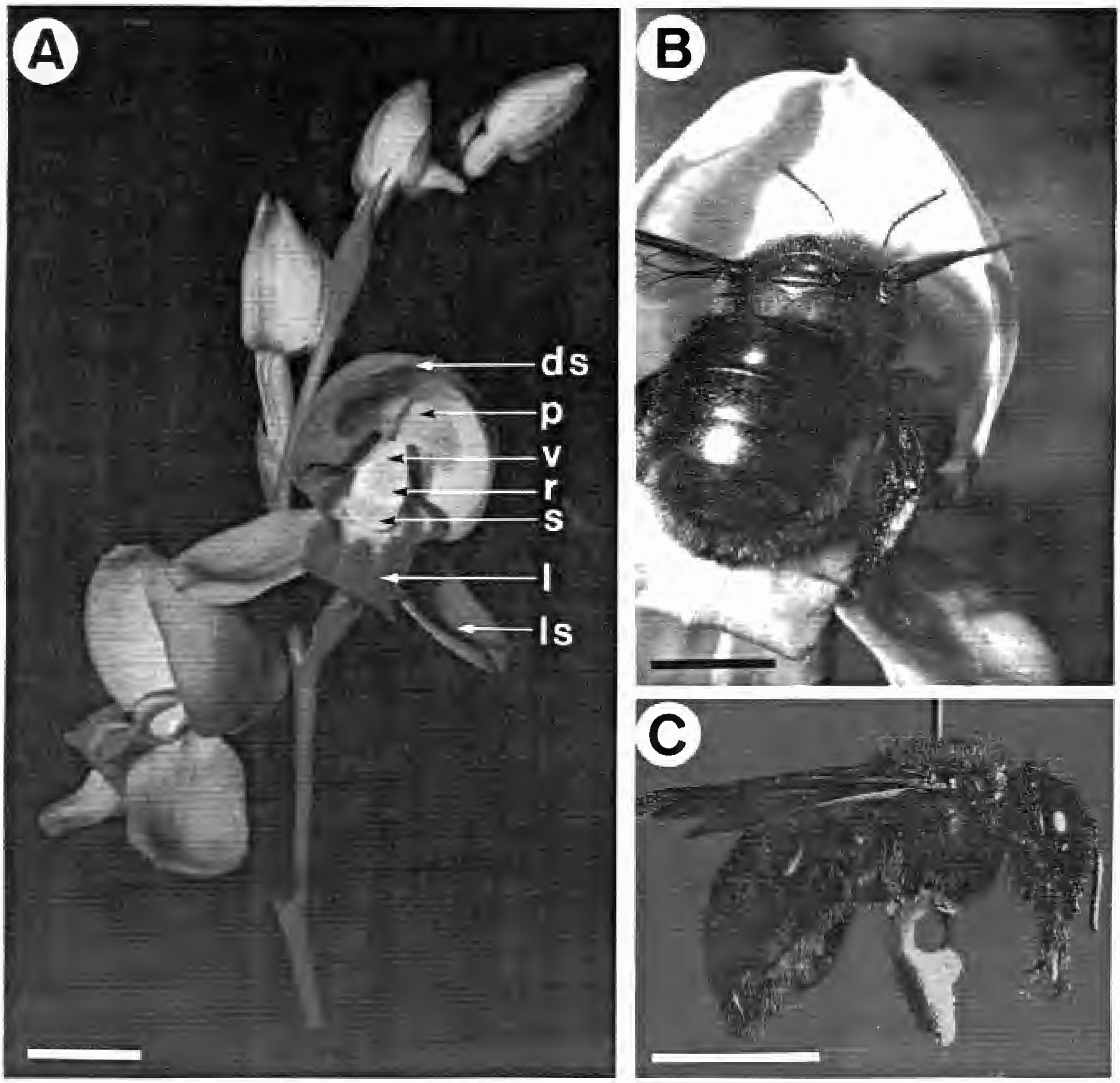


Figure 3.16. **A**, Inflorescence of *Herschelianthe graminifolia*. Abbreviations: **ds** - dorsal sepal; **p** - petal; **v** - viscidium; **r** - rostellum; s - stigma; **l** - lip; **ls** - lateral sepal. Scale bar 10 mm. **B**, The carpenter bee *Xylocopa rufitarsus* visiting a flower of *H. graminifolia*. Scale bar 5 mm. **C**, Close up of the bee *Xylocopa rufitarsus* showing two pollinaria attached to the ventral side of the thorax. Scale bar 5 mm.

Observations

Despite more than 30 hours of observation over nine days, only ca.15 visits to the flowers of *H. graminifolia* were observed. All the visits were by carpenter bees which removed pollinaria from the flowers (Fig 3.16B). The bees attempt to crawl into the flower, but their entrance is blocked by the robust column. As the bee presses against the column, the two pollinaria, which are joined by fused viscidia, become attached as a single unit to the ventral surface of the bee's thorax (Fig 3.16C). There is no nectar in the flower, consequently visits usually last fewer than four seconds.

Twelve carpenter bees carrying pollinaria of *H. graminifolia* were caught. Eleven of these bees (10 females and 1 male) were identified as *Xylocopa rufitarsus* Lepeletier (Anthophoridae). A single female *Xylocopa caffra* L. was found with pollinaria of *H. graminifolia*. Both these carpenter bees are common and widespread throughout the Cape floral region (Eardley, 1983).

The number of pollinaria carried by the bees varied between two and six ($\bar{x}$ = 3.16, S.D. = 1.38, n = 12). As the fused viscidia are so large (5 mm by 4 mm), all the available surface on the base of the thorax is used up by two or three pairs of pollinaria.

Pollination success - Although 58% of the flowers sampled on Table Mountain and 46% at Silvermine had pollinaria removed (indicating that they had been visited), only c. 31 % of the flowers at both sites had pollen deposited on the stigma (Table 7.1). The flowers are long-lived, however, and pollination success probably improved towards the end of the season as a sample of 28 flowers from six plants at Silvermine showed 63% fruiting success.

Discussion

Flowering in *H. graminifolia* takes place in late summer during the dry season, when very few other Cape plants are in flower (Johnson, 1993b). Food-source mimicry in *H. graminifolia* is probably facilitated by flowering at this time when long-lived carpenter bees have a shortage of food sources. During the spring carpenter bees feed mainly on mass-flowering leguminous shrubs (pers. obs.). But during this late-summer study, carpenter bees were forced to feed primarily on minute quantities of nectar found in the flowers of *Erepsia gracilis* (Haw.) L. Bolus (Mesembryanthemaceae).

No evidence was found that *H. graminifolia* mimics the flowers of other plants. Pollination through sexual deception can also be excluded as both male and female bees visit the orchid. Instead, the visits to *H. graminifolia* appeared to be opportunistic attempts at exploring new food-sources. Exploitation of the instinctive foraging behaviour of insects, particularly bees, appears to be a common pollination strategy in Orchidaceae (Ackerman, 1983, 1986; Johnson, 1992b; Johnson & Steiner, 1994a). This form of deception has been variously termed naivetè exploitation (Ackerman, 1986), mistake pollination (Dafni, 1984), and nonmodel mimicry (Dafni, 1986).

The pollination success data (Table 7.1) shows that a relatively high proportion of flowers had pollinaria removed, yet pollination rates were low. This indicates that many pollinaria are "wasted" when bees remove pollinaria without pollinating other flowers. It also indicates that many visits are by bees not already carrying pollinaria. There are three possible explanations for the pattern of high pollinaria removal and low pollination rates: (1) Bees may groom pollinaria off before visiting other flowers, (2) bees may visit flowers and remove pollinaria, but then avoid the orchid as it does not offer nectar and (3) bees may remove pollinaria and then not encounter another orchid.

It seems unlikely that the wastage of pollinaria is due to grooming as the viscidium of *H. graminifolia* adheres very strongly to the thorax of bees. Several unsuccessful attempts by carpenter bees to remove pollinaria with their legs were observed at the study sites.

The second explanation, that bees quickly learn to avoid the unrewarding flowers of *H. graminifolia*, is supported by the low numbers of pollinaria carried by the bees. But as explained above, there is limited space on the thorax for only two or three pairs of pollinaria, thus physical factors rather than avoidance behaviour by the bees could explain the low numbers of pollinaria carried.

The most likely explanation for pollinaria wastage in *H. graminifolia* is the sparse population structure and lack of constancy by the bees. As the plants are widely spaced, some bees probably remove pollinaria and then do not encounter another flower of the same species, resulting in wastage of pollinaria. Nilsson *et al* (1986) described similar pollinaria wastage in *Cymbidiella flabellata* (Orchidaceae) which is pollinated by wasps with a low flower constancy (see also Dafni & Calder, 1987).

Although pollinaria wastage in *H. graminifolia* may occur because the plants are widely spaced, it is probably true that even more pollen wastage would occur if the pollen were loose as in most angiosperms. Pollinaria, which remain attached to insects for long periods of time, facilitate cross pollination between widely spaced plants, even if the pollinator does not show flower-constancy.

Summary

Field observations at two sites on the Cape Peninsula, South Africa, showed that carpenter bees (*Xylocopa*: Anthophoridae) pollinate the orchid *Herschelianthe graminifolia* (Spreng)Dur. & Schinz. The orchid does not produce nectar and appears to function as a food-source mimic. Opportunistic male and female bees in search of new nectar-sources are attracted to the bright blue flowers. While attempting to enter the flower, the bee pushes against the column and the large pollinaria are attached to the ventral surface of the thorax. Pollinaria removal rates from flowers exceeded pollination rates at two sites, indicating that many pollinaria do not reach the stigmas of other flowers. This can be ascribed to the large distances between plants.

INCOMPLETE CASE HISTORIES

Below I give a brief account of case histories I regard as incomplete, meaning that the observational data is not yet sufficient to reach confident conclusions about the pollination system of each species.

Disa caulescens

This orchid is common along streambanks in the western Cape where it flowers in midsummer. The flowers are very small (c. 10 mm in diameter) and have a striking banded pattern on the petals. At both Du Toits Kloof and Riviersonderend (Fig 3.2) I have observed pollination by small flies. Pollinaria of *D. caulescens* were attached to the underside of the thorax of 5 individual blow flies (Muscidae) and one bee fly (Bombyllidae).

Disa pillansii

This orchid has small pink flowers c. 10 mm in diameter which do not contain nectar. I have made scattered observations of this species in the mountains above Betty's Bay (c. 2 hrs) and also at Pilaarkop (c. 3 hrs). At Pilaarkop (Fig 3.2), I observed visits by small halictid bees which crawled into the chamber formed by the dorsal sepal. Three bees were captured, each of which carried several pollinaria of *D. pillansii* attached to the underside of the thorax.

Disa fasciata

Disa fasciata is one of the most fascinating Cape orchids on account of its apparent mimicry of the flowers of *Adenandra* species (Rutaceae). The orchid is non-rewarding, while *Adenandra* species produce viscous nectar (unpublished data). The similarity between these species has often been cited as an example of mimicry (Dafni, 1986), but there have been no observations of pollination of the species. I have carried out more than 30 hours of observations (over a total of 10 days) of populations of *D. fasciata* at Constantiaberg in the Silvermine Nature Reserve during 1990-1991, and Pilaarkop, near Riviersonderend during 1994. Flowering in *D. fasciata* occurs only in the first two seasons following fire. I have confirmed Marloth's (1908) observation that *Adenandra* species are pollinated by long-proboscid flies; the fly *Prosoeca westermanii* (Nemestrinidae) was a regular visitor to *Adenandra* at both Constantiaberg and Pilaarkop. On two occasions (both at Pilaarkop) I have also seen this fly visit several flowers of *D. fasciata*, though I was unable to capture the flies. The pollination of *D. fasciata* has thus proven to be one of the most elusive interactions in this study, but the preliminary observation suggest that the hypothesis that this orchid is mimic of *Adenandra* is worth examining further.

Disa sagitallis

Several visits to this orchid by hoverflies (Syrphidae) were seen during a brief (c 2 hrs) observation period at a population on the Montagu Pass (Fig 3.1E). One of the hoverflies was captured and found to have pollinaria of *D. sagitallis* attached to the underside of its mouthparts.

Disa rhodantha

Disa rhodantha has long-spurred pink flowers which contain nectar. An anthophorid bee has been captured with pollinaria of this orchid in the Natal Drakensberg (Stewart & Manning, 1982), but the bee had mouthparts which were much shorter than the spur of the orchid. I studied *D. rhodantha* in a marsh at the Verloren Valei Nature Reserve, near Dullstroom (Fig 3.2) on 1-2 and 19-21 January 1994. The flowers were regularly visited by long-proboscid flies (unidentified *Prosoeca* spp. - Nemestrinidae) (Fig 3.1F). Eight flies with pollinaria of *D. rhodantha* attached to the underside of the head were captured. As in the bees observed in the Drakensberg (Stewart and Manning, 1982), the proboscis of the flies was only about half as long as the orchid spur. I suspect that larger long-proboscid flies may be the primary pollinators of this orchid.

4 The phylogeny of *Disa* and adaptive radiation for pollination

Adaptive radiation for different pollinators was studied in *Disa*, a large orchid genus. A phylogeny of 27 species was constructed using 46 morphological characters. Known pollination systems were mapped onto the phylogeny in order to analyze pathways of floral evolution. It was found that shifts from one pollination system to another were a major feature of the evolutionary diversification of *Disa*. Unlike many plant genera which are pollinated mainly by a single group of insects eg. bees, radiation in *Disa* has encompassed nearly all the major groups of pollinating insects; in all, 19 different specialised pollination systems were found in the 27 species which were studied. Another striking pattern is the repeated evolution of broadly similar pollination systems in unrelated clades. For example, large red flowers pollinated by the butterfly *Meneris tulbaghia* have evolved twice, showy deceptive flowers pollinated by carpenter bees twice, long-spurred flowers pollinated by long-tongued flies four times, night-scented flowers pollinated by moths three times and auto-pollination three times. This suggests that a few dominant pollinator species in a region may be sufficient to generate diversification in plants through repeated floral shifts which never retrace the same pathways.

INTRODUCTION

Evolutionary biologists often ask how many times a particular trait evolved in their study group and which pathways were followed. To address these questions it is essential to have an explicit phylogenetic hypothesis for the study group. Mapping of ecological traits onto phylogenies has become one of the most popular and successful methods of studying pathways of adaptive evolution (Wanntorp, 1983; Midgley, 1988; Sillén-Tullberg, 1988; Coddington, 1988; Donoghue, 1989; Funk & Brooks, 1990; Mickevitch & Weller, 1990; Linder, 1991a; Wanntorp *et al*, 1990; Bremer & Eriksson, 1992; Manning & Linder, 1992; Armbruster, 1992, 1993; McDade, 1992; Crisp, 1994; Steiner, 1994). This method is particularly suited to plant-pollinator systems, because there are few insights into the origin of these relationships from the fossil record (Armbruster, 1992).

Cladistics has developed into the method of choice for phylogeny estimation. Refinement of cladistic methods and appropriate software has made the estimation of phylogeny possible for most well studied taxa. Provided its limitations are properly understood, cladistic methodology is the single most powerful tool available for inferring the evolutionary history of ecological traits.

The main contribution of cladistics to the study of adaptive radiation has been to clarify (1) the monophyly of lineages and (2) the patterns and sequences of changes in traits within lineages. Cladistics *per se* is not informative about the processes which generate radiation (Wanntorp *et al*, 1990). If we hypothesise that adaptive evolution has driven radiation in floral morphology in a group, it needs to be demonstrated through time-consuming observation and experimentation that pollinators select among floral characters. Cladistics has another use to the evolutionary biologist, though, and that is to identify derived traits - which reflect adaptation to relatively recent conditions - for special study (Wanntorp, 1983; Coddington, 1988).

The seminal study of adaptive floral radiation in plants was the Grants' (1965) study of pollination in the Polemoniaceae (phlox family). In this work they gave extensive examples of floral shifts from one method of pollination to another (summarized by Stebbins, 1974). The importance of their study was the realization that pollinator shifts had resulted in floral radiation in the Polemoniaceae, but many of their interpretations were hampered by the lack of a phylogeny based on modern cladistic methods.

There have been remarkably few other studies of adaptive radiation to different pollinators within genera or families (Dodson 1962b; Raven, 1979; Armbruster, 1992, 1993; Manning & Linder, 1992). This is probably not due to the paucity of examples of adaptive radiation, but rather the tendency of pollination biologists to study single species in detail, without a comparative perspective. In southern Africa, there are numerous examples of genera which have radiated within a limited region eg. the 600 Cape species of *Erica* which are characterised by enormous diversity in floral form (Rebelo & Siegfried, 1985). These genera arouse a natural curiosity as to the role of adaptive evolution in generating such diversity. In this chapter I focus on *Disa*, a large orchid genus which has speciated extensively in the Cape region where about 120 species occur (an estimate which includes *Herschelianthe* and *Monadenia*, see below). While the systematics of *Disa* has received considerable attention, almost nothing was known about pollination in the genus until recently when we began intensive field studies of the pollination of these spectacular orchids (Johnson, 1992b, 1993a, 1994abc; Johnson & Steiner, 1994abc; Johnson *et al*, 1994; Steiner *et al*, 1994).

The aim of this study was to show adaptive radiation to different pollinators in *Disa*. The two important questions asked are (1) What are the pathways of evolution in pollination systems in *Disa*, eg. how many times did various pollination systems evolve, and (2) Does history play an important role in determining the pollination biology of a species, or are pollination systems evolutionarily labile (ie easily shifted in the face of selection)?

METHODS

In order to interpret the history of pollination systems in *Disa*, a phylogeny was derived by cladistic analysis of those species for which pollinators are known (see previous chapter). The minor genera, *Herschelianthe* and *Monadenia*, were included in the analysis because all the available evidence indicates that these are embedded in *Disa* and thus form part of the same monophyletic lineage (Linder, 1986; Linder & Kurzweil, 1990, 1994). The taxonomy of *Disa* will be changed to include some of the minor genera (HP Linder, pers. comm.), but in the meantime I have used the current nomenclature throughout this chapter. *Satyrium* was used as an outgroup to root the cladogram, on the basis of evidence that this genus may be a sister taxon of the Disinae (which includes *Disa* and the minor genera) (Linder & Kurzweil, 1994; H Kurzweil pers. comm.). *Schizodium*, *Brownleea* and *Corycium* are also possible candidates for outgroups, but were rejected either because they may be embedded in *Disa* (*Schizodium*), may be of hybrid origin (*Brownleea*) or may be more distantly related to *Disa* than *Satyrium* (*Corycium*) (see Linder & Kurzweil, 1994).

A total of 10 vegetative and 36 inflorescence and floral characters for each species were used in the cladistic analysis (Table 4.1, 4.2). Some of these characters have been used in previous studies (Linder, 1981abc, 1986; Linder & Kurzweil 1990, 1994), while others were found through additional morphological studies. The outgroup was included as a root and the principle of global parsimony was used which makes no *a priori* assumptions about the primitive or derived state of any one character (cf. Maddison & Maddison, 1992). The data set was analyzed using the ie* routine in Hennig86 (Farris, 1988) which finds the complete set of shortest (most parsimonious) trees. To help choose among several equal length trees, a successive weighting procedure ("xsteps w" in Hennig86) was applied which locates trees which are best supported by characters with a high consistency index (Carpenter, 1988). A strict consensus tree, which reveals areas of agreement between the fundamental trees, was calculated using the "nelsen" routine in Hennig86. The data were also analyzed using the bootstrap procedure in Paup vs 3.1 to determine how many of the nodes in the cladogram are retrieved when characters are randomly selected, with replacement, from the data set (Felsenstein, 1985). Although it is not considered to be a statistical test, the bootstrap does provide a measure of support for each node in the cladogram (Sanderson, 1989; Linder, 1991c).

Table 4.1. Characters used in the cladistic analysis. Multistate characters which were coded as additive are indicated by an asterisk.

1. Stolons: absent (0); present (1).
2. Tubers: monostelic (0); polystelic (1).
3. Sterile vegetative shoots: absent (0); present (1).
4. Plants: glabrous (0); hairy (1).
5. Leaves: radical (0); basal (1); cauline (2).
6. Leaves: firm (0); soft (1).
7. Leaf mesophyll: homogenous (0); heterogenous (1).
8. Leaves: hysteranthous (0); synanthous (1).
9. Leaf vascular bundles: no sclerenchyma (0); sclerenchyma caps (1).
10. Leaves: linear (0); expanded (1).
11. Inflorescence: racemose (0); corymbose (1).
12. Bracts: green (0); dry (1).
13. Flowers: resupinated (0); not resupinated (1).
14. Flowers: facing down (0); facing straight (1).
15. Flowers: small (0); large (1).
16. Lateral sepals: keeled (0); not keeled (1).
17. Lateral sepal orientation: straight (0); reflexed (1); forward (2).
18. Lateral sepal basal lobe: absent (0); present (1).
19. Dorsal sepal: galeate (0); flat (1).
20. Dorsal sepal: flat or galeate (0); navicular (1).
21. Dorsal sepal galea: sessile (0); shortly pedicellate (1).
22. Dorsal sepal: rounded (0); obtuse to acute (1).
23. Spur: present (0); absent (1).
24. Spur length to dorsal sepal: shorter (0); longer (1); twice as long (2)*.
25. Spur entrance: round (0); laterally compressed (1).
26. Spur: distinct from galea (0); continuous with galea (1).
27. Nectar: present (0); absent (1).
28. Spur apex: slender (0); clavate (1).
29. Petal chamber: absent (0); elongate (1); square (2).
30. Petals: ovate (0); elongate (1).
31. Petals: straight (0); twisted facing forward (1).
32. Petal apex: lobed (0); simple (1).
33. Petals: straight or bilobed (0); serrated (1).
34. Petal anticous lobe: absent or small (0); large (1).
35. Petal barring: absent (0); present (1).
36. Petal front edge: thin (0); thickened (1).
37. Lip: linear (0); lorate (1); ovate (2)*.
38. Lip at base: orthotropous (0); deflexed (1).
39. Stigma: pedicellate (0); subpedicellate (1).
40. Rostellum: three-lobed (0); one-lobed (1).
41. Rostellum lobes horn-like (0) square (1).
42. Rostellum lobes: diverging (0); parallel (1); converging (2)*.
43. Caudicles: short (0); long (1).
44. Anthers: globose (0); elongate (1).
45. Pollen: hamulate (0); other (1).
46. Seeds: small (0); large (1).

TABLE 4.2. Distribution of the characters among the taxa. Missing values are coded as "?" and inapplicable characters as "-". The pollination system for each taxon is coded in the right margin, but was not used to generate the cladogram. Pollinator codes: LTF - long-tongued fly; STF - short-tongued fly; AUT - auto-pollination; BUT - butterfly; BEE - bee; MOT - moth; HMO - hawkmoth; WAS - wasp.

	5	10	15	20	25	30	35	40	45		Pollinator
	|	|	|	|	|	|	|	|	|		
Satyrium	01?02	00101	00110	00010	001-?	-0000	01000	0?000	??001	0	?
draconis	00001	0?0-1	01011	00010	00020	01001	01000	01100	12000	0	LTF
harveiana	00001	0?0-1	01011	00010	00020	01001	01000	01100	12000	0	LTF
sagittalis	00001	01001	01010	00010	00000	01001	01000	01100	1?000	0	STF
glandulosa	0?012	01101	00010	00000	00000	0?000	01000	01100	11000	0	AUT
vaginata	0?012	0?101	00010	00000	00000	0?000	01000	01100	11000	0	AUT
ferruginea	01000	11010	01010	00000	00020	11000	00000	02100	12000	0	BUT
porrecta	0?000	1?010	01010	00000	00020	11000	00000	02100	12000	0	BUT
oreophila	0?001	10110	01010	02000	00010	01000	00000	01100	11000	0	LTF
H. graminifolia	?0001	?0100	10110	00000	01000	10000	00000	21000	20000	0	BEE
M. bracteata	00002	00101	00010	01000	00010	00100	01000	01101	1-000	0	AUT
M. ophrydea	00002	00101	00010	01000	00010	00100	01000	01101	1-000	0	MOT
salteri	01000	00000	01010	00000	00020	00000	01000	01100	1?000	0	MOT
cooperi	00102	00101	00000	01000	00020	00100	11000	01100	12000	0	HMO
rhodantha	0?102	0?101	00000	01000	00020	00100	11000	01100	01000	0	LTF
versicolor	00102	0?101	00000	0?000	00010	00100	11000	01100	11000	0	BEE
obtusa	00002	00100	00010	00000	00000	01000	01000	01100	01000	0	STF
caulescens	1?002	0?101	00010	00000	00001	01000	01001	10110	01000	1	STF
filicornis	0?001	01100	00010	00110	11101	01010	01001	10110	00110	0	BEE
tenuifolia	00001	0?100	00010	00110	11101	01010	01000	10110	00110	0	BEE
atricapila	0?001	00101	10110	10111	01101	01010	01100	10110	00010	0	WAS
bivalvata	00001	00101	10110	10111	01101	01010	01100	10110	00010	0	WAS
uniflora	1?002	00101	00011	00100	01001	00110	01001	10110	00010	1	BUT
racemosa	0?002	0?101	00011	00100	01?01	01020	01001	10110	00010	0	BEE
venosa	0?002	0?101	00011	00100	01?01	01020	01001	10110	00010	0	BEE
fasciata	0?002	0?101	10110	00010	00001	01000	01011	02100	0?000	0	LTF
rosea	00002	01101	10110	00000	00101	01000	01010	02110	01000	0	AUT
pillansii	0?001	01101	10110	02000	00101	01000	01001	02110	0?000	0	BEE

A potential problem with using floral characters to generate a phylogeny for interpreting the evolution of pollination systems is that if whole suites of characters are closely coupled to the pollination systems, then the application of the principle of minimum evolutionary change (parsimony) will also result in a conservative estimate of change in pollination systems (Armbruster, 1992). To test whether the pollination systems mapped onto the cladogram were independent of the characters used to generate the cladogram, I used Armbruster's (1992) method of comparing the average consistency index for the cladogram characters with the consistency index of the pollination systems (coded 1-8 as a multistate character). If the consistency index for pollination is far lower (ie shows more homoplasy) than the cladogram characters, then it can be assumed that the pollination system is more labile, and thus reasonably independent of the characters used to generate the tree.

RESULTS

Cladistic analysis

Parsimony analysis of the data set yielded four equal length trees (length 95, consistency index 54, retention index 80), of which one was selected by the successive weighting procedure (Fig 4.1). This cladogram was used as a working phylogeny for the study group. Most of the disagreement among the four fundamental trees was located in the positions of the basal nodes, as evidenced firstly by the low bootstrap values for these nodes (Fig 4.2), and secondly by the topology of the strict consensus tree which preserved nearly all the structure within the major clades, but collapsed at the basal nodes (Fig 4.2). In general, the cladogram shows far more resolution (ie fewer polychotomies) than any previous attempts at an infrageneric cladistic analysis of *Disa* (Linder, 1986; Linder & Kurzweil, 1990). This is probably because species were used as terminal taxa, unlike previous analyses which were based on sections with many variable characters (Linder & Kurzweil, 1990).

The evolutionary lability of pollination systems in *Disa* is reflected in a very low consistency index (23) for this "character" compared with values of 20-100 (average 73) for the morphological characters used to generate the cladogram.

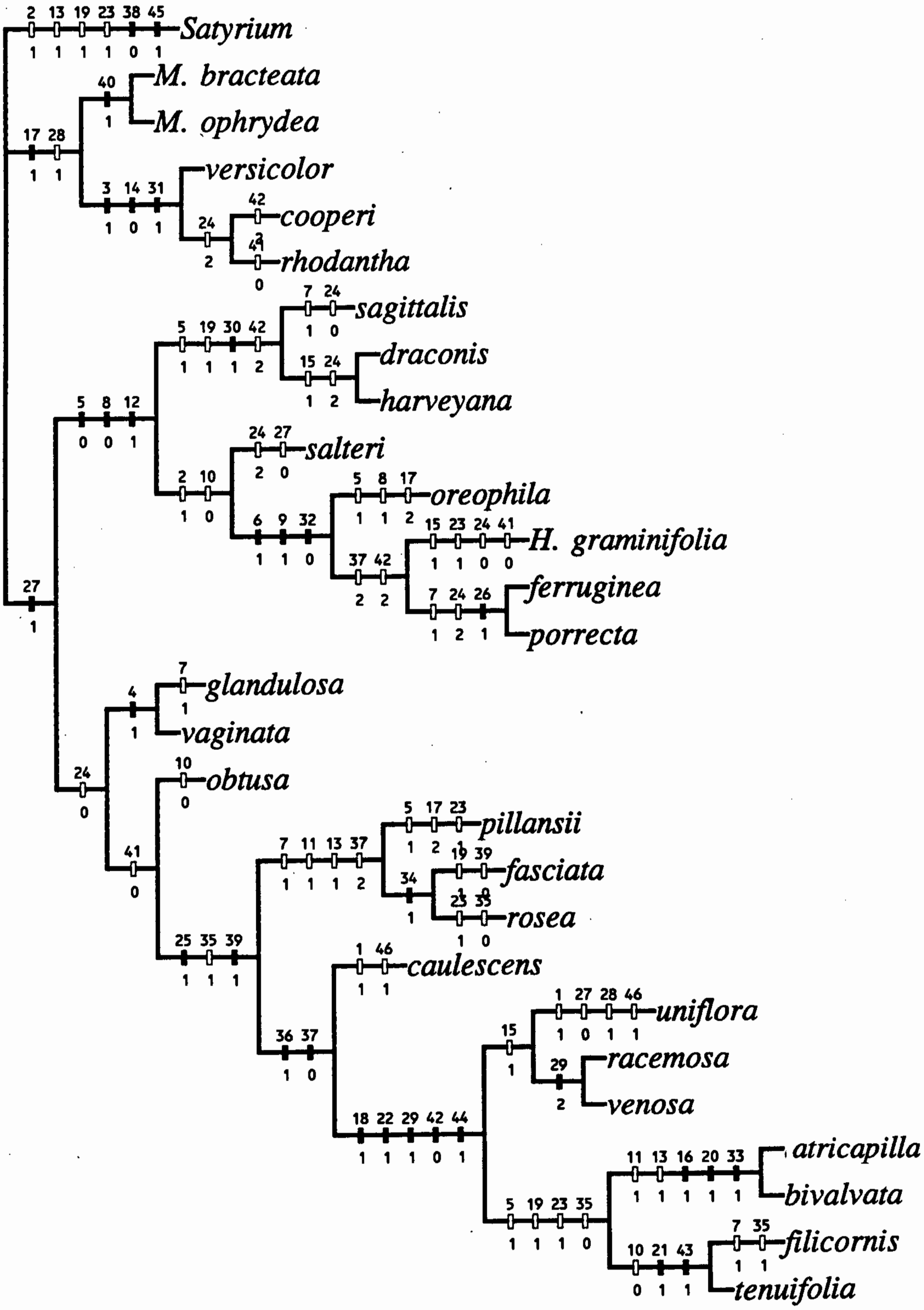


Figure 4.1. Cladogram of *Disa* species. This tree was selected by a succesive weighting procedure from the four equally parsimonious trees obtained by analysis of the data set. Solid bars represent apomorphies which evolved only once, while hollow bars represent characters which are homoplasious, ie appear to have evolved more than once. The number above each bar refers to the character in Table 4.1, while the number below the bar refers to the character state.

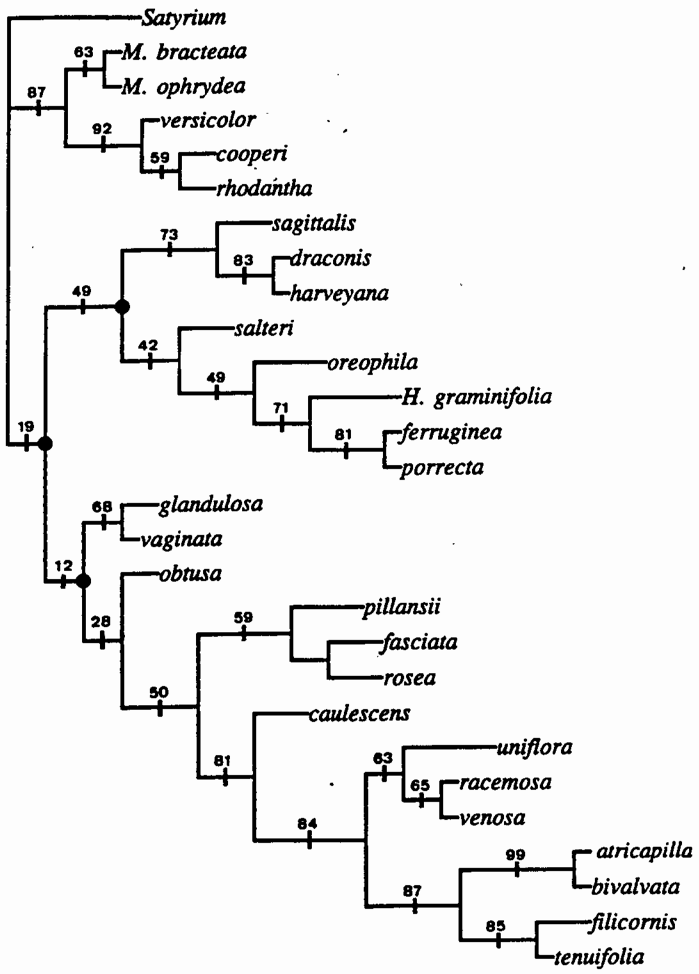


Figure 4.2. Topology of the tree in Fig 4.1, but without the characters. The number at each node refers to the bootstrap value, a measure of support for the node (see text) and the solid dots indicate nodes which collapsed on the strict consensus tree. Note that nodes which collapsed usually have bootstrap values of less than 50.

Trends in floral evolution

Before turning to consider the evolution of pollination systems, it is useful to summarize the major trends in floral evolution in *Disa* (Fig 4.3).

Dorsal sepal and spur - The most important diagnostic feature for the Disinae as a whole is the modification of the dorsal sepal to form a posterior spur. The spur appears to have been lost at least twice in *Disa*; once in "*Herschelianthe*" (clade 2) and once in a group of species in clade 4 (Fig 4.3). Nectar production in *Disa* occurs basally in clade 1 (consisting of "*Monadenia*" and the species belonging to *Disa* sect. Hircicornes). The absence of nectar is a synapomorphy for clades 2-5, but a reversal to nectar production occurs in *D. salteri* (clade 2) and *D. uniflora* (clade 4). Many of the long-spurred species do not produce nectar, and rely on various deceptive mechanisms to achieve pollination (see Chapters 2 & 3).

Petals (excluding the lip) - The petals of *Disa* are complex and play a very important mechanical role in pollination. In most of the species, the petals physically guide the insect into the correct position to receive and deposit pollinaria. In some of the long-spurred species such as *D. cooperi* and *D. oreophila*, the petals are modified to block the entrance of the galea, leaving only a tiny entrance to the spur (Fig 3.9, 3.11A). This results in a funnel-like arrangement which forces the insect proboscis to contact the column at the entrance to the flower so that pollinaria are attached firmly to the proboscis. In *D. draconis* the petals are overlapping and curled to form a channel leading into the spur. This channel guides the extraordinarily long proboscis of its pollinator into the flower (Chapter 5). A similar channel-like arrangement is found in *Monadenia ophrydea*, where it guides the proboscis of settling moths into the spur. In the species which lack a spur, the petals often form a secondary chamber (Fig 3.6C). These species are typically pollinated by bees or wasps which grasp the petal chamber and insert the head into the secondary petal chamber eg. *D. racemosa*, *D. venosa*, *D. filicornis*, *D. tenuifolia*, *D. atricapilla* and *D. bivalvata*. The petal is usually thickened in these species, particularly where it is grasped by the insect.

Lip (labellum) - In most *Disa* species, the lip is a simple linear structure, often reduced to a filament which probably serves no function at all. Interesting exceptions are the evolution of complex, scent-producing lips in two different clades within *Disa* (Fig 4.3): an autapomorphic spathulate scent-producing lip occurs in the hawkmoth-pollinated *D. cooperi* (clade 1) and complex dissected (fringed) lips evolved independently in *Herschelianthe* (clade 2).

Column - The basic *Disa* column is compressed and consists of two pollinaria located in a horizontal anther cell, two viscidia cradled in lateral rostellum arms and an anterior stigma. Column morphology is critical to the successful deposition of orchid pollinaria onto insects. It is not surprising, therefore, that this organ has been highly modified in some *Disa* species. A single viscidium, instead of two, has evolved at least twice within the genus (Fig 4.3): *Monadenia* (clade 1) has a single viscidium derived from an unlobed rostellum and *Herschelianthe* (clade 2) has a massive single viscidium derived from fusion of two viscidia from the lateral rostellum lobes (Linder & Kurzweil, 1990).

Pollinaria have to be placed where they will adhere properly. For instance, long hornlike rostellum lobes have evolved in clade 4 where they function to place pollinaria on the legs of insects. In other clades, closely adjacent rostellum lobes function to place pollinaria on the narrow mouthparts of insects (Fig 3.9, 3.11).In at least three Cape *Disa* species, the rostellum lobes are highly reduced, facilitating auto-pollination by flipping of the pollinaria onto the stigma (Fig 3.8). This modification has occurred independently in *D. glandulosa* - *D. vaginata* (clade 5) and *D. rosea* (clade 4).

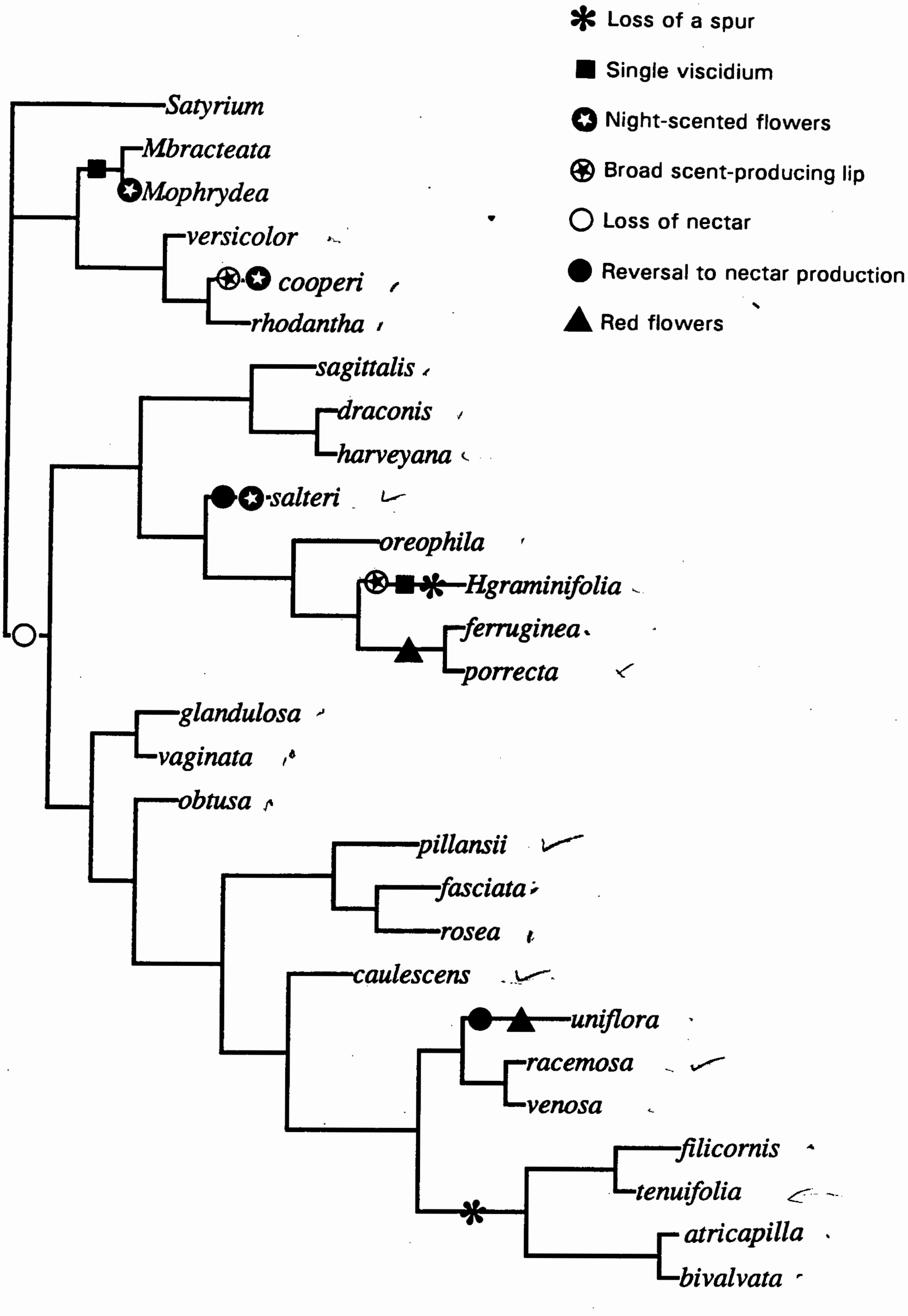


Figure 4.3. Major reversals and convergences in the evolution of floral traits in *Disa*. The major clades are numbered 1-5 to facilitate reference in the text.

Mapping the natural history data from the previous chapter onto the cladogram clearly revealed the extent of radiation in pollination systems in *Disa* (Fig 4.4). Each of the major clades (labelled 1-5 for clarity) in Fig 4.4, are characterised by major shifts in pollination systems. I have used dashed lines for the basal portion of the phylogeny in Figure 4.4 to indicate that the relationships between clades 1-5 are relatively weakly supported; however, the basal topology of the cladogram does not affect the conclusions which are made below.

A striking pattern is the independent evolution of similar pollination systems in different clades.

Large red flowers pollinated by the Mountain Pride butterfly have evolved twice in *Disa* (Fig 4.3, 4.4) The sister species *D. ferruginea* and *D. porrecta* probably evolved from long-spurred ancestors which were preadapted to butterfly pollination. But remarkably, the large butterfly-pollinated flowers of *D. uniflora* appear to have evolved in a clade of small-flowered species which mostly lack spurs (Fig 4.4).

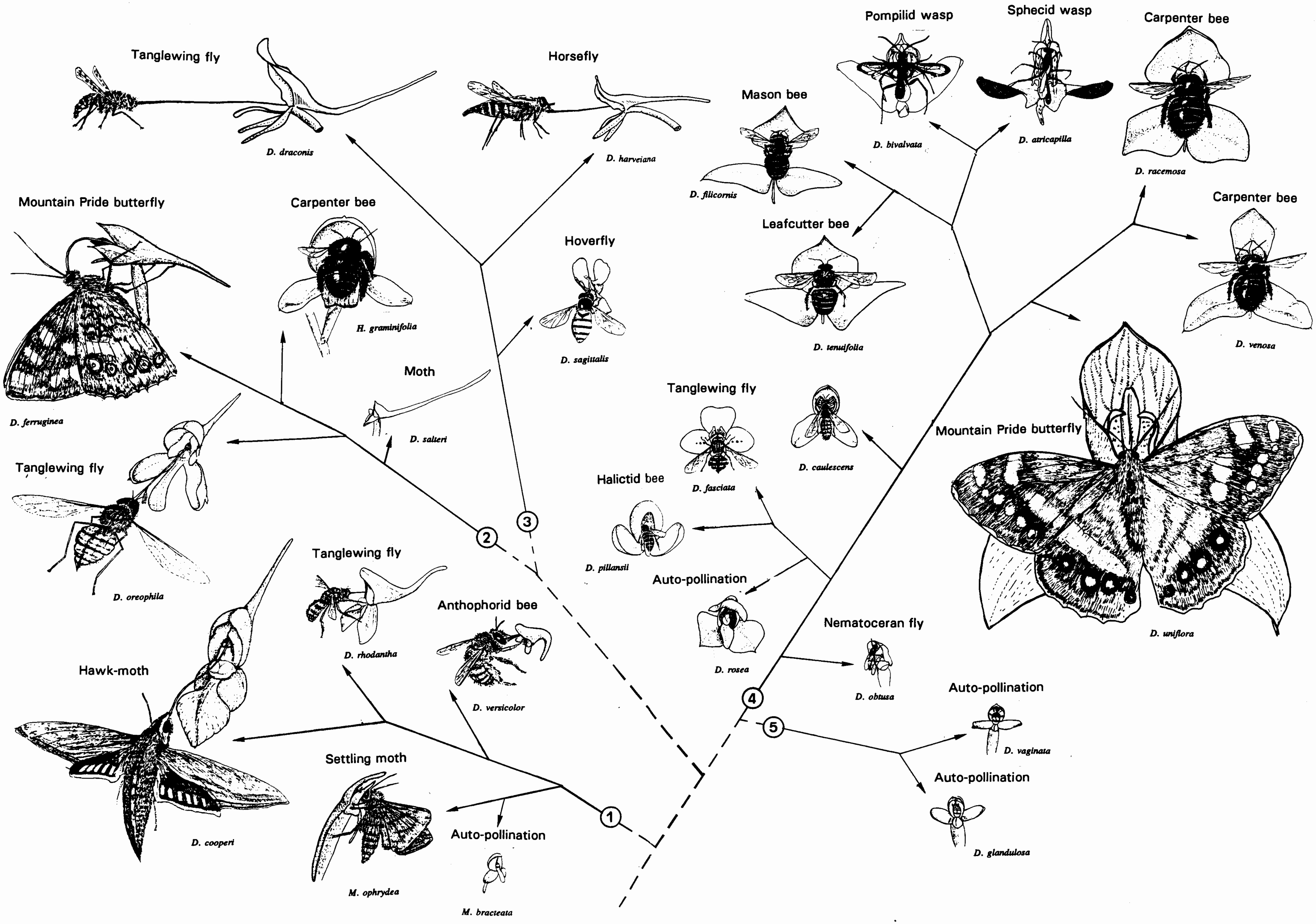
Large brightly coloured flowers pollinated by carpenter bees evolved independently in *H. graminifolia* (clade 2) and *D. racemosa* - *D. venosa* (clade 4). In both instances, the flowers lack nectar and rely on food deception mimicry to attract bees (Chapter 3). Pollination by smaller anthophorid bees, megachilid bees and halictid bees also occur in *Disa* (Fig 4.4). Bee pollination in the broad sense has evolved at least five times in the genus. Wasp pollination has a singe origin in the *D. bivalvata* - *D. atricapilla* species pair. The highly unusual flowers of these species are sexually deceptive to pompilid and sphecid wasps respectively (Steiner *et al*, 1994; Appendix B).

Long-proboscid fly pollination is an extraordinary phenomenon which is fairly widespread among Cape plants. In *Disa*, these flies pollinate *D. rhodantha* (clade 1), *D. oreophila* (clade 2) *D. draconis* - *D. harveiana* (clade 3) and *D. fasciata* (clade 4). Thus long-proboscid fly pollination has evolved at least four times. The divergence in spur length between *D. draconis* and *D. harveiana* appears to be directly attributable to adaptation to flies with differing tongue lengths (Chapter 5). Pollination by short-tongued flies occurs in *D. sagittalis* (clade 2 3), *D. obtusa* (clade 4) and *D. caulescens* (clade 4).

Night-scented flowers pollinated by moths have at least three independent origins (Fig 4.3, 4.4): *D. cooperi* (clade 1), *M. ophrydea* (clade 1) and *D. salteri* (clade 2).

Finally, methods of pollination which do not require pollinators have evolved several times in *Disa*. Auto-pollination through pollinia flipping onto the stigma occurs in *D. vaginata* - *D. glandulosa* (clade 5) and *D. rosea* (clade 4). A different method of auto-pollination in which the pollinaria break up and fall onto the stigma has evolved in *M. bracteata* (clade 1). Because self-pollinating flowers are often highly reduced, their phylogenetic affinity is notoriously difficult to determine (Wyatt, 1988). This is evident in the present analysis: clade 5, consisting of two auto-pollinating species, does not strongly group with any of the other clades, largely because of the lack of specialized features in their flowers.

Figure 4.4. Phylogeny of pollination systems in *Disa* species. The phylogeny is based on the cladogram in Fig 4.1. Numbers for each clade are the same as the previous figures. Dashed lines indicate that the basal branching patterns of the phylogeny were weakly supported by the cladistic analysis.


Tanglewing fly
D. draconis
Horsefly
D. harveiana
Mason bee
D. filicornis
Pompilid wasp
D. bivalvata
Sphecid wasp
D. atricapilla
Carpenter bee
D. racemosa
Carpenter bee
D. venosa
Leafcutter bee
D. tenuifolia
Mountain Pride butterfly
D. ferruginea
Carpenter bee
H. graminifolia
Hoverfly
D. sagittalis
Moth
D. salteri
Tanglewing fly
D. oreophila
Tanglewing fly
D. fasciata
D. caulescens
Halictid bee
D. pillansii
Auto-pollination
D. rosea
Mountain Pride butterfly
D. uniflora
Nematoceran fly
D. obtusa
Tanglewing fly
D. rhodantha
Anthophorid bee
D. versicolor
Hawk-moth
D. cooperi
Settling moth
M. ophrydea
Auto-pollination
M. bracteata
Auto-pollination
D. vaginata
Auto-pollination
D. glandulosa
①
②
③
④
⑤

DISCUSSION

The most striking pattern in the phylogeny of *Disa* is the extent to which so many different types of pollination have evolved. I am not aware of any other genus for which comparable diversity in pollination systems has been demonstrated. Studies of *Disperis*, another large orchid genus in southern Africa by Steiner (1989) and Manning and Linder (1992), for example, show that most of the radiation has involved adaptation to a few species of oil-collecting bee. Other similar examples of relatively constrained radiation include *Pedicularis* - a large genus that is pollinated mostly by bumblebees (Macior, 1982) - and the numerous tropical orchid genera pollinated solely by euglossine bees (Dressler, 1993).

Adaptive radiation as a process

Diversity in flower form within a genus is readily observable. It is more difficult to show that an underlying process has caused the pattern of diversity. Although it may seem obvious that floral diversity in *Disa* is a result of adaptation to pollinators, alternative explanations such as genetic drift or pleiotropy, improbable as they seem, must be addressed. Cladistic pattern is informative about which features of a species or clades are likely to be adaptations (Coddington, 1988). A cladogram by itself does not, however, explain which selective factors resulted in adaptation. To use a trivial example, the repeated evolution of night-scented flowers in many plant lineages is a pattern which suggests that this feature is an adaptation; the observation that flowers with night-scent are visited by moths, though now well known, was required before this character was considered an adaptation to moth pollination.

Evidence for the adaptive nature of at least some floral traits in relation to pollination in *Disa* comes from (1) the observation that flowers of many *Disa* species attract and accommodate only specific pollinators (see Chapter 3), (2) convergence in floral traits in unrelated species which share the same pollinator, eg. evening-scented flowers in moth-pollinated species, (3) experimental evidence that certain derived floral traits, eg. the large red flowers of *D. uniflora*, are essential for the attraction of pollinators (Chapter 1) and (4) evidence that differences in the floral characters of closely related taxa are attributable to selection by pollinators. Examples of the latter include flower colour of *D. ferruginea* which varies from orange to red in response to the foraging preferences of the Mountain Pride butterfly in different regions (Johnson, 1994a), and differences in spur length between the closely related *D. draconis* and *D. harveiana*, corresponding to the proboscis length of their respective fly pollinators (Chapter 5). The process of floral evolution, and its relationship to speciation, is explored more fully in the final chapter of this thesis.

"Constraints" in the evolution of pollination systems

Phylogenetic constraints are a broad class of factors which have the effect of restraining the evolutionary change within a lineage (cf. Antonovics & van Tiederen, 1991; Perrin & Travis, 1992; Appendix A). The problem with the term "constraints" is that it is often invoked as an *ad hoc* explanation when evolution does not conform to an expected pattern. Despite the dramatic radiation in pollination systems in *Disa*, there are nevertheless some "constraints" evident in Fig 4.4; clades 1-3 are dominated by long-spurred flowers (pollinated by long-tongued flies, butterflies and moths) and clade 4 is dominated by short spurred flowers (pollinated by small flies, bee and wasps). Is it possible to isolate the underlying causes of these patterns?

NB [illegible]

In his influential paper on adaptive radiation in pollination systems, Stebbins (1970) introduced the term "lines of least resistance" which is helpful when thinking about shifts between pollination systems. Stebbins argued that preadaptation (= "exaptation" in cladist terminology, cf. Gould & Vrba, 1982) plays an important role in determining the direction of shifts between pollination systems. Preadaptation is evident on a cladogram when a character evolved before its current function. To give an example from this study, *Disa cooperi* has long spurs which play a vital role in hawkmoth pollination; however long spurs are plesiomorphic in this clade and are an "exaptation" for hawkmoth pollination. The utility of long spurs in many different pollination systems (eg hawkmoth, butterfly and moth pollination) is therefore possibly the "line of least resistance" which explains the dominance of long-spurred flowers in clades 1-3. Preadaptation in this manner must be a common reason for channelled evolution in plant pollination systems. In animals and some plants, which have a higher level of organization, phylogenetic constraints in the sense of "burden", ie characters which cannot readily be modified because they maintain other characters, may be a more common reason for evolutionary conservatism than preadaptation *per se*.

[illegible]

Limitations of the data set

The cladogram was constructed using only taxa for which I had data on pollination. It is important to stress that these species were chosen opportunistically, according to my "luck" with field work, and not because they supported a particular cladistic structure. The 28 species represent only c. 20% of the total number of species in *Disa* (including *Herschelianthe* and *Monadenia*) in deriving the cladogram. It is possible that by adding other species, the topology of the cladogram would change, particularly at the weakly supported basal nodes; however, many of the middle and terminal nodes are very well supported and therefore less sensitive to addition of taxa. Judging from the tremendous variety in flower form of the species which are not included here (cf. Stewart *et al*, 1982), it is likely that adding taxa would make the patterns of radiation more, rather than less, impressive.

There has been concern about the problem of "circularity" in using floral characters to derive cladograms which are then used to interpret the evolution of pollination systems (eg Wyatt, 1988; Chase & Hills, 1992; Armbruster, 1992). Some of these authors suggest that a cladogram used for interpretation should be generated using characters which are "independent" of the ecological traits (eg Coddington, 1988; Bremer & Eriksson, 1992). Proponents of this approach are usually systematists who have based their phylogenies on chloroplast DNA data (eg Chase & Hills, 1992; Bremer & Eriksson, 1992). However, as I will explain below, the notion of circularity is often a red herring based on a misunderstanding of phylogenetic methods (see Maddison, 1990; Maddison & Maddison, 1992; Kluge & Wolf, 1993).

Floral characters can be included in a cladistic analysis for interpreting pollination systems, provided the consequences and potential problems are properly understood (Maddison & Maddison, 1992). Firstly, the independent evolution of a pollination system in unrelated taxa may result in convergent floral features being treated as homologous (see Wyatt, 1988; Goldblatt, 1990). It is unlikely, however, that the independent evolution of a pollination system will result in convergence in all floral characters. Those characters which are convergent should be manifest as homoplasies which contradict the general cladistic pattern (Wanntorp *et al*, 1990). Convergence may also occur in "independent" characters sets, thus by eliminating floral characters, the problem of homoplasy is not circumvented. Vegetative characters such as leaf shape, for example, are often convergent in plants growing in similar habitats (see Eckenwalder & Barret, 1986) and thus result in a misleading cladogram if treated as homologous. Thus, because no one character set is likely to generate a perfect cladogram, the only viable approach is to include as many characters as possible (including the ones which are interpreted) in order to generate the best possible estimation of phylogeny (cf. Kluge & Wolf, 1993; Deleporte, 1993).

Finally, any potential problems of circularity depend on the questions being asked. In this chapter, for example, I have asked how many times particular pollination systems have evolved. If anything, convergence in floral characters would make it more difficult to detect separate origins of a pollination system. Thus by using floral characters in a study of adaptive radiation, the researcher is using a conservative method that is likely to diminish rather than increase the chances of showing that particular pollination systems evolved independently.

Concluding remarks

Adaptive radiation is often interpreted, particularly by zoologists, as the consequences of an "innovation" which allows a lineage to radiate within a new "adaptive zone" (Wanntorp *et al*, 1990; Guyer & Slowinski, 1994). The diversity of birds, for example, is frequently attributed to the innovation of flight. Wanntorp *et al* (1990) even suggested that the word "adaptive" be dropped from adaptive radiation, until a phylogenetic analysis had demonstrated that radiation in a group was associated with a particular trait. However this is a narrow view which places all the emphasis in adaptive radiation on the synapomorphy which is presumed to have somehow promoted speciation. A more tractable meaning is given to the term by botanists such as Grant and Grant (1965) and Stebbins (1970) who equated adaptive radiation with the pattern (epiphenomenon) resulting from a multiple series of adaptations in a particular structure or organ.

Consequently, there may be no special innovation which favoured the radiation to numerous pollination systems in *Disa*. Orchids with a similar "Bauplan" in other regions have tended to be conservative with respect to the method of pollination. The extensive patterns of radiation to numerous major pollinator groups found in *Disa* appears to exist in other Cape genera eg. *Erica*, *Pelargonium* and *Lapeirousia* (Johnson, 1992c; P Goldblatt, pers. comm.). Thus radiation in *Disa* is perhaps not due to an intrinsic innovation, but rather to something unusual about the pollination environment of the Cape region. In the following chapters, I develop the argument that floral diversification in many Cape lineages is promoted by an environment in which pollinator availability limits fitness and where pollinator abundance varies both in time and space.

Helo

Part 4

The process of floral shifts

5 Spur length divergence in the *Disa draconis* complex: a response to pollinators with different proboscis lengths

The variation in spur length among populations in the *Disa draconis* complex was studied in relation to pollinator proboscis length. Spur length varied enormously between populations in the southern mountains (mean = 35 mm), lowland sandplain (mean = 48 mm) and northern mountains (mean = 65 mm). Flowers in the complex appear to be pollinated exclusively by hovering flies with long mouthparts. The short-spurred plants in the southern mountains were pollinated by the horsefly *Philoliche rostrata* (Tabanidae) which has a medium long proboscis (mean = 28 mm), while long-spurred plants on the sandplain were pollinated by the fly *Moegistorynchus longirostris* (Nemestrinidae) which has a very long proboscis (mean = 56 mm). This latter fly is endemic to the sandplains. A one month difference in flowering time between the sandplain and southern mountain populations corresponds to a similar difference in the flight period of the two long-proboscid fly species. Despite extensive observations, pollinators were not found for the very long-spurred populations in the northern mountains. These are probably pollinated by an undescribed fly with a proboscis approximately 70 mm long.

"Where adaptive radiation is in its initial stages we can study it as a process rather than as an historical event. Particular interest therefore attaches to cases of racial and specific differentiation in methods of pollination." Grant and Grant (1965).

INTRODUCTION

Most studies of the adaptive significance of floral traits have dealt with selection by pollinators among floral forms, or artificial flowers, in a single population (eg Kay, 1978; Galen *et al*, 1987; Dafni *et al*, 1990; Johnston, 1991a; Herrera, 1993; see also Chapter 1). While these studies demonstrate the potential for selection to modify floral traits, they do not show that selection by pollinators has resulted in divergence between populations. Remarkably few studies have been attempted to show that variation in floral traits between populations is a consequence of adaptation to different pollinators (Grant & Grant, 1965; Cruden, 1972a; Miller, 1981; Pellmyr, 1986; Kendrick *et al*, 1987; Robertson & Wyatt, 1990; Chapter 2). This paucity of evidence for ecotypic differentiation is surprising given the widely held view that plants which colonize new regions may undergo selection for a shift to a "more effective" pollinator in the new habitat (Stebbins, 1970). It remains a major challenge, therefore, to determine whether spatial variation in floral traits reflects the spatial mosaic of pollinator availability.

The depth of floral spurs is one floral trait which often varies geographically within a species (cf. Miller, 1981; Robertson & Wyatt, 1990). The evolution of this trait can readily be understood in terms of a mechanism which was suggested by Darwin (1862) and later developed and tested by Nilsson (1978, 1988): selection will favour longer corolla tubes when they cause the pollinator to insert its entire proboscis into the flower and thus pick up and deposit pollen firmly with its face. Conversely, selection will favour the evolution of long mouthparts in the pollinator enabling it to reach deeply concealed nectar. Empirical support for selection on spur length was given by Nilsson (1988) who showed that moth-pollinated *Platanthera* flowers with artificially shortened spurs received less effective pollination. Reciprocal coevolution between flower depth and pollinator proboscis length could result in the evolution of extreme traits, evident for example in the very long orchid spurs and proboscides of some moths in Madagascar (Darwin, 1862; Nilsson *et al*, 1985, 1987, 1992; see also Steiner & Whitehead, 1990, 1991).

In this chapter, I consider the geographical variation in spur length and flowering time within *Disa draconis*. The current concept of *D. draconis* species defines a group of Cape orchids with large unscented cream, rarely mauve, flowers with a long slender spur, varying in length from 30-80 mm (Linder, 1981a). The flower spur does not contain nectar. Following extensive analyses of morphological variation between populations of *Disa draconis sensu* Linder (1981a), we have proposed that the species be split into four diagnosable taxa (Johnson & Linder, submitted). These four proposed taxa form a closely related complex. Thus depending on which taxonomic position is adopted, this chapter can be interpreted either as a study of intraspecific variation (existing taxonomy) or variation between closely related species (proposed taxonomy).

The only historical record of pollination in *D. draconis* is a painting housed in the Bolus Herbarium in Cape Town which clearly shows a horsefly (probably *Philoliche gulosa*) carrying pollinaria of *D. draconis* attached to its proboscis (see Johnson & Johnson, 1993). Horseflies (Tabanidae), together with tanglewing flies (Nemestrinidae), are important pollinators of many Cape plants (Johnson, 1992c; Goldblatt & Bernhardt, 1990; Chapter 3). These flies are stout insects, often the size of large bees and with highly elongated mouthparts.

The aim of this study was to determine whether the geographical variation in spur length in the *Disa draconis* complex can be attributed to adaptation to different long-proboscid fly pollinators. The alternative hypothesis would be that floral variation simply reflects different climatic and edaphic regimes, as was suggested for another Cape orchid *Disa uniflora* by Balfour & Linder (1990)

METHODS

Morphological measurements were taken from a single flower on 6-20 plants in each of six populations of *Disa draconis* (*sensu* Linder, 1981a), during October-December 1993. Additional measurements were taken from herbarium specimens, reconstituted where necessary, in K, BOL, STEL, PRE and NBG. Means for the various populations were compared by one-way analysis of variance, followed by the Tukey multiple range test to identify populations which differ significantly in spur length.

Pollinator observations were made at the following seven sites during 1990-1993: Table Mountain and Du Toits Kloof in the southern mountains; Silverstroomstrand and Ysterfontein on the sandplains, and Tulbagh and the Skurweberg Pass in the northern mountains (Fig 3.2). The total period of the observations, which were usually carried out between 1000 h and 1500 h on warm sunny days, was approximately 30 hours in the southern mountains, 25 hours on the sandplain and 15 hours in the northern mountains. Insects carrying pollinaria of the orchid were captured and identified and their proboscides measured. Additional proboscis measurements were made from specimens in the South African Museum, Cape Town and the Natal Museum, Pietermaritzburg. Plant and insect vouchers are deposited in the Bolus Herbarium and the South African Museum, Cape Town, respectively.

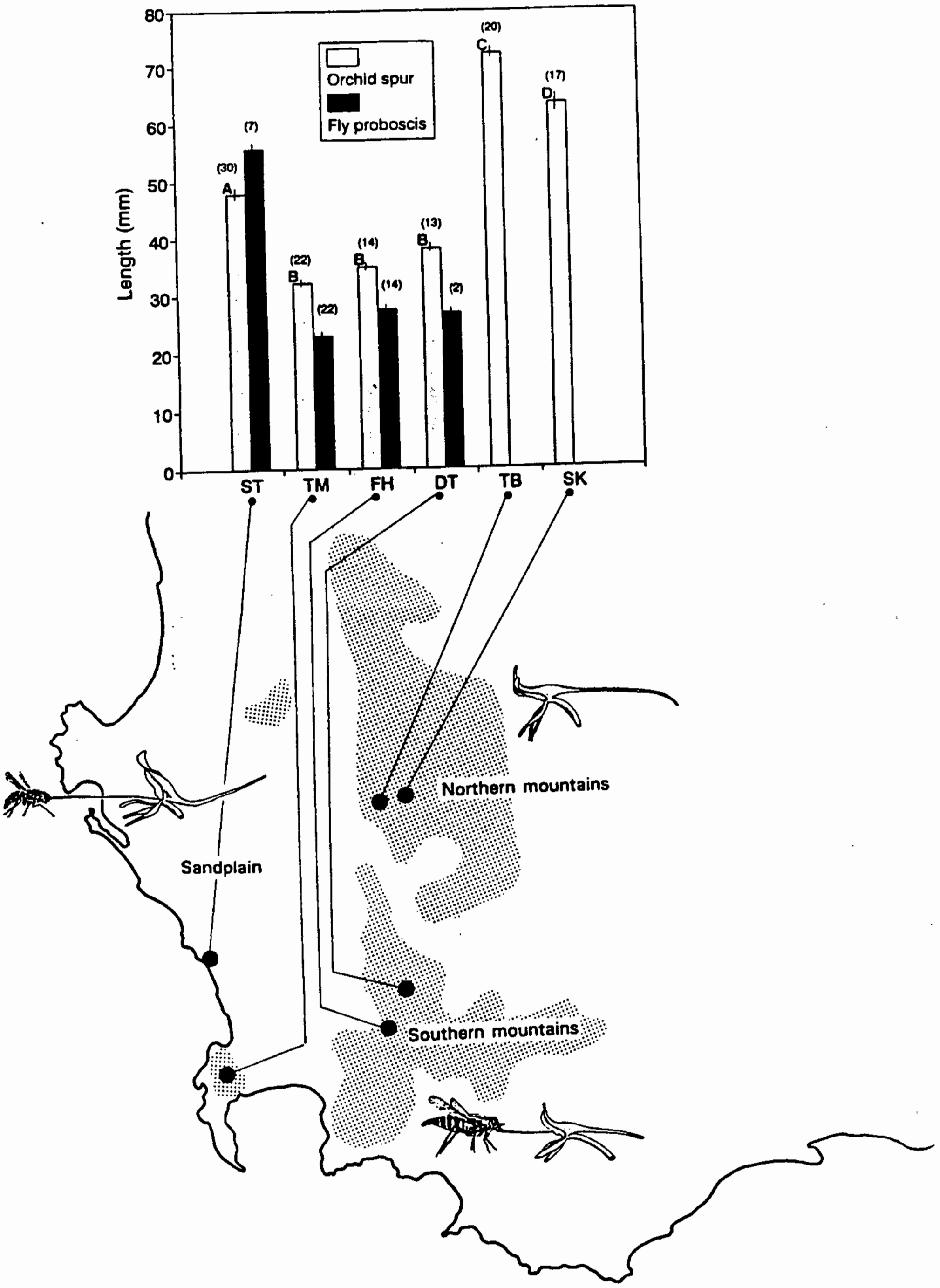


Figure 5.1 Localities of the study sites in the south western Cape, South Africa. The graph shows the mean spur length and pollinator proboscis length at each site. Vertical lines indicate standard error. Sample sizes are given above each bar. Bars which share the same letter are not significantly different (Tukey multiple range test). Abbreviations: ST - Silverstroom Strand, TM - Table Mountain, FH - Franschhoek, DT - Du Toits Kloof, TB - Tulbagh, SK - Skurweberg Pass. Note that no pollinators were found at the sites in the northern mountains.

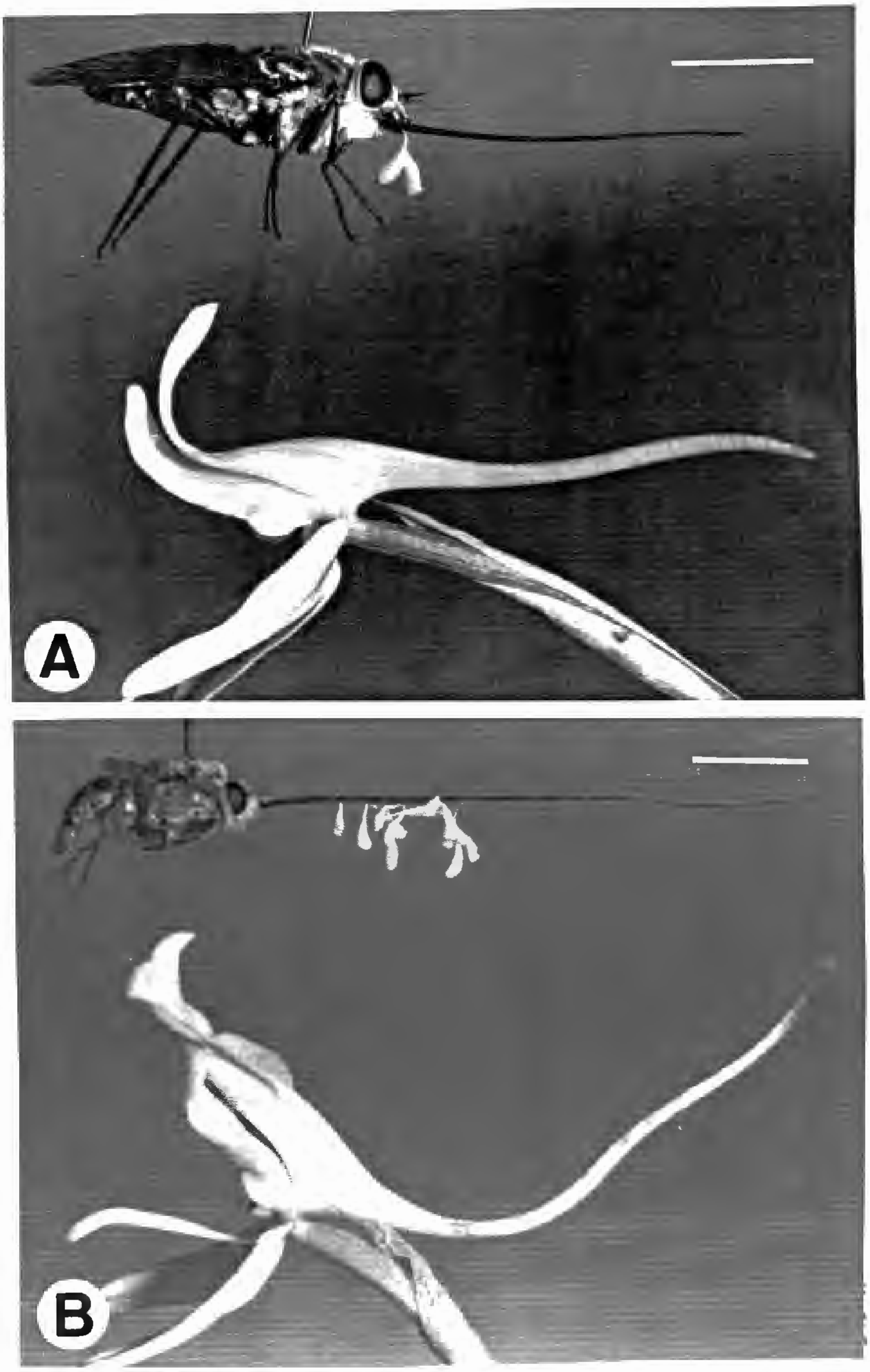


Figure 5.2. **A**, Short-spurred form of *Disa draconis* from Table Mountain, with its pollinator, the horsefly *Philoliche rostrata*. **B**, Longer-spurred form of *Disa draconis* from the sandplains near Cape Town, with its pollinator, the tanglewing fly *Moegistorynchus longirostris*. Scale bars 10 mm.

RESULTS

I found significant differences in spur length between all three of the regions: the southern mountain populations had the shortest spurs (32-38 mm), those on the sandplains were intermediate (c. 48 mm), while the longest spurs (63-72 mm) were found at the two northern mountains sites (Fig 5.1).

No pollinators were found during 1991, despite extensive observations at the study sites. During three days of observations on Table Mountain in December 1992, however, I managed to capture four individuals of the horsefly *Philoliche rostrata* Linnaeus (Tabanidae), carrying the distinctive pollinaria of *D. draconis* attached to the ventral base of the proboscis (Fig 5.2). This was the only long-proboscid fly species observed at the site. Like most long-proboscid flies, *P. rostrata* is incredibly swift and almost impossible to capture unless surprised at a flower. During 1993, I also captured a single individual of the same species of horsefly with pollinaria of *Disa draconis* at the Du Toits Kloof site during 1993. K. Steiner (pers. comm.) has found this horsefly with pollinaria of *D. draconis* at the Franschhoek site. Mean proboscis lengths were obtained by combining measurements for the captured flies with those of museum specimens that had been captured in the same localities. The proboscis length of *P. rostrata* ranged from 23-27 mm in the southern mountains which is slightly shorter than the corresponding orchid spur lengths (32-38 mm) (Fig 5.1). Since the entire proboscis of the fly is inserted into the flower, the pollinaria are attached to the basal portion of the proboscis (Fig 5.2)

Observations of the sandplain form of *D. draconis* proved as difficult as the mountain form. Only a few populations of this form remain, the rest having been destroyed by development around Cape Town. Observations at the undisturbed Ysterfontein and the nearby Silverstroomstrand sites in 1992 were unsuccessful, but during October 1993 I managed to capture four individuals of the extraordinary fly *Moegistorynchus longirostris* Wiedemann (Nemestrinidae) with a large number of pollinaria of *D. draconis* attached to their proboscides (Fig 5.2). Another three captured individuals did not carry pollinaria. No other long-proboscid fly species were observed on the sandplain. The mean proboscis length of the seven flies was 55.7 mm, which is slightly longer than the orchid spur at these sites (mean = 47.8 mm). As a consequence, the proboscis is not fully inserted into the spur and pollinaria are placed along its length, rather than at its base as in the mountain form (Fig 5.2).

The function of the curious upturned shape of the spur in the sandplain form (Fig 5.2) is not clear, but it would have the effect of causing the proboscis to press firmly against the column as it is forced into the curvature of the spur. As an *ad hoc* adaptive explanation, it could be surmised that this firm contact improves the attachment of pollinaria along the length of the proboscis (cf. Nilsson *et al*, 1985 for a slightly different interpretation of spur curvature in the malagasy orchid *Angraecum arachnites*).

The phenology of the southern mountain and sandplain populations of *D. draconis* coincides closely with the short adult flight periods of their pollinators. Flowering in the southern mountain populations occurs mainly in December when *P. rostrata* is most common in the Cape mountains (Oldroyd, 1957; Johnson, unpublished), while flowering in the sandplain populations occurs more than a month earlier (late October - early November) during the short flight period of *M. longirostris*. I have never observed *M. longirostris* after early November and all of the specimens in the Cape Town Museum were collected in October or early November.

Flowers in the *D. draconis* complex are non-rewarding and therefore rely on deception to achieve pollination. At both the sandplain and southern mountain sites, the flies obtained nectar from other long-tubed flowers. On Table Mountain, *P. rostrata* most commonly obtained nectar from the flowers of *Pelargonium cucullatum* L'Her (Geraniaceae), while on the sandplain *M. longirostris* visited the flowers of *Pelargonium* spp. and *Lapeirousia anceps* (L.f) Ker Gawler (Iridaceae) (see frontispiece). Most of the flies carrying orchid pollinaria were caught on these nectar-producing flowers, rather than on the orchids.

Despite a total of more than 15 hours of observation at two populations in the northern mountains (Fig 5.1) during 1992-1993, I failed to find any insects carrying pollinaria of the very long-spurred form of the orchid.

DISCUSSION

These preliminary results suggest that variation in spur length and flowering time in the *D. draconis* complex is an adaptive response to selection by various long-proboscid flies. This hypothesis is supported by significant differences in spur length among populations in different regions which correspond to the different proboscis lengths of sandplain and mountain fly species.

The extraordinary long mouthparts found in members of the dipteran families Tabanidae and Nemestrinidae in South Africa probably evolved through diffuse coevolution with long-tubed nectar-producing flowers, as was suggested for long-tongued hawkmoths and orchids in Madagascar by Nilsson *et al* (1985, 1987). At both the Table Mountain and Silverstroomstrand sites, distinctive guilds of plants visited by these flies could be identified. The non-rewarding flowers in the *D. draconis* complex could not have influenced the evolution of fly proboscis length. It can be assumed, therefore, that spur length in *D. draconis* is an adaptation to fly proboscis length, rather than the reverse. Unfortunately, the direction of evolution in spur length cannot be determined with certainty due to the lack of phylogenetic resolution within the complex (Johnson & Linder, submitted).

The potential for directional selection to act on floral traits is greatly reduced in plants which have generalist pollination systems (Herrera, 1988). It is easier to isolate selective pressures when a plant has a specialised pollination system. For example, Robertson and Wyatt (1990) were able to attribute spur length variation in *Platanthera ciliaris* in the southeastern United States to differences in the proboscis length between two butterfly species which are the primary pollinators of this orchid at mountain and lowland sites, respectively. Similarly, I found that both the spur length of the orchid *Disa ferruginea* and the proboscis length of its exclusive pollinator - the butterfly *Meneris tulbaghia* - were significantly longer on Table Mountain than at a site in the Langeberg Mountains (Johnson, 1994a; Chapter 2).

In both *D. ferruginea* and *D. draconis*, the pollinaria are placed along the length of the proboscis (Fig 2.1C, 5.2). It is thus not essential for pollination that the spurs exceed the proboscis length of the pollinators, unlike the many orchids which attach pollinaria to the face or eyes of the pollinator (Nilsson, 1978, 1983b, 1988; Robertson & Wyatt, 1990; Nilsson *et al*, 1987). Why then have longer spurs in the two *Disa* species apparently evolved in areas where the pollinator proboscis is relatively long? One selective pressure, acting through male fitness, may be that longer spurs ensure that pollinaria are attached firmly to the thickened base of the proboscis rather than at the slender distal end. On the other hand, long spurs may pose disadvantages for male fitness, because pollinaria placed nearer the base of the proboscis are not in a position to pollinate short-spurred plants. Selection for longer spurs, therefore, probably occurred mainly through female function by increasing the number of matings as follows: short-spurred plants will only be pollinated by pollinaria from short-spurred plants, whereas long-spurred plants will be pollinated by pollinaria from both long- and short-spurred plants.

The lack of studies of intraspecific variation in floral traits may be partly due to taxonomic practice. Populations which have shifted to a different pollinator are often given species status, and are therefore not considered for studies of intraspecific variation. In the orchid genus *Ophrys*, for example, populations are often given species status on the sole criterion that they attract a distinct pollinator (Paulus & Glack, 1990). This practice raises a difficult philosophical problem: should intraspecific taxa be maintained as examples of "incipient speciation" (eg. Pellmyr, 1986), or should species be recognised on the basis of diagnosibility alone? I follow the view that species are no more "real" than subspecies or any other rank for that matter, thus the choice of ranking may be an arbitrary one (cf. Nelson, 1989; Chapter 8). Perhaps the subspecies rank should be retained to easily identify allopatric ecotypes. Subspecies are instantly recognisable as having closest relationship (Mayr, 1992). For example, we have used the subspecies concept to describe the northern and southern mountain populations which differ in spur length and flowering time, but are otherwise indistinguishable (Johnson & Linder, submitted).

A full understanding of the adaptive significance of floral traits in the *D. draconis* complex is not possible until the elusive pollinator of the very long-spurred populations in the northern mountains is discovered. Based on the very long floral spurs (c. 30 cm) of the orchid *Angraecum sesquipedale* in Madagascar, Darwin (1862) predicted the existence of a very-long tongued hawkmoth on this island. He was vindicated when the hawkmoth *Xanthopan morganii* ssp. *praedicta* - which has a tongue matching the spur length of this orchid - was later discovered. Given this precedent, it seems quite possible that with further observations, a fly with a proboscis measuring approximately 70 mm will be found to pollinate the northern mountain form of *D. draconis*. The only South African fly known to have a proboscis approaching this length is *M. longirostris* which has been collected exclusively from the sandplain. The existence of an undescribed giant fly species in the northern mountains therefore seems a distinct possibility.

6 Spatial heterogeneity in pollinator availability: implications for pollination success of a Cape orchid[1]

The pollination success of *Disa uniflora* (Orchidaceae) was studied in two habitats on Table Mountain over a four year period. Plants occurring in a rocky gorge were far more successful than plants occurring in an adjacent open valley. Each year, 30-80% of flowers in the gorge were pollinated and set fruit compared with 9-25% of flowers in the valley. These differences are explained by the preference of *Meneris tulbaghia* (Nymphalidae: Satyridae), the exclusive pollinator of the orchid, for rocky, sheltered habitats. Fruit set of hand-pollinated flowers did not differ significantly between the two habitats, indicating that resources did not account for the variation in fruiting success.

"Although fruits (of Disa uniflora) are rare along streams, they occur often on cliffs, where the large mountain butterfly Meneris tulbaghia is free to visit its flowers, this beautiful but shy insect not readily venturing in between the banks of streams" Marloth (1915)

Reproductive success in plants has often been shown to be regulated, within a season, by the availability of pollinators (Zimmerman & Aide, 1989; Montalvo & Ackerman, 1987). Little is known, however, about spatial variation in the pollination success of plants occurring in different habitats (Campbell, 1987). In this study it is shown that the reproductive success of an orchid is substantially higher in the habitat preferred by its pollinator.

The orchid *Disa uniflora* is typically found on stream banks and wet cliff faces at high elevation in the south western Cape Province of South Africa. It usually produces a single flower, although up to six have been recorded. The spectacular red flowers (among the largest known in orchids) are pollinated exclusively by the butterfly *Meneris tulbaghia* (Nymphalidae: Satyrinae) (Marloth, 1895). This butterfly visits only red-flowered species (Johnson & Bond, 1992a, 1994; Chapter 1). It has a well known preference for sheltered habitats where it spends much of the day resting in the shade of rocks (Swanepoel, 1953).

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[1] Adapted from Johnson & Bond (1992b)

Table 6.1. A comparison of reproductive success in adjacent gorge and valley populations of *Disa uniflora*.

		Gorge		Valley		X^2	P
		%	n	%	n		
At least one pollinarium removed	1990	60	(42)	12	(42)	18.7	<0.001
	1991	62	(77)	9	(75)	43.9	<0.001
	1992	30	(69)	9	(81)	10.6	<0.01
	1993	51	(49)	9	(57)	30.4	<0.001
Pollen deposited on stigma	1991	73	(77)	9	(75)	60.3	<0.001
	1992	32	(69)	9	(81)	49.9	<0.001
	1993	61	(49)	9	(43)	21.8	<0.001
Fruit set (natural)	1990	83	(6)	17	(6)		N.S.*
	1991	59	(61)	23	(77)	16.7	<0.001
	1993	77	(39)	35	(63)	15.4	<0.001
Fruit set (hand-pollinated)	1990	70	(10)	59	(17)		N.S.*
	1991	87	(15)	80	(10)		N.S.*

* Fishers exact test (two tailed)

The consequences of the habitat preference of the butterfly was examined by comparing the pollination success of orchids in adjacent sheltered and open localities. The study site consisted of an 800 m stretch of "Disa Stream" on the summit of Table Mountain. The stream flows for 400 m through a rocky gorge habitat and then a further 400 m along a broad open valley with deep sandy soils before entering a reservoir. The orchid occurred at similar densities along the stream in both habitats. No other plants visited by *M. tulbaghia* were in flower, therefore nectar resources for the butterfly were practically identical at both sites. During 1990-1993 a census was made of pollinaria removal, pollen on stigmas and fruit set of plants in both habitats. In addition, a number of flowers at each site were hand-pollinated in order to simulate the effect of equal pollinator availability at both sites. Plants were randomly selected and one flower per plant (most plants have one flower) was hand-pollinated by brushing the stigma with pollinaria from a plant not less than five metres away.

The results show clearly that plants growing in the gorge were far more successful than their valley counterparts in terms of number of pollinia removed, pollen received and fruits set (Table 6.1). The difference in natural fruit set between the two populations cannot be ascribed to differences in resource availability as hand-pollinated flowers showed no significant differences in fruit set between populations.

While *D. uniflora* is normally confined to wet cliffs or streams in rocky areas favoured by the butterfly, its existence in the valley, despite low fruit set, probably reflects the propensity of the orchid for clonal reproduction. Its distribution may have also been extended beyond the gorge by seed and ramets washed downstream to the valley.

This study demonstrates that variation in reproductive success between populations may be due to differences in pollinator availability (Campbell, 1987). Uneven spatial distribution of pollinators (cf. Ehrlich & Wheye, 1984) may influence not only plant distribution patterns, but also floral evolution. If a plant invades a habitat not favoured by its pollinator, selection may rapidly modify the flower, making it more attractive to pollinators in the new habitat (Stebbins, 1970; Robertson & Wyatt, 1990; Chapter 5).

7 Evidence for pollen-limited fruiting success in Cape wildflowers

Hand-pollination experiments were used to test whether fruiting success was limited by pollen availability in 12 herbaceous Cape wildflower species. Natural fruiting success was low (median = 32% of flowers per plant) and in some populations the majority of plants failed to set any fruit. Hand-pollinations resulted in significant increases in fruit production at a whole plant level in 13 out of the 14 study populations, indicating that pollen limitation was the primary cause of fruiting failure. This was corroborated by the finding that species with low fruit set also had low natural levels of pollen deposition onto stigmas. Many of the study species flower soon after fire when pollinator populations may be most depleted.

Local botanists as well as entomologists have repeatedly noticed that often there appears to be an entire absence of insect life, although the fields or the hillsides may be aglow with flowers" Marloth (1908), commenting on the Cape flora

INTRODUCTION

It is a commonly held view that female reproductive success in most plants is limited by resources (Willson & Price, 1980; Bell, 1985; Willson, 1991). However, a recent review of the literature showed that pollen, rather than resources, limited reproductive success of most angiosperm species which have been studied (Burd, 1994). If generally applicable, these findings have important implications for our understanding of sexual selection and its role in floral evolution. The problem, however, is that many of the studies reviewed by Burd (1994) had inappropriate experimental designs (see below) and most dealt only with single species. It is thus difficult to know whether pollen limitation of fecundity is an unusual occurrence or widespread in certain regions (Johnston, 1991b).

Only a handful of studies have dealt with determinants of fecundity in more than one species in a particular region. Motten (1986), for example, showed that fecundity of wildflowers in a temperate forest in North America was primarily limited by resources; hand-pollinations increased fruit and seed set in only 3 of the 12 study species (see also McCall & Primack, 1985). On the other hand, fecundity was pollen-limited in most temperate montane wildflower populations studied by Campbell (1987). Fruiting in neotropical orchids is generally pollen-limited, though long-term costs of increased fruiting may mitigate the benefits of hand-pollination in some species (Janzen *et al*, 1980; Montalvo & Ackerman, 1987; Ackerman, 1989; Ackerman & Montalvo, 1990; Zimmerman & Aide, 1989). Burd (1994) reanalysed fruit and seed set data from published breeding systems surveys and showed that pollen-limited fruit and seed set may be widespread in tropical trees (Bawa, 1974) and sclerophyllous vegetation in Chile (Arroya & Uslar, 1993). However, caution should be applied when inferring pollen limitation from the results of breeding system surveys as hand-pollinations are seldom carried out on a whole plant level (see below).

The method used in nearly all studies of pollen limited reproductive success is to perform supplementary hand pollinations on an experimental group of plants and to compare the fruit and or seed set of this group with a control group of unmanipulated plants. While this may seem straightforward, the methodology has come under close scrutiny in recent years. Flowers on a plant are not independent as they share a common resource pool. Therefore, average fruit or seed set values for whole plants should be used in any statistical analysis in order to avoid pseudoreplication. Relatively few studies have demonstrated pollen limitation of reproduction at a whole plant level (Johnston, 1991b). In shrubs, particularly, it is generally very difficult to use whole plants as replicates because of the large number of flowers which have to be pollinated (cf. Zimmerman & Pyke, 1988; Ayre & Whelan, 1989).

Herbaceous wildflowers form spectacular displays in the Cape region of South Africa, particularly in the first season after a fire. Flowering in many of these wildflowers, especially Iridaceae, Amaryllidaceae and Orchidaceae, is strongly fire-stimulated and plants may remain sexually dormant for up to 30 years until the vegetation is burnt once again. The implications of this unusual life history for pollination success had not been studied. The purpose of this study was to test whether reproductive success of herbaceous Cape wildflowers, especially those dependent on fire for flowering, is limited by pollen availability.

METHODS

Study species

The study species were chosen opportunistically over a four year period from 1990-1993 by searching for large wildflower populations in the mountains near Cape Town. Most of the populations large enough for adequate sampling were located in recently burnt areas. The populations were chosen at the flowering stage, thus any bias towards species with especially low fruiting success was avoided. The study species are representative of a wide range of pollination systems, including sunbird, butterfly, bee, wasp and even horsefly pollination (Chapter 3).

Pollination success

Low levels of fruit set in plants could conceivably result from genetic load (Charlesworth, 1989), or simply from deposition of incompatible pollen. To determine whether fruiting failure was due to the lack of pollen *per se*, rather than the quality of pollen, the presence of pollen on the stigmas of a large sample of orchids was recorded. Unlike most other plants, orchids have pollen aggregated into massulae that can easily be observed on the stigma.

Hand-pollinations

Plants in each of the study populations were tagged and then randomly assigned to either the control (unmanipulated) group or the experimental (hand-pollinated) group. I pollinated as many flowers on each of the experimental plants as possible with pollen collected from a source at least 10 metres away. A different source of pollen was used to pollinate each of the plants in the experimental group. The study species all produce a single inflorescence, even a single flower per plant in some cases, making them ideal for hand-pollination studies at a whole plant level.

The percentage of flowers which developed fruits was determined for each of the control and experimental plants. Since most of the study species were terrestrial orchids which have microscopic seed, seed number per fruit was determined only in two of the amaryllid species. The large number of zero values made it difficult to normalize the fruit and seed set data, hence the treatments were compared using the nonparametric Mann-Whitney U test (Zar, 1984).

RESULTS

Pollen was deposited on only a small percentage of stigmas (median = 22.8%) in each of the 34 orchid populations sampled (Table 7.1). The median natural pollination success of the seven orchid species which were hand-pollinated was 16.5%. Although these values show that only a small fraction of flowers were pollinated at the height of the flowering season, pollination success of open flowers is not always an accurate predictor of final fruit set as pollination can take place after the flowers are checked in the field, particularly in orchids which have long-lived flowers.

Table 7.1. The percentage of naturally pollinated flowers and flowers with pollinaria removed in various Cape orchid populations. The data were collected opportunistically between 1991 and 1994.

Species	Study site	No. plants examined	No. flowers examined	Flowers pollinated (%)	Flowers with pollinaria removed (%)
*Disa tenuifolia**	Cape Point	45	45	15.5	15.0
	Silvermine	53	53	39.6	26.4
*Disa obtusa**	Silvermine	10	301	84.0	93.3
Disa oreophila	Naudes Nek	25	125	31.2	27.2
Disa draconis	Silverstroomstrand	36	122	5.7	13.9
Disa harveiana ssp. harveiana	Table Mountain	17	49	7.6	24.4
Disa harveiana ssp. longicalcarata	Tulbagh	20	62	8.1	6.5
	Skurweberg Pass	8	23	0.0	4.3
*Disa racemosa**	Silvermine	59	59	10.1	33.8
	Cape Point	81	81	4.9	22.2
	Swartberg	44	44	47.7	63.6
	Franschhoek	41	41	7.3	4.9
	Bettys Bay	42	42	9.5	4.8
Disa salteri	Oudtsthoorn	24	114	22.8	38.5
*Disa venosa**	Bainskloof	36	57	19.3	48.2
Disa caulescens	DuToits Kloof	17	37	51.4	21.6
Disa longicornis	Table Mountain	31	31	6.5	12.9
Disa tripetaloides	Kogelberg	15	45	15.5	37.4
*Disa elegans**	Swartberg Pass	18	30	46.7	63.3
*Disa subtenuicornis**	Langeberg	13	39	76.9	82.0
*Disa filicornis**	Bains Kloof	33	33	64.0	82.0
*Disa bivalvata**	Swellendam	35	68	21.4	35.7
*Disa fasciata**	Constantiaberg	24	47	34.0	40.4
Herschelianthe spathulata	Worcester	12	39	7.6	10.25
Herschelianthe graminifolia	Table Mountain	23	44	31.8	61.4
	Table Mountain	17	30	30.0	35.3
	Silvermine	16	28	32.1	46.4
Herschelianthe purpurescens	Cape Point	43	56	7.1	48.2
*Monadenia ophrydea**	Pilaarkop	18	179	93.2	94.4
Disperis capensis	Table Mountain	13	13	53.8	61.5
	Silvermine	30	30	20.0	26.6
Satyrium bracteatum	Constantia Nek	25	80	62.5	63.8
Satyrium stenopetalum	Grabouw	6	71	64.8	70.4
		MEDIAN		22.8	35.7

* Species which are fire dependent for flowering

Table 7.2. The effects of hand-pollination on fruit set in Cape wildflower species. The sample size *n* refers to the number of plants sampled.

Species	Locality (year)	Fls/plant	Pollinator	Fruit set (%)		Z	P
				Control	Hand-pollinated		
		$\bar{X}$ ± S.D. (*n*)		$\bar{X}$ ± S.D. (*n*)	$\bar{X}$ ± S.D. (*n*)		
Amaryllidaceae							
Haemanthus rotundifolius[#]	Cape Point (1991)	97.5 ± 44.6 (61)	Bees, butterflies, sunbirds[1]	4.6 ± 12.3 (50)	24.2 ± 23.7 (11)	3.75	***
Cyrtanthus ventricosus#	Table Mountain (1990)	3.8 ± 2.35 (115)	Sunbirds[1]	13.2 ± 27.7 (29)	42.0 ± 38.7 (86)	3.59	***
Cyrtanthus guthrieae[#]	Bredasdorp (1991)	1.0 ± 0.0 (33)	Butterflies[2]	5.9 ± 23.5 (17)	44.4 ± 49.7 (18)	2.55	**
Nerine sarniensis	Betty's Bay (1990)	9.4 ± 2.5 (30)	Butterflies[2]	61.7 ± 25.4 (14)	76.3 ± 16.2 (16)	1.18	N.S.
Orchidaceae							
Disa bivalvata#	Swellendam (1991)	6.5 ± 4.3 (32)	Wasps[3]	56.6 ± 39.7 (16)	96.1 ± 5.9 (16)	3.21	***
Disa fasciata#	Cape Peninsula (1991)	2.8 ± 1.2 (49)	Unknown	28.8 ± 26.8 (26)	60.6 ± 33.2 (25)	3.12	***
Disa racemosa[#]	Franschhoek (1993)	2.6 ± 0.8 (47)	Bees[4]	3.5 ± 14.8 (19)	63.4 ± 31.2 (28)	5.17	***
	Silvermine (1993)	6.5 ± 1.6 (66)		32.2 ± 19.8 (33)	47.2 ± 18.5 (33)	2.95	**
Disa tenuifolia[#]	Cape Point (1993)	1.9 ± 0.9 (107)	Bees[5]	48.8 ± 45.6 (54)	79.7 ± 32.8 (53)	3.58	***
Disa tenella	Malmesbury (1992)	7.3 ± 2.0 (21)	Bees[1]	63.5 ± 25.8 (11)	83.2 ± 12.5 (9)	1.68	*
Disa harveiana	Table Mountain (1992)	4.4 ± 1.6 (21)	Horseflies[6]	11.5 ± 16.1 (10)	77.8 ± 26.8 (11)	3.72	***
Disa uniflora	Table Mountain: Disa valley (1991)	1.3 ± 0.5 (68)	Butterflies[2]	20.7 ± 36.0 (58)	70.0 ± 40.0 (10)	3.42	***
	Table Mountain: Disa gorge (1991)	1.4 ± 0.6 (57)	Butterflies	58.3 ± 47.5 (42)	83.3 ± 34.9 (15)	1.78	*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

\# Species which are fire dependent for flowering

[1] S.D. Johnson (unpublished)

[2] Johnson & Bond (1994)

[3] Steiner *et al* (1994)

[4] Johnson & Steiner (1994a)

[5] Johnson & Johnson (1993)

Natural levels of fruit set per plant varied from 4% to 64% (median = 32.2%) among the 13 study populations (Table 7.2). Hand-pollination resulted in significant increases in fruit set in 12 out of 13 populations. Fruit set in the hand-pollinated treatments varied from 24% to 96% (median = 70%). Fruit set was increased more than 100% by hand-pollination in about half of the study populations and more than 500% in populations of *Haemanthus rotundifolius*, *Cyrtanthus guthrieae* and *Disa racemosa* (Franschhoek population) (Table 7.2).

Hand pollination also resulted in significant increases in the number of seeds per fruit in *Cyrtanthus ventricosus* and *Nerine sarniensis.* In the former, the mean capsule seed set per plant was 1.76 (± 3.4) in the controls versus 8.07 (± 9.5) in the experimental group ($Z = 3.59$, $P < 0.001$), while in the latter it was 4.41 ± 3.8 in the controls versus 7.11 ± 3.6 in the experimental group ($Z = 1.99$, $P < 0.01$). Interestingly, *N. sarniensis* was the only species which did not show a significant increase in fruit set following hand-pollination (Table 7.2)

DISCUSSION

Fruiting success in all but one of the study species was limited by pollen availability as evidenced by the large increases in fruit set after pollen supplementation to stigmas and the low levels of pollen deposition on stigmas. These results are consistent with the casual observations by naturalists that pollinating insects are scarce in the Cape region, particularly after fire (Marloth, 1908). Almost all of the species tested are fire-dependent for flowering.

Three possible adaptive outcomes of pollen-limited reproductive success are: (1) a change in breeding system which allows the plant to be independent of pollinators (cf. Wyatt, 1983); (2) allocation of resources to produce more attractive flowers to secure more visits by the same pollinator (cf. Haig and Westoby, 1988); (3) shift to another more effective pollinator (cf. Stebbins, 1970). I will consider each of these outcomes below.

A change to a breeding system that does not require pollinators appears to have occurred frequently in harsh environments where pollinators are scarce, such as alpine Patagonia and the Arctic Circle (Arroya & Squeo, 1990). The available evidence indicates that the Cape flora has a relatively low percentage of selfing and autogamous species and a relatively high percentage of obligate outcrossers such as dioecious species (Steiner, 1987). Among the c. 350 terrestrial orchid species in the Cape flora, for example, there are only about four species known to be capable of auto-pollination, despite the evidence for chronic pollen limitation of reproductive success in some of the Cape orchids (Johnson *et al*, 1994).

The evolution of large expensive flowers is a feature of Cape wildflowers, particularly geophytic species. Haig and Westoby (1988) explored the theoretical consequences of allocation to attractive structures in pollen limited plants. They argued that selection will favour allocation of resources to floral attractants, until pollen is no longer limiting. It remains to be shown, however, whether visits by pollinators can be assured simply by more allocation to floral attractants. Many of the plants in this study have enormous flowers; *Disa uniflora*, for example, has possibly the largest flowers of all Orchidaceae and several others, such as *Cyrtanthus guthrieae*, are highly sought after by horticulturists for their showy flowers. Yet even these species failed to attract pollinators to most of their flowers.

Shifts to more effective pollinators may have been the most common outcome of pollen limited reproduction in Cape wildflowers. I have shown that *Disa*, a genus in which pollen limited fruiting success appears to be common (Table 7.2), is characterised by frequent shifts in pollination systems (Chapter 4). Stebbins implicitly invoked pollen limitation as a cause of floral shifts when he argued that plants which colonize regions in which their pollinators are scarce or absent might shift to other, more *effective* pollinators (Stebbins, 1970). Thus spatial and temporal mosaics in pollinator availability (Chapters 5 & 6) may result in some populations responding to pollen limitation by shifting to more effective pollinators.

Caveats of this study

Ideally, studies of pollen limitation should continue over several seasons to monitor the effects of hand-pollinations on lifetime fitness. Several studies show that increased fruit production may result in a future cost to reproduction; thus, resource limitation may limit plant reproduction over a lifetime (Janzen *et al*, 1980; Montalvo & Ackerman, 1987; Ackerman, 1989; Ackerman & Montalvo, 1990; Snow & Whigham, 1989; Zimmerman & Aide, 1989; but see Horvitz & Schemske, 1988). Calvo (1993), however, points out that the costs of reproduction which have so far been demonstrated are relatively negligible compared with the benefits of hand-pollination. Thus long-term costs may mitigate the effects of increased fruiting, but are unlikely to outweigh the benefits.

It was obviously impossible to monitor the costs of fruiting on subsequent reproduction in most of the study species due to the long intervals (5-30 years) between flowering. Attempts at long term monitoring of *Disa uniflora*, the only study species to flower each year, were thwarted by floods. Heavy fruiting episodes are, however, unlikely to have future reproductive costs in most of the study species due to the long vegetative period between flowering.

Studies, even of single species, should preferably be carried out at several sites to monitor the interpopulation variation in pollen availability. For example, *D. racemosa* showed a tenfold difference in natural fruiting success between two populations, and fruit set in *D. uniflora* varied twofold between closely adjacent populations (Table 7.2).

In the final analysis, the question of whether pollinators are more limiting in particular environments may not be answered adequately by hand-pollinations experiments. As Arroya and Squeo (1990, pg 221) aptly stated, " To some extent this doubt [about whether fruit set reflects pollinator availability] is logically redundant, because, unlike in an experimental situation, we are dealing with organisms that might already have evolved some kind of compensatory adaptations for the extreme conditions for pollination" (see also Bond, 1994). A similar sentiment was expressed by Haig and Westoby (1988), only they limited their discussion to resource allocation as a compensation mechanism.

Despite the above problems, hand-pollination experiments remain the best way of determining whether plants are pollinator-saturated or pollinator-limited. The implications of pollen-limitation for floral evolution are explored further in the following chapter.

8 Pollination, adaptation and speciation models

The findings in previous chapters are discussed in the context of current ideas about species and speciation. Recently, there has been a trend to define species on typological criteria ("diagnostic characters"), rather than isolating mechanisms. Pollination biology has much relevance to this new species concept because floral modifications are often used to diagnose plant species. The floral radiation evident in many Cape genera indicates that adaptation to pollinators has played a major role in diversification of the species. Based on the finding that fecundity of Cape wildflowers is often pollen-limited, it is suggested that selection for more efficient mating systems, rather than isolating mechanisms, may have driven adaptive floral evolution.

INTRODUCTION

Perhaps the greatest challenge in evolutionary biology is to understand the relationship between adaptation and speciation. For Darwin, speciation was a simple epiphenomenon of adaptation. Later, Ernst Mayr and other proponents of the biological species concept (BSC) developed a more rigid view of speciation - the acquisition of isolating mechanisms (Mayr, 1963). Ironically, ideas about speciation have come full circle and many evolutionists (particularly botanists and cladists) have abandoned the BSC in favour of a revitalised typological species concept (Cracraft, 1989; Nelson, 1989). In this view, the essential feature of taxa is their monophyly and possession of diagnostic features, rather than isolating mechanisms (Cracraft, 1989; Baum, 1992). The problem of speciation, then, can be rephrased: instead of asking which processes give rise to isolating mechanisms, we could simply ask which process (or processes) of differentiation gives rise to diagnostic features. A focus on the origin of diagnostic characters, rather than isolating mechanisms, brings studies of adaptive character evolution and speciation closer together than they have been in the post-Darwinian era.

ESTIMATING DETERMINANTS OF SPECIATION

Stebbins (1970, 1974) drew attention to the fact that some plant families, such as the Euphorbiaceae, have radiated primarily in vegetative characters (reflecting adaptations to the physical environment), while others, such as the Orchidaceae, have radiated mainly in floral characters (reflecting adaptations to pollinators). Similarly, Carson (1985) has suggested that the environmental determinants of speciation in plants are reflected in the patterns of radiation in vegetative and floral character sets, respectively. I attempted to use this approach to explore the relative contributions of the physical and pollination environment in the diversification of Cape plants (Table 8.1). In practice, I found that most taxonomists are not explicit about which characters are used to delimit species; therefore, the importance of vegetative versus floral characters in most genera can only be assessed qualitatively.

Growth environment-driven speciation

Numerous studies have shown how vegetative characters are correlated with parameters of plant growth and survival such as light, moisture, soil fertility, fire regime and herbivory. For example, leaf pubescence and small leaves are associated with aridity (Ehleringer & Clark, 1988). Clearly, if heterogeneity in the growth environment is responsible for plant diversification, this should be evident in radiation of vegetative characters (cf. Eckenwalder and Barret, 1986). Some plant adaptations to the growth environment are physiological, but these do not contribute to taxonomic diversity unless they are expressed in the plant morphology.

Table 8.1. A protocol for examining speciation in plants (after Carson, 1985).

Mode of differentiation	Evidence from plant characters	Possible Cape examples
Growth-environment driven	Radiation in vegetative characters eg. leaf shape, size, anatomy, spinescence, growth form, mode of nutrition, secondary compounds	*Cliffortia*, *Leucospermum*, *Rhodocoma*, *Aspalathus*, *Muraltia*
Pollinator- driven	Radiation in floral characters eg. Flower shape, size, colour, flowering time, nectar chemistry	*Disa*, *Satyrium*, *Erica*, *Gladiolus*, *Watsonia*

The most widely cited explanation for the extraordinary plant species richness of the Cape region is that taxa have differentiated in a mosaic of growth environments (Cowling, 1987; Cowling *et al*, 1990; Linder, 1985). This idea, which I will refer to as the "growth-environment hypothesis", is based on phytosociological data which have revealed high levels of species turnover between communities, and a perception that the physical environment in the Cape is unusually heterogenous. Cowling and coworkers have strongly advocated the role of substrate gradients in the differentiation of Cape taxa (Cowling, 1987; Cowling *et al*, 1990; Cowling & Holmes, 1992; Thwaites & Cowling, 1988). For instance, Cowling *et al* (1990) suggested that the rampant speciation in *Erica* (c. 600 species in the Cape) might reflect adaptive changes in host/root symbiont interactions across gradients of soil fertility.

The growth-environment hypothesis is supported by vegetative radiation in Cape genera such as *Rhodocoma* (Restionaceae), *Cliffortia* (Rosaceae), *Leucospermum* (Proteaceae), *Aspalathus* (Fabaceae) and *Muraltia* (Polygalaceae) (Rourke, 1972; Linder & Vlok, 1991). On the other hand, many Cape genera are characterised by a low diversity of growth forms and leaf types. The suggestion of Cowling *et al* (1990) that speciation in *Erica* is driven by adaptation to substrates is difficult to reconcile with the vegetative uniformity and floral diversity of the genus. Selective pressures at the root level alone could not have resulted in such a plethora of (taxonomically useful) floral characters as are found in *Erica* (cf. Rebelo *et al*, 1985; Oliver, 1991).

Pollinator-driven differentiation

A feature of the Cape flora is the diversity of flower forms within large genera such as *Erica* (Ericaceae), *Pelargonium* (Geraniaceae), *Geissorhiza*, *Gladiolus*, *Lapeirousia*, *Watsonia*, *Tritoniopsis* (all Iridaceae), *Disa* and *Satyrium* (Orchidaceae) (Vogel, 1954; Linder, 1981a; Goldblatt, 1991; Oliver, 1991). This suggests that adaptation to pollinators has been an important factor in the evolution of certain components of the flora.

Intraspecific variation in floral characters provides further evidence for the active role of pollinators in the diversification of Cape taxa. Some examples include variation of spur length in the *Disa draconis* complex (Chapter 5), red and orange forms of *Disa ferruginea* (Chapter 2), white-scented and yellow-unscented forms of *Hesperantha falcata* (Goldblatt, 1991), brown-scented and reddish-unscented forms of *Gladiolus maculatus* (Goldblatt, 1991) and allopatric yellow, white and red forms in the *Disa tripetaloides* complex (Linder, 1981a).

DETERMINANTS OF FLORAL SHIFTS

It is commonly accepted that divergence in floral characters results from adaptive shifts in the pollination system (Grant & Grant, 1965; Ornduff, 1969; Stebbins, 1970; Pellmyr, 1986; Paulus & Gack, 1990; Robertson & Wyatt, 1990). In this thesis I have shown through studies of convergence and mimicry that certain floral traits in the Cape flora are adaptive to specific pollinators (Chapter 2 & 3). I have also shown, through a detailed study of natural history and phylogeny, that cladogenesis in the genus *Disa* is associated with shifts between pollinators (Chapter 4 & 5). The actual processes involved in floral evolution are subtle, however, and not yet fully understood. Generally, it is thought that floral shifts require both a spatial mosaic in the pollination environment and also selective pressure on floral characters (cf. Stebbins, 1970; Arroya *et al*, 1982; Cruden, 1972b; Herrera, 1988; Robertson and Wyatt, 1990; Chapters 5 & 6).

Spatial heterogeneity in pollinator availability has been demonstrated in several studies (Arroya *et al*, 1982; Cruden, 1972b; Herrera, 1988; Robertson and Wyatt, 1990). The prediction that similar pollinator gradients occur in the mountainous Cape region is upheld by the few existing studies (Chapter 5 & 6) and this would be an interesting topic to explore further. Oleson (1992) has reviewed evidence which supports Stebbin's (1970) hypothesis that shifts to other pollinators often take place when plants face a different pollination environment at the edge of their distribution range.

I now turn to consider the selective pressures that lead to floral shifts between pollinators. There are a number of competing hypotheses for such shifts, including (1) selection for isolating mechanisms, (2) selection through male fitness and (3) selection through female fitness.

The isolation hypothesis

The literature on pollination and plant speciation has been heavily influenced by the biological species concept (Carson, 1985; Grant, 1993, 1994). This has led to a preoccupation with the role of flowers in maintaining genetic isolation among congeners (Grant, 1948, 1993, 1994; Whalen, 1978; Chase, 1986). The traditional concept of plant speciation, though accepting a plurality of species concepts, has been heavily influenced by Dobzhansky's notion of "reinforcement" (Dobzhansky, 1937). According to this view, speciation occurs as follows: allopatric populations first adapt to a novel growth environment; then, following secondary contact of the populations, flowers are modified to prevent erosion of the adapted genotype through hybridization. Proctor (1978), for instance, considered reproductive isolation a "potent factor in the evolution of floral diversity". It is interesting to note, however, that in a recent review Grant (1994) concluded that the majority of floral isolating mechanisms arise as incidental by-products of allopatric divergence, whereas in an earlier paper he had placed heavy emphasis on the role of reinforcement (Grant, 1948).

Is selection for isolating mechanisms important in floral evolution or is it a special case? In the Cape, it is not difficult to find examples where the integrity of sympatric sister species depends on floral divergence. For instance, floral features which attract distinct pollinators prevent hybrids between the sympatric species *Disa bivalvata* and *Disa atricapilla* (Steiner *et al*, 1994, Appendix B) as well as between *Crassula coccinea* and *Crassula fascicularis* (Johnson *et al*, 1993). These examples are ambiguous with respect to reinforcement, however, as it is not clear whether the "isolating mechanisms" evolved directly through selection or, alternatively, as a pleiotropic consequence of other selective pressures on the pollination system (cf. Grant, 1994).

The Achilles heel of the isolation hypothesis is that it cannot account for floral shifts during allopatric speciation (Paterson, 1985, 1986; Willson, 1991). Clearly, explanations other than selection for isolating mechanisms have to be sought to explain the enormous floral diversity in Cape families such as the Iridaceae which have mainly speciated in the allopatric mode (Goldblatt, 1991). Mayr and other proponents of the biological species concept recognized that isolating mechanisms may originate in allopatric populations, but they usually invoked pleiotropic explanations (cf. Mayr, 1992). Paterson (1985), however, has pointed out that direct selection for more effective mating systems, rather than pleiotropy, may explain the divergence in reproductive features of allopatric animal populations (see also Carson, 1985). Below, I consider Paterson's proposal for mating systems shifts from a botanical viewpoint by examining how selection might favour increased efficiency of the male and female function of flowers.

The male fitness hypothesis

The male fitness hypothesis for floral shifts has had a strong influence on evolutionary pollination ecology over the last 15 years (Charnov, 1979; Willson, 1991). There have been various attempts to show that selection favours floral traits which improve pollen export, but these have often been inconclusive (Wilson *et al*, 1994). Recently, Willson (1991) attempted to demonstrate the importance of male fitness indirectly, by showing that many plant genera show radiation in male rather than female floral traits. A weakness of her study was that the important perianth characters of hermaphrodite plants cannot be considered exclusively male or female, despite Bell's (1985) generalization that the display of hermaphrodite flowers is primarily male in function. Selection on floral traits through male fitness probably occurs mainly in plants growing in pollinator-saturated situations where plants compete for pollen access to a limited number of ovules.

The female fitness hypothesis

There is increasing evidence that pollen-limitation of fecundity is widespread among plants (cf. Bierzychudek, 1981b; Burd, 1994; Chapter 7). This has important consequences for our understanding of floral evolution: if plant fitness is limited by pollen availability, then selection will favour traits which maximise pollination effectiveness above traits which protect genetic integrity or facilitate pollen export (cf. Levin, 1971; Kiester *et al*, 1984; Wilson *et al*, 1994). Pollen limitation of fecundity may be due to several factors including natural low abundance of pollinators, competition between plants for attraction of pollinators and reproductive interference, such as pollen wastage and stigma clogging between sympatric plants as a result of pollinator infidelity (Waser, 1983; Armbruster, 1985). Levin (1971) was among the first to recognise that reproductive interference, rather than erosion of the genome through hybridization, may have been an important factor in floral evolution, particularly the trend towards specialization.

Experiments have shown that there may be direct selection for traits which improve the effectiveness of pollination (Campbell, 1991; Herrera, 1990; Johnston 1991a). This information, combined with the amazing results of artificial breeding of horticultural varieties, suggests that floral characters can be rapidly influenced by selection when pollinator visits determine fitness (cf. Gill, 1989).

The finding that pollination success is low in Cape orchids and that 11 out of 12 herbaceous wildflower species showed chronic pollen limitation of fruit set (Chapter 7), indicates that the female fitness hypothesis for floral shifts may be most applicable to speciation in the non-shrubby component of the Cape flora. It is also possible that fecundity of Cape shrubs is frequently pollen-limited, but I have no data to support this.

Conclusion

Adaptive shifts in pollination systems probably explain the floral diversity evident in many large Cape genera. Various hypotheses for floral shifts were discussed, of which selection for increased efficiency of the female function of flowers was held to be most appropriate for explaining floral shifts in herbaceous genera such as *Disa*. Pollinator-driven speciation has certainly been underrated in the Cape flora. However, the conventional view that speciation is promoted by adaptation to the physical environment may still be applicable to those genera, mostly shrubs, which have radiated in vegetative characters. The method of distinguishing between vegetative and floral character radiation (Table 8.1) is an exploratory, heuristic tool for inferring the environmental determinants of speciation when other data are not available. It should not, therefore, be seen as a substitute for detailed process studies of character evolution at both an intraspecific and interspecific level.

SUMMARY

1. The aim of this thesis was to show how patterns of convergence, mimicry and radiation in the floral traits of Cape plants result from adaptation to pollinators.

2. The adaptive significance of the large, red flowers which have evolved in several Cape lineages was studied. The only observed pollinator of these species was the Mountain Pride butterfly, *Meneris tulbaghia*. It is shown that the convergent floral traits in these species reflect the morphology and foraging preferences of the Mountain Pride butterfly, which chose large, red model flowers over other sizes and colours.

3. Floral mimicry is often invoked to explain cases of floral similarity, but has seldom been verified. The hypothesis that the non-rewarding orchid *Disa ferruginea* secures pollinator visits by imitating flowers of the nectar-producing species *Tritoniopsis triticea* and *Kniphofia uvaria* was tested explicitly. The Mountain Pride butterfly - which pollinates the orchid - relies on the models for nectar, and it was shown by experiment that the butterfly is unable to discriminate between the non-rewarding orchid and the rewarding models. The fitness of the orchid is also higher in the presence of one of the models than when it grows alone.

4. One of the most striking features of the Cape flora is the floral diversity evident in many of the large genera. The hypothesis that radiation in the genus *Disa* (Orchidaceae) has resulted from floral shifts between pollinators was examined. Very little was previously known about pollination in this genus, hence detailed new descriptions of pollination in nearly 20 *Disa* species are provided. This demonstrated the extraordinary diversity of pollination systems in the genus, including pollination by butterflies, settling moths, hawkmoths, bees, wasps, short-proboscid flies, long-proboscid flies and also autogamy. Many of the species were highly specialised, being pollinated by a single insect species.

6. A cladogram, based on morphological characters, was used to establish a phylogenetic hypothesis for the evolution of pollination systems in *Disa*. Mapping pollination systems onto this phylogeny showed that the pollination systems in *Disa* are very labile - unlike many genera which are pollinated mainly by one group of insects, the cladogenesis of *Disa* is dominated by floral shifts between pollinators. Another feature of the phylogeny was the convergent evolution of similar pollination systems in different clades within *Disa*.

8. Adaptive evolution of a single floral trait, spur length, was studied in populations of the *Disa draconis* species complex. It was found that a shift between a short-tongued fly in the mountains and a long-tongued fly on the lowlands explains some of the variation in spur length, but no pollinator was found that might account for the very long spurs of populations in the northern mountains.

7. Floral shifts may occur when a plant colonizes habitats which are unfavourable for its existing pollinator. The implication of habitat preference of a pollinator for the reproductive success of a Cape orchid, *Disa uniflora*, was studied over a four year period. Fruiting success of the orchid was shown to be significantly lower in a habitat which is avoided by its butterfly pollinator, relative to a habitat which is preferred by the butterfly.

8. The hypothesis that fecundity in Cape wildflowers is commonly limited by pollinator availability was examined. Pollination success of a large sample of terrestrial orchid species was low (c. 25% of flowers) and fruiting success of eleven out of twelve herbaceous species was found by hand-pollination experiments to be pollen-limited. Limited pollinator availability may explain why shifts in pollination system have occurred frequently in many Cape genera.

9. It is concluded that floral diversification through pollinator shifts is promoted by environments in which there is spatial heterogeneity in pollinator availability and pollen-limited plant fecundity.

10. The extensive floral diversity found in many large Cape genera provides indirect evidence that pollination system shifts have been an important mode of speciation in the region.

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Appendices

Appendix A

Journal of Ecology 1992,
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Climatic and phylogenetic determinants of flowering seasonality in the Cape flora

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Summary

1 Flowering patterns in the species-rich and largely endemic flora of the southern and south-western Cape Province, South Africa, are described.

2 Analysis of the flowering times of 7075 Cape species (83% of the flora) showed that flowering peaks in spring and then gradually tails off, reaching a trough in autumn and early winter.

3 Flowering seasonality varies along a climatic gradient from winter rainfall in the west, where species show a spring peak, to nonseasonal rainfall in the east, where species show an early summer flowering peak.

4 At least 20% of the flora in the winter rainfall half of the Cape region is in flower at any time of the year. This contrasts with other winter rainfall regions worldwide where very little flowering takes place outside of spring.

5 Strong differences in flowering seasonality between some families and some genera were found, particularly in monocotyledons.

6 Differences in flowering time among lineages are often attributable to the degree to which flowering is constrained by the timing of other phenophases such as growth, seed dispersal and seed germination. Lineages in which these phenophases are uncoupled show more flexibility in flowering time.

Keywords: Cape flora, fynbos, Mediterranean type ecosystems, phenology, phylogenetic constraints.

Journal of Ecology 1993, **81**, 567–572

Introduction

Flowering time is a trait which could be critical to plant success through its effect on reproductive processes such as pollination and the timing of seed dispersal. Optimal flowering time may be a trade-off between a variety of selective factors including pollinator availability (Waser 1979), pollinator competition (Rathke & Lacey 1985), moisture availability (Bell & Stephens 1984; Zimmerman, Roubik & Ackerman 1989) and conditions for seed germination and seedling establishment (Burtt 1970; Pierce 1984). In addition there is evidence that flowering time may be conservative within a lineage, reflecting shared 'phylogenetic constraints' which limit the ability of directional selection to influence flowering times (Kochmer & Handel 1986; Zimmerman, Roubik & Ackerman 1989).

The purpose of this study was to investigate the patterns of flowering in the Cape flora in relation to rainfall seasonality and phylogenetic affinity of species. The distinctive phytogeography of the Cape flora (68% of the 8504 species are endemic to an area of only 90 000 km^2) has led to its recognition by some as one of the world's six floral kingdoms (e.g. Takhatajan 1986).

Methods

This study was based on a descriptive catalogue of the Cape flora (Bond & Goldblatt 1984). Flowering patterns in the flora were analysed using the beginning and end dates of flowering which are provided for most of the 8504 species listed in the catalogue. Species with incomplete flowering information were excluded, as were the graminoids (Restionaceae, Poaceae and Cyperaceae) because records are sparse and unreliable for these wind-pollinated families (H. P. Linder, pers. comm.).

Flowering patterns of the remaining 7075 species (83% of the flora) were analysed using two different methods. The first ('ranges method') simply involves calculating the number of species in flower during each month (cf. Specht *et al.* 1981; Bell & Stephens 1984; Pierce 1984). In this method the entire range of flowering of each species contributes to the overall pattern. The second ('means method') involves estimating the flowering peak of each species (by calculating the midpoint between the beginning and end dates of flowering) and then determining the number of species in *peak* flower during each month (cf. Anderson & Hubricht 1940; Kochmer & Handel 1986). In this method, only the flowering midpoint of each species influences the curve. An assumption of the second method is that the midpoint and peak of flowering in a species are equivalent, this is not always the case (cf. Thompson 1980).

RAINFALL SEASONALITY AND FLOWERING TIME

The Cape floristic region includes two climatic regions: the south-western Cape, which has a strictly winter rainfall (mediterranean) climate, and the southern Cape, which has a nonseasonal rainfall climate (Deacon, Jury & Ellis 1992). The boundary between these regions is taken here to be 21°3′E, a line of longitude which passes through the Riversdale and Ladismith districts (Fig. 1). In order to determine the flowering patterns in these two climatic regions, separate flowering curves were calculated for 5781 species which occur west of 21°3′E (winter rainfall), and 2742 species which occur east of 21°3′E (nonseasonal rainfall). An additional analysis excluded the 1429 species which occur in both rainfall regions. This analysis thus involved subsets of 4352 and 1313 species restricted to the regions west and east of 21°3′E, respectively.

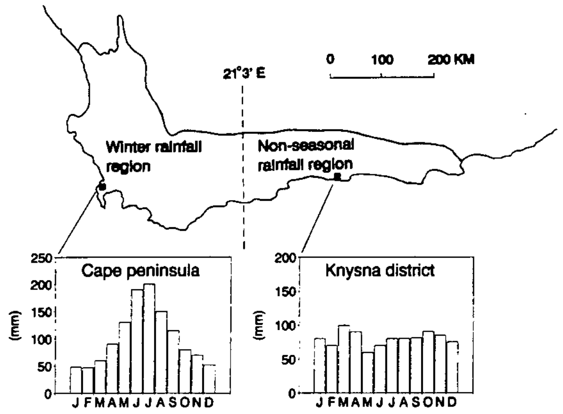

Fig. 1 The Cape floristic region (indicated by a solid line) with representative rainfall patterns for the two climatic regions (after Cowling & Holmes 1992)

PHYLOGENY AND FLOWERING TIME

To determine the influence of phylogeny on flowering time, the data set was analysed at a number of taxonomic levels. These included comparing (i) monocotyledons with dicotyledons, (ii) major families, (iii) genera within one family, the Amaryllidaceae and (iv) a detailed analysis of flowering within the genus *Gladiolus* (Iridaceae). Lewis & Obermeyer (1972) was consulted for additional data on the phenology of *Gladiolus*.

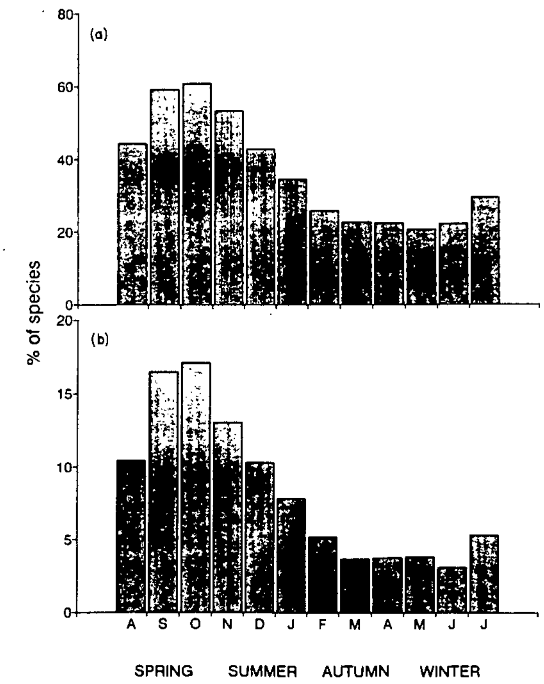

Fig. 2 Flowering seasonality in the Cape flora based on two different methods of analysis (see text): (a) ranges; (b) means.

Results

RAINFALL SEASONALITY AND FLOWERING TIME

The Cape flora as a whole shows a flowering peak in spring (Fig. 2). Subdivision of the flora into species occurring either side of 21°3′E revealed a 1-month difference in the peak of flowering: the flora of the winter rainfall region shows a peak in September–October (spring), while the flora of the nonseasonal rainfall region shows a peak in October–November (late spring/early summer) (Fig. 3). When species restricted to either side of 21°3′E were analysed separately, the effect of rainfall seasonality was more apparent. This is seen particularly in the means-based analysis which showed that the percentage of species in peak flower in the nonseasonal rainfall region remains high throughout late spring and summer (October–January), unlike the spring flowering peak (September-October) of species restricted to the winter rainfall region (Fig. 4). Because most of the Cape flora occurs in the winter rainfall region, the spring flowering peak for the Cape flora as a whole is due mainly to the contribution of species from this region.

PHYLOGENETIC AFFINITY

Monocotyledons in the Cape flora flower for shorter periods than do dicotyledons (Fig. 5), although the seasonality of flowering in these groups is similar (Fig. 6a). Analysis at the family level revealed striking differences in flowering times. For example, the near exclusive spring flowering of Proteaceae is in

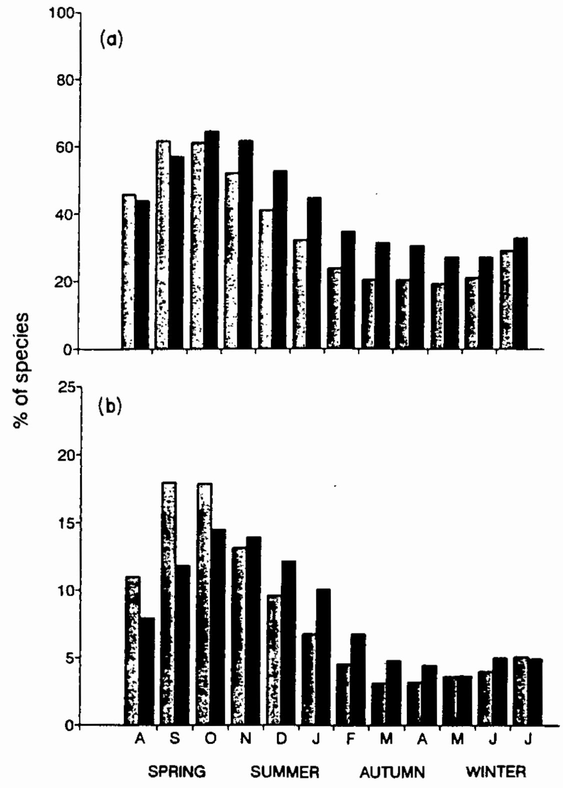

Fig. 3 Flowering patterns in the (□) winter rainfall region (west of 21°3′E; $n = 5781$) and (■) the nonseasonal rainfall region (east of 21°3′E; $n = 2742$): (a) data from ranges; (b) data from means.

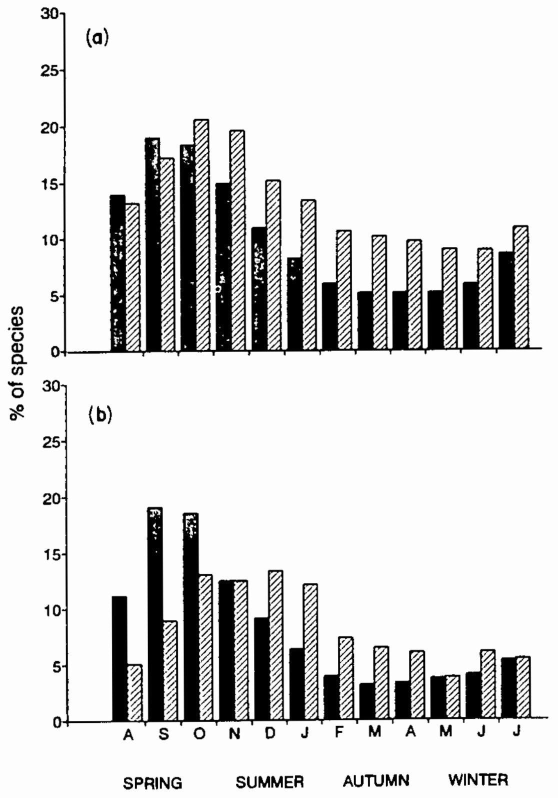

Fig. 4 Flowering patterns of species restricted to (□) the winter rainfall region (west of 21°3′E; $n = 4352$) and (▨) the nonseasonal rainfall region (east of 21°3′E; $n = 1313$): (a) data from ranges; (b) data from means.

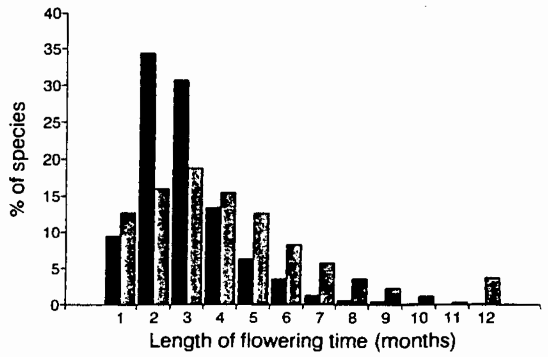

Fig. 5 A frequency distribution of the length of flowering i (□) dicotyledons ($n = 1304$) and (■) petaloid monocotyledons ($n = 5771$) in the Cape flora.

marked contrast to the year-round flowering of Ericaceae which show a mild peak in spring (Fig. 6b) Monocotyledon families show a clear sequence o flowering from Iridaceae in spring, through Orchidaceae in early summer, to Amaryllidaceae in autumr (Fig. 6c). Flowering seasonality differs at a generi level within the Amaryllidaceae – most genera flower in autumn, but summer flowering occurs in th dry-seeded *Cyrtanthus* and in *Gethyllis*, which ha an underground ovary (Fig. 7). The flowering patter in *Gladiolus* is bimodal; species with synanthous foliage (leaves present with flowers) are restricted t flowering in spring, but species with hysteranthou foliage (leaves not present with flowers) are capable of flowering in autumn (Fig. 8).

Discussion

RAINFALL SEASONALITY AND FLOWERING TIME

The Cape Floristic Region is one of five regions world-wide which have a predominately winter rainfall climate. The spring flowering peak of the Cape flora is typical of these regions, but flowering never drops to the low summer levels evident in south-western Australia or both low summer and winter levels evident in Israel, Chile and southern California (Bell & Stephens 1984; Shmida & Dafni 1989; Keeley 1992). At least 20% of the Cape flora is in flower during each month of the year (Fig. 2).

Insufficient moisture for physiological activity is probably the major factor which limits summer flowering in mediterranean climate regions. Plants which flower well into the dry season in the Cape are often those which occupy marsh or streambank communities (personal observation). Summer flowering is relatively common in the nonseasonal rainfall region where at least 30% of the flora is in flower during any particular month of the year (Figs 3 and 4). The evidence for the greater phenological flexibility afforded by the nonseasonal rainfall of the southern Cape is reinforced by intrageneric comparisons in

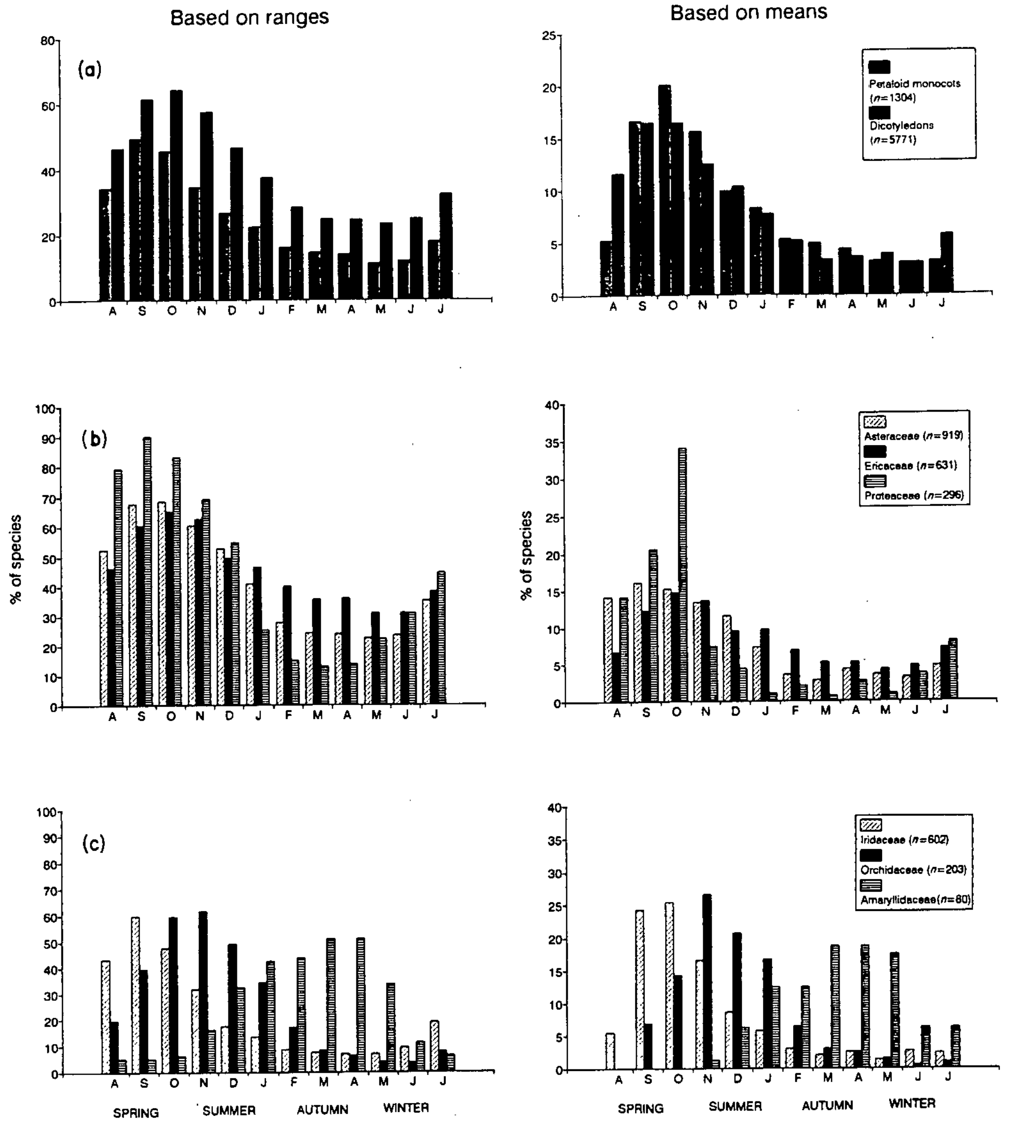

Fig. 6 Flowering seasonality in important taxonomic groups in the Cape flora: (a) dicotyledons and petaloid monocotyledons; (b) representative dicotyledon families; (c) representative monocotyledon families.

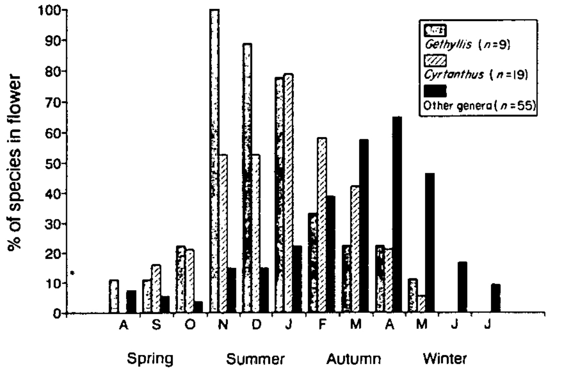

Fig. 7 Flowering patterns of genera of the Cape Amaryllidaceae showing that *Gethyllis* and *Cyrtanthus* manage to flower well before the advent of the winter rains. The analysis was based on the range of flowering in the species (see text).

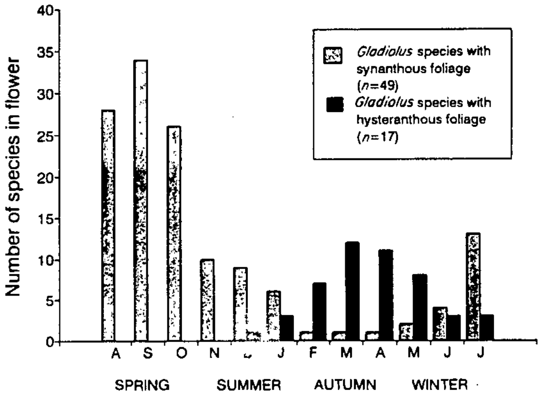

Fig. 8 A comparison of flowering seasonality in *Gladiolus* species with synanthous foliage (leaves present when flowering) and those with hysteranthous foliage (leaves absent when flowering). The analysis was based on the range of flowering in the species (see text).

Protea, *Leucospermum*, *Leucadendron* (all Proteaceae) and *Erica* (Ericaceae), all of which show less flowering seasonality in the southern Cape relative to the western Cape (Pierce 1984).

It could be argued that the relatively high percentage of the Cape flora in flower during summer simply reflects the influence of the nonseasonal rainfall region. However, the flora of the western half of the region (which has strictly winter rainfall) shows a similar high percentage of summer flowering (Figs 3 and 4). Even species restricted to the Cape winter rainfall region show less seasonality in their flowering than species of other winter rainfall regions. Summer cloud condensation on Cape mountains during south-easterly winds has long been thought to be a major factor which allows the relatively high proportion of species to flower during summer, despite the low summer rainfall (Marloth 1905). Fynbos plants do not generally suffer summer water stress as severely as plants in other mediterranean climate regions (Stock, Van der Heyden & Lewis 1992).

There is evidence that the difference in flowering seasonality between the winter and nonseasonal rainfall regions of the Cape influences the reproductive phenology of insect pollinators. According to Hepburn & Jacot Guillarmod (1991) colony reproduction in the Cape honeybee (*Apis mellifera capensis*) occurs in early spring in the winter rainfall region and in early summer in the nonseasonal rainfall region, thus coinciding with the flowering peaks in these two regions.

PHYLOGENY AND FLOWERING TIMES

Optimistic adaptive explanations for traits of organisms have given way, in recent years, to a more cautious consideration of the role of design and developmental constraints in determining the outcome of selection (Gould & Lewontin 1979; Kochmer & Handel 1986). Related species are expected to show similar flowering patterns as they share similar inherited design constraints which limit directional selection on flowering time.

The tendency for taxonomically related species to show similar flowering times and conversely for unrelated species to show differences in flowering time has been reported for Kwongan species (Bell & Stephens 1984) and temperate North American species (Kochmer & Handel 1986). The results of this study show the strong influence of taxonomic affinity on flowering time in the Cape flora.

For example, monocotyledons and dicotyledons were found to differ strongly in the length of flowering time (Fig. 5). The short flowering times of monocotyledons is readily explained by a design constraint: their greatly reduced underground stem usually results in the production of a single inflorescence with relatively few buds. Dicotyledons, on the other hand, usually have an elongated and richly branched shoot system on which bud development can be adjusted so that flowering is extended over many months or a full year in some species.

In the discussion that follows, an attempt is made to identify tangible design constraints which may explain the differences in flowering patterns between taxa. The approach taken is to consider the degree to which flowering is coupled to the other phenophases of growth, seed dispersal and seed germination. The ability of plants to track pollinator abundance or escape pollinator competition may be restricted if flowering is coupled to other phenophases (cf. Zimmerman, Roubik & Ackerman 1989).

Coupling of flowering and growth

Uncoupling of flowering and growth is most readily observed in those petaloid monocotyledons which flower in a leafless condition during the dry season. This phenomenon is common in geophytes of the Cape flora and Israel (Dafni, Shmida & Avishai 1981). The storage organs (bulbs, tubers or corms) of geophytes facilitate the separation of leafing and flowering in time. The flowering flexibility afforded by uncoupling of leafing and flowering is clearly evident in *Gladiolus*; those species capable of flowering in a leafless condition, do so in autumn at the end of the dry season (Fig. 8).

Uncoupling of the leafing and flowering phenophases may permit geophytes to flower when pollinators are in peak abundance or to escape pollinator competition in the spring (Dafni & Werker 1982). In the fynbos, the seasonal occurrence of the butterfly *Meneris tulbaghia* (Satyridae) at the end of the dry season is successfully 'tracked' by many geophytes which are pollinated exclusively by this butterfly (Johnson 1992). Many of these geophytes grow in dry habitats and manage to flower in late summer by uncoupling leafing and flowering.

Coupling of leafing and flowering may, on the other hand, restrict flowering to the wet season. Zimmerman *et al.* (1989) invoked growth form constraints to explain why the neotropical orchid *Catesetum viridiflavum* does not flower in synchrony with the peak abundance of its euglossine bee pollinator in the dry season. Unlike many other orchids in Panama which flower in the dry season from pseudobulbs produced the previous year, the constraint of flowering from a pseudobulb produced from the current year's growth restricts *Catesetum viridiflavum* to flower at the end of the wet season.

Coupling of flowering with seed dispersal and germination

Coupling of flowering with seed dispersal and germination is readily apparent in the Amaryllidaceae. The autumn flowering peak for Cape Amaryllidaceae contrasts sharply with other petaloid monocotyledon families which flower mostly in spring (Fig. 6c). This difference can be ascribed to the lack of dormancy in the fleshy seeds of most Amaryllidaceae (Markotter 1936), a design constraint which restricts flowering and seed dispersal to the autumn period shortly before the winter rains. Two genera within the Amaryllidaceae, however, have evolved traits which facilitate uncoupling of flowering and seed germination. In *Cyrtanthus* the seeds are covered with a thin crust of phytomelan. This derived trait (Dahlgren & Rasmussen 1983) permits dormancy and therefore flowering can occur in summer, rather than autumn (Fig. 7). In *Gethyllis* flowering is possible in midsummer as the fleshy seeds are protected in an underground ovary until the start of the rainy season; a ripe berry then emerges above ground and releases the seeds. An underground ovary is found in many monocotyledon genera and is nearly always associated with storage of seeds over the dry season (Burtt 1970).

CONCLUDING NOTES

It has been possible to attribute only some of the phylogenetic patterns of flowering time in the Cape flora to obvious design constraints. For most of the flora, particularly dicotyledons, more research into phenophase coupling and pollinator interactions is required before the flowering patterns can be adequately explained.

The strong effects of phylogenetic affinity on flowering time have several implications for the interpretation of community flowering patterns. Staggered flowering times in plant communities are often considered to reflect adaptive displacement of flowering times due to competition for pollinators (Cole 1981; Rathke & Lacey 1985). An alternative hypothesis, however, is that staggered flowering times simply reflect the wide range of phylogenies represented in a community. Competition for pollinators could result in a process of species sorting among fairly inflexible lineages, rather than natural selection on flowering time.

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FLORAL AND POLLINATOR DIVERGENCE IN TWO SEXUALLY DECEPTIVE SOUTH AFRICAN ORCHIDS[1]

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Two sister species in *Disa* section *Disa* were studied to evaluate the effect that minor differences in floral color, shape, and scent have on pollination. *D. atricapilla* and *D. bivalvata* have overlapping distributional ranges, occupy similar habitats, flower at the same time, and often occur sympatrically. Observations at several sites indicate that each species is pollinated almost exclusively by male wasps. *D. atricapilla* is pollinated by *Podalonia canescens* (Sphecidae), while *D. bivalvata* is pollinated mainly by *Hemipepsis hilaris* Smith (Pompilidae). Both wasp genera appear to exhibit mate-seeking behavior when approaching and visiting flowers. This together with the absence of a floral reward suggests that *D. atricapilla* and *D. bivalvata* are pollinated through sexual deception. Pollination success often appears low, but because of long floral life spans, overall fruiting success is generally high.The occasional presence of hybrids in areas of sympatry is attributed to secondary beetle pollinators. The rarity of these hybrids indicates that prefertilization barriers between the species are usually strong. This is the first report of pollination through sexual deception for southern Africa.

Some of the most fascinating examples of floral adaptation are found among the orchids (van der Pijl and Dodson, 1966; Nillson, 1988; Peakall, 1989; Borg-Karlson, 1990; Dafni and Bernhardt, 1990; Paulus and Gack, 1990b, Robertson and Wyatt, 1990). The diversity of ways in which orchids are pollinated, even within monophyletic groups, makes them particularly well suited for studies linking changes in floral morphology to pollinator shifts and speciation.

The relatively large number (ca. 391 spp.) of terrestrial orchids in South Africa provides a rich potential source of new information about adaptive radiation in particular and about orchid pollination in general. Pollinators are known for less than 7% of the species. In *Disa*, the largest South African orchid genus (90 spp. or ca. 21% of the orchid flora), pollinators are known for only six species and these include butterflies, various types of bees, and small midgelike flies (Marloth, 1895; Stewart and Manning, 1982; Johnson, 1992a, b, in press; Johnson and Steiner, unpublished data). Considering the variation in floral size, color, scent, morphology, and flowering time within *Disa*, these pollination studies reflect only a fraction of the diversity in pollinator adaptations present within the genus.

To understand the adaptive nature of floral variations within *Disa*, it is necessary first to factor out those morphological traits or characters that are due to shared ancestry (historical factors sensu Brooks and McLennan, 1991). This is best accomplished by identifying sister species or small monophyletic assemblages where divergence in floral characters among the species are likely to be the result of selection by different pollinators or groups of pollinators.

Closely related species pairs that differ in floral features are fairly common in *Disa*. We chose to study *D. atricapilla* (Lindl.) Bolus and *D. bivalvata* (L.f.) Dur. and Schinz, the only two species in series *Complanae* of *Disa* section *Disa*, because they form a clade defined by the presence of a compressed galeate dorsal sepal. Differences between these species are minor, yet striking (Figs. 1, 2). Linder (1981) was aware that differences between the two species might be considered trivial, but he emphasized that these could "be of great importance to the pollinating agents" (see below).

The purpose of our study was to determine whether these small floral differences between the species were reflected in different pollinator profiles and, if so, to determine whether pollinators restrict their visits to a single *Disa* species in areas where the two species occur in sympatry.

Disa bivalvata and *D. atricapilla* are terrestrial geophytic orchids endemic to fynbos vegetation in the Cape region of South Africa. Flowering in both species is strongly stimulated by fire, with the largest number of individuals flowering on sites burned the previous summer or autumn. Flowering populations of both species can be quite small (one to 20 individuals) or very large (>1,000 flowering individuals), but large populations are more common for *D. bivalvata* than for *D. atricapilla*. *D. bivalvata* populations flower almost entirely in the first season after fire (Hall, 1959; Johnson, personal observation), whereas *D. atricapilla* populations can flower in subsequent seasons as well (Steiner, personal observation).

Disa bivalvata and *D. atricapilla* have overlapping geographic ranges, but *D. bivalvata* extends further eastward and *D. atricapilla* is more common in the inland mountains to the north (Fig. 3). The two orchids occupy very similar habitats, usually seasonally wet seeps or swamps, and where their distributions overlap, they often grow intermixed. Both species flower from October to February, and even within a particular site, flowering times can be strongly overlapping.

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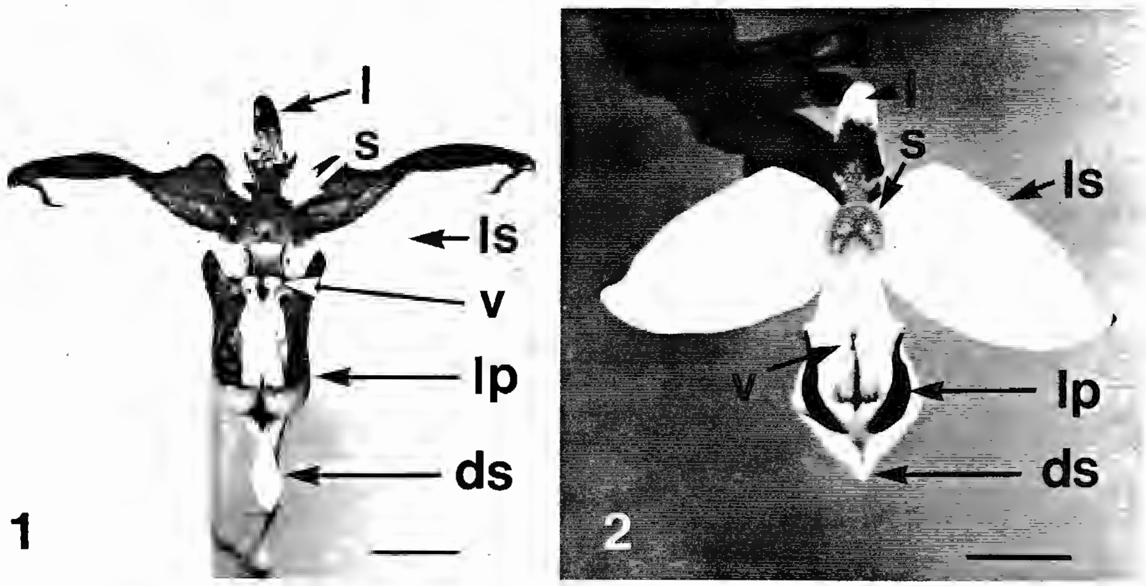
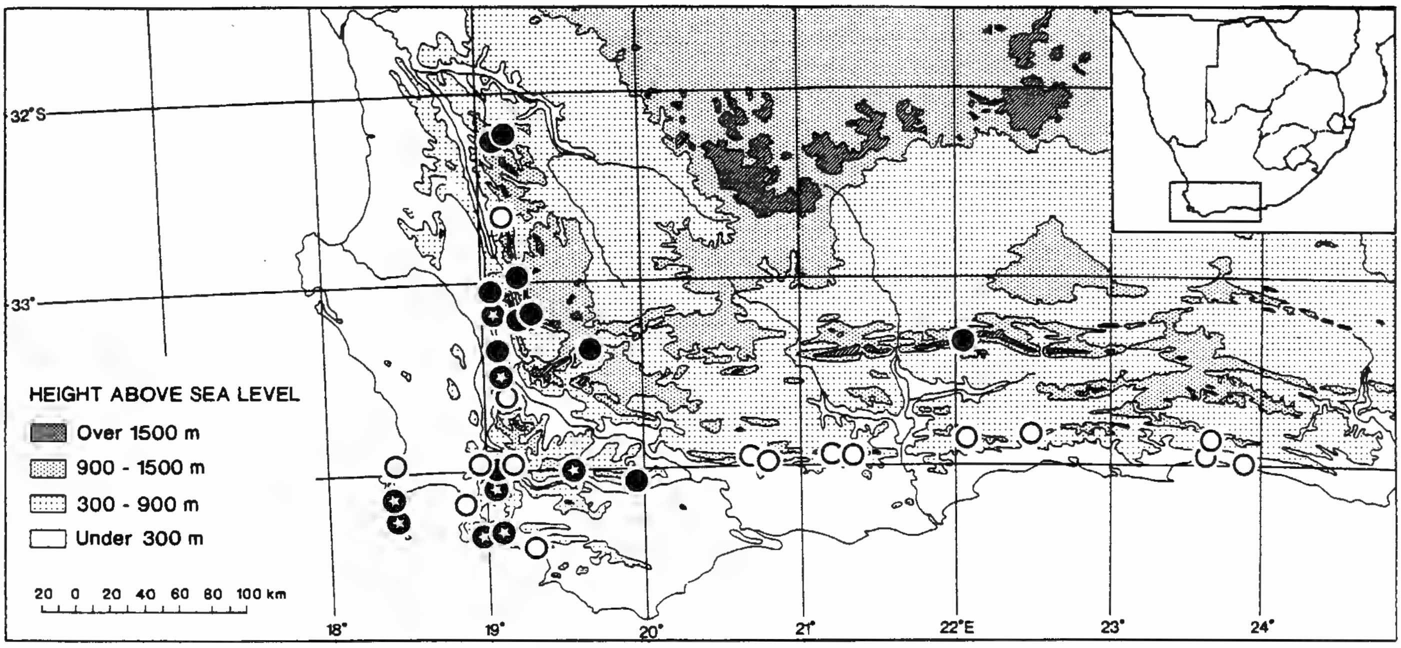


Figs. 1, 2. Flower close-ups of **1.** *D. atricapilla* and **2.** *D. bivalvata.* ds = dorsal sepal; l = lip; lp = lateral petal; ls = lateral sepal; v = viscidium; s = stigma. Bars = 1 cm.

The two orchids vary in height from 50 to 450 mm and are almost identical vegetatively, but differences primarily in shape, orientation, and color of the lateral sepals make them quite distinct. In *D. bivalvata,* the lateral sepals are flat or shallowly concave and deflected away from the lip. They are uniformly white or white with a green or brown midrib on the upper surface and white with brown or dark maroon tips on the lower surface (Fig. 2). In *D. atricapilla* the lateral sepals are more concave, have a distinct twist in the distal half, and are perpendicular to or inclined toward the lower lip. The upper surface is split longitudinally into two colors, red or maroon and white or greenish-white. The lower surface is divided horizontally into a very shiny bluish-black distal half and a greenish-white basal half (Fig. 1). The shiny black surface is not very conspicuous when the flower is open, because it faces downward or inward, but when the flower is closed (either in bud or after flowering), it is very conspicuous (Fig. 4). It is this feature, together with the bicolored lateral sepals, that makes *D. atricapilla* strikingly different from *D. bivalvata.*

The coloration of the winglike lateral petals is also usu-



Figs. 3. Distribution of *D. atricapilla* (closed circles) and *D. bivalvata* (open circles) in the Cape of South Africa. Closed circles with stars are locations where the two species are known to co-occur. Insert shows location of main map in relation to southern Africa.

TABLE 1. Collecting localities for wasp pollinators of *Disa atricapilla* and *D. bivalvata*.

Location	Coordinates		Pollinator[a]	Host plant
Farm Onderboskloof	32°58′48″S	19°12′36″E	*P. canescens*	*D. atricapilla*
Bain's Kloof, Montagu Rocks	33°36′12″S	19°07′30″E	*P. canescens*	*D. atricapilla*
			H. hilaris	*D. bivalvata*
Bain's Kloof, Waterfall	33°37′30″S	19°08′24″E	*H. hilaris*	*D. bivalvata*
Viljoen's Pass	34°04′54″S	19°03′30″E	*P. canescens*	*D. atricapilla*
			H. hilaris	*D. bivalvata*
Muizenberg Plateau	34°05′42″S	18°27′00″E	*P. canescens*	*D. atricapilla*
			H. hilaris	*D. bivalvata*
			H. capensis	*D. bivalvata*
Ysterklip	34°19′00″S	19°07′12″E	*P. canescens*	*D. atricapilla*
Cape Point	34°16′36″S	18°27′48″E	*P. canescens*	*D. atricapilla*
Swellendam	33°59′30″S	20°25′40″E	*H. hilaris*	*D. bivalvata*

[a] *P* = *Podalonia*; *H* = *Hemipepsis*.

ally different. In *D. bivalvata*, the petal is mostly maroon to black both inside and out with a white base and sometimes a white tip. The petals of *D. atricapilla* are greenish-white with small maroon spots on the inside surface that coalesce into irregular maroon patches at their distal tips (Figs. 1, 2). Less conspicuous are differences in lengths of the anther sacs and rostellum arms which cause the pollinaria to be noticeably larger in *D. atricapilla* than in *D. bivalvata*.

MATERIALS AND METHODS

Floral scent and UV pattern—Since floral scent has been shown to play an important role in orchid pollination biology, this was assessed qualitatively for both species and a hybrid by direct smelling of flowers and by smelling a concentrated aroma made by storing fresh flowers of each species for 30 minutes in small glass jars. To help visualize the scent-producing regions of the flower, *D. atricapilla* flowers were additionally stained in an aqueous solution of neutral red (1:10,000) for several hours (cf. Vogel, 1990).

In order to determine possible differences in ultraviolet reflectance, inflorescences of both species were photographed through a Corning 7-60 filter which transmits only long-wave ultraviolet light. A grey scale was made up as in Kevan et al. (1973) to control for proper exposure. Photographs were made on Ilford 400 ASA black and white film with an Olympus OM-4 camera equipped with a ring flash and a Zuiko 55 mm macro lens.

Floral development and breeding system—Floral development and breeding system of *D. atricapilla* were monitored throughout the 1992–1993 flowering season at Cape Point. For breeding system experiments, plants were bagged and flowers were either left unmanipulated as a control ($N = 11$), self-pollinated ($N = 37$), or cross-pollinated ($N = 38$). Seed set was evaluated by checking the presence of embryos in a sample of approximately 500 seeds per capsule from those capsules setting seed. Differences in percentages of embryo-filled seed between the two treatments that resulted in fruits (i.e. self- and cross-pollination) were checked with a one-way ANOVA after arcsine-transformation of the data.

Natural levels of pollination and fruit set—Natural levels of pollination in different populations and at different times during the season were checked. These values were compared to the fruit set determined at the end of the flowering period.

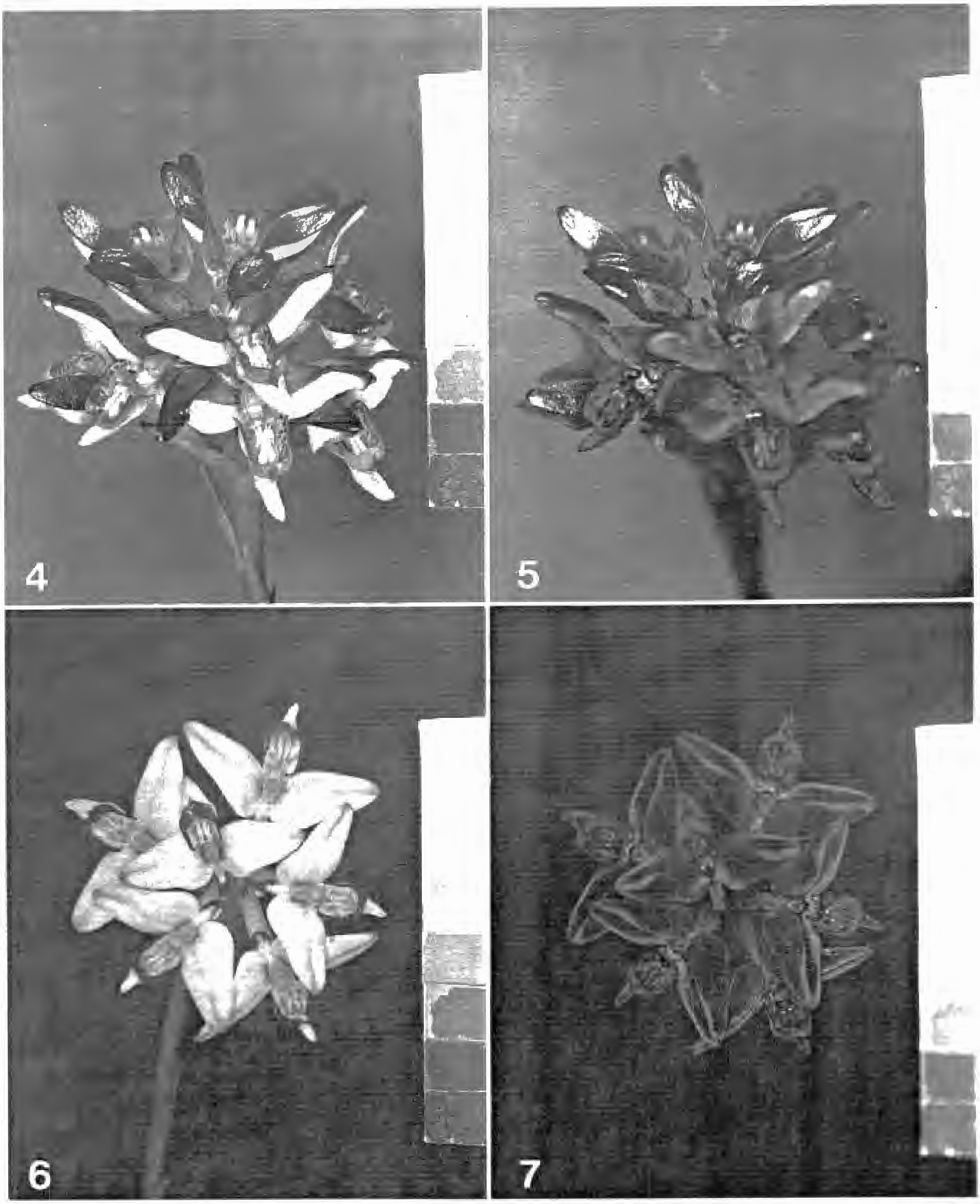
Pollinator observations—Observations and collections of *D. atricapilla* pollinators were made over a 3-year period in 1990–1991, 1991–1992, and 1992–1993 at six different sites in the Cape Province of South Africa (Table 1). Most of these observations were made on the slopes above Montagu Rocks in Bain's Kloof and on the Farm Ysterklip near the village of Kleinmond. At Montagu Rocks, observations were made weekly during the peak flowering period from mid-November to mid-December, 1990; at Ysterklip observations were made once in mid-December and three times during January, while at Cape Point observations were made biweekly during December and January 1992–1993. Pollinator observations of *D. bivalvata* were made mainly at Swellendam, Bain's Kloof, and the Muizenberg Plateau sites (Table 1).

Vouchers—Vouchers of two *Disa* species, a hybrid between them, and associated nectar plants utilized by the pollinators have been deposited in the Compton Herbarium, National Botanical Institute of South Africa. Insect vouchers are in the South African Museum, Cape Town.

RESULTS

Floral scent—Neither species produces any obvious food reward, but both give off a floral scent. The strong perfumy scent of *D. bivalvata* has been characterized as "very fragrant with the odour of ripening apples" (Bolus, 1918, p. 91). The scent of *D. atricapilla* is spicy with a hint of peppermint, but is very weak and difficult to detect unless concentrated in a small glass vial or jar. Flowers of the hybrid had an odor most similar to that of *D. bivalvata*. Three areas in the flower of *D. atricapilla* stained strongly with neutral red: the stigma, the auricles (flanking the base of the anther sacs), and the inner surface of the petals in the distal half where they come together above the anthers. Cutting up these three areas and isolating them in glass vials revealed that the floral scent was produced mainly by the petals

UV pattern—Both *D. atricapilla* and *D. bivalvata* are very strongly and uniformly ultraviolet absorptive (Figs.



Figs. 4–7. *Disa* inflorescences as they appear in the visual and UV spectrums. **4, 5.** *D. atricapilla.* **6, 7.** *D. bivalvata.* Grey scale on right of each photo made up as in Kevan et al. (1973) to control for proper exposure. All photographs were underexposed in printing to make the flowers more clearly visible. Bars = 4.9 cm.

TABLE 2. Pollination and fruiting success of *D. atricapilla* and *D. bivalvata*. Pollination success is the proportion of open flowers in the population that have been pollinated on a given date. It is based on a sample of one flower per plant. Since individual flowers remain open for 2–3 weeks, if unpollinated, pollination success is usually much less than fruiting success (the total percentage of fruits set per plant).

	Pollination success			Fruiting success			
Location	% Open flowers pollinated	(*N*)	Date	% Fruit set/plant ± SD	Plants (*N*)	Date	Approx. number of flowering individuals
			Disa atricapilla				
Bain's Kloof—M. R. 1	75.9	29	27 Dec. 1990	71.2 ± 24.8	29	3 Jan. 1991	50
—M. R. 2	—	—	—	90.8 ± 10.5	25	3 Jan. 1991	70
Viljoen's Pass	34.9	43	10 Dec. 1992	71.3 ± 30.4	32	25 Jan. 1993	40
Ysterklip	18.2	302	13 Dec. 1991	72.6 ± 27.9	20	13 Jan. 1992	150
Cape Point	36.0	75	2 Dec. 1992	48.0 ± 30.0	63	7 Jan. 1993	75
Muizenberg Plateau—Pop. 2	16.8	101	31 Dec. 1992	34.5 ± 31.0	15	1 Feb. 1993	150
			Disa bivalvata				
Swellendam	24.0	154	5 Dec. 1991	56.2 ± 38.5	17	5 Jan. 1992	2,000
Viljoen's Pass	43.0	93	27 Nov. 1992	82.7 ± 28.8	30	25 Jan. 1993	150
Muizenberg Plateau—Pop. 1	48.8	125	2 Dec. 1992	51.8 ± 29.4	36	1 Feb. 1993	400
Muizenberg Plateau—Pop. 2	52.7	110	8 Dec. 1992	56.7 ± 22.2	33	1 Feb. 1993	1,000

4–7), but the shiny spectral reflectance so prominent on the back of the sepals of *D. atricapilla* is lacking in *D. bivalvata*. This reflectance is obvious both in the visible and the UV and presumably mimics the shine from a folded pair of insect wings, analogous to the "mirror or speculum" on the lip of some *Ophrys* orchids (e.g., *O. vernixia* Brotero = *O. speculum* Link) (Palus and Gack, 1990b). In order to have the flowers show up clearly under UV light, negatives had to be underexposed when printed (cf. grey scales in Figs. 4–7).

Floral development and longevity—The corymbose inflorescences of *D. atricapilla* and *D. bivalvata* bear from three to 15 ($\bar{X}$ = 8–9) and from three to 22 ($\bar{X}$ = 6–10) flowers, respectively. The average number open at any one time was two to four for *D. atricapilla* populations and three to seven for *D. bivalvata* populations.

The flower buds of *D. atricapilla* have the lateral sepals tightly appressed to the dorsal sepal with their black shiny backs facing outward or upward. At anthesis, the sepals spread apart and the tip of the dorsal petal moves downward. Sepals and petals are relatively thick and fleshy, and if unpollinated, flowers will remain open and fresh-looking for 2–3 weeks. Eventually, however, the sepals move back together and fold over the petals and dorsal sepal, the position they occupy in bud. Even after pollination, it takes several days before the flowers have closed sufficiently to prevent further pollination. *D. bivalvata* exhibits a similar pattern of opening and closing, but the backs of the sepals are not black and shiny.

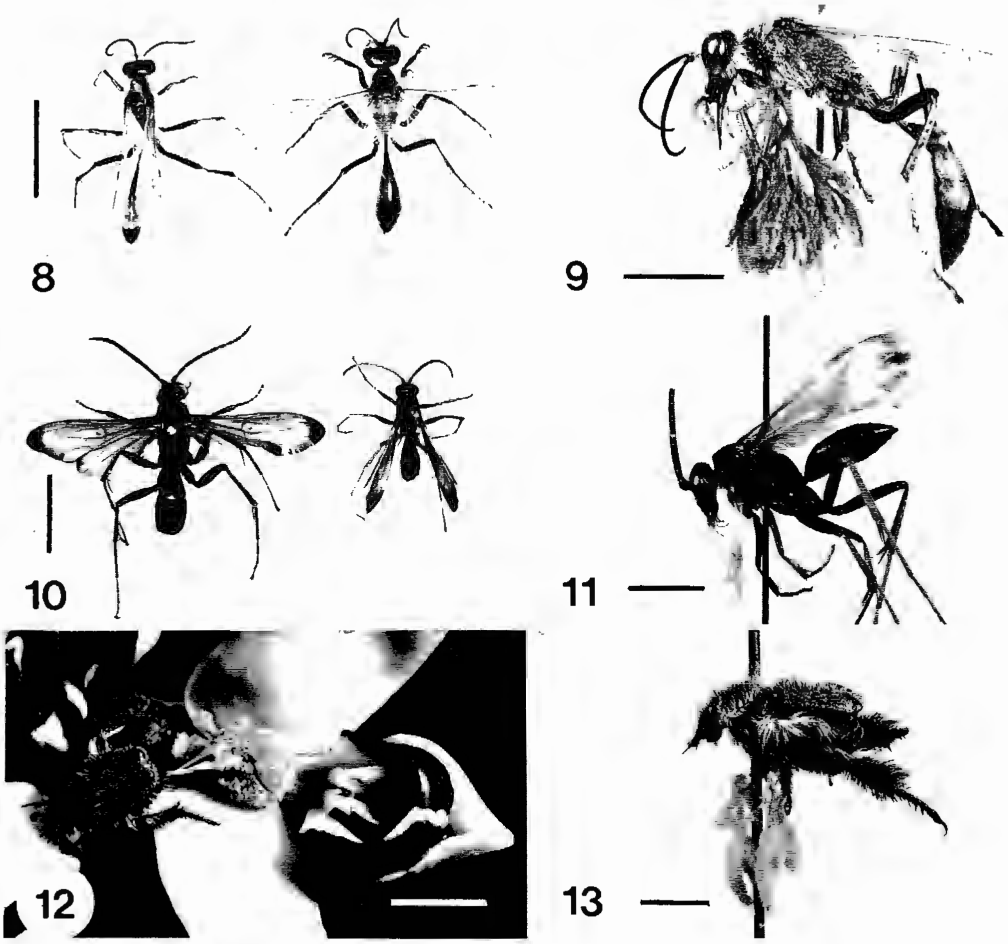
Breeding systems—*Disa atricapilla* is fully self-compatible. Fruit set for both self ($N = 37$) and cross pollinated ($N = 38$) flowers was 100%. Although the average percentage of embryo-filled seed was higher among cross-pollinated seed (94.7 ± 5.8% vs. 82.2 ± 18.0%), this difference was not quite significant ($F = 4.04$, $P = 0.057$). Most of the unmanipulated flowers were destroyed by animals. Nevertheless, it is clear from the three flowers that survived and the regular censusing of marked plants, that flowers are not capable of autopollination or apomixis.

Natural levels of pollination and fruit set—Natural levels of pollination and fruit set are presented in Table 2. In four of the pollinations (Bain's Kloof, Viljoen's Pass, Cape Point, and Muizenberg Plateau—Pop. 2), pollination success (the number of open flowers that had been pollinated) was checked more than once during the season, and in all cases it was highest early in the season. Since individual flowers remain open for 2–3 weeks without pollination and several days postpollination, estimates of final fruit set based solely on single date assessments of pollination rates are usually much too low (Table 2).

Pollination of D. atricapilla—The main pollinator of *D. atricapilla* is the wasp *Podalonia canescens* (Sphecidae) (Figs. 8, 9). This wasp was caught carrying pollinaria and visiting flowers of *D. atricapilla* at each of six different sites (Table 1). The average body length of wasps caught on *D. atricapilla* was 17.1 mm (SD = ±1.9 mm; range: 11.0–19.5). *Podalonia* individuals were unusually abundant on a first year burn in Bain's Kloof during 1990–1991. At this site, 22 wasps were caught on or patrolling around *D. atricapilla* flowers over a 1-month period. All but two of these were males of *Podalonia canescens* (Sphecidae), one was a female of *Hemipepsis hilaris* with *D. atricapilla* pollinaria (Pompilidae), and one was a male of *Scolia hottentota* Saussura (Scoliidae) without pollinaria.

Only two other floral visitors, both beetles (*Peritrichia*, Hopliini, Scarabaeidae), were observed on *D. atricapilla* flowers, and their visits were rare and occurred only at two of the six sites. Nevertheless, they both carried pollinaria (four and one, respectively), and the one (at Ysterklip) was observed pollinating a flower.

Visitation behavior—*Podalonia canescens* males exhibited typical mate-seeking behavior when visiting *Disa atricapilla* flowers. This consisted of patrolling plants repeatedly and in many cases inspecting inflorescences by hovering or circling briefly without landing. In other cases, wasps landed in the center of an inflorescence and looked around very briefly before flying off (<2 seconds), and in still other cases the wasps stayed on the inflorescence long



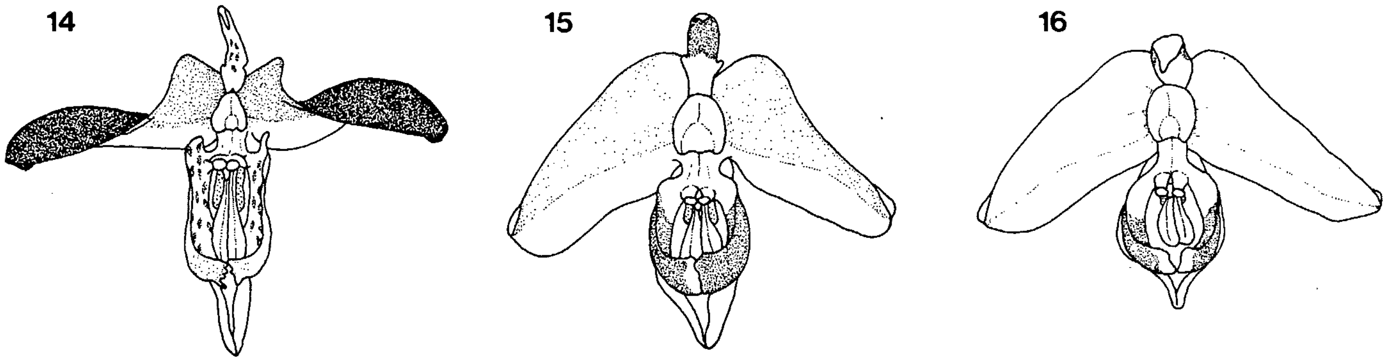
Figs. 8–13. Pollinators of *D. atricapilla* and *D. bivalvata*. **8.** *Podalonia canescens* male (left) and female (right). **9.** *Podalonia canescens* male with pollinaria of *D. atricapilla*. **10.** Males of *Hemipepsis*. *H. capensis* (left) and *H. hilaris* (right). **11.** *Hemipepsis hilaris* male with pollinarium of *D. bivalvata*. **12.** *Peritrichia* sp. on flower of *D. bivalvata*. **13.** *Peritrichia* sp. with pollinaria of *D. bivalvata*. Bar = 1 cm for Figs. 8, 10; 0.5 cm for Figs. 9, 11, 12; and 0.25 cm for Fig. 13.

enough (<5 seconds) to move from the center of the inflorescence out toward the tip of an open flower. When this occurred, they would probe the point where the two petals come together over the anthers (the site of maximum scent production). Although probing visits were rarely observed, such behavior would have been necessary in order to bring wasps into contact with the pollinaria. The large number of pollinaria carried by some individuals suggests that this type of behavior was much more frequent than observed, while the constancy of this visitation behavior can be inferred from pollinarium placement on the ventral surfaces of the wasps (Fig. 9). The visitation behaviors we observed were very similar to those exhibited by the pollinators of *Ophrys* species (Kullenberg and Bergström, 1975), except we never observed attempted copulation.

The mate-seeking behavior exhibited by *P. canescens* males visiting *D. atricapilla* contrasted sharply with normal food-seeking behavior. Wasps visiting nectar flowers for food did not repeatedly approach inflorescences without landing and when they did land, they stayed on inflorescences for much longer periods of time and often probed more than one flower before leaving.

Of the 20 *P. canescens* males caught on or around *D. atricapilla* at Bain's Kloof, 18 carried pollinaria. An additional 12 males of *P. canescens* with *D. atricapilla* pollinaria were caught on nearby nectar-secreting flowers (*Corymbium glabrum* L. [Asteraceae], *Lobelia jasionoides* [A. DC.] E. Wimm. [Campanulaceae], *Geissorhiza ramosa* Ker. ex Klatt. [Iridaceae], and *Ornithogalum* sp. [Hyancinthaceae]). For those individuals with pollinaria (N = 30), 86.7% carried more than two pollinaria. The average number of pollinaria carried was 11.2 (SD = ±8.9; range: 1–35).

Pollination of Disa bivalvata—The principal pollinator of *D. bivalvata* is the wasp *Hemipepsis hilaris* (Pompilidae) (Figs. 10, 11). This insect was observed visiting or patrolling the flowers of *D. bivalvata* at five different locations. It was most abundant at a site near Swellendam, where on a single day, 14 male wasps were collected on the flowers. Seven of these carried pollinaria of *D. bivalvata*. Males of a second species of *Hemipepsis*, *H. capensis*, were commonly observed patrolling *D. bivalvata* flowers on Muizenberg Plateau and occasionally seen carrying *D. bivalvata* pollinaria. *H. capensis* is about twice as large as



Figs. 14–16. *Disa* flowers from Muizenberg Plateau—Pop. 2. **14.** *D. atricapilla.* **15.** Hybrid between *D. atricapilla* and *D. bivalvata.* **16.** *D. bivalvata.*

H. hilaris (body length = 22.9 mm vs. 12.5 mm) (Fig. 10). Only one female of *H. hilaris* visited *D. bivalvata,* and it landed briefly on two inflorescences while foraging for nectar on a sympatric population of *Itasina filifolia* (Thunb.) Rafinesque (Apiaceae). Neither visit resulted in the extraction of a pollinarium.

The only other floral visitors were hopliinid beetles (*Peritrichia* spp.) (Figs. 12, 13) similar to those captured on *D. atricapilla,* but different species from the one observed on *Disa elegans* (Bolus, 1918). Beetles were observed at three different populations on the Cape Peninsula and at one population on Garcia's Pass near Riversdale in the southern Cape. During a short portion of the flowering period, beetles were very common on *D. bivalvata* inflorescences in population 1 on the Muizenberg plateau. In this same population, two different species of hopliinid beetles were caught on the flowers, one with five pollinaria and one with a single pollinarium.

Visitation behavior—Visitation behavior of *Hemipepsis* on *D. bivalvata* was very similar to that of *Podalonia canescens* on *D. atricapilla.* Wasps exhibited the same patrolling behavior, and most of the observed visits were of very short duration (<1 second).

Beetle visits to flowers were much longer in duration. Because of their small size relative to the flowers, beetles contacted pollinaria less frequently and more haphazardly than the wasp pollinators. Beetles also showed an interest in the scent-producing region of the lateral petals and effected pollination as they roamed over the flower with pollinia trailing behind them (Fig. 12). At one site, beetles were observed mating on *D. bivalvata* infloresecenses.

Hybridization—Hybrids between *D. atricapilla* and *D. bivalvata* were reported by Bolus (1918), but without details of where they were observed. Our observations coupled with herbarium specimen notes and a personal communication (E. G. H. Oliver, National Botanical Institute, Stellenbosch) have revealed at least four separate localities where hybrids between *D. atricapilla* and *D. bivalvata* occur. These include two sites in Bain's Kloof, one on the Cape Peninsula, and one near Franschhoek Pass. In at least one locality (Viljoen's Pass) where the two species occurred together, there were no hybrids. Hybrids are readily identified because their flowers look more like *D. bivalvata* in form and more like *D. atricapilla* in color (Figs. 14–16). The portion of the lateral sepal that is normally dark red or maroon in *D. atricapilla* is usually light red or pink in the hybrids. The petals in the hybrids have the coloration and scent closest to that of *D. bivalvata.* The pollinaria of *D. atricapilla* (ca. 10.3 mm) are more than 1.5 times as large as those of *D. bivalvata* (ca. 6.4 mm), and those in the hybrid are intermediate in length (ca. 7.2 mm). In a mixed population of ca. 1,000 *D. bivalvata* plants and ca. 100 *D. atricapilla* plants at Muizenberg Plateau, approximately ten to 20 hybrid individuals were observed. In addition to possible F1s, it appeared that many of the hybrids were backcrosses to *D. bivalvata.* Although hybrids were present at several different locations, they were generally rare and only occurred where the two species were sympatric. At Franschhoek, only one hybrid individual was found in a mixed population dominated by *D. bivalvata* (E. G. H. Oliver, personal communication).

DISCUSSION

Evidence for sexual deception—The absence of a floral reward, the exclusive presence of male wasps as primary pollinators, and the patrolling and visitation behavior of the wasps and their repeated visits to nonrewarding flowers (as shown by the large number of pollinaria carried) all suggest that these two orchids are pollinated by sexual deception. It is difficult to rule out the possibility of generalized food deception, but it is very doubtful considering the lack of female visitors. It is also unlikely that either *Disa* species is mimicking a specific nectar plant, since no obvious models were observed among the nectar plants visited by the wasps or among other plants in the community. Females of *Podalonia canescens* were caught at three sites and females of *Hemipepsis hilaris* at two sites, but they were very rare and only observed near the end of the flowering periods of the two orchids. Thus these wasps fit the pattern of many aculeate Hymenoptera, especially those involved in sexual deception, with emergence of males preceding the emergence of females (Paulus and Gack, 1990b).

The primary pollinators of *D. atricapilla* and *D. bivalvata* are wide-ranging species in southern Africa. *Podalonia canescens* (Dahlbom) occurs throughout the African subcontinent and Madagascar (Bohart and Menke, 1976), while both *Hemipepsis hilaris* and *H. capensis* occur throughout southern Africa (Arnold, 1932). Their ranges extend far beyond that of the two orchids, which indicates that they are not in any way dependent on or adapted to the orchids. The involvement of spider-hunting wasps (Pompilidae) in the pollination of sexual deceptive orchids has not been reported before, but sphecid wasps (*Argogorytes* spp.) are well-known pollinators of *Ophrys* spp. (Paulus and Gack, 1990b).

Visits by beetles to the flowers of *D. bivalvata* were more common than to *D. atricapilla,* which may have been due to differences in relative attractiveness of the flowers, abundance of flowering individuals, or simply to site-specific differences. The use of open inflorescences, like that of *D. bivalvata,* for mating is common among hopliinid beetles and is not necessarily tied to the presence or absence of a food reward (Steiner, unpublished data). *Disa elegans,* a sister species to the *D. atricapilla*/*D. bivalvata* clade (Steiner, unpublished data), has flowers similar in coloration (white sepals with maroon petals) to *D. bivalvata* and it too has been reported to be visited by a hopliinid (*Peritrichia*) beetle (Bolus, 1918). Although beetles may be important pollinators in certain locations or at certain times during the flowering season, their lack of abundance and visitation behavior argue against a primary role in pollination for *D. atricapilla* and *D. bivalvata.* Beetles (scarabaeid and elaterid) have also been implicated as secondary pollinators of those *Ophrys* species, whose primary pollinators are solitary bees (Kullenberg, 1973a; Paulus and Gack, 1990b).

Unlike most orchids pollinated by sexual deception, the flowers of *D. atricapilla* and *D. bivalvata* do not resemble a female wasp, at least to the human eye (Fig. 8). Instead it is the entire inflorescence that appears to be the attractive unit and especially the black shiny lateral sepals that are so prominent in bud. The buds and old closed flowers of *D. atricapilla* probably provide the greatest optical stimulus to males seeking mates.

Not all orchid flowers that are pollinated by sexual deception bear a close resemblance to a specific female insect (Stoutamire, 1975; Bino, Dafni, and Meeuse, 1982). The optical and textural components of the flower, even in pseudocopulatory orchids such as *Ophrys,* are only secondary in importance to the odor for attracting pollinators (Kullenberg, 1961, 1973a, b; Bergström, 1978; Borg-Karlson, 1990). Even in the presence of the appropriate odor, male bees do not require an exact image of the female in order to be sexually deceived. Rather, only certain key optical features such as the shine, color, and/or markings on the lip and overall size of the flower contribute to the effectiveness of pollinator attraction (Paulus and Gack, 1990b).

Evolution of sexual deception in the Cape—Do *D. atricapilla* and *D. bivalvata* represent a transitional stage between food deception and sexual deception with vestiges of both? Neither orchid has flowers that are close optical mimics of their pollinators, yet both attract male wasps for pollination. *D. bivalvata* has no obvious visual cues that distinguish it from a simple food deception flower, which may explain why it is more attractive than *D. atricapilla* to secondary less-specialized food-seeking pollinators (e.g., beetles). The additional morphological and visual characteristics (autapomorphies) of *D. atricapilla* that make the inflorescences more like an aggregation of insects, may be the result of selection to make the flowers less attractive to beetles or nonsphecid wasps and more attractive to sphecid wasps (i.e., a further shift away from food deception and a refinement of the sexual deceit syndrome). Further evidence that sexual deception in *D. atricapilla* and *D. bivalvata* is not yet fully refined is the absence of pseudocopulatory behavior by the pollinating wasps. However, despite this, reproductive success appears relatively high for both orchid species.

Only a single species in *Disa* section *Disa, D. uniflora,* secretes floral nectar (Johnson, unpublished data), and this is clearly an autapomorphic character (Steiner, unpublished data). *D. atricapilla* and *D. bivalvata* along with the remaining 19 species in the section produce no floral reward, which suggests that sexual deception has evolved from ancestors whose flowers were nonrewarding generalized food mimics. This has apparently been the case for *Orchis* and *Ophrys* in the Mediterranean region (Dafni, 1987; Paulus and Gack, 1990b) and for most of the pseudocopulatory genera in Australia (Dafni and Bernhardt, 1990).

Coexistence of D. bivalvata and D. atricapilla—Artificial hybridizations in *Disa* section *Disa* have shown a general lack of strong postfertilization barriers (Vogelpoel, 1989, 1992), but natural hybrids are very rare (Linder, 1990). Only seven cases of natural hybridization have been documented within the genus (Linder, 1990), not including the brief mention of hybridization between *D. bivalvata* and *D. atricapilla* made by Bolus (1918). This suggests that prefertilization barriers within *Disa* such as habitat or pollinator partitioning are strong or that postzygotic barriers prevent germination and/or establishment of hybrids in the parental habitat.

The strong monophily of *Hemipepsis* and *Podalonia* males suggests that absence of extensive hybridization between *D. atricapilla* and *D. bivalvata* is due primarily to prefertilization barriers and not postzygotic barriers. No males of *Podalonia* have been caught on *D. bivalvata,* and no males of *Hemimpepsis* have been found on the flowers of *D. atricapilla.*

If males of *Hemipepsis* and *Podalonia* are normally monophilic, how can one explain the presence of hybrids in at least four different localities? We cannot rule out the possibility that hybrids are the result of occasional mistakes by the primary pollinators, but it seems more likely that hopliinid beetles are responsible. Both primary pollinator mistakes and interspecific movements by secondary beetle pollinators are known to cause hybridization among *Ophrys* species (Borg-Karlson, 1990; Paulus and Gack, 1990b).

Orchids that are pollinated through sexual deception are not visited by females (Kullenberg and Bergström, 1973; Paulus and Gack, 1990a). Therefore, it is very surprising that a female of *H. hilaris* was caught on *D. bivalvata* at one site and on *D. atricapilla* at another site. We can only assume that these females were searching for nectar and visited the inflorescences by mistake.

Floral divergence and speciation—The differences in floral morphology (mostly shape and coloration of the sepals) between *D. atricapilla* and *D. bivalvata* are minor, but the effect produced is striking. Together with differences in floral scent, they are sufficient to attract pollinators from two different families of wasps. Thus, the implicit prediction that these two species should have different pollinators (Linder, 1981) is borne out. However, it is not clear whether morphological or olfactory differences are more important in the shift from the one pollinator to the other. If examples from *Ophrys* are any indication, it is the scent profile that is most critical. The morphological differences probably serve only to reinforce the isolation precipitated by the change in odor profile.

Orchids pollinated by sexual deception and those providing volatile chemicals as rewards provide some of the best potential examples of true sympatric speciation (Dressler, 1968; Paulus and Gack, 1990b). For such plants, a simple mutational or recombinational event could change the composition of the complex odor profile and thereby attract an entirely new pollinator. This would effectively isolate that plant from other members of the population, while self-pollination would allow additional plants with the new profile to become established (cf. Dressler, 1968). The morphological differences between the two species could have been linked to the same mutational or recombinational event or they could have developed subsequently in response to selection for enhanced attractiveness to the new pollinator and/or selection for decreased attractiveness to the original pollinator. Alternatively, morphological shifts may have been selected to decrease attractiveness to less efficient pollinators such as beetles.

Among sexually deceptive orchids there is some evidence for sympatric speciation among *Ophrys* species with large- and small-flowered forms that have different pollinators and for a few closely related species pairs with different pollinators (Paulus and Gack, 1990b).

D. atricapilla and *D. bivalvata* are also likely products of sympatric speciation, because of their close relationship (i.e., sister species), their broad geographical overlap, their occurrence in the same habitat in a number of populations, their adaptation to different pollinators through sexual deception, and their overlapping flower times.

Sexual deception in southern Africa has obviously arisen independently from pseudocopulatory orchids of the Mediterranean region and Australia, yet there are many features that they have in common. Additional work on floral odors of *D. atricapilla* and *D. bivalvata* coupled with experimental work analogous to that done on *Ophrys* (Kullenberg, 1961, 1973b; Tengö, 1979; Borg-Karlson, 1990) may help determine the relative importance of optical and olfactory cues in floral divergence and speciation. Unfortunately, low pollinator abundances, low visitation rates, and fire-dependent flowering conspire against quick solutions to these questions.

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