Variation in pollination across a fragmented landscape at the Cape of Africa

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Statement

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

Chapter 7 describes a study conducted in collaboration with Dr. Julie Hawkins and Dr. Yvonne Charters. Their contributions were mainly through genetic laboratory work (Charters) and discussion on how to improve the manuscript (Hawkins).

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Abstract

In this thesis I assessed the ability of established conservation areas to conserve plant-pollinator interactions. The study area was the species rich, but highly fragmented West Coast Lowlands of the Cape of Africa. I focused on the *Nectarinia* sunbird pollination system and the oil-collecting *Rediviva* bee pollination system. Before exploring the consequences of habitat fragmentation, it was necessary to study the natural history of the plant-pollinator interactions.

Among 16 conservation areas, I studied variation in the pollination of *Brunsvigia orientalis* (Amaryllidaceae) by *Nectarinia* sunbirds. Pollen supplementation experiments and censuses of sunbirds showed that the observed failure of seed set could be attributed to the lack of sunbirds. Three factors: 1) the post-fire successional stage of the vegetation, 2) the number of flowering *B. orientalis* plants, and 3) the size of the conservation area, explained 75 % of variation in seed set. Seed set failed in small conservation areas (22 - 30 ha), and was reduced in medium-sized conservation areas (811 - 1113 ha) compared with large conservation areas (> 15 000 ha).

Among 27 conservation areas, I studied variation in the pollination of an oil-secreting orchid, *Pterygodium catholicum*, by the oil-collecting bee, *Rediviva peringueyi*. Variables that significantly influenced the ability of nature reserves to conserve the orchid-bee mutualism were: 1) the soil type, 2) the post-fire successional stage of the vegetation and 3) the interaction between reserve size and the nature of the surrounding land-use. A lack of pollinators led to reproductive failure in consecutive years in small conservation areas (4 - 385 ha) in an urban matrix. Correlated patterns of variation in seed capsule production suggested that the results obtained for *P. catholicum* could be extrapolated to several additional plant species which are also pollinated by *R. peringueyi*.

Are these apparent anthropogenic declines in pollination real? Declines can only be detected with certainty by comparison with historical rates. I reconstructed the historical pollination landscape by detecting the genetic echo of past pollination, and by determining historical pollination rates from rehydrated herbarium specimens. Thus, I demonstrated that plant-pollinator mutualisms have recently collapsed in small, urban conservation areas.

Does a decline in pollination really matter? Patterns of variation in the composition of the community of oil-secreting plants suggest that the anthropogenic extirpation of oil-collecting bees from small conservation areas has been followed by the extirpation of dependent plant species. Patterns of infraspecific variation in the floral traits in sunbird and oil-bee pollinated plants suggest an alternative to extinction. Pollinator loss may ultimately result in the evolution floral traits that decrease dependence on the absent pollinator.

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Introduction

In an aerial view, it is evident that large parts of the globe are dominated by human activity with natural areas reduced to a series of small fragments. Many of these fragments are now proclaimed conservation areas, on which we depend as the last refuges for the earth's biodiversity. The conservation of biodiversity in these reduced ecosystems is potentially threatened by the breakdown of mutualistic relationships between plants and animals. Recent reviews emphasize the probable vulnerability of plant-pollinator relationships, but point to the paucity of large scale studies (Hobbs & Yates, 2003; Kearns & Inouye, 1997; Kearns et al., 1998; Renner, 1998). To date, lower levels of pollination have been detected only in very small (< 10 ha) fragments of natural vegetation (Aizen & Feinsinger, 1994; Cunningham, 2000b; Donaldson et al., 2002; Jennersten, 1988; Lennartsson, 2002; Lindberg & Olesen, 2001; Powell & Powell, 1987; Robertson et al., 1999). Are similar declines occurring at a larger geographical scale, relevant for established conservation areas?

Here I explore patterns of variation in pollination across a fragmented landscape at the Cape of Africa. The study area is the West Coast Lowlands, a coastal plateau ~200 km long and ~70 km wide, bound by inland mountains. The plateau is a mosaic of two fundamentally different soil types: coarse-grained, sandy soils, poor in essential plant nutrients; and finer grained, clay soils, richer in nutrients (Goldblatt & Manning, 2002). The vegetation is a fine-leaved, fire-prone shrubland with exceptionally high levels of species diversity and endemicity.

Today 78% of the lowlands are transformed. What remains are about 18 000 remnants of natural habitat in an urban or agricultural matrix (von Hase et al., 2004). I selected 50 of these remnants for inclusion in various parts of this study and made additional comparisons with areas outside of the coastal lowlands. Both "islands" and "mainlands" are, with few exceptions, proclaimed conservation areas, which are actively managed for the conservation of biological diversity. Many additional remnants, without formal conservation status, are currently being prioritized for inclusion in a network of conservation areas (Heijnis et al., 1999; von Hase et al., 2004).

The Cape flora has a preponderance of highly specialized pollination systems (Johnson & Steiner, 2000). Two of these, namely the sunbird pollination system and the oil-collecting bee pollination system, are studied here (Section 1 and Section 2 respectively). *Nectarinia* sunbirds and oil-collecting *Rediviva* bees pollinate two large groups of plant species, which are specially adapted for these respective modes of pollination. I chose to study specialized pollination systems because they are more likely to be sensitive to anthropogenic disruption than more generalized pollination systems (Bond, 1994).

The nature of plant pollinator interactions within the *Rediviva* and *Nectarinia* pollination systems is still relatively unexplored. Before exploring the consequences of habitat fragmentation, I first studied the natural history of the plant-pollinator interactions (Chapter 1, 4 & 5). I was then in a position to ask: Does the current reserve system in the lowlands adequately conserve these plant-pollinator interactions?

To answer this question, I quantified patterns of variation in pollination across the study area and related them to physical features of the landscape (Chapter 2 & 6). The response variables were: 1) the composition of the pollinator community, 2) pollinator visitation rate, 3) pollination rate and 4) seed set. Because the study area is heterogeneous, I included both anthropogenic and natural features of the landscape as predictor variables. The predictor variables used in this study were: 1) the size of conservation area, 2) the matrix in which it is located, 3) the soil type and 4) the post-fire successional stage of the vegetation.

Where low pollination rates were found to be associated with anthropogenic factors (matrix characteristics and size of the conservation area) there is the strong inference of recent, human-caused declines in pollination services. However, to confirm the occurrence of recent anthropogenic declines it is necessary to make comparisons with historical, base-line pollination rates. To do so, I reconstructed the historical pollination landscape by detecting the genetic echo of past pollination, and by determining pollination rates from rehydrated herbarium specimens (Chapter 7).

What are the long-term consequences of pollinator loss for plant species conservation in established conservation areas? In one scenario, interacting species in an ecosystem can be likened to a closely woven fabric. The loss of a pollinator and the services that it provides cause a tear in the fabric, triggering the loss of other species (Gilbert, 1981). In contrast, it has often been suggested that pollination may be weakly related to plant population growth and persistence (Addicott, 1986; Bond, 1994; Calvo, 1993). To assess the role of pollinators in maintaining plant populations, I looked for evidence for plant extinction triggered by pollinator loss, by relating patterns of variation in plant community composition to patterns of pollinator abundance (Chapter 8).

What are the alternatives to plant extinction following pollinator loss? Johnson (1996) has suggested that selection for more efficient pollination systems in a pollinator-limited context, is the primary driving force behind floral evolution in the Cape flora. In the long-term, will the loss of sunbirds and oil-collecting bees result in the evolution of new floral features which promote alternative means of pollination? In this thesis I addressed this question by analyzing infraspecific variation in floral traits in relation to long-term stable patterns of pollinator abundance across the landscape (Chapter 3 & 8).

Conventions and nomenclature

The following conventions are used:

- Sites are named in accordance with the Reader's Digest Atlas of Southern Africa (1984), which is a compilation of official maps prepared by the South African Directorate of Surveys and Mapping.
- Plants are named in accordance with Goldblatt & Manning (2000).
- Breeding system jargon follows Barrett & Harder (1996) and Barrett, Jesson & Baker (2000).
- Means are reported ± 1 Standard Error

SECTION 1

The *Brunsvigia—Nectarinia* pollination mutualisms: sunbirds and candelabra plants.

1 Reproductive dependence of *Brunsvigia orientalis* (Amaryllidaceae) on *Nectarinia* sunbirds (Nectarinidae)

Reproduction in *Brunsvigia orientalis* (Amaryllidaceae) is found to be dependent on cross-pollination, a service provided only by sunbirds (*Nectarinia spp.*). A suite of specialized floral characters, including peculiarly recurved flowers and obstructed nectar, exclude insects from the flowers. This set of traits combines with a lack of vegetative reproduction to make *B. orientalis* populations potentially vulnerable to the loss of sunbirds. Comparisons are made with the "generalist" pollination system of *Brunsvigia bosmaniae*.

Brunsvigia orientalis (L.) Aiton ex Eckl. (Amaryllidaceae) is one of the most striking plants of the Cape of Africa, where it grows in the deep sand of coastal lowlands and valley bottoms. Despite being one of the first Cape plants to be formally described (in the year 1753)(Dyer, 1950, 1951), the pollination biology of *B. orientalis* has not been studied. The specific aims of this study were: (1) to determine how *B. orientalis* is pollinated, (2) to describe the floral morphology and floral phenology, as well as the nectar properties and nectar phenology in relation to pollination, (3) to test how dependent seed set is on cross-pollination, and (4) to determine whether *B. orientalis* is capable of vegetative reproduction. This study is a necessary preliminary to the following two chapters in which I explore natural and anthropogenic sources of variability in the reproduction of *B. orientalis*.

Materials and methods

Study species

B. orientalis plants of flowering age have spherical bulbs measuring ~8 cm in diameter with ~6 strap-shaped leaves in a prostrate rosette that measures ~80 cm across (Fig. 1.1a). Leaves are present during the wet winter (May-Sept.); plants are leaf-less during the dry summer (Oct.-April). A spherical, umbellate inflorescences, ~35 cm in diameter, is produced at the end of the summer (Feb.-April), while the plants are leaf-less (Fig. 1.1b). Inflorescences bear about ~40 large, scarlet flowers, each of which produces a winged, trilocular capsule. After flowering, the inflorescence detaches from the bulb and is rolled over the ground by the wind, dropping large, green seeds in its course. The seeds germinate immediately, just at the time when the first winter rain falls.

Study sites

In 2000, I studied the pollination biology of *Brunsvigia orientalis* at 12 sites. Additional observations and experiments, described below, were conducted at a subset of the sites in 1998 and 2001.

Flower morphology, nectar characteristics and phenology

At Wiedourivier I determined patterns of anthesis by tagging 50 mature buds and recording the number of open flowers at ~3-h intervals for 12 hrs. Observations started when the first bud opened. In addition, I counted the number of buds, open flowers and wilted flowers on a sample of 10 inflorescences, to determine the size of the floral display.

At the same site, daily rhythms in nectar properties were determined by sampling the standing crop of nectar from one flower on 20 randomly selected plants at ~3-h intervals for 16 hrs. The nectar volume was measured with 5 μL capillaries (Drummond Scientific Company, Broomall, Pennsylvania, USA) and nectar concentration was determined with a 0–50% field refractometer (Bellingham and Stanley, Tunbridge Wells, UK). I avoided sampling the same flower twice. At Langebaanweg and Vermont I measured the distance between the nectar and the anthers and the stigma to the nearest 0.5 mm.

Breeding system

To determine the breeding system of *B. orientalis*, I enclosed 12 budding inflorescences at Langebaanweg in gauze-covered cages that did not make contact with the flowers. The apertures in the gauze measured 1mm^2. Later, 9 female-stage flowers on each plant were selected and randomly assigned to one of three treatments: 1) unmanipulated to test for autogamy, 2) pollinated by hand with self-pollen to determine whether plants are self-compatible, and 3) pollinated by hand with a pollen mixture from five different plants. The number of seeds per fruit was determined for each treatment. To determine whether pollination treatment affected seed size, the seeds were sieved into three classes: large (seeds > 5.6 mm); medium (5.6 mm > seeds > 4.6 mm); small (4.6 mm > seeds > 2.83 mm).

Alternative modes of reproduction

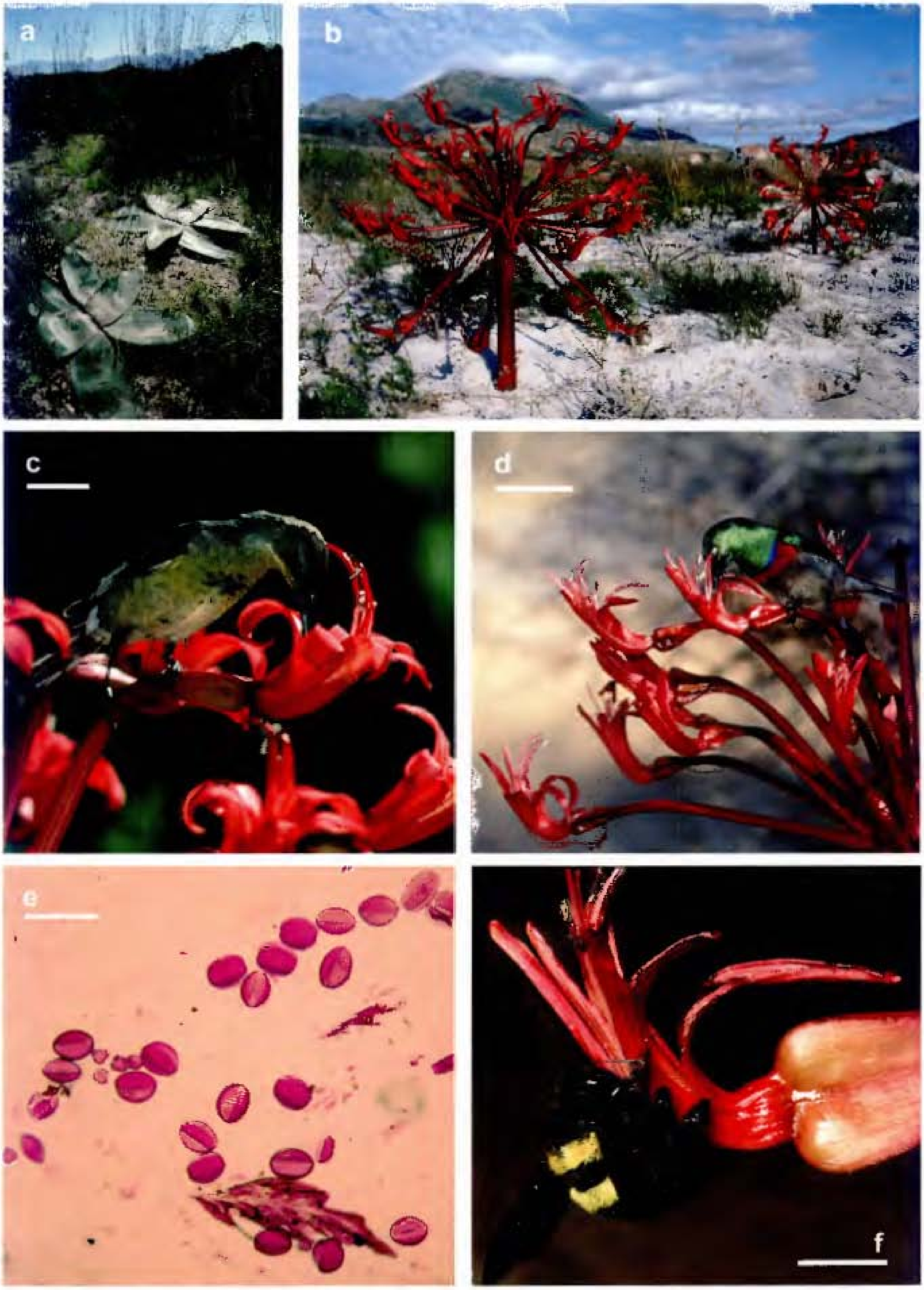
I investigated the vegetative morphology of *B. orientalis* to determine whether vegetative reproduction occurs. On the site of a planned development at Milnerton, I excavated 50 bulbs of various sizes, concentrating on areas where bulbs occur in clusters. I studied and described the bulb and root morphology.

Pollinators

In 2000 I conducted detailed flower visitor censuses at all 12 sites during peak flowering. At each site I observed between 3 and 35 inflorescences (median = 15) from a single vantage point for two separate 0.5 h periods. Observations were made from a distance of ~10 m. Visitation rates were quantified by dividing the number of visits observed by the number of open flowers in the census area. Detailed observations of pollinator behaviour were made from a distance of ~4 m aided by close focusing 8 x 32 binoculars. The behavior of small flower visitors was observed at closer range.

I captured birds in mist nets at Vermont, Miller's Point and Wiedourivier. Pollen was sampled from bird heads by pressing a broad strip of transparent adhesive tape along the length of the upper mandible, across the top of the head, to the nape of the neck. The tape was cut into sections and mounted sticky-side-up on microscope slides in fuchsin gelatin (Beattie, 1971). Pollen grains were compared with *B. orientalis* reference pollen and counted.

Fig. 1.1 Reproduction in *Brunsvigia orientalis*. **a,** Two six-leafed *B. orientalis* plants at Rondevlei. Plants flower for the first time when they have four or five leaves. Width of leaf rosettes, 1 m. **b,** Two flowering *B. orientalis* plants at Scarborough in the second year after a fire. Inflorescence height, 40 cm. **c,** A juvenile male Malachite Sunbird, *Nectarinia famosa*, sipping nectar from a female stage flower of *B. orientalis* at Vermont. The stigma contacts the crown of the sunbird. Scale bar, 2 cm. **d,** A male Southern Double-Collared Sunbird, *N. chalybea*, sips nectar from a male stage *B. orientalis* flower at Wiedourivier. Scale bar, 4 cm. **e,** Contact pollen sample from the forehead of *N. famosa* showing the density and spatial distribution of the pollen load. Large oval grains are *B. orientalis*, small triangular grains are an unidentified dicotyledon. There is a feather fragment bottom right. Scale bar, 0.1 mm. **f,** A carpenter bee, *Xylocopa caffra*, visits a flower of *B. orientalis* at Langebaanweg, but does not make contact with the anthers nor stigma. Scale bar, 1 cm.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Results

Functional floral morphology and phenology

The entire inflorescence of *B. orientalis* is deep red. The flowers are large, lack visible nectar guides and were found to be scentless during the day and night. The pedicels are sturdy enough to support the weight of a perched bird. The flowers are widely spaced, allowing access to the pedicels. The corolla is recurved to face towards the perch provided by the pedicle. In the uppermost flowers, the pedicels ascend vertically, then bend at the end to provide a horizontal perch (Fig. 1.1b-d). The top-most tepals of the flowers are rolled back; the lowermost tepals form a curved hood, which supports the similarly curved anthers and style. The anthers and style are stiff and wiry (Fig. 1.2a, b). The bases of the filaments and tepals are fused into a broad, 5 mm long tube (Fig. 1.2c). The style passes through the center of the tube. The mouth of the tube is almost sealed by the inward projection of the filaments at the point where they become free from the tepals. The sturdy bases of the filaments, which constrict around the style, need to be forced apart to gain access to the nectar in the tube (Fig. 1.2c). The filaments are roughly grouped into two height classes, with three anthers in each group. The attachment of the anthers to the filament is versatile. The pollen grains are large and sticky (Fig. 1.1e).

The plants in a population were found to flower synchronously. At peak flowering 58.5 ± 4.57 % of the flowers on an inflorescence were open; the others were wilted or in bud. The flowers opened predominantly during the morning (Table 1.1). Anthers dehisced before the stigma unfurled minutely. Style curvature increased with flower age from almost straight in male-phase flowers, to sickle-shaped in the female phase. As style curvature increased, the distance between the stigma and the nectar decreases and the stigma moved from above the height of the anthers into the anther region (Fig1.2a, b). Graphs of the relative lengths of floral parts provided no evidence for stylar polymorphism within populations.

The maximum nectar volume recorded in unbagged flowers was 27 μL. At sunrise, the volume of available nectar was the highest and the concentration was the lowest. Nectar volume declined sharply during the period of sunbird activity, while

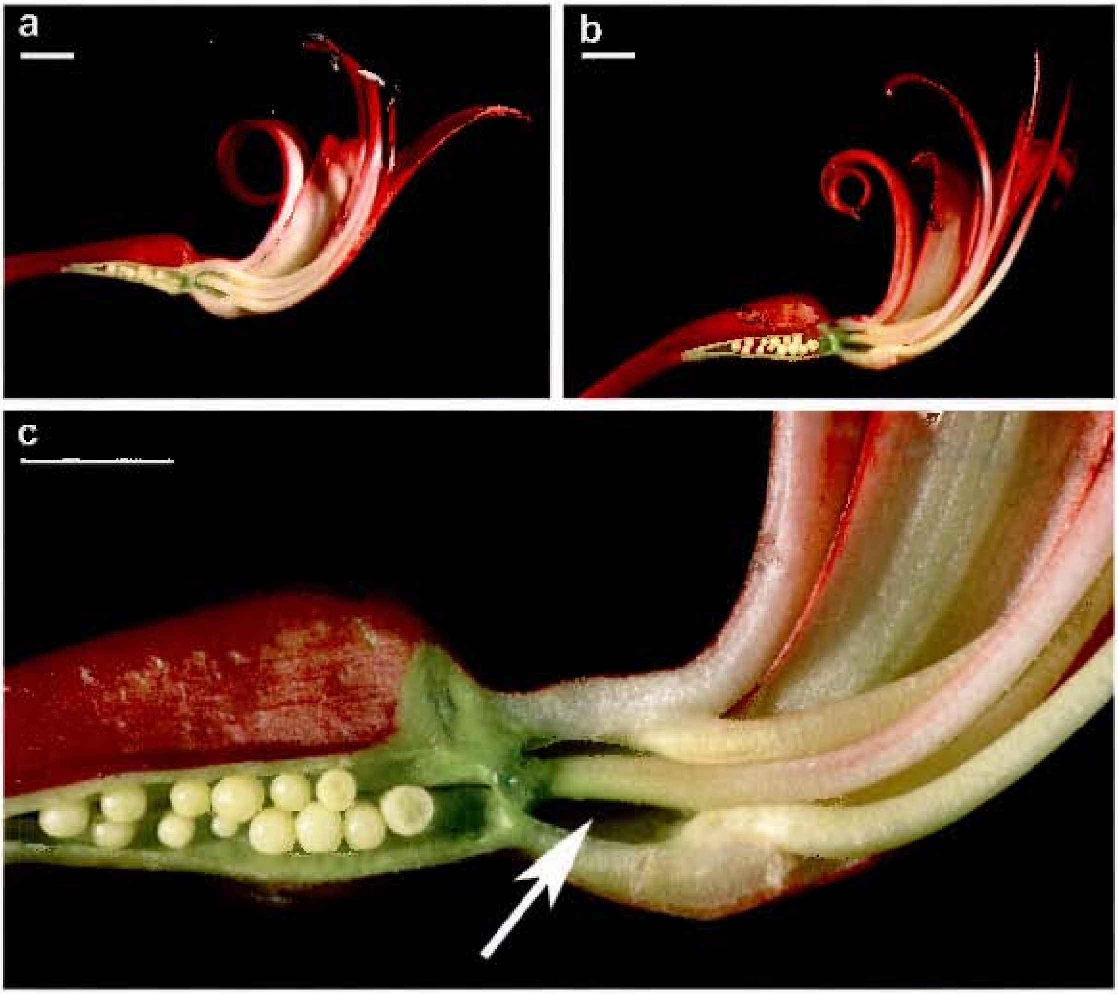

Fig. 1.2 Half-flowers of *B. orientalis*.**a**, A male phase flower with curved, pollen bearing anthers. Scale bar, 1 cm. **b**, A female phase flower with curved style and minutely unfurled stigma. Scale bar, 1 cm. **c**, Base of the corolla. The arrow indicates the sealed nectar chamber. Scale bar, 5 mm.

concentration increased slightly as the day progressed (Fig. 1.3a, b). The epithelial cells of the flower release bitter sap if damaged.

Breeding system

Flowers exposed only to air-borne pollen and ant visitation set no or very few seeds (Table 1.2). Seed set did not increase significantly from these low levels following self-pollination, but increased ~20 times following cross-pollination. Cross pollination resulted in larger seeds than self- or pollinator-excluded treatments, but too few seeds were produced in the latter two treatments to determine if this difference is statistically significant.

Alternative modes of reproduction

The bulbs of *B. orientalis* were found to be discrete units, never linked together by roots. I found no bulbs that were in the process of fission, and there were no bulbils attached to root tips or base plates.

Pollinators and flower visitors

At all the study sites, sunbirds (*Nectarinia* spp.) were the only regular flower visitors (Fig. 1.1c, d). During 2 795 flower-hours of pollinator censusing, I observed 720 visits by *N. chalybea* (Lesser Double-Collared Sunbirds) and 507 by *N. famosa* (Malachite Sunbirds). Visitation rates by sunbirds averaged 0.35 ± 0.12 visits per flower per hour (range = 0-0.66, *N* = 12 sites). On one occasion, outside of the formal observation period, *N. violacea* (Orangebreasted Sunbird) was observed visiting the flowers of *B. orientalis* at Miller's Point.

Sunbirds entered between the flowers and perched on the pedicels, while sipping nectar from the flowers. At Vermont, where plants had 19.5 ± 7.5 open flowers, *N. famosa* visited 11.9 ± 6.1 flowers and *N. chalybea* visited 6.9 ± 1.1 flowers before flying to the next inflorescence. Direct contact between the top of the head and the anthers and/or the stigma was observed at all sites. White pollen was often visible on the crown feathers of birds. On five occasions at Vermont, I observed *N. famosa* nipping at the exposed anthers of *B. orientalis*, apparently feeding on pollen.

Table 1.1 Daily pattern of flower opening in *B. orientalis*. The number of flowers that opened per time period differs significantly from values expected if opening was random ($\chi^2 = 10.08$, df = 2, $p = 0.006$).

	No. of flowers that opened per time period		
	8:45-11:00	11:00-15:00	15:00-20:30
Observed	6	7	1
Expected	2.7	4.7	6.7

Table 1.2. Results of an experiment to determine the breeding system of *B. orientalis*. Treatment had significant effect on the number of seeds set per capsule (Kruskal-Wallis $H = 52.04$, $p < 0.00001$). Treatments sharing the same superscript did not have significant differences in the number of seeds set per capsule. Methodology for identifying homogenous groups follows Siegel and Castellan (1988, p 213-214).

Treatment	N (flowers)	%Capsules with seeds (%)	Seeds per capsule	Large seeds (%)	Medium seeds (%)	Small seeds (%)
Outcrossed	34	85.3	8.82 ± 1.47^a	45	32	23
Selfed	34	23.5	0.38 ± 0.15^b	23	31	46
Pollinator-excluded	35	20	0.26 ± 0.09^b	22	22	56

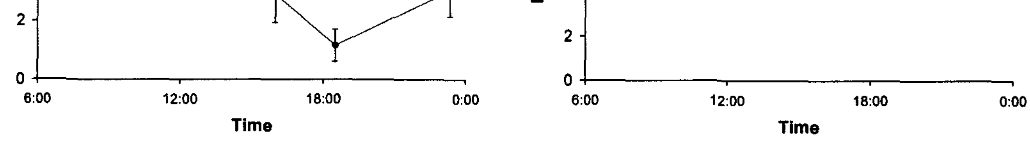

Fig. 1.3 Daily rhythms in nectar properties of *B. orientalis* over a 16 hr period. **a**, Volume **b**, Concentration (% w/w).

I captured two *N. chalybea* (both male) at Wiedourivier, four *N. famosa* (two male; two female/juvenile) at Vermont and one *N. famosa* (female/juvenile) at Miller's Point. All captured *Nectarinia* carried large quantities of *B. orientalis* pollen (Table 1.3, Fig. 1.1e). Most of the *B. orientalis* pollen load was located on the head feathers (*N. famosa*, 89 ± 7.8 %; *N. chalybea*, 93 ± 2.8 %) with the remainder on the bill. *Zosterops pallidus*, a less specialized nectarivore, carried no *B. orientalis* pollen.

Table 1.3 Pollen loads carried by three species of nectarivorous birds caught at the study sites.

Species	No. of birds caught	No. with *Brunsvigia* pollen	*Brunsvigia* pollen load on bird (mean ± *SE*)	*Brunsvigia* pollen as a % of total pollen load
Nectarinia chalybea	2	2	984 ± 166	84
Nectarinia famosa	5	5	341 ± 170	68
Zosterops pallidus	2	0	0	0

The populations of *B. orientalis* are relatively dense and discrete, with very few or no other bird-visited flowers scattered in between. Additional nectar sources utilized by sunbirds in the close vicinity of the study populations included: *Lycium afrum* (Solanaceae) and *Haemanthus coccineus* (Amaryllidaceae) at Miller's Point; *Cotyledon orbiculata* (Crassulaceae) at Vermont; and *Microloma sagittatum* (Apocynaceae) at Wiedourivier. At Miller's Point the upper, recurved tepals of *B. orientalis* were dusted with dark yellow pollen from *H. coccineus*, which was transferred between plant species on the throats of sunbirds.

In addition to sunbirds, sugar ants, *Camponotus niveosetosus,* visited some flowers at Miller's Point. And a carpenter bee (*Xylocopa caffra*), visited 9 flowers at Langebaanweg (Fig. 1.1f). Neither species made contact with the anthers or stigma.

Discussion

The results of this study show that two species of sunbird, *Nectarinia famosa* and *N. chalybea* (Nectarinidae), are the only pollinators of *Brunsvigia orientalis* in the study region. They feed on the nectar in a non-destructive way and frequently make contact with the anthers and/or stigma. They fly between plants carrying pollen on the top of their heads, and thus effect cross-pollination. Very few other animals visit the flowers of *B. orientalis*, and none was seen to make contact with the reproductive parts. Wind pollination is precluded by large, sticky pollen grains. Self-fertilization is and spatial separation of male and female functions inside the flower.

A suite of specialized floral features limit the pollinators of *B. orientalis* to a very small subset of the flower-visiting fauna of the area. The function of most of these specialized features is apparently to make the flowers unattractive to insects, thus excluding the major portion of potential pollinators. The role of floral features in limiting the pollinator fauna of *B. orientalis* is most evident in a comparison with the more "generalist" flowers of *Brunsvigia bosmaniae*, a species which frequently occurs in close proximity to *B. orientalis* (Table 1.4 & Fig. 1.4). *B. bosmaniae* is pollinated by honeybees, carpenter bees and bee-flies by day, and by settling moths and hawkmoths by night (Raimondo and Pauw unpublished). The flowers of *B. bosmaniae* are ignored by sunbirds. The carpenter bee, *Xylocopa caffra*, is the only flower visitor that the two *Brunsvigia* species have in common. A comparison of the behaviour of *X. caffra* on the flowers of *B. orientalis* and *B. bosmaniae* demonstrates the importance of flower orientation in determining the pollinator specificity of *B. orientalis* (Fig. 1.1f & Fig. 1.4a). The importance of red coloration in limiting the pollinator fauna of *B. orientalis* is suggested by the observation that old flowers of *B. bosmaniae*, which presumably no longer need to attract insects, turn deep pink (Raimondo and Pauw unpublished).

In addition to features that serve to exclude insects, *B. orientalis* has a range of features which promote pollination by birds, and by long-billed passerines in particular (Table 1.5). Unrelated bird-pollinated plants in many parts of the world have much in common with the flowers of *B. orientalis*, suggesting that many of the features listed in Table 1.4 & Table 1.5 are adaptations that arise under the selective pressure of bird pollinators and nectar robbing insects (Faegri & Pijl, 1979; Vogel, 1954). However, in *Brunsvigia* the inflorescence is also the dispersal unit, and selection for enhanced dispersal is also likely to have played a role in shaping inflorescence morphology.

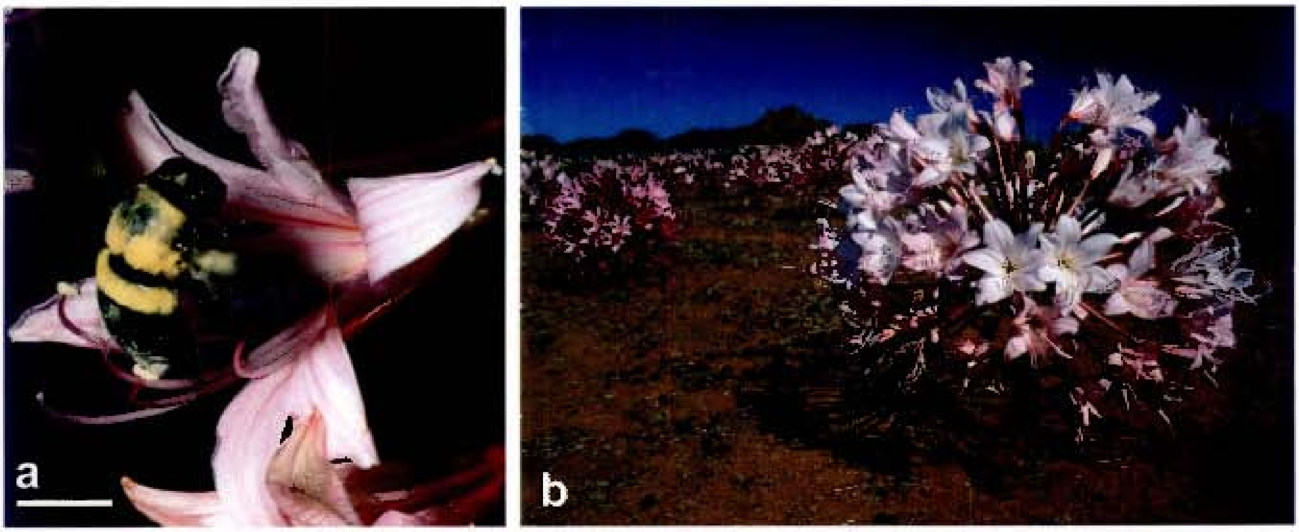

Fig. 1.4 Floral biology of B. bosmaniae. a, Xylocopa caffra contacts the anthers and is dusted with pollen. Scale bar = 12 mm. b, Inflorescence morphology.

Table 1.4 Floral features likely to be important in determining the degree of specialization of the pollination system in two *Brunsvigia* species. The solely sunbird pollinated *B. orientalis* is compared with *B. bosmaniae*, which is pollinated by honey bees, carpenter bees, bee-flies, hawkmoths and settling-moths (Raimondo and Pauw unpublished). Measurement were taken at Wiedourivier (*B. bosmaniae*) and Nieuwoudtville (*B. orientalis*). The two sites are separated by 50 km.

Plant feature	*B. orientalis*	*B. bosmaniae*	Probable role of the feature in determining the breadth of the pollinator fauna
Mouth of nectar tube	Obstructed by anther bases	Unobstructed	Most insects have insufficient strength to access obstructed nectar; sharp bills of sunbirds can force floral parts apart.[1]
Maximum nectar concentration	16 %	50 %	Insects can not forage efficiently on weak nectars; birds can.[2]
Daily peak in nectar volume	Morning only	Morning and evening	Low nectar volumes at sunset discourage moths.[3]
Flower colour	Red	Brilliant white, turning light pink	Most insects are insensitive to red; birds have high retinal sensitivity to red.[4]
Display	39 ± 1.7 flowers per inflorescence; 59 % open at peak flowering.	74 ± 6.0 flowers per inflorescence; 68 % open at peak flowering.	Insects have a strong preference for larger displays; birds are adept at finding inflorescences with smaller displays.[2]
Nectar guides	Absent	Present, bold	Insects use nectar guides; birds learn to find nectar without guidence.[4]
Odour	None	Sweet, strongest at sunset	Moths and diurnal insects use scent to find flowers; nectivorous birds have a poorly developed sense of smell.[4]
Corolla orientation	Recurved	Straight	Recurved flowers face away from oncoming insects but towards the perched bird.[5]
Stamen and style orientation	Resupinate	Non-resupinate	Resupinate stamens/styles overarch the flower visitor and can not serve as landing platform for insects.[4]
Petal width	Narrow	Broad	Broad petals funnel insects towards the anthers/stigma.[4]
Anther attachment	Mobile	Mobile	Mobility hinders pollen-collecting bees.

[1](Pauw, 1998), [2](Bolten & Feinsinger, 1978), [3](Luyt & Johnson, 2001; Nilsson et al., 1992), [4](Faegri & Pijl, 1979), [5](Rebelo, 1987).

Table 1.5 Features of *Brunsvigia orientalis* likely to promote pollination by sunbirds. Information for *B. bosmaniae*, an insect pollinated species, is included for comparison. Measurement were taken at Wiedourivier (*B. bosmaniae*) and Nieuwoudville (*B. orientalis*).

Plant feature	*B. orientalis*	*B. bosmaniae*	Probable role of the feature in promoting pollination by *Nectarinia*
Maximum nectar volume	47 µL	8 µL	Birds have larger energy requirements than insects and can process larger volumes.[1]
Pedicel width	5.1 ± 0.15 mm	3.6 ± 0.17 mm	Thick pedicels provide sturdy perches for birds·[1]
Distance between neighbouring flowers	41 ± 7.5 mm	14 ± 2.5 mm	Widely spaced flowers allow birds to enter the inflorescence and perch on the pedicels.
Corolla curvature	Curved	Straight	*Nectarinia* have curved bills that move in a arc when feeding.[2]
Robustness of floral parts	Robust	Less robust	Birds apply considerable force when visiting flowers.[1]
Anthesis	Morning	Morning	*Nectarinia* are diurnal and most active in the mornings.[3]
Inflorescence lifespan	Longer	Shorter	Sequential flower opening prolongs the flowering period allowing birds to become established in area.[4]

[1](Faegri & Pijl, 1979), [2](Westerkamp, 1990), [3](Rebelo, 1987), [4](Stiles, 1981).

The poverty of the bird fauna of the area contributes further to the specialization of bird pollination systems at the Cape. Only seven long-billed nectivorous birds occur within the distribution range of *B. orientalis* (Goldblatt & Manning, 2000; Harrison et al., 1997a). The distribution ranges of Sugarbirds (*Promerops cafer*), and Orangebreasted Sunbirds (*N. violacea*), broadly overlap the distribution of *B. orientalis*, but they seldom visit areas of coastal thicket where *B. orientalis* occurs (Rebelo, 1987). *N. fusca* enters the distribution of *B. orientalis* along its north-western margin, but I did not observe it at flowering populations in this region. *N. afra* and *N. amethystina* enter the range of *B. orientalis* in the east, just outside the study area. It seems likely that they play a role in the pollination of *B. orientalis* in that region. *Zosterops pallidus*, a short-billed, facultative nectar feeder, was common at some study sites. It feeds destructively on the nectar of many sunbird pollinated flowers, but was not observed to visit the flowers of *B. orientalis*, which has nectar protected by a thick perianth that releases bitter sap when damaged.

There are no nectarivorous bats in the region, and very few insect that are large enough to span the distance between the nectar and the anthers or stigma. Frazer and McMahon (1994) observed the mountain pride butterfly *Aeropetes tulbaghia* visiting the flowers of *B. orientalis*. The presence of this satyrid, which normally occurs at higher altitudes, can be attributed to the proximity of coastal cliffs. Frazer and McMahon (1994) do not provide detailed observations, but judging by the morphology of the flowers, it seems unlikely that the butterfly would make contact with the stigmas and anthers of *B. orientalis*. The butterfly is a specialist robber of red, bird pollinated plants (Johnson, 1994b).

Predictions

Large parts of the former distribution range of *B. orientalis* have been transformed by agriculture and the urbanization of the lowlands. As a result, many of the remaining populations occur in small remnants of natural vegetation, which might not be large enough to support the needs of sunbirds. The results of this study suggest that the floral morphology and nectar properties of *B. orientalis* prevent pollination by all vectors except sunbirds, while breeding system data show that seed set is dependent on cross-pollination. On the basis of these observations, I predict that sexual reproduction in *B. orientalis* will fail in areas that are not able to support sunbirds. The further observation that *B. orientalis* is incapable of vegetative reproduction, leads to the prediction that *B. orientalis* populations will decline in the absence of sunbirds. Some of these predictions are explored in the following chapters.

2 Disruption of bird-pollination by anthropogenic and natural environmental transformation

Sixteen conservation areas at the Cape of Africa were assessed for their ability to conserve a bird-pollination mutualism between *Brunsvigia orientalis* (Amaryllidaceae) and *Nectarinia* sunbirds. Seed set in *B. orientalis* varied among conservation areas from an average of 311 seeds per plant to an average of 2 seeds per plant. Pollen supplementation experiments and censuses of sunbirds showed that the failure of seed set in some conservation areas could be attributed to the lack of sunbirds. Three factors: 1) the post-fire successional stage of the vegetation, 2) the number of flowering *B. orientalis* plants, and 3) the size of the conservation area, explained 75 % of the variation in the number of seeds produced per capsule among sites in 2000. The number of seeds per capsule was reduced to near zero in recently burned vegetation and was somewhat reduced in small populations. Anthropogenic factors overlay this natural pattern of variation. The number of seeds per capsule was strongly depressed in small conservation areas (21.7 - 29.9 ha), and somewhat reduced in medium-sized conservation areas (811 - 1113 ha) compared with large conservation areas (> 15 000 ha). In a multi-year data set (1998, 2000, 2001), the size of the conservation area and the number of years since fire were significant predictors of variation in the number of seeds per capsule. Calendar year and number of flowering plants were non-significant factors in this analysis. Among-population differences in the number of seeds per capsule translated directly into among-population differences in whole plant seed production, suggesting that differences in the number of capsules per plant (caused by infraspecific morphological variation and herbivory) played a relatively minor role in determining whole plant seed set. I suggest that both habitat fragmentation and fires indirectly cause the failure of the *Brunsvigia-Nectarinia* pollination mutualism by reducing nectar resources, and hence sunbird abundance, in the plant community to which *B. orientalis* belongs.

One way to view an ecosystem is as a web of interacting organisms (Bascompte et al., 2003; Gilbert, 1981). From this perspective, the loss of a species means the loss of a set of interactions, which could lead to linked extinctions of other dependent species (Gilbert, 1981). Such cascades of linked extinctions could accelerate the current process of human-caused biodiversity loss.

Birds are particularly sensitive to environmental degradation, and are among the first species to be lost from human impacted ecosystems (Debinski & Holt, 2000; Saunders et al., 1991; Turner, 1996). Because of their mobility, birds interact with many other species and fulfill a range of ecological roles, among which pollination is one of the more specialized and irreplaceable (Gilbert, 1981; Kearns et al., 1998; Renner, 1998). In some areas of the world, for example Neotropical rainforests and the Mediterranean climate shrublands of Australia, more than 10% of the flora is dependent on birds for pollination (Bawa, 1990; Keighery, 1982; Rebelo, 1987). What happens to plants when they lose their mutualistic bird pollinators?

Surprisingly, a large percentage of the studies to date suggest that bird-pollination systems rarely break down (Aizen & Feinsinger, 1994; Bruna & Kress, 2002; Cox, 1983; Feinsinger et al., 1982; Kelly et al., 2000; Smith et al., 1995). Most understorey hummingbirds (Trochilidae) seem able to persist in a matrix of forest patches, secondary growth and cleared areas (Stouffer & Bierregaard, 1995). Hummingbirds are attracted to forest edges and gaps because these areas have more flowers than less disturbed forest (Stouffer & Bierregaard, 1995). Trap-line foragers typically travel hundreds of meters between the flowers they visit (Kress, 1985). Thus, Aizen & Feinsinger (1994) and Bruna & Kress (2002) found no response, or higher pollination levels in forest remnants. In both studies, the smallest remnants (~1 ha) were probably too small to support hummingbird populations, but the ~100 m of clearcut that separated the remnants from continuous forest apparently posed no barrier to hummingbirds. Similarly, Kelly *et al.* (2000) report that New Zealand honeyeaters (Meliphagidae) were attracted to forest edges and isolated trees in pastures, resulting in higher pollination rates for mistletoes in these settings.

Ecological redundancy seems to provide resilience in speciose bird-pollination systems. Feinsinger *et al.* (1982) found that bird-pollination of eight plant species was only subtly affected by the natural reduction in hummingbird diversity from 16 species on the larger Trinidad to 5 species on the smaller Tobago. A similar lack of pollinator specificity has saved two bird-pollinated Hawaiian plants. Formerly they were pollinated by now rare or extinct nectivorous birds; today, the introduced *Zosterops japonica* (Zosteropidae) and the indigenous but previously subordinate *Vestiaria coccinea* (Drepanididae), respectively perform that function (Cox, 1983; Smith et al., 1995).

Evidence for a decline in bird pollination comes only from areas of very high human impact. Two Australian plant species, which have features suggestive of pollination by honeyeaters, had lowered fruit set in small remnants surrounded by extensive cleared areas (Cunningham, 2000a, 2000b; Paton, 2000). Hand pollination of selected flowers or branches on plants in the remnant populations suggested that the deficit in seed set might be due to a paucity of pollinators. Nevertheless, reproduction did not fail in either plant species (~17% fruit set in *Astroloma*; and ~30% in *Eremophila*).

Extreme specialization combines with extreme landscape transformation in the Andean highlands. The sword-billed hummingbird (*Ensifera ensifera*) sips nectar from a guild of exceptionally long-tubed flowers (Lindberg & Olesen, 2001). One of the long-tubed plants, *Passiflora mixta*, set few or no fruits in road-side hedges in cleared areas, compared with 0.4 fruits per branch in forest remnants. The authors suspect that the fruit set deficit results from lower *Ensifera* visitation in open areas. Introduced honeybees (*Apis mellifera*) were frequent visitors to the flowers in open areas, but they are apparently ineffective pollinators.

Introduced honeybees have the potential to disrupt bird-pollination mutualism on a continent-wide scale (Huryn, 1997; Roubik, 1996). Honeybees are normally ineffective pollinators of bird-flowers and reduce the effectiveness of bird-pollination by robbing nectar and pollen (Paton, 1993; Vaughton, 1996) (England et al., 2001; Hansen et al., 2002). Ironically, honeybees might sometimes be important pollinators of bird-flowers in areas where birds have been lost (Paton, 1997). It is possibly for this reason, that no studies document major impacts on seed set when honeybees displace birds.

Here I present the results of a study of habitat fragmentation on bird-pollination at the Cape of Africa, an area characterized by exceptional botanical diversity (Goldblatt & Manning, 2002) and a high proportion of bird-pollinated plants (Rebelo, 1987).The large number of bird-pollinated plant species (4 % or 318 species) is dependent on only nine species of insectivorous birds (Rebelo, 1987), of which about three co-occur at any one site. These high levels of specificity potentially make the system more vulnerable than bird-pollination systems which involve more bird species (Bond, 1994). To test whether disruption of bird-pollination is occurring at the Cape, I focused on the lowlands, where about 90 % of the area has been transformed by agriculture and urbanization (Heijnis et al., 1999; Kemper et al., 2000; McDowell & Moll, 1992). Within this matrix, small nature reserves have been proclaimed to conserve the endemic lowland flora (Hanks, 2003; Maze & Rebelo, 1999). I tested the ability of these proclaimed conservation areas to conserve a bird-pollination mutualism involving *Brunsvigia orientalis* (Amaryllidaceae), a conspicuous member of the endemic lowland flora, and *Nectarinia* sunbirds. In Chapter 1, I found that *B. orientalis* is self-incompatible, incapable of vegetative reproduction and dependent on pollination by only two species of sunbird (*Nectarinia famosa* and *N. chalybea*). Flowering occurs in autumn, when nectar resources for sunbirds are the most limited.

I addressed the following questions:

- What is the relationship between bird visitation rate and seed set?
- Is seed set limited by bird availability or by resource availability (e.g. nutrient, water)?
- How does seed set vary across space and time in relation to:
    - calendar year
    - successional stage of the plant community
    - number of flowering plants
    - size of the conservation area
- Do herbivores significantly influence seed set?

Materials and methods

Study sites

The study region of 2 500 km^2 is located in the western half of the Cape Lowlands and encompasses most of the known populations of *B. orientalis*. Within this region, I chose 16 conservation areas (Fig. 2.1, Table 2.1). Four of the areas were small (range, 21.7 - 29.9 ha), two medium sized (range, 811 - 1113 ha) and nine large (continuous with > 15 000 ha of untransformed land).

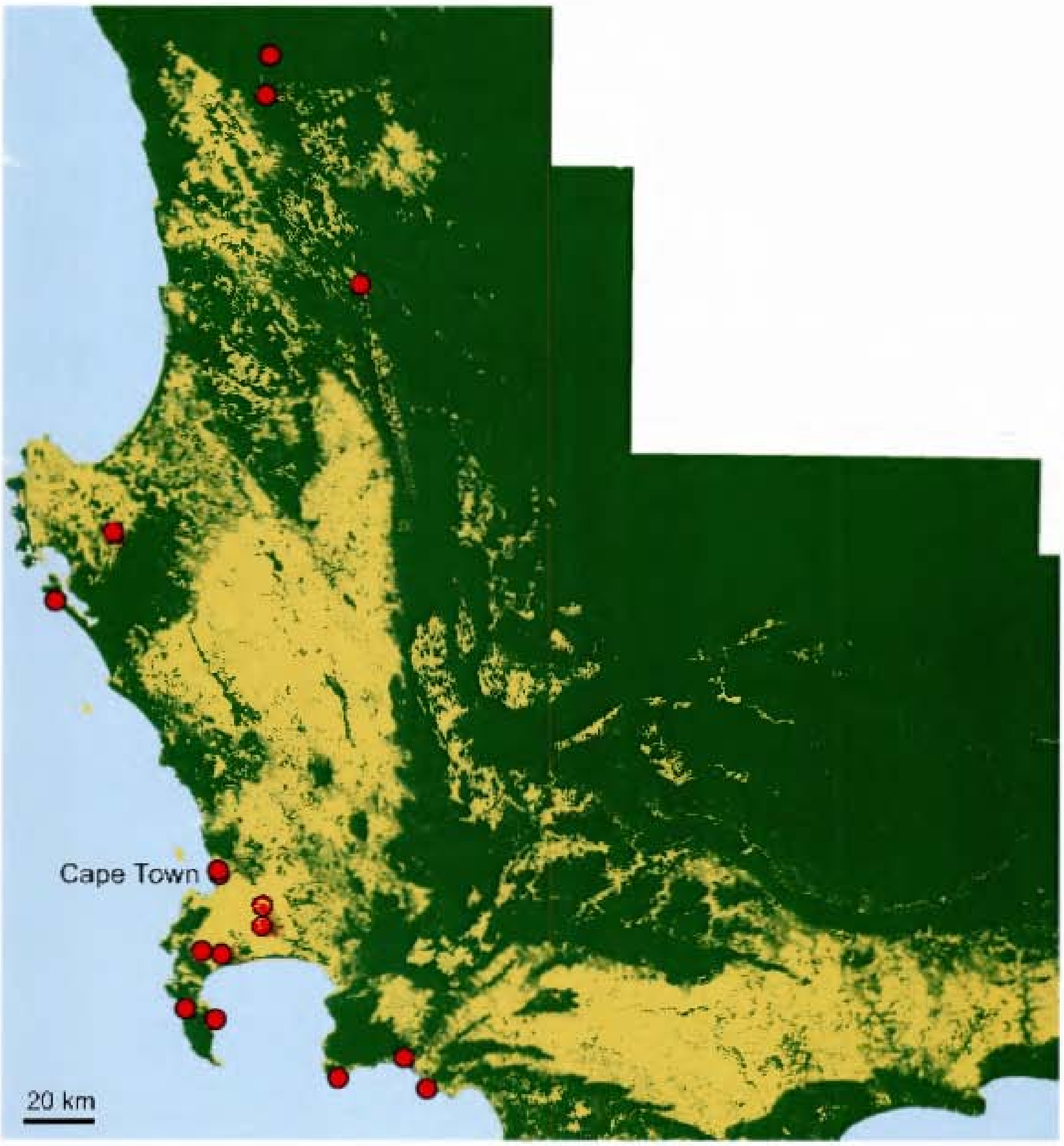

Fig. 2.1 Location of study sites (red dots) where variation in pollination rate and seed set in *Brunsvigia orientalis* was studied. Green indicates natural areas; yellow indicates areas that have been transformed by urbanization or agriculture.

The listed size of each conservation area includes all untransformed areas contiguous with the formally proclaimed protected area. Small and medium-sized reserves were located within the Cape Town Metropolitan area. Two of the sites were burnt in wildfires two months prior to the study (Table 2.1). None of the other sites were burnt in the preceding five years. Fire is a natural and regular phenomenon in the shrublands of the Cape (Bond & Van Wilgen, 1996).

Pollinator visitation rate

In Feb. and March 2000 I quantified pollinator visitation rates at the flowers of *B. orientalis* at 10 sites (indicated in Table 2.1). Bird visitation rates were determined by observing between 88 and 812 flowers (median = 185) at each site for two separate 0.5 h periods during peak flowering. I avoided inclement weather and the midday lull in bird activity. The number of visits observed was divided by the number of open flowers observed and by the number of hours of observation to get visits per flower per hour.

Pollen limitation experiment

I supplemented natural levels of pollination with hand-pollination to determine whether seed set was limited by pollinator availability or by the availability of resources such as nutrients or water. I used a small paintbrush to apply mixed pollen from five sympatric pollen parents to the stigmas of all open flowers on between 2 and 8 plants (median = 3.5) at 8 sites. At each site between 10 and 44 control plants (median = 22) were left unmanipulated. Seed set for pollen-supplemented and control plants was quantified on a whole plant basis because the question of resource limitation can only be addressed at the whole plant level (Johnson & Bond, 1997; Zimmerman & Pyke, 1988). The list of sites used in this study appears in Fig. 2.3.

Determinates of seed set across sites in 2000

I returned after flowering and recorded the level of seed set per capsule at all 16 study sites by opening all the capsules on between 4 and 44 (median = 22.5) randomly selected plants at each site. Each ovary develops into a winged capsule regardless of whether or not it contains seeds. The capsules are never shed, and mature seeds are retained within the capsules for some time. In 2000 I recorded seeds set for 12 708 flowers on 346 plants. In this portion of the study I focused on pollination-related differences in seed set between sites, therefore, I controlled for the effect of differences in plant size and for the effect of herbivores (which removed entire flowers along with their ovaries) by dividing total seed set per plant by the number of remaining capsules. This gave the number of seeds per capsule (seed set), which is the main dependent variable used in this study.

A problem with working at large geographical scale is that classical island-mainland comparisons might be confounded by naturally occurring spatial variability. Thus, I attempted to simultaneously evaluate the significance of natural and anthropogenic sources of variation. I visually assessed normal probability plots and histograms of log-transformed seed set data for normality and homoscedasticity. A preliminary one-way ANOVA was run to test whether seed set differed significantly between sites. Then, a range of predictors (anthropogenic and natural), which seemed most likely to be important in determining seed set, were included in a general linear model of seed set. Some of the predictors are continuous; others are categorical. The model was built in Statistica (StatSoft Inc., Version 6.1) by the step-wise addition of the predictors. Coding for the predictors is as follows: (1) size of conservation area [large; medium; small]; (2) successional stage of the plant community [burnt in the last year; not recently burnt]; (3) number of flowering *B. orientalis* plants [continuous variable, log-transformed]. Number of flowering plants is the number of co-occurring flowering plants.

Multi-year comparison of seed set

At six of the sites I recorded additional seed set data in 1998 and/or 2001 (for sites and years used see Fig. 2.6). Seed set data were log-transformed. The significance of the following predictor variables was assessed in a general linear model of seed set across sites and years: (1) calendar year [1998, 2000, 2001]; (2) size of conservation area [large, medium, small]; (3) successional stage of the plant community [continuous variable, log-transformed]; (4) number of flowering *B. orientalis* plants [continuous variable, log-transformed]. For recently burnt sites the time since the last fire was known; for sites that were not burnt recently, the time since the last fire was estimated from the condition of the vegetation. The number of years since fire was log-transformed, because most vegetation change occurs in the first few years.

Whole plant seed production

Whole plant seed production is the product of seed set per capsule (the main dependent variable used in this chapter) and the number of capsules per plant. Pearson correlation was used to determine how well site related differences in seed set per capsule translated into differences in whole plant seed production.

Results

Pollinator visitation rate in relation to seed set

The sites could be divided into two groups on the basis of sunbird visitation rates: sites where sunbirds were abundant (> 0.25 visits per flower per hour, $N = 6$); and those where sunbirds were rare (< 0.04 visits per flower per hour, $N = 4$, Fig. 2.2). *B. orientalis* populations with abundant sunbirds set significantly more seeds than populations where sunbirds were rare (Fig. 2.2).

Pollen limitation experiment

The addition of pollen to the stigmas of *B. orientalis* flowers increased the number of seeds set per plant at 7 of 8 sites, and significantly so at 5 of 8 sites. At one site, which had the highest levels of natural seed set, pollen-supplemented plants set significantly fewer seeds than unmanipulated controls (Fig. 2.3). Determinates of seed set variation across sites in 2000

Average seed set varied between sites from 311 seeds per plants to 1.9 seeds per plant. A preliminary one-way ANOVA indicated that the variation in seeds set among sites was significant (Table 2.1). A general linear model of the form log(seed per capsule + 1) = $\beta_0 + \beta_1$(size of conservation area) + β_2(successional stage of the plant community) + β_3(log(number of flowering plants)) explained 75.54 % of the variability in seed set across 16 sites in the year 2000 (Table 2.2). The addition of each predictor stepwise improved the predictive power of the model. The overall model was highly significant. The size of the conservation area and the successional stage of the plant community were the best predictors of seed set (Fig. 2.4). The predictive power of the model was only slightly improved by adding the number of flowering plants as a variable (Fig. 2.5)

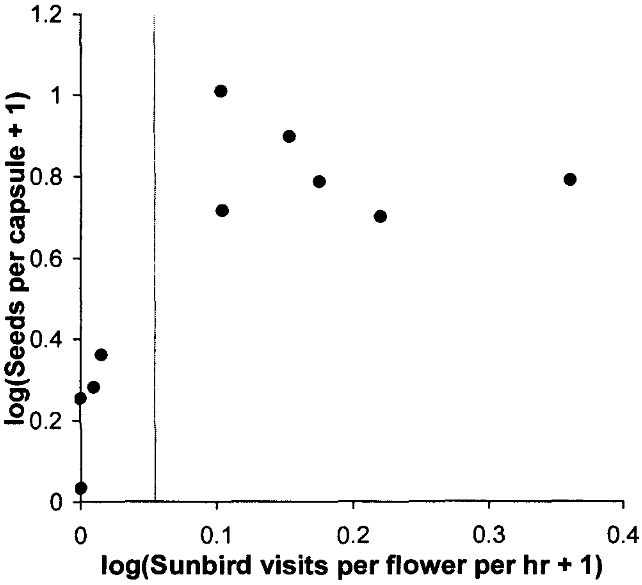

Fig. 2.2 Relationship between sunbird visitation rates and seed set in *Brunsvigia orientalis* at 10 sites. A weak relationship is expected because seed set reflects the sum total of pollen receipt over the lifetime of the inflorescence, whereas bird censuses are snapshots in time. For statistical testing, sites were divided along the dotted line into sites with high visitation rates and sites with low rates. Mann-Whitney U Test, $Z = -2.56$, $N = 6$ and 4, $p < 0.01$.

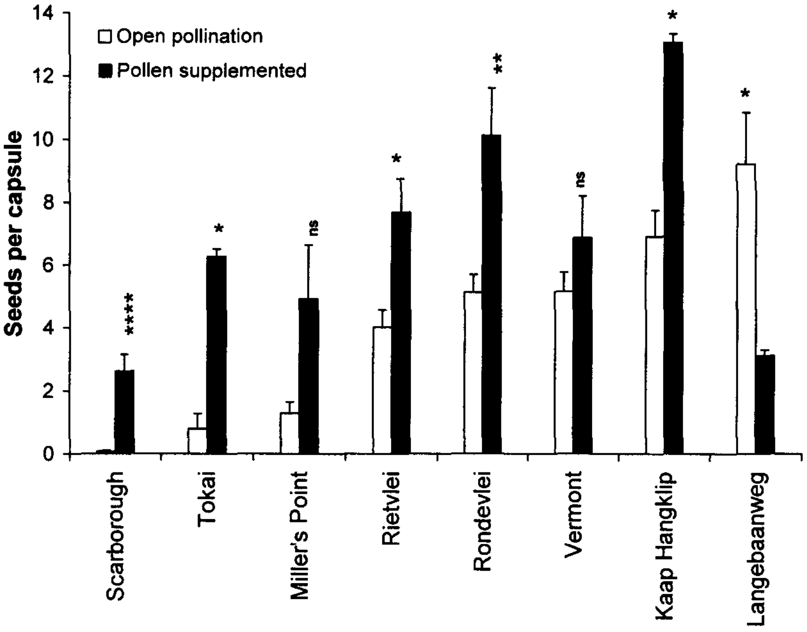

Fig. 2.3 The effect of supplementary hand-pollination on seed set in *Brunsvigia orientalis* at 8 sites. Mann-Whitney U Test results are displayed as: ns not significantly different, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

Table 2.1 Seed set in *B. orientalis* in the year 2000, and the three predictor variables that were used to explain variation in seed set. A preliminary ANOVA indicated that there was significant variation in seed set between sites ($F = 49.7$, df = 15, 330, $P < 0.001$).

Site	Size of conservation area*	Successional stage of the plant community	Number of flowering plants	Seeds per capsule ± SE
Vermont[§]	large	not burnt	60	5.15 ± 0.61
Kleinmond[§]	large	not burnt	24	4.19 ± 0.69
Kaap Hangklip[§]	large	not burnt	596	6.90 ± 0.84
West Coast National Park	large	not burnt	50	6.69 ± 1.74
Langebaanweg[§]	large	not burnt	85	9.20 ± 1.63
Citrusdal	large	not burnt	150	9.14 ± 0.67
Klawer	large	not burnt	300	8.41 ± 0.47
Wiedourivier	large	not burnt	400	9.18 ± 0.76
Scarborough[§]	large	burnt[†]	150	0.08 ± 0.02
Miller's Point[§]	large	burnt[‡]	85	1.29 ± 0.36
Swartklip Nature Reserve	medium	not burnt	8	2.17 ± 1.25
Rondevlei[§]	medium	not burnt	88	5.12 ± 0.56
Tokai[§]	small	not burnt	9	0.79 ± 0.48
Cape Flats Nature Reserve[§]	small	not burnt	11	0.91 ± 0.36
Woodbridge	small	not burnt	60	0.34 ± 0.14
Rietvlei[§]	small	not burnt	60	4.01 ± 0.55

* Small and medium sized reserves are located within the Cape Town Metropolitan Area

[†] Burnt in January 2000

[‡] The population was not burnt, but most of the peninsula on which it is situated was burnt in January 2000

[§] Sites where pollinator visitation rates were determined (see Fig. 2.2).

Multi-year comparison of seed set

A general linear model of the form log(seeds per capsule + 1) = $\beta_0 + \beta_1$(calendar year) + β_2(size of conservation area) + β_3(log(years since fire + 1)) + β_4(log(number of flowering plants)) explained 74.31 % of the spatial and temporal variation in seed set in a data set that spanned three years and six sites (Table 2.3). Years since fire and size of conservation area had the strongest influence on seed set; number of flowering plants and calendar year marginally improved the overall predictive power of the model. Fire depressed seed set dramatically, followed by a partial recovery in the following year (Fig. 2.6). In the absence of fire, seed set remained relatively stable across years, and differences between sites were maintained (Fig. 2.6).

Whole plant seed production: the role of pollinators, herbivores and inflorescence size

The average number of flowers (capsules) per inflorescence varied between sites from 21 to 60 (Table 2.4). This range was reduced by herbivores, particularly steenbok (*Raphicerus campestris*),.which removed flowers (usually along with their ovaries and pedicles) (Table 2.4). There was no predation on mature seeds or capsules. Visual inspection of the data revealed no clear pattern of variation in flower number in relation to reserve characteristics or population size. The magnitude of variation between sites in the number of capsules per plant was overshadowed by pollination related differences in seed set across sites (Table 2.1). Thus, per capsule seed set data translated well into whole plant seed production despite the potentially confounding influence of herbivores and differences in inflorescence size (Fig. 2.7). Only at one site (Langebaanweg) did herbivores have a major impact on whole plant seed production (Fig. 2.7). The differences in seeds set between large and small reserves, and between burnt and unburnt reserves, translate into similar differences in whole plant seed production. Only the difference between medium sized reserves and large, unburnt reserves is lost due to the confounding effect of herbivores and differences in inflorescence size (Fig. 2.8).

Table 2.2 The ability of a range of predictor variables to explain variation in seeds set in *Brunsvigia orientalis* at 16 sites in the year 2000. The predictor variables were combined in a general linear model of the type log(seed per capsule + 1) = $\beta0 + \beta1$(size of conservation area) + $\beta2$(fire) + $\beta3$(log(number of flowering plants)). Adjusted $R^2 = 0.57$, $p < 0.001$, type III sum of squares. For a list of sites used see Table 2.1.

Predictor	Variable type	SS	df	MS	F	p
Size of conservation area	categorical	8.78047	2	4.39023	64.2305	0.000000
Successional stage of the plant community	categorical	20.41398	1	20.41398	298.6627	0.000000
Log(number of flowering plants)	continuous	1.30139	1	1.30139	19.0397	0.000017
Error		23.30778	341	0.06835		

Table 2.3 The ability of a range of predictor variables to explain variation in seed set in *B. orientalis* across three years and six sites. The predictor variables were combined in a general linear model of the type log(seed per capsule) = $\beta_0 + \beta_1$(calendar year) + β_2(size of conservation area) + β_3(log(years since fire + 1)) + β_4(log(number of flowering plants + 1)). Adjusted $R^2 = 0.54$, $p < 0.001$, type III sum of squares. For a list of sites and years used see Fig. 2.6.

Predictor	Variable type	SS	df	MS	F	p
Calendar year	categorical	0.35672	2	0.17836	2.5481	0.079722
Size of conservation area	categorical	11.79719	2	5.89860	84.2691	0.000000
Log(years since fire + 1)	continuous	11.81115	1	11.81115	168.7376	0.000000
Log(number of flowering plants + 1)	continuous	0.01195	1	0.01195	0.1707	0.679793
Error		23.79904	340	0.07000		

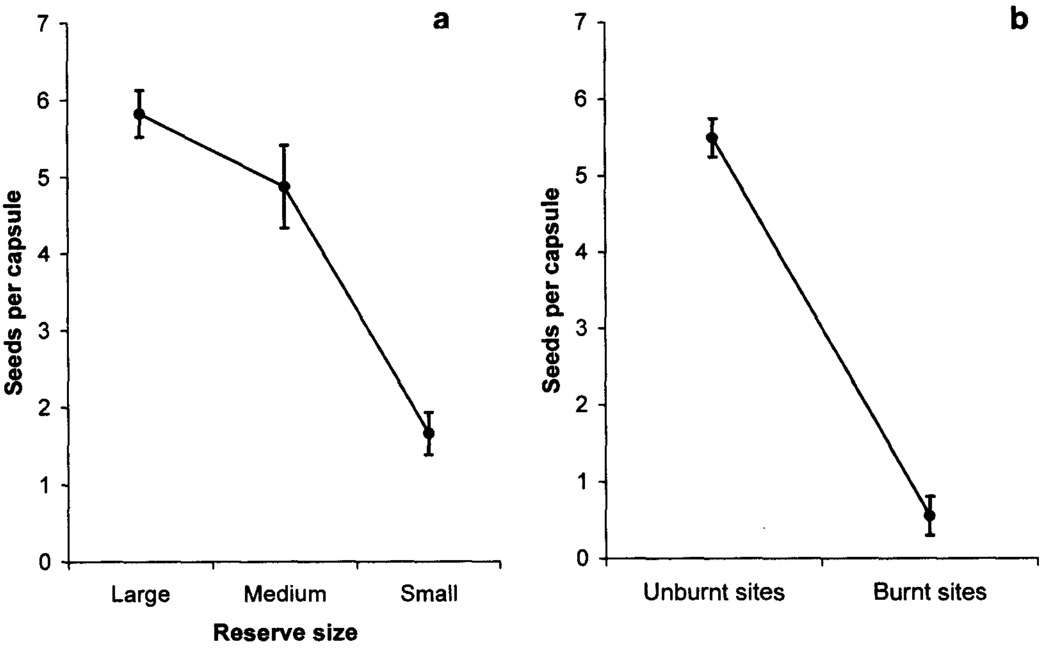

Fig. 2.4 Variation in seed set in *Brunsvigia orientalis* in relation to the characteristics of the conservation area. **a**, Size of the conservation area. **b**, Post-fire successional stage of the vegetation.

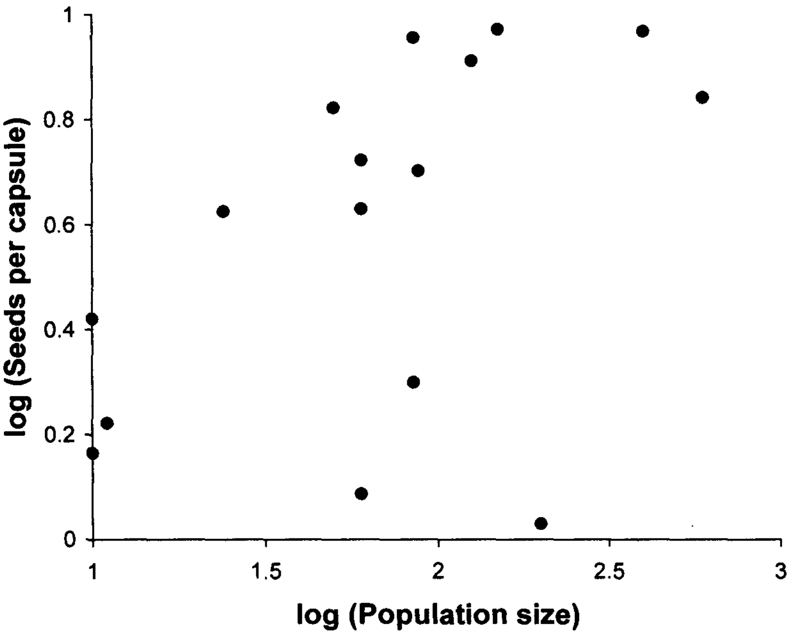

Fig. 2.5 Variation in seed set in *Brunsvigia orientalis* in relation to the number of flowering plants in the population.

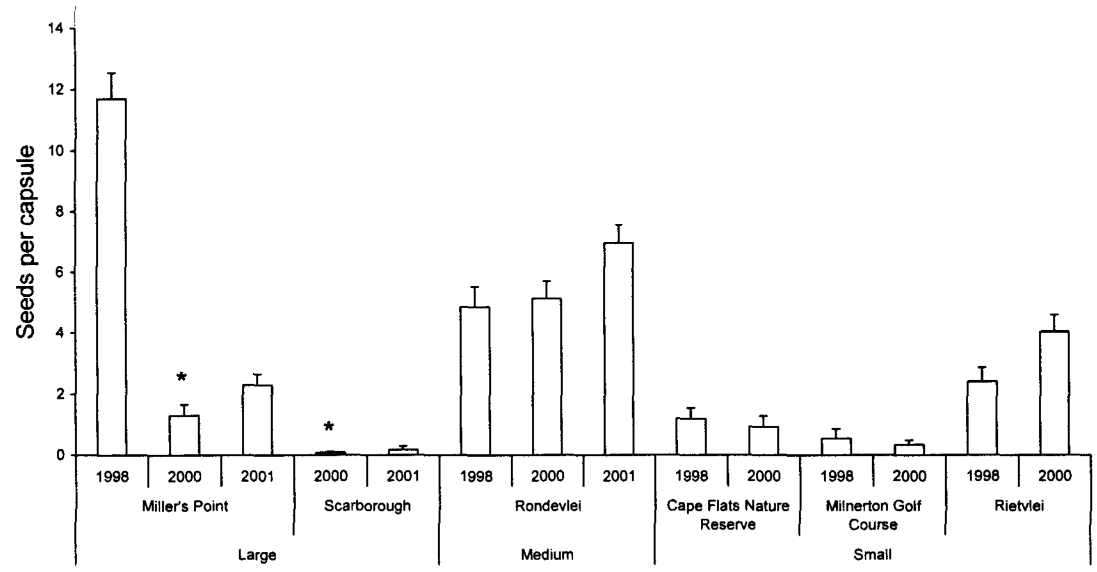

Fig. 2.6 Temporal variation in seed set in *Brunsvigia orientalis* in relation to the size of the conservation area and the occurrence of fire. Conservation areas are grouped according to size as Large, Medium and Small. * indicates the occurrence of a fire. Only conservation areas for which more than one year of data were available are included here.

Table 2.4 Variation between sites in the number of capsules per plant. Differences result from variation in the initial number of flowers produced and variation in the number of flowers (capsules) removed by herbivores. All flowers produce capsules regardless of whether or not they contain seeds.

Site	Flowers produced per plant	Flowers/Capsules destroyed per plant	Capsules remaining
Vermont	42.1	0.14	42 ± 2.01
Kleinmond	28.1	0.43	27.6 ± 2.04
Kaap Hangklip	32.0	4.30	27.7 ± 1.91
West Coast National Park	32.0	0.00	32 ± 7.90
Langebaanweg	60.0	49.00	11 ± 1.77
Citrusdal	34.0	0.00	34 ± 0.71
Klawer	33.7	0.00	33.7 ± 1.53
Wiedourivier	24.1	0.00	24.1 ± 1.57
Scarborough	21.4	0.00	21.4 ± 1.49
Miller's Point	37.6	0.68	36.9 ± 3.40
Swartklip Nature Reserve	41.8	25.50	16.3 ± 1.80
Rondevlei	49.5	0.86	48.6 ± 2.15
Tokai	31.7	0.00	31.7 ± 5.13
Cape Flats Nature Reserve	51.1	2.09	49 ± 6.48
Woodbridge	47.9	3.67	44.2 ± 2.35
Rietvlei	41.6	1.83	39.8 ± 3.49

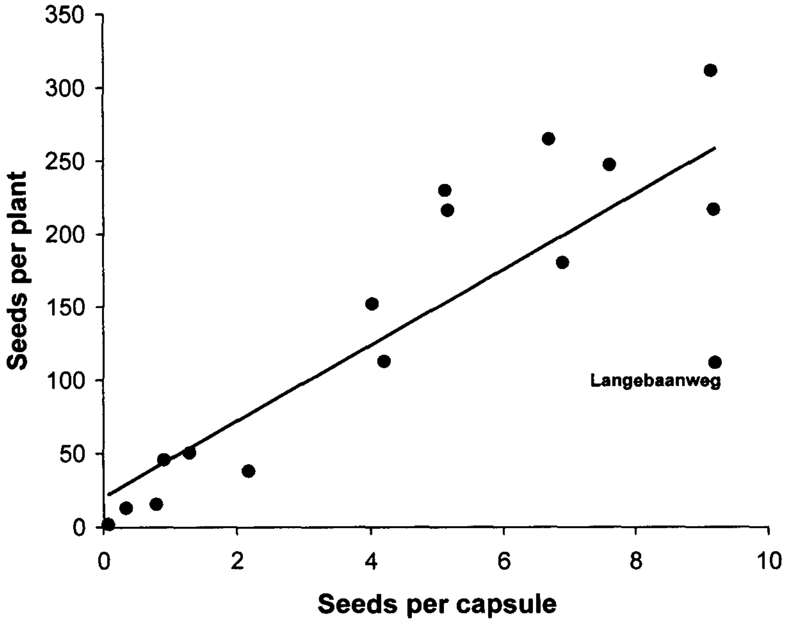

Fig. 2.7 Relationship across sites between seed set per capsule and total plant seed production in *Brunsvigia orientalis*. Residual variation can be attributed to differences in the number of flowers per plant (Table 2.4). At one site (Langebaanweg) seed set per capsule is a poor predictor of whole plant seed set, because herbivores have a large impact by reducing the number of flowers per plant. Pearson's correlation coefficient $r = 0.84$, $P < 0.01$.

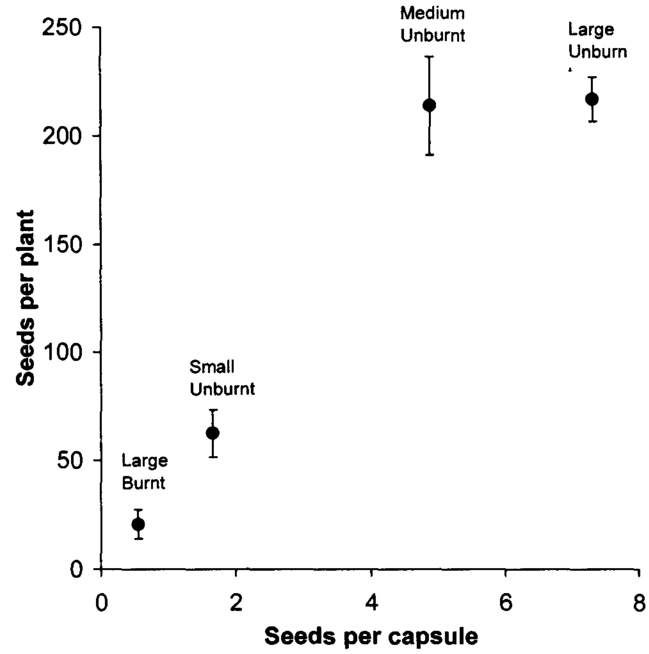

Fig. 2.8 Relationship across four classes of sites between seed set per capsule and total plant seed production in *Brunsvigia orientalis*. The differences in seeds set between large and small reserves, and between burnt and unburnt reserves, translate into similar differences in whole plant seed production.

Discussion

Why does seed set fail?

Seed set in *Brunsvigia orientalis* varied between populations from an average of more than 300 seeds per plant to a population average of 2 seeds per plant (Table 2.1, Fig. 2.7). Reproduction in plants commonly fails due to a lack of genetic resources (i.e. unrelated mates) or growth resources (i.e. nutrients, water or light) (Wilcock & Neiland, 2002). However, the following three lines of evidence suggest that reproductive failure or depression in *B. orientalis* is not caused by the lack of these resources, but rather by the failure of the *Brunsvigia-Nectarinia* pollination mutualism. (1) *Nectarinia* sunbirds, the sole pollinators of *B. orientalis* (Chapter 1), were very rare or absent at sites with low seed set and common at sites with high seed set (Fig. 2.2). (2) Low levels of seed set could be restored in *situ* by artificially substituting the lost pollination services with hand-pollination using locally available pollen (Fig. 2.3). (3) High levels of seeds set could be artificially depressed to near zero by excluding sunbirds from inflorescences with gauze cages placed over plants in the field (Chapter 1).

Where does the Brunsvigia-Nectarinia *pollination mutualism fail?*

A substantial proportion of the variation in seeds set between sites can be explained by the natural properties of the areas. Seed set failed in early successional plant communities (Fig. 2.4) and was depressed in small populations (Fig. 2.5). The influence of habitat fragmentation, brought about by the recent urbanization of the Cape Lowlands, overlies this natural pattern of spatial and temporal variation in seed set — seed set was very low in small conservation areas and was somewhat depressed in medium sized areas (Fig. 2.4). These natural and anthropogenic factors together explained 75 % of the observed variation in seed set in *B. orientalis* (Table 2.2).

When does the Brunsvigia-Nectarinia *pollination mutualism fail?*

The geographical pattern of variation in seed set did not remain constant across years, but fluctuated considerably at some sites. A high proportion of the temporal variability is brought about by fire-driven vegetation change (Table 2.3). Miller's

Point provides an illustration. Seed set at this site dropped from 12 seeds per capsule in 1998 to 1 seed per capsule after an extensive fire (Pauw, 2001). Some recovery was already evident in the first year after the fire (Fig. 2.6). Calendar year is a poor predictor of seed set because fire is a local occurrence that does not affect all sites simultaneously (Table 2.3). In contrast with the temporary effect of fires, habitat fragmentation brings about a permanent depression of seed set, with little inter-annual fluctuation evident in small unburnt reserves (Fig. 2.6).

Whole plant seed set: the effect of pollinators, herbivores, and inflorescence size

In this chapter, I have been primarily interested in pollination related variation in seed set in *B. orientalis*. I have used seed set per capsule as the primary dependent variable, because it is a good measure of pollination success in a self-incompatible, pollen-limited plant. However, from the point of view of plant demography and population persistence, whole plant seed production is a more meaningful variable.

Whole plant seed production is the product of seed set per capsule and the number of capsules per plant. The number of capsules (flowers) per plant increases with plant age (unpublished data), decreases with herbivore activity (Table 2.4), and can be expected to vary between sites in relation to genotype and growth environment differences. Thus, site related differences in the number of capsules per plant have the potential to modify or reverse the effect of difference in seed set per capsule between sites. For example, Steffan-Dewenter *et al.* (2001) found that the negative effect of anthropogenic landscape transformation on pollination were counterbalanced by lower seed predation rates in more transformed landscapes (see also Kelly et al., 2000). In this study herbivory and inflorescence size differences had a relatively minor impact, so conclusions about the effect of natural and anthropogenic factors on capsule set also apply to whole plant seed production (Fig. 2.7 & Fig. 2.8).

Why does the Brunsvigia-Nectarinia pollination mutualism fail?

I suggest that both habitat fragmentation and fires indirectly bring about the failure of the *Brunsvigia-Nectarinia* pollination mutualism by directly acting on other sunbird-visited plants. Of seven sunbirds captured in a previous study, six were carrying the pollen of several other plant species in addition to the pollen of *B. orientalis* (Chapter

1). This result suggests that sunbirds depend on an extensive group of plant species to fulfill their nectar requirements. Although the members of the group may be scattered over a large area, sunbirds maintain the integrity of the group by acting as mobile links (sensu Gilbert, 1981). Corbet (2000) describes such sub-groups of closely interacting plants and their pollinator as "compartments in pollination webs" – there are more interactions within a compartment than across its boundaries.

Fire offers a unique opportunity to examine how dependent *B. orientalis* is on other plant species in the sunbird-pollination compartment. The most important nectar resources in the sunbird-pollination compartment at the Cape are large shrubs (Proteaceae and Ericaceae) (Rebelo, 1987), which are typically killed by fire and flower only in late successional communities (le Maitre & Midgley, 1992; Van Wilgen, 1981). In contrast, *B. orientalis* is not directly affected by fire because its bulbs are deeply buried and leafless during the fire season. Thus, *B. orientalis* populations flower normally after a fire, but in the absence of other members of the sunbird-pollination compartment. Under these conditions, they experienced very low sunbird visitation rates and near complete reproductive failure (Table 2.1, Fig. 2.6). Waser and Real (1979) demonstrated similar mutualistic interdependence between plants in a hummingbird-pollination compartment.

I suggest that habitat fragmentation acts much as fire does, by altering the community context in which *B. orientalis* occurs. *B. orientalis* populations in small conservation areas are effectively cut off from the greater community of which they were formally an integral part. These islands of remaining vegetation contain a small subset of the plants in the *Nectarinia*-pollination compartment, and this subset may not be sufficient to sustain a sunbird population.

Conserving the Brunsvigia-Nectarinia *pollination mutualism*

The interpretation of the results of this study suggests that the *Brunsvigia-Nectarinia* pollination mutualism is functional in *large* (< 15 000 ha) and *medium*-sized (range, 811 - 1113 ha) conservation areas, but non-functional in *small* conservation areas (range, 21.7 - 29.9 ha)(Fig. 2.4). Thus, a reduction in reserve size from medium to small can be expected to result in the collapse of the *Brunsvigia-Nectarinia* pollination mutualism. For example, Rondevlei (106 ha) is a medium-sized reserve by virtue of its continuity with 705 ha of untransformed, but not formally protected, land. The *Brunsvigia-Nectarinia* pollination mutualism will breakdown if the unprotected land is lost to proposed urban expansion; but will be conserved if this land is protected in the proposed False Bay Coastal Park.

Fires are a regular and natural source of disturbance in fynbos plant communities (Bond & Van Wilgen, 1996). Today, however, almost all fires at the Cape are started and/or extinguished by humans. High fire frequency will have a long-term negative effect on the *Brunsvigia-Nectarinia* pollination mutualism because the vegetation will be maintained in an early successional stage (Fig. 2.4). Management schemes aimed at maintaining low fire frequencies will benefit the *Brunsvigia-Nectarinia* pollination mutualism, especially if "block burning" is designed so that at any one time there is a large enough area of mature vegetation (> 1000 ha) to maintain sunbirds within the boundaries of the conservation area.

B. orientalis is a member of an integral sunbird-pollination compartment which includes two sunbird species and several nectar-producing plants species. Thus, conservation planning and management strategies might be most effective if they aim to conserve the sunbird-pollination compartment as a whole, rather than focusing on individual members of the compartment in isolation. For example, the boundaries of conservation areas can be drawn to include several members of the pollination compartment, either by using detailed population level distribution data, or by using vegetation type as a proxy.

The pollination compartment view has the advantage of allowing generalization. By monitoring seed set in a few long-lived, self-incompatible, large-seeded species (e.g. *B. orientalis*), it should be possible to monitor the reproductive "health" of the entire sunbird-pollination compartment. Prediction is another advantage. The members of a pollination compartment often share convergent morphologies by virtue of co-evolution with their pollinators (Faegri & Pijl, 1979). Thus, the morphology of the extinct *Erica verticillata* predicts that it is a member of the *Nectarinia*-pollination compartment. Successful pollination in re-established population of *E. verticillata* in small lowland conservation areas (Gibbs, 2003; Milton et al., 1999), will depend on the conservation of the entire sunbird-pollination compartment, of which *B. orientalis* is an integral member.

3 Flower size variation in *Brunsvigia orientalis* in relation to the historical and modern distribution of sunbird species

Broad-scale geographical patterns of variability in flower size in *Brunsvigia orientalis* (Amaryllidaceae) were found to reflect geographical variability in the community of sunbird pollinators. Small-flowered populations occur in the NW Cape; large-flowered populations occur in the SE. Both large sunbirds (*Nectarinia famosa*) and small sunbirds (*N. chalybea*) occurred throughout the study region, but a combination of seasonal migration and competitive interspecific interactions polarized the pollinator community so that small sunbirds were the only pollinators in the NW and large sunbirds were the primary pollinators in the SE. The adaptive significance of variation in flower size was evident from field observations of pollination: small sunbirds were ineffective pollinators of the large-flowered form. In a finer scaled geographical study, flower size accurately predicted the pollinator fauna at seven out of eight *B. orientalis* populations. At one population, located in an urban nature reserve, large flowers were mismatched with small sunbirds, suggesting anthropogenic disruption of the nectarivorous bird community. An analysis of bird species occurrence in gardens supported this interpretation by showing greater sensitivity to urbanization in the large sunbird species. The results are interpreted as evidence for human-caused disruption of the evolutionary process of plant pollinator adaptation in small conservation areas.

In Chapter 1 I explored the pollination biology of *Brunsvigia orientalis* (Amaryllidaceae) in the West Coast Lowlands and found that seed set is dependent on pollination by only two sunbird species, Malachite Sunbirds (*Nectarinia famosa*) and Lesser Double-Collared Sunbirds (*N. chalybea*). In Chapter 2 I found that a range of natural and anthropogenic environmental factors (successional stage of the plant community, population size and the size of the conservation area) impacted on sunbird abundance and hence reproductive success (seed set) in *B. orientalis*.

Here I look for more subtle natural and anthropogenic impacts on the *Brunsvigia-Nectarinia* mutualism by examining geographical patterns of variability in the *composition* of the sunbird community. I investigate the short-term ecological consequences of variation in sunbird community composition by examining impacts on seed set in *B. orientalis*; and investigate long-term evolutionary consequences by examining spatial patterns of variation in flower size in relation to the size of the primary pollinator.

Much of the spectacularly diversity of Cape plants is expressed in floral rather than vegetative anatomy (Goldblatt et al., 2000; Goldblatt et al., 1995, 2001; Goldblatt et al., 2002; Johnson et al., 1998; Rebelo et al., 1985). This observation has lead to the suggestion that floral adaptation to a geographically variable pollinator fauna has been a major force in the genesis of plant diversity at the Cape (Johnson, 1996). Studies of incipient speciation in bird pollinated plants are particularly illuminating because they might provide insights into the origin of the impressive diversity of bird pollinated flowers at the Cape (4% of the flora)(Rebelo, 1987).

Methods

Variation in flower size and pollinator size

I studied geographical variation in flower size in *B. orientalis* along a 320 km transect that spanned from Wiedourivier in the arid NW to Vermont in the mesic SE (Fig. 3.1). At 12 sites along the transect, I measured the shortest distance from the stigma to the entrance of the nectar chamber on one female-phase flower on each of 15 plants. Measurements were taken to the nearest millimeter with a steel ruler. This distance, measured across the arc of the curved style, can be directly related to pollinator size.

At five sites (Vermont, Scarborough, Rietvlei, Langebaanweg, Wiedourivier) I captured sunbirds in mist nests and measured the lengths of their heads and bills with calipers. These measurements were graphically related to the flower size measurements.

Effect of variation in pollinator size on pollination

To examine how variation in flower size affects pollination by large and small sunbirds, I made detailed observations of bird-flower interactions at three sites. Pollination of the large-flowered form of *B. orientalis* was observed at Vermont and Rondevlei; pollination of the small-flowered was observed at Langebaanweg. Observations were made from a distance of 5-10 m using close-focusing 8X magnification binoculars. If a sunbird visit could be clearly observed, it was assigned it to one of the following classes: contact with anthers and stigma; contact with stigma only; contact with anthers only; no contact with anthers or stigma. Most visits could not be clearly observed. Observation were recorded using a tape recorder and microphone and later transcribed.

Broad-scale study of bird distribution

I analyzed data collected by the South African Bird Atlas Project to examine the relationship between geographical variation in flower size and the geographical distribution of Malachite and Lesser Double-Collared Sunbirds. Bird Atlas Data consists of presence/absence checklists compiled at a 15' x 15' resolution (Harrison et al., 1997a). Data for the two study species were pooled by degree-square and month, and converted to a reporting rate by dividing the number of occurrences by the number of checklists available for the particular degree square. Reporting rates were plotted to reveal patterns of distribution and migration, which could be related to the geographical pattern of variation in flower size in *B. orientalis*. Generalized linear models were fitted to the patterns of seasonal abundance as described in Harrison *et al.* (1997b).

Sunbird interaction

In areas where the two sunbird species co-occur, interactions between the species are likely to have an impact on their role as pollinators. To study these interactions, 32 *B. orientalis* plants were randomly selected from the population at Vermont, numbered and observed for 30 min. For each observed visit, the species of bird and the plant's identification number were noted. If the sunbird species repelled each other, few plants would be visited by both species, and most would be visited by either one or the other species. The observed spatial pattern of sunbird visitation at *B. orientalis* inflorescences was compared with random expectations in a 2 x 2 contingency table. In addition to these observations, the occurrence of aggressive encounters between the species was documented.

Fine-scale study of sunbird distribution

Bird atlas data provides a reliable picture of sunbird distribution patterns at a coarse scale. However, at the fine scale of individual conservation areas, sunbird abundance and species composition can vary dramatically in relation to anthropogenic and natural factors which determine habitat size and quality (Chapter 2). To investigate how these factors modify the ecological and evolutionary interaction between sunbirds and flowers, I determined the exact species composition of the sunbirds community at twelve point locations (*B. orientalis* populations) along the NW-SE transect (sites are listed in Table 3.3). At each population I observed between 184 and 812 flowers (median = 280) for two separate 0.5 h periods during peak flowering (Feb-April 2000). The sunbird species and the number of visits were recorded. The composition of the sunbird fauna at these point locations was related to 1) seed set 2) the size of *B. orientalis* flowers at the site and 3) the broad-scale bird atlas data.

To determine the effect of urbanization on fine-scale patterns of sunbird abundance within the Cape Town Metropolitan Area, I analyzed data collected by the members of the Cape Bird Club for the Birds in Gardens Project. Participants recorded presence/absence checklists for the birds in 46 gardens. Each checklist spanned one day. Between 12 and 381 checklists were available for each of the 46 gardens (median = 139). Presence/absence checklists data were converted to a reporting rate by dividing the number of occurrences by the number of checklists available for the particular garden. Reporting rates were arcsine-transformed and related to the log-transformed distance between the garden and the urban edge using linear regression.

Relationship between the pollinator community and seed set

I returned after flowering and recorded the level of seed set at a subset of the *B. orientalis* populations by opening all the capsules on between 10 and 44 (median = 26) randomly selected plants at each site. Each ovary develops into a winged capsule regardless of whether or not it contains seeds. The capsules are never shed, and mature seeds are retained within the capsules for some time. The number of seeds per plant was divided by the number of capsules.

Results

Geographical variation in flower size in relation to variation in pollinator size

Two morphological forms of *B. orientalis* can be identified: a large-flowered form, which occurs in the SE, has a stigma-to-nectar distance of 41.6 mm ± 0.62 mm; and a small-flowered form, which occurs in the NW, has a stigma-to-nectar distance of 27.1 ± 0.54 mm (Fig. 3.1). Plants growing at one geographically intermediate site (Cape Flats Nature Reserve) are morphologically intermediate between these two forms.

Captured Malachite Sunbirds were found to have longer bills and heads than Lesser Double-Collared Sunbirds (Table 3.1). The difference between sexes and sites was small relative to the difference between species. The distance between the bill tip and crown feathers of the Malachite Sunbirds coincided with the stigma-nectar distance of the large-flowered form of *B. orientalis*; while the distance between the bill tip and crown feathers of Lesser Double-Collared Sunbirds coincided with the stigma-nectar distance of the small-flowered form (Fig. 3.1).

Effect of variation in pollinator size on pollination

The morphometric matching of flowers and pollinators is supported by direct field observations of the mechanics of pollination (Fig. 3.2). Malachite Sunbirds make frequent contact with the anthers and/or stigma when sipping nectar from the large-flowered form of *B. orientalis*. Lesser Double-Collared Sunbirds very seldom contact the anthers and/or stigma when visiting the large flowered form, but make frequent contact when visiting the small-flowered form.

Broad-scale study of bird distribution

Bird atlas data showed that both species of sunbird occur throughout the study area, but Malachite Sunbirds migrate out of the NW region during the flowering time of *B. orientalis* (autumn) (Fig. 3.1). Thus, Lesser Double Collared Sunbirds are the only species present in the NW region during the flowering time of *B. orientalis*, while in the SE both sunbirds species co-occur (Fig. 3.1).

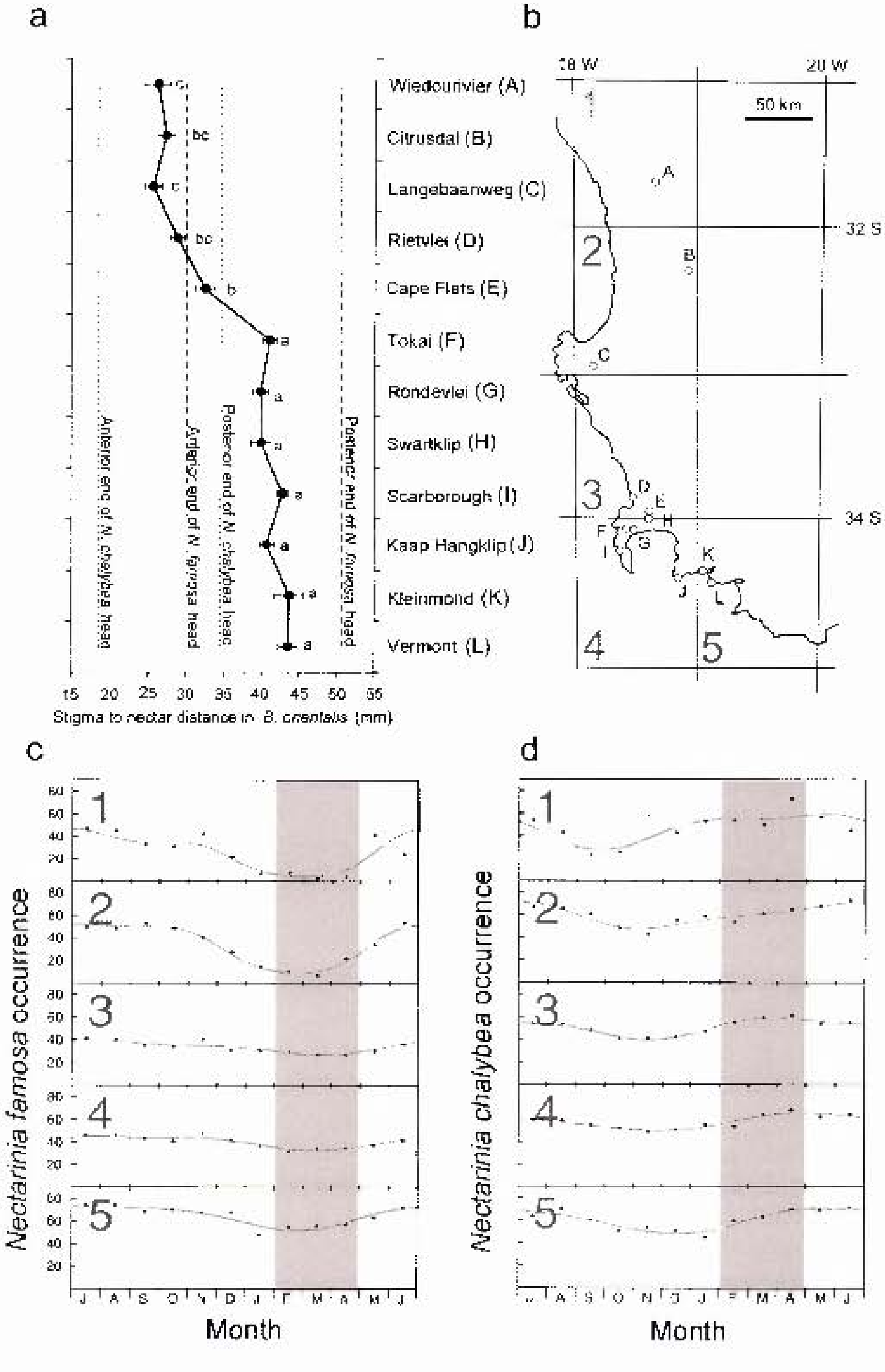

Fig. 3.1 Geographical variation in flower size in *Brunsvigia orientalis* in relation to the size and seasonal migration patterns of the two primary sunbird pollinators *Nectarinia famosa* and *N. chalybea*. **a,** Along a NW-SE transect there was significant variation in the stigma-to-nectar distance between sites ($F = 36$, df = 11, 155, $P < 0.001$). The Tukey post hoc test identified two homogeneous groups: the large-flowered form (superscript a) and the small-flowered form (superscript c). Dashed lines indicate that the stigma-to-nectar distance of the large-flowered form matched the distance between the bill tip and the crown feathers of *N. famosa*; while the small-flowered forms matched the bill length of *N. chalybea*. Letters in brackets correspond with locations on the map. **b**, Small-flowered populations of *B. orientalis* occurred in the NW; large-flowered populations occurred in the SE. Numbered cells correspond with graphs of sunbird abundance below. **c**, During the flowering period of *B. orientalis* (gray). *N. famosa* migrates out of the NW, but remains in the SE. **d**, *N. chalybea* remains resident at all sites during the flowering of *B. orientalis* and is thus the exclusive pollinator of populations in the NW.

Table 3.1 Morphometrics of captured *Nectarinia* sunbirds (mm).

Species	Tip of bill to first feathers of forehead	Tip of bill to back of head	*N*
Lesser Double-Collared Sunbirds (*N. chalybea*)	18.5 ± 0.40	34.6 ± 0.81	5
Malachite Sunbirds (*N. famosa*)	30.1 ± 1.06	50.6 ± 1.72	5

Table 3.2 Territorial interaction between Malachite Sunbirds (*Nectarinia famosa*) and Lesser-Double Collared Sunbirds (*N. chalybea*) at a flowering population of *Brunsvigia orientalis*. The pattern of visitation suggests repulsion, resulting in less than expected overlap in the individual plants utilized by each species. Fisher's exact two-tailed p = 0.18. Expected frequencies are in brackets.

	Plants visited by *N. chalybea*	Plans not visited by *N. chalybea*
Plants visited by *N. famosa*	3 (6.3)	17 (13.8)
Plants not visited by *N. famosa*	7 (3.8)	5 (8.3)

Table 3.3 Predicted and observed pollinators of large- and small-flowered forms of *Brunsvigia orientalis*, and the effect of variation in the pollinator fauna on seed set.

Sites (arranged from SE to NW)†	Area of natural vegetation	Flower size	Predicted primary pollinator*	*N. famosa* visits observed	*N. chalybea* visits observed	Seeds per capsule
Vermont	large	large	*N. famosa*	286	118	5.15 ± 0.61
Kleinmond	large	large	*N. famosa*	50	0	4.19 ± 0.69
Kaap Hangklip	large	large	*N. famosa*	168	0	6.90 ± 0.84
Rondevlei	**small**	**large**	***N. famosa***	**0**	**331**	**5.12 ± 0.56**
Rietvlei	small	small	*N. chalybea*	0	153	4.01 ± 0.55
Langebaanweg	large	small	*N. chalybea*	0	109	9.20 ± 1.63
Citrusdal	large	small	*N. chalybea*	0	~100	9.14 ± 0.67
Wiedourivier	large	small	*N. chalybea*	0	~300	9.18 ± 0.76

*Predictions are based on the size of *B. orientalis* flowers.

†At an additional four sites (Cape Flats Nature Reserve, Scarborough, Swartklip Nature Reserve, Tokai) the composition of the pollinator fauna could not be established because fewer than five visits by sunbirds were observed.

Sunbird interaction

Individual Malachite Sunbirds defended feeding territories consisting of about ~10 *B. orientalis* inflorescences. On 17 occasions Malachite Sunbirds were observed to chase Lesser Double-Collared Sunbirds away from the inflorescences of *B. orientalis*. The reverse situation was not observed. Repulsion between the two sunbird species was also evident from the spatial pattern of resource use at a site where they co-occurred (Table 3.2).

Fine-scale study of sunbird distribution

At seven out of eight *B. orientalis* populations the composition of the pollinator fauna concurred with the broad-scale distribution patterns in the Bird Atlas data and matched the expectations based on the size of *B. orientalis* flowers (Table 3.3). Three large-flowered populations in the SE were visited exclusively or most frequently by Malachite Sunbirds; four small-flowered populations in the NW were visited exclusively by Lesser Double-Collared Sunbirds. At Rondevlei, a small urban nature reserve, Bird Atlas data and the size of *B. orientalis* flowers predicted the presence of Malachite Sunbirds, but only Lesser Double-Collared Sunbirds occurred. At four additional sites the composition of the sunbird fauna could not be determined because fewer than five visits by sunbirds occurred during the census period.

The analysis of census data from the Birds in Gardens Project revealed that Lesser Double-Collared Sunbirds penetrate further into urban areas than Malachite Sunbirds (Fig. 3.3). There is a weak relationship between the reporting rate of Lesser Double-Collared Sunbirds and distance from the urban edge. Malachite Sunbirds occur at lower abundance at the urban edge, and their abundance drops off more rapidly with increasing distance from the urban edge. There is a strong relationship between the reporting rate of Malachite Sunbirds and distance from the urban edge.

Relationship between the pollinator community and seed set

Seed set varied across sites from about four seeds per capsule to about nine seeds per capsule (median = 6 seeds per capsule). At Rondevlei, where small sunbirds are mismatched with large flowers, seed set ranked the third lowest out of eight sites (5.12 ± 0.56 seeds per capsule).

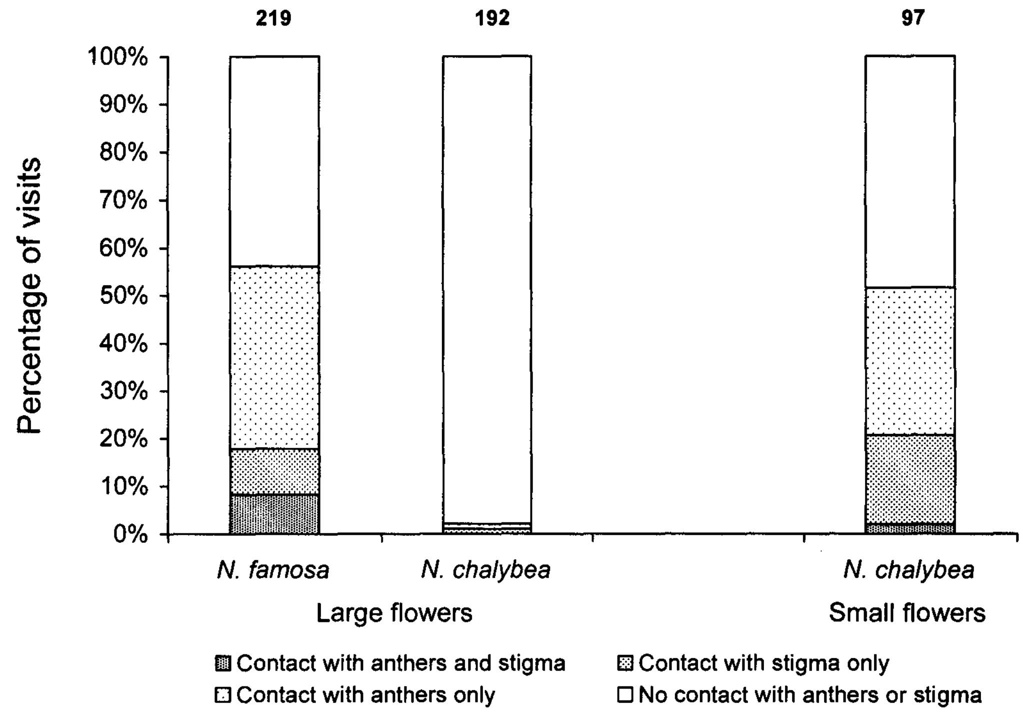

Fig. 3.2 Pollination efficiency of Malachite Sunbirds and Lesser Double-Collared Sunbirds during visits to large- and small-flowered forms of *Brunsvigia orientalis*. Numbers above bars indicate the number of clearly observed visits.

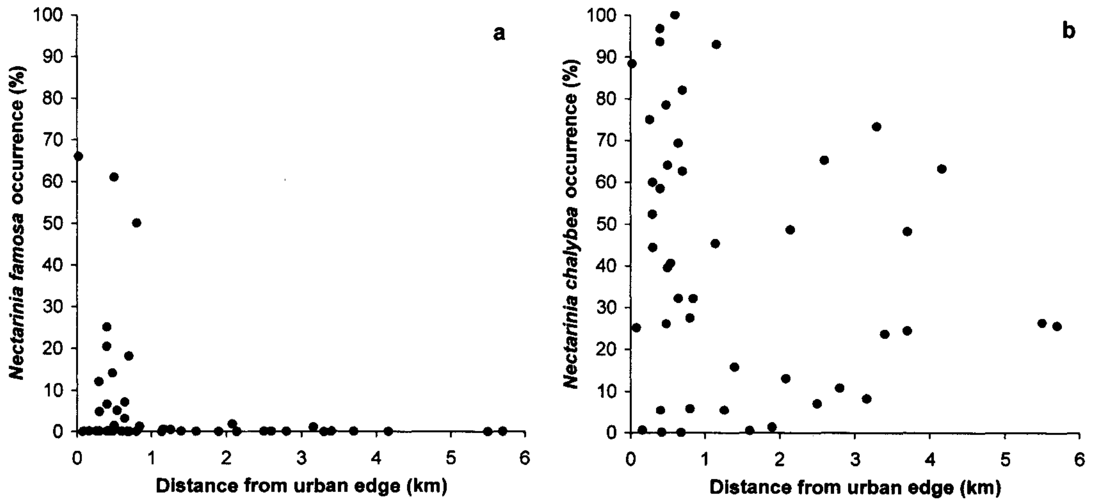

Fig. 3.3 Relationship between the reporting rate for sunbirds in urban gardens in the Cape Town Metropolitan Area and the distance between the garden and the urban edge. **a,** Malachite Sunbirds (*N. famosa*), arcsine((sqrt(y)) = 0.13-0.23*log(km from urban edge), $R^2 = 0.22$, df = 44, $p < 0.001$ **b,** Lesser Double-Collared Sunbirds (*N. chalybea*), arcsine(sqrt(y)) = 0.65-0.17*log(km from urban edge), $R^2 = 0.04$, df = 44, $p > 0.1$.

Discussion

The ancient pattern

On the basis of the data presented here, I suggest the existence of bird-pollination ecotypes in *Brunsvigia orientalis*. Large-flowered populations have a stigma-to-nectar distance which results in effective pollination by the large Malachite Sunbird (*Nectarinia famosa*); small-flowered populations have a stigma-to-nectar distance which results in effective pollination by the smaller Lesser Double-Collared Sunbird (*N. chalybea*) (Table 3.1, Fig. 3.1 & Fig. 3.2).

The distribution of large- and small-flowered populations of *B. orientalis* matches the broad distribution patterns of large and small sunbirds. Large-flowered populations of *B. orientalis* occur in the SE, while small-flowered populations occur in the NW. Both Malachite and Lesser Double-Collared Sunbirds occur throughout the study area, but Malachite Sunbirds migrate out of the NW during the flowering time of *B. orientalis* (autumn). Thus, Lesser Double Collared Sunbirds are the only species present in the NW during the flowering time of *B. orientalis*, while both sunbirds species co-occur in the SE (Fig. 3.1). The migration of Malachite Sunbirds out of the arid NW is most likely driven by nectar shortages during autumn (the driest time of the year). Studies of bird energetics and migration suggest that seasonal nectar shortages will more strongly affect large-bodied insectivores because of their greater nectar requirements (Carpenter, 1983; Keast, 1968).

Body size differences have another consequence. In the SE, where the two species co-occur, the larger Malachite Sunbirds were found to competitively displace Lesser Double-Collared Sunbirds from feeding territories (Table 3.2). Similar size structured pecking-orders are well known in communities of nectarivorous birds (Feinsinger, 1976; Ford, 1981; Skead, 1967). At the Cape, competitive interactions between insectivores combine with seasonal migration to polarizes the pollinator community of *B. orientalis* along a NW-SE axis.

Geographical variation in the growth environment, rather than the pollination environment, provides an alternative explanation for the existence of geographical variation in flower size in *B. orientalis*. The occurrence of smaller flowers in the NW fits the predicted response to a more arid environment in the NW, but the existence of two discrete flower size groups and the rapid geographical transition between these groups does not fit the gradual NW-SE aridity gradient.

A number of studies have demonstrated population differentiation in floral features associated with a shift between different families on insect pollinators (Galen, 1996; Inoue, 1993; Johnson & Steiner, 1997; Steiner, 1998). Such case studies provide strong evidence of pollinator-driven speciation (Grant & Grant, 1965). This study presents some evidence for pollinator-driven population differentiation associated with a shift from one species of *Nectarinia* sunbird to another. Speciation driven by shifts from one bird pollinator to another may help to explain some of the rich species diversity within bird-pollination systems.

Specialization for pollination by particular bird species might be a more widespread phenomenon. Examples of bird-pollinated Cape plants with large stigma-to-nectar distances include *Watsonia tabularis* and *Chasmanthe floribunda* in the Iridaceae, and *Brunsvigia josephinae* and *Cyrtanthus angustifolius* in the Amaryllidaceae. I have observed Lesser-Double Collared Sunbirds obtaining nectar from these four species exclusively by piercing the corolla tube. Hence, the classification of plants as "bird-pollinated" may be simplistic because it hides the diversity that might commonly exist with in the bird-pollination category.

The modern pattern

The sunbird community at a particular *B. orientalis* population is determined not only by the broad patterns of bird migration and inter-specific interactions, but also by local factors such as habitat size and quality (Chapter 2). The lowland habitat of *B. orientalis* has recently been transformed by agricultural and urban expansion (Heijnis et al., 1999; Maze & Rebelo, 1999), and as a result many *B. orientalis* populations have either been lost, or are restricted to small remnants of natural vegetation. In Chapter 2 I considered all sunbird species together and showed that recent anthropogenic landscape transformation has had a major impact on sunbird abundance and hence pollination and seed set in *B. orientalis*. Thus, an ecological process has been disrupted. The data presented here suggests a possible additional impact: a human-caused disruption of an evolutionary process through the disruption of the composition of the bird community.

At Rondevlei, a small urban nature reserve (811 ha in total; 106 ha protected), broad-scale bird atlas data and the size of *B. orientalis* flowers predicts the presence of Malachite Sunbirds, but only Lesser Double-Collared Sunbirds visit *B. orientalis* flower at this site (Table 3.1). The mismatch between flower size and the observed pollinator fauna suggests recent disruption of the composition of the pollinator fauna, through the loss of the normally dominant Malachite Sunbirds. Historical records and the analysis of bird occurrence data collected in urban gardens support this interpretation. Malachite Sunbirds do not penetrate as far into urban areas as Lesser Double-Collared Sunbirds (Fig. 3.3). The records of the Rondevlei Nature Reserve record the historical presence of breeding pairs of Malachite Sunbirds during 1950. These records predate the establishment of the surrounding suburbs (Heathfield and Grassypark), which, as their names indicate, were established on potential Malachite Sunbird habitat. Although the four bird hides (blinds) and the observation tower at Rondevlei are frequented by bird watchers on a daily basis, Malachite Sunbirds have not been seen there more than 5 times in the last ten years (Dalton Gibbs, Reserve Manager, pers. com.).

Interestingly, this possible human-caused disruption of the pollinator fauna, and hence the disruption of an evolutionary interaction, has not been accompanied by a complete loss in ecological functionality. Seed set at Rondevlei ranks low relative to other sites, but does not fail completely (Table 3.3). *B. orientalis* is self incompatible, thus, the presence of seed set indicates that Lesser Double-Collared Sunbirds are contributing to cross-pollination. However, pollen supplementation experiments indicated that seed set is not maximal at this site (Chapter 2). Thus, in the absence of Malachite Sunbirds, selection for smaller flowers in *B. orientalis* can potentially occur through enhanced pollen export as well as enhanced seed set..

Smith *et al.* (1995), found that the anthropogenic disruption of bird pollination systems in Hawaii has led to a recent reduction in the bill length of the nectar feeding I'iwi. Such adaptation to modern conditions has apparently not yet occurred in the long-lived Brunsvigias – their anachronistic flowers still echo the loss of large sunbirds.

SECTION 2

The *Rediviva* pollination system: oil-collecting bees and oil-secreting flowers

4 Up-down stylar polymorphism: a new stylar polymorphism in an oil-secreting plant, *Hemimeris racemosa* (Scrophulariaceae)

A new kind of stylar polymorphism, provisionally called *up-down stylar polymorphism*, is described from the annual herb *Hemimeris racemosa* (Scrophulariaceae). Most populations were found to be dimorphic for style position: styles alternate with the two stamens and are either in the up or in the down position. Flowers on one plant are of the same morph. Pollination is by oil-collecting bees in the genus *Rediviva*, which carry the pollen of the two morphs in discrete anterior and posterior locations on the underside of the thorax. Almost all dimorphic populations have slightly higher proportions of the style-down morph. In contrast with dimorphic populations, monomorphic populations of *H. racemosa* have the style and the stamens clustered together in the down position. Monomorphic populations are autogamous and are no longer dependent on oil-collecting bees for pollination.

Stylar polymorphism is one of the most interesting sexual systems known in living organisms. Individuals are bisexual, but populations consist of two or three morphological types which differ in the length or orientation of the sex organs. These differences, often coupled with genetic self-incompatibility, promote inter-morph mating and limit intra-morph pollen transfer and fertilization. Their function is to promote cross-pollination (Barrett et al., 2000; Jesson & Barrett, 2002b).

Darwin's (1877) pioneering work on stylar polymorphism has blossomed into a rich literature (reviewed in Barrett, 1992; Barrett et al., 2000), which describes four types of polymorphism. Populations of *distylous* and *tristylous* species (together referred to as heterostylous) are composed, respectively, of two and three floral morphs that differ in stigma and anther lenghts. *Stigma height dimorphism* is a less common condition in which populations consist of two morphs that differ only in the length of the stigma, but not the length of the anthers. *Enantiostyly* is a form of floral asymmetry in which the style is deflected away from the central axis of the flower either to the left or right side.

Here I describe a new type of stylar polymorphism, provisionally referred to as *up-down stylar polymorphism*. The polymorphism occurs in most populations of *Hemimeris racemosa*, a member of the Scrophulariaceae that is specialized for pollination by female oil-collecting bees. Of particular interest in the context of this thesis is the discovery of monomorphic populations of *H. racemosa* in which the dissolution of the up-down stylar polymorphism occurs through the formation of a third morph in which the anthers and stigma are in contact.

Hemimeris is known to be the major oil source for several species of oil-collecting bees in the genus *Rediviva* (Whitehead & Steiner, 2001), but details of the pollination biology of *Hemimeris* have not been described. Hence, I explore the functional floral morphology and describe the nature of the interaction between the flowers and their pollinators. Finally, I investigate how monomorphic and dimorphic populations differ in their response to variation in pollinator abundance. Throughout, I make comparisons with the closely related *H. sabulosa*. Later chapters, which deal with the consequences of pollinator loss, rely on the information on floral biology, which is presented in this chapter.

Methods

Study species and sites

This study deals primarily with *Hemimeris racemosa* (Houtt.) Merrill (incl. *H. montana* L. f.) (Scrophulariaceae, tribe Hemimerideae), but frequent comparisons are made with *H. sabulosa* L. f.. The study was conducted at 17 sites (listed where relevant) in an area bound by Langebaan, Tulbagh, Paarl and the Cape Peninsula.

Floral morphology

H. racemosa and *H. sabulosa* were examined and photographed in the field, and specimens were collected into FAA for later comparison of three dimensional morphology. Pressed voucher specimens were collected at all sites and placed in the Bolus Herbarium.

Electronmicrographs of liquid-preserved material were taken using a Cambridge Stereoscan S 200 scanning electron microscope. Before viewing, flowers were critical-point-dried and splutter-coated with Au/Pd.

Tests for oil secretion

The Sudan IV test was used to test for the presence of floral oil in the study species. The crystals dissolve in oil, staining all the liquid red; the crystals do not dissolve in aqueous solutions, which remain unstained. Crystals were sprinkled onto floral parts and the reaction examined under magnification. The swollen trichomes inside the flower were mechanically agitated before testing.

Frequency of style morphs

To determine the relative frequencies of up- and down-styled morphs in populations of *H. racemosa* I surveyed 17 populations by checking the style position on between 71 and 244 plants along a random wandering transect (median = 95.5 plants). Biased selection of individuals is prevented by the fact that the style position can only be determined at very close range. Plants were sampled at ~4 m intervals, because a pilot study revealed spatial clustering of same style morphs within small neighbourhoods (< 4m). Replicated goodness-of-fit tests (*G*-statistics) were calculated as in Sokal and Rohlf (1995) to determine 1) whether populations deviated from the 1:1 expectation of morph ratios, 2) whether morph ratios were homogenous across populations and 3) whether the pooled data from all populations deviated from the expected 1:1 frequency.

Variation in self-fertility

To compare autogamous pollination rate among floral morphs of *H. racemosa* and *H. sabulosa*, I excluded insects by enclosing budding plants in wire cages over which fine gauze bags were stretched. The edges of the bag were pinned down on the ground. There was no contact between gauze and flowers. Sites and sample sizes are listed in Table 4.2. I recorded capsule set as the dependent variable because seeds in *Hemimeris* are minute and numerous. Capsules mature and dehisce sequentially over a prolonged period both during and after flowering, but remain attached to the plant after the seeds are released. The withered remains of aborted ovaries along with their peduncles and calyxes also remain attached to the plant after flowering, indicating failed seed set. The Mann-Whitney U test was used to determine the statistical significance of differences in capsule set between treatments.

Flower visitors, pollen loads and pollinator behaviour

I observed pollinators at 19 flowering populations of *Hemimeris racemosa* and 4 populations of *H. sabulosa* on a total 33 days between mid August and mid October of 1998-2000. Pollinators were captured and identified and their pollen loads examined. Detailed observation of pollinator behaviour were made from photographic series taken mostly at Joostenberg.

Variation in visitation rate among populations

Pollinator visitation rate was quantified in x populations by observing ~5 m^2 patches of flowering plants for two 15 min intervals from a distance of ~1.5 m. Visitation rate observations were restricted to warm (>20 °C), windless hours between 10:30 and 15:30. The rate was calculated as visits per flower per hour.

Variation in capsule set among populations

I recorded capsule set as a measure of reproductive success by marking flowering *H. racemosa* and *H. sabulosa* plants with adhesive jewellery tags and returning after flowering to determine the proportion of flowers that had produced seed capsules. Capsule set was recorded in x population for between x and x plants. I tested for an interaction between capsule set and visitation rate using regression.

Results

Morphology and oil secretion

Hemimeris racemosa and *H. sabulosa* seedlings were first observed during mid winter (June-July). By Spring, both species had grown to herbs 10-25 cm in height (Fig. 4.1). Flowering in *H. racemosa* started at the beginning of August and ended at the end of October. Flowering in *H. sabulosa* started in July and ended in October. After seed production, both species die during the middle of the dry season (November-December).

Fig 4.1 Morphology of *Hemimeris* (Scrophulariaceae). **a,** Typical form of *H. racemosa* showing the dorsal petal hinged closed over the stigma (Tygerberg). **b,** Up-down stylar polymorphism in two adjacent plants of the typical form of *H. racemosa*. The dorsal petal lobe has been hinged open (Chapman's Peak). **c,** Dish-shaped flowers of *H. sabulosa* with style and stamens in the down position (Rondevlei). **d,** Monomorphic, autogamous form of *H. racemosa* with style and stamens in the down position (Cape Point). **e,** Floral display of the typical, dimorphic form of *H. racemosa* (Chapman's Peak). **f,** Floral display of the monomorphic, autogamous form of *H. racemosa* (Cape Point). a-d scale bars = 5 mm; e-f scale bars = 5 cm. P, pouch lined with oil-secreting trichomes; A, stamen; S, style.

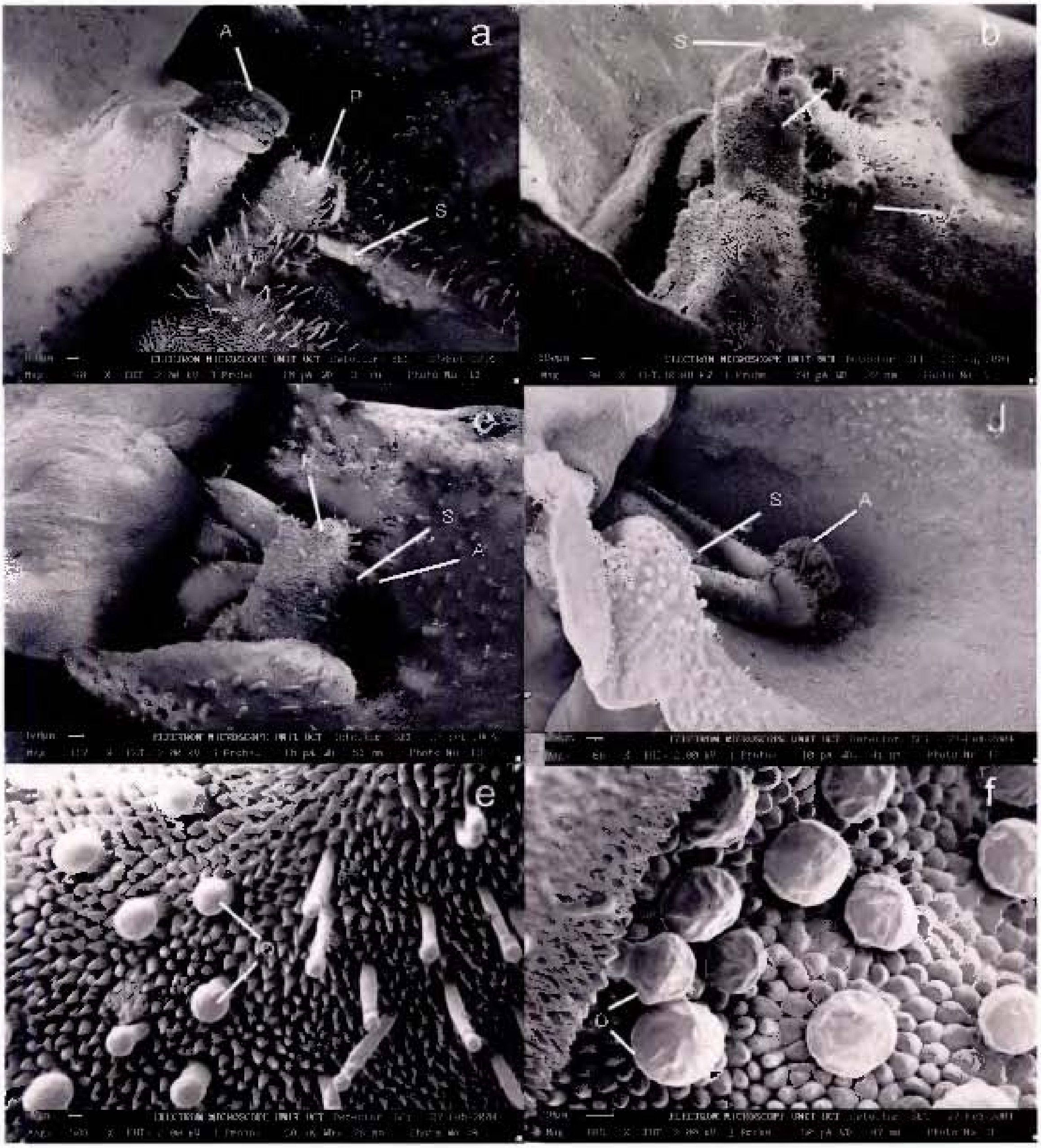

Fig. 4.2 Microscopic morphology of *Hemimeris* (Scrophulariaceae). **a,** Style-down morph of the typical form of *H. racemosa*. **b,** Style-up morph of the typical form of *H. racemosa*. **c,** Monomorphic, autogamous form of *H. racemosa* with style and stamens in the down position. **d,** Dish shaped flowers of *H. sabulosa* with style and stamens in the down position. **e,** Variation in trichome structure in *H. racemosa* at the transition from petal blade (right) to pouch (left). **f,** Oil secreting trichomes inside the pouch of *H. racemosa*. A, stamen; S, style; P, intrusion of petal; O, oil-secreting trichomes.

The flowers of both species are unscented and bright yellow, with roughly circular areas of small magenta spots on the dorsal petal lobe (Fig. 4.1). Immediately below each of the spotted areas, on either side of the centrally placed stamens and style, is a shallow pouch. In *H. racemosa* the pouches are about 1.5-3 mm deep. Access into the twin pouches of *H. racemosa* is hindered by the spring-loaded dorsal petal lobe which snaps shut over the entrances to the pouches. The lower lip forms a flat landing platform, which extends out in front of the entrances to the pouches. In contrast, the corolla of *H. sabulosa* is open and dish-shaped with shorter (1-2 mm) pouches (shallow depressions), a concave lower lip and a dorsal petal lobe which is not hinged and does not close over the entrances to the pouches.[1]

In both *H. racemosa* and *H. sabulosa* the twin pouches, and the entrances to the pouches, are coated with specialized trichomes (Fig. 4.2). The trichomes are multicellular, consisting of a base, a narrow stalk and a swollen, spherical head. Testing with Sudan IV confirmed that the trichomes contain and release oil.

Frequency of style morphs

Most (12/17) populations of *H. racemosa* were found to consist of roughly equal ratios of two floral morphs. The style alternates with the two stamens and is either in the up or in the down position (Fig. 4.1). Thus, the flowers of the dimorphic populations are herkogamous with the stigma separated from the anthers by a distance of 2-4 mm. Two outgrowths of the petals intrude between the stamen and style and act as a physical barrier between them (Fig. 4.1). Flowers on the same plant are of the same morph. Almost all dimorphic populations have a slight bias in favour of the style-down morph (Fig. 4.3). The deviation from the expectation of equal ratios was not statistically significant in any of the populations when considered separately (maximum *G*-value = 2.01, df = 1). When the data from all 12 populations were pooled, the ratio of up to down-style plants in the total sample deviated significantly from the 1:1 expectation (Table 4.1). The significant deviation did not result from heterogeneity among populations, but rather from the increase in power obtained by pooling samples (Table 4.1).

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[1] The morphological descriptions provided in this chapter are necessary because the current treatment (Grant 1938) does not provide sufficient detail.

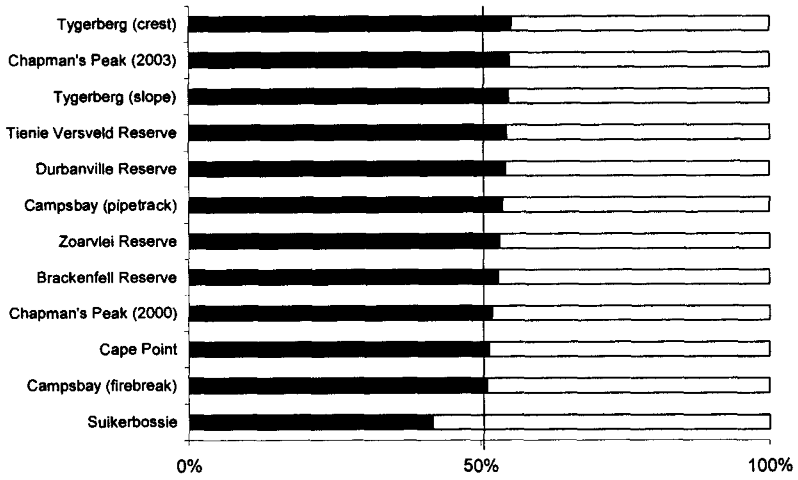

Fig. 4.3 Frequencies of style-down (black) and style-up plants (white) in dimorphic populations of *Hemimeris racemosa*. From top to bottom *N* = 72, 88, 103, 77, 66, 169, 65, 173, 244, 71, 140, 121. Floral morphology suggests hybridization with adjacent monomorphic, autogamous populations at Suikerbossie.

Table 4.1 Replicated goodness-of-fit tests of observed ratios of style-down and syle-up morphs against a 1:1 expected morph ratio in 12 populations of *Hemimeris racemosa*. Populations are listed in Fig. 4.3.

Test	df	*G*	p
Pooled	1	5.45	< 0.025
Heterogeneity	11	4.93	ns
Total	12	10.28	ns

Table 4.2 Effect of excluding pollinators on capsule set in *Hemimeris racemosa* at 9 sites. Significant Mann-Whitney U Test results are displayed as: * p < 0.05, ** p < 0.01, *** p < 0.001.

Species	No. of morphs in population	Site	Pollinator excluded capsule set	*N* (bagged plants)	Open pollination capsule set	*N* (open plants)	*Z*
H. racemosa	2	Darling	1 ± 2 %	6	69 ± 17 %	25	3.75***
H. racemosa	2	Joostenberg	17 ± 7 %	8	55 ± 3 %	37	3.43***
H. racemosa	2	Camps Bay	19 ± 3 %	13	83 ± 3 %	28	5.04***
H. racemosa	2	Chapman's	16 ± 4 %	19	67 ± 2 %	75	6.16***
H. racemosa	1	Chapman's	73 ± 5 %	24	84 ± 3 %	47	1.73
H. racemosa	1	Glencairn	93 ± 1 %	38	89 ± 2 %	54	1.22
H. racemosa	1	Cape Point	84 ± 2 %	37	44 ± 2 %	59	6.95***
H. sabulosa	1	Rondevlei	74 ± 9 %	10	51± 10 %	14	1.49
H. sabulosa	1	Churchaven	81 ± 4 %	7	--	--	--

Five of the seventeen populations of *H. racemosa* were found to be monomorphic. Plants in these populations differ from both of the two morphs in the dimorphic populations in having the two stamens and the style clustered together in the down position (Fig. 4.1 & Fig. 4.2). Thus, plants in monomorphic populations are not herkogamous and there is direct contact between the anthers and stigma. Plants in monomorphic populations are smaller and have fewer and considerably smaller flowers. In other details of floral and vegetative morphology the monomorphic form of *H. racemosa* clearly resembles the typical dimorphic form. At Cape Point, Chapman's Peak and Suikerbossie, monomorphic populations occurred in close proximity (5-100 m) to dimorphic populations, but occupied wetter and shadier micro-habitats. At these three sites, flowers of the dimorphic form were approximately twice as large as those of adjacent monomorphic populations (10 mm vs. 5 mm wide)(Fig. 4.1).

In contrast with *H. racemosa*, all the examined populations of *H. sabulosa* were found to be monomorphic with the two stamens and the style clustered together in the down position (Fig. 4.1 & Fig. 4.2). In this respect the flowers of *H. sabulosa* resemble the flowers of the monomorphic, autogamous form of *H. racemosa*. The dish shaped flowers and vegetative features (Grant, 1938) nevertheless distinguish this species from the autogamous form of *H. racemosa*. There was considerable variation in plant and flower size among 12 populations of *H. sabulosa*.

Variation in self-fertility

Pollinator exclusion significantly reduced capsule set in dimorphic (herkogamous) populations of *H. racemosa* at all four sites (Table 4.2). In contrast, in three monomorphic populations of *H. racemosa*, pollinator exclusion treatments did not have a significant effect on capsule set, or resulted in a significant increase in capsule set (Table 4.2). Similarly, in *H. sabulosa*, high levels of capsule set were maintained in pollinator excluded plants (Table 4.1).

Flower visitors

Insects captured on the flowers of *Hemimeris racemosa* at the study sites consisted of three species (20 individuals) of small pollen-eating beetle (Nitidulidae, Melyridae

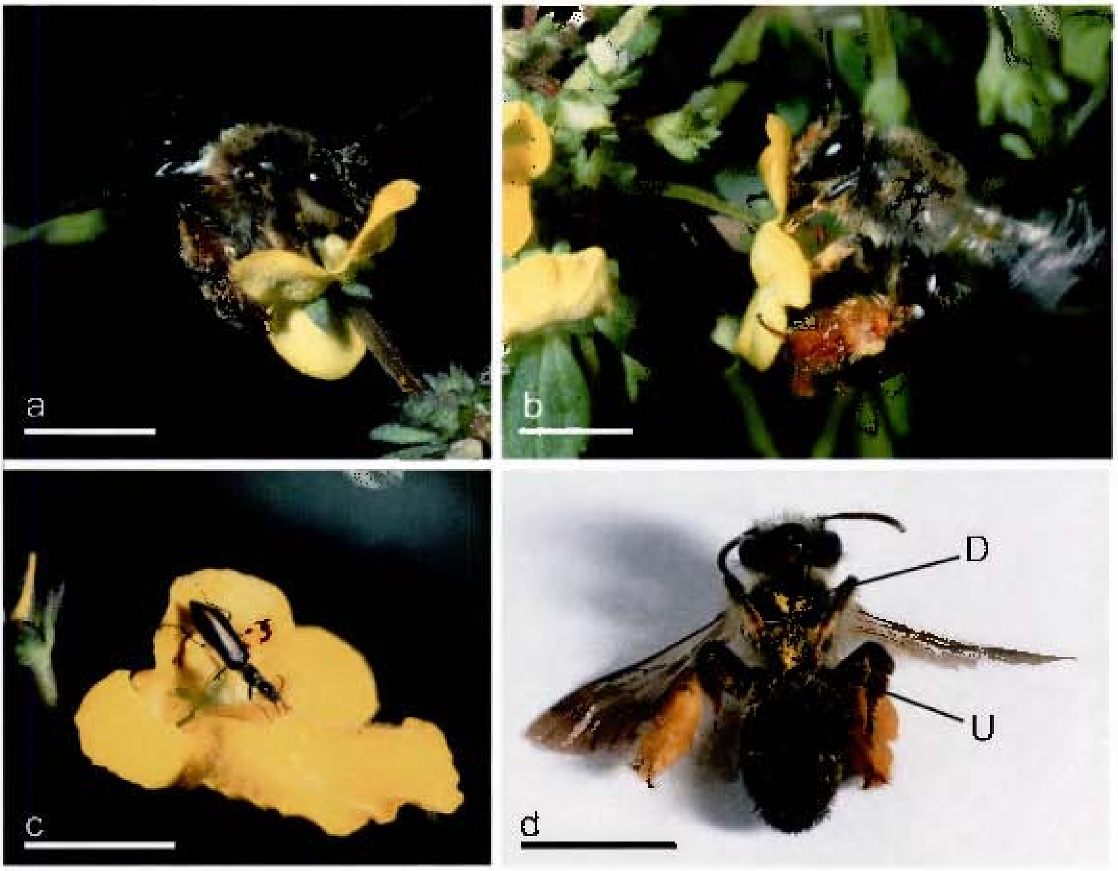

Fig. 4.4 Pollination in *Hemimeris racemosa*. **a,** *Rediviva bicava* collects oil from *H. racemosa* by hinging the flower open and inserting its front and middle tarsi into the shallow spurs (Joostenberg). **b,** *Rediviva peringueyi* collects oil from *H. racemosa*. Oil glistens in the scopae (Joostenberg). **c,** A beetle (fam. Melyridae) feeds on the pollen of *H. racemosa* (Zoarvlei). **d,** *H. racemosa* pollen on the underside of the mesosoma of *Rediviva parva*. U, pollen cluster probably derived from style-up morph; D, pollen cluster probably derived from style-down morph (Chapman's Peak). Scale bars = 5 mm.

and Scarabaeidae) and four species (32 individuals) of *Rediviva*. The maximum number of *Rediviva* species captured on *H. racemosa* at one site was 3 (four sites). *R. bicava* was captured at six sites, *R. intermixta* at three sites, *R. parva* at seven sites and *R. peringueyi* at four sites. All captured *Rediviva* were female.

In general, foraging *Rediviva* could not be identified to species level. However, it was possible to distinguish *R. peringueyi* from other *Rediviva* species with reasonable certainty on the basis of its larger size and dull, non-reflective thorax. *R. peringueyi* visited *H. racemosa* at 7 of the 16 census sites and accounted for 29 % of 1 885 *Rediviva* visits observed at *H. racemosa*.

Limited data were obtained on seasonal changes in the *Rediviva* community. On 26 Aug 1999 two 15 min censuses were conducted at Joostenberg and no *R. peringueyi* were observed, while 207 and 223 visits by smaller bees were observed. On 22 Sept 1999 *R. peringueyi* accounted for 52% of 63 visits observed during a 15 min census conducted at the same site.

Pollen loads

The beetles carried little or no *H. racemosa* pollen on their bodies. The *Rediviva spp.* all carried large quantities of *H. racemosa* pollen on the sternum (underside) of the mesosoma (thorax) and the coxae (bases of the legs) as well as smaller quantities on the post genial area (underside of the head)(Fig. 4.4). The pollen was concentrated in two areas on the body of the bee, which appear to correspond to the position of the anthers in the up- and down stylar morphs. Pollen derived from the style-down morph is clustered on the sternum of the mesosoma anterior to the coxa of the front legs; pollen derived from the style-up morph is clustered on the sternum of the mesosoma immediately anterior to the coxa of the middle legs (Fig. 4.4). Some individuals carried unidentified pollen in their scopae (pollen baskets). Large quantities of yellow oil could be drawn from the pollen masses in the scopae using filter paper.

Pollinator behaviour

Beetles were most often observed to be resting inside the flowers. Occasionally, mating and pollen feeding was observed (Fig. 4.4c). Low levels of beetle visitation were recorded during censusing, because beetles seldom moved between flowers (0.03 ± 0.02 visits per flower per hour, range = 0 – 0.31, 17 sites). In contrast, the average visitation rate by *Rediviva* was 0.78 ± 0.22 visits per flower per hour (range = 0-3.60, 17 sites). All *Rediviva* were actively engaged in collecting oil from *Hemimeris* flowers (Fig. 4.4a & b). They did so by hinging the dorsal petal lobe open and probing inside the pouches with their front legs. The tibia of the middle legs were typically seen to rest in the entrances to the pouches. The hind tibia hooked onto the edges of the bilobed lower lip of the corolla. In this position, the stigma and anthers were brought into contact with the bases of the legs (coxa) and the underside of the head and thorax (mesosoma). A foraging *R. parva* was observed to visit between 17 and 19 flowers per minute. A maximum of 84 flowers were visited in one foraging bout.

Smaller bees (*R. bicava* and *R. parva*) appear to match the flower morphology more closely. In these species, the position of the hind tibia coincides with the notch between the lateral and ventral petal lobes (Fig. 4.4a). In the larger *R. peringueyi* the hind tibia are placed further back and grip the sides of the bilobed lower lip (Fig. 4.4b). In some photographs small *Rediviva* appear to be holding on to the anthers or style (whichever is vertical) with their front tarsi and/or jaws, while the middle legs and/or front legs are inside the pouches.

Nectar plants observed to be visited by *Rediviva* in the vicinity of *H. racemosa* include *Moraea gawleri*, *Moraea tricolor*, *Oxalis obtusa*, *Cysticapnos vesicarius* and its introduced relative *Fumaria muralis*.

Capsule set and visitation rate

Average capsule set varied from 26 % (Zoarvlei) to 81 % (Camps Bay) in dimorphic populations of *H. racemosa*. In monomorphic populations of *H. racemosa* capsule set varied from 48 % (Cape Point) to 92 % (Suikerbossie). In two populations of *H. sabulosa* capsule set was 84 % (Milnerton Racecourse) and 86 % (Rietvlei).

In dimorphic populations of *H. racemosa*, low levels of capsule set are associated with low pollinator visitation rates (Fig. 4.5). In contrast, relatively high levels of capsule set were observed in monomorphic populations of *H. racemosa* and in populations of *H. sabulosa* despite the absence of *Rediviva* (Fig. 4.5).

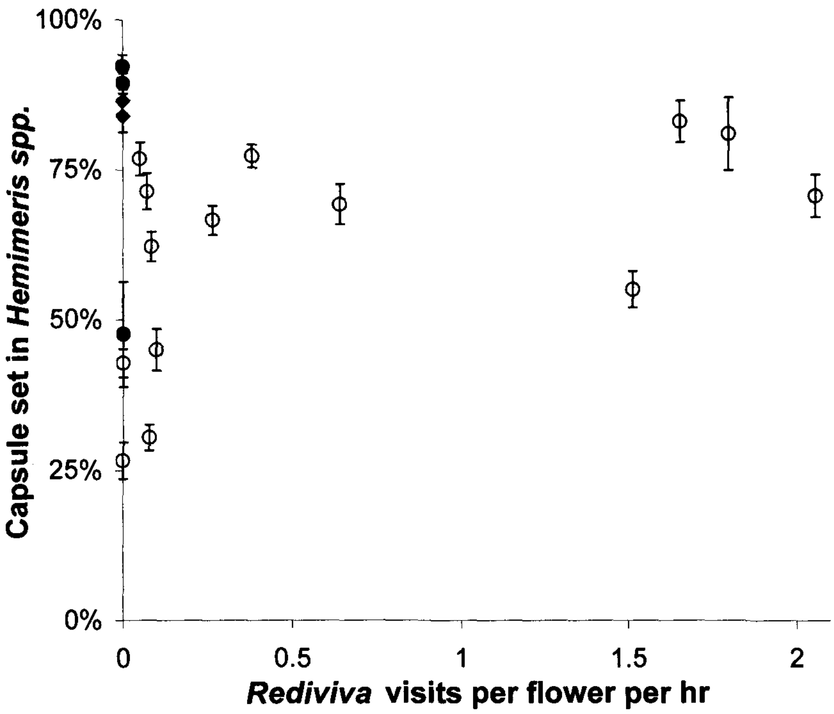

Fig. 4.5 Relationship between capsule set and visitation rate by oil-collecting bees (*Rediviva spp.*) in three types of *Hemimeris*. Self pollination provides reproductive assurance in *H. sabulosa* (diamonds) and in monomorphic populations of *H. racemosa* (filled circles). In contrast, in dimorphic populations of *H. racemosa* (unfilled circles), low pollinator visitation rates are associated with low levels of capsule set, which increase sharply as visitation rate increases. Bars show one SE.

Discussion

Pollination by oil-collecting bees

In the West Coast Lowlands of the Cape, where this study was conducted, *Hemimeris racemosa* was found to be pollinated by at least four species of oil-collecting bee in the genus *Rediviva* (*R. bicava*, *R. intermixta*, *R. parva* and *R. peringueyi*)(Fig. 4.4). In other parts of its distribution range, additional species of *Rediviva* are known to visit *H. racemosa* (Whitehead & Steiner, 2001). *Hemimeris* is one of seven genera of Scrophulariaceae which form the cornerstones of the extensive *Rediviva* pollination system, which consists of 24 *Rediviva* species and about 140 oil-secreting plant species in 14 genera (Manning & Goldblatt, 2002; Manning & Brothers, 1986; Steiner, 1989; Steiner & Whitehead, 1988, 1996, 2002; Vogel, 1984; Whitehead et al., 1984).

The flowers of *H. racemosa* offer only oil as a reward to pollinators. The oil is secreted by trichome elaiophores, which are concentrated inside and around twin pouches. The trichome elaiophores of *H. racemosa* resemble those described from *Colpias mollis*, another member of the Scrophulariaceae (Steiner & Whitehead, 2002). While alighting on the flowers, female *Rediviva* insert their front (and sometimes middle) legs into the twin pouches. There are plumose oil-collecting hairs on the tarsi of these legs (Whitehead & Steiner, 2001). Floral oil is not known to be used as an adult food by oil-collecting bees, but is used by female bees as a larval food and in nest construction (Cane et al., 1983; Vinson et al., 1997; Vogel, 1974). Thus, the fact that only female bees were caught on the flowers of the study species confirms the finding that the reward is oil rather than nectar. The presence of large quantities of oil in the scopae (pollen baskets) of female bees indicates that the reward was collected successfully. While accessing the oil, the centrally placed anthers and stigma make contact with the underside of the mesosoma (thorax) of the bee.

Up-down stylar polymorphism

Close examination of the flowers of *H. racemosa* revealed the existence of a new kind of stylar polymorphism. Styles alternate with the two anthers, and are deflected either up or down. The up-down stylar polymorphism is distinct from heterostyly (distyly and tristyly), in which floral morphs differ in stigma and anther lengths, and is distinct from enantiostyly (mirror-image flowers), in which flowers are asymmetrical, with the style deflected either to the left or right (Barrett et al., 2000). Stylar polymorphisms are particularly well represented in the South African flora (Ornduff, 1974; Steiner, 1987), but have not been reported for *Hemimeris*, and are not known from other Scrophulariaceae (Barrett et al., 2000).

The distribution of pollen on the underside of captured *Rediviva* suggested anterior/posterior separation of the pollen load from up- and down-style morphs. Pollen from the style-down morph appeared to be clustered on the sternum of the mesosoma anterior to the coxae of the first pair of legs; pollen from the style-up morph appeared to be clustered posterior to the coxae of the first pair of legs (Fig. 4.4). Styles alternate with the anthers, so the morph that places pollen anteriorly collects pollen posteriorly and vice versa. Since all flowers on a plant are of the same morph, this arrangement will promote cross pollination by limiting geitonogamy (Jesson & Barrett, 2002b). The precise positioning of the oil-collecting bee on the flower must be an important factor in the development of the up-down stylar polymorphism, since mis-positioning of the bee by only 3 millimeters will result in intra-morph pollen transfer.

Morph ratios in dimorphic populations

Equal morph ratios are expected in plants with polymorphic sexual systems because frequency dependent selection will favour the rarer morph (Charlesworth & Charlesworth, 1979; Fisher, 1941). In this light, the subtle but consistent bias in favour of style-down morphs in dimorphic populations of *H. racemosa* is interesting (Fig. 4.3).

In heterostylous and enantiostylous species, unequal morph ratios have been suggested to result from founder effects and genetic drift, clonal propagation and, in species without heteromorphic incompatibility, morph-specific differences in selfing or assortative mating (Barrett et al., 1997; Eckert & Barrett, 1992; Husband & Barrett, 1992; Jesson & Barrett, 2002a). Morph-specific differences in selfing is a probable explanation for morph ratio deviation in *H. racemosa* because pollen movement by gravity is likely to result in more frequent self-fertilization in the style-down morph. Morph-specific differences in assortative mating may result from disruption of the pattern of pollen placement on the bee by the passive or active movement of pollen from the anterior to the posterior.

Monomorphic populations

Five out of seventeen populations of *H. racemosa* were found to be monomorphic for style and stamen position. Floral morphology in these populations differed from both the style-up and the style-down morph of dimorphic populations in having the two stamens and the style clustered together in the down position (Fig. 4.1 & Fig. 4.2). Plants in monomorphic populations are not herkogamous -- there is direct contact between the anthers and stigma. In this respect, monomorphic populations of *H. racemosa* resemble *H. sabulosa* (Fig. 4.1 & Fig. 4.2).

Pollination exclusion experiments clearly demonstrated that seed set in monomorphic population of *H. racemosa* and in *H. sabulosa* does not require pollinators (Table 4.2). It seems likely that high levels of capsule set in these populations resulted from autonomous self-pollination rather than apomixis, but the possibility of apomixes can not be ruled out in this study. The observation that capsule set was higher inside enclosures than outside, suggests that resource limitation, rather than pollinator availability, limits seed capsule production in monomorphic *H. racemosa* and *H. sabulosa*. Herbivore damage was insignificant on control plants, suggesting that the observed effect was not created by herbivore exclusion. Rather, the shading and wind-breaking effect of the gauze bags probably created a more favourable microclimate for growth and seed production. The small size of the flowers and floral display in monomorphic populations of *H. racemosa* compared with nearby dimorphic populations is in accord with a lack of dependence on pollinators (Fig. 4.1).

Homostylous, self-pollinating populations or taxa, that have originated through the breakdown of the floral polymorphism, are commonly observed in heterostylous clades (Baker, 1966; Barrett, 1989; Barrett & Shore, 1987; Darwin, 1877; Ernst, 1955; Ornduff, 1972).

Response to variation in pollination rates in monomorphic and dimorphic populations

Pollinator visitation rates varied among *Hemimeris* populations from zero visits to about two visits per flower per hour. In dimorphic *H. racemosa*, variation in pollinator abundance influenced capsule set. In populations with low pollinator visitation rates, capsule set was relatively low (minimum 27 %), from where it increased sharply to ~70 % as pollinator visitation rate increased (Fig. 4.5).

In contrast, variation in pollinator abundance did not appear to influence capsule set in *H. sabulosa* and monomorphic populations of *H. racemosa*. These populations are characterized by very low pollinator visitation rates, but capsule set is nevertheless high (Fig. 4.5). In the absence of pollinators, self-pollination apparently provides reproductive assurance (sensu Baker, 1955) to these populations (Fig. 4.5).

The distylous *Primula sieboldii* provides an interesting point of comparison. In a small urban conservation area in Japan both the pin and thrum morphs set relatively few seeds due to the lack of pollinators (Washitani et al., 1994). In contrast a single homostylous genet, which also occurs at this site, has high levels of seed set, leading to the prediction that the homostyle morph will increase in frequency in this population (Washitani, 1996).

Conclusion and prediction

Hemimeris has a relatively specialized pollination system due to the specialized nature of the reward (oil). However, the reward is brightly advertised and secreted in shallow pockets, making it accessible to the full size range of oil-collecting bees (*Rediviva spp.*). Pollinator exclusion experiments demonstrated varying degrees of self fertility among populations and species of *Hemimeris*. Monomorphic populations of *H. racemosa* and all tested populations of *H. sabulosa* set high levels of seed in the absence of pollinators. Dimorphic populations of *H. racemosa* required pollination for full seed set, but also set low to medium levels of seed in the absence of pollinators (minimum 27%). Populations regenerate annually from seeds, which germinated in response to disturbance and are apparently able to survive in the soil for several years.

How will *Hemimeris* respond to pollinator loss? Given the floral and vegetative features listed above, it can be predicted that *H. sabulosa* and monomorphic populations of *H. racemosa* will be insensitive to pollinator loss. Dimorphic population of *H. racemosa* will be more vulnerable, but pollination by multiple species of *Rediviva* and a partial capacity for autonomous self-pollination will buffer even these populations against pollinator loss.

5 Pollination of a guild of oil-secreting orchids (Coryciinae) by Peringueyi's bee, *Rediviva peringueyi* (Melittidae)

Five co-occurring oil-secreting orchids (*Pterygodium alatum, P. cafrum, P. catholicum, P. volucris* and *Corycium orobanchoides*) were found to be pollinated solely by the oil-collecting bee *Rediviva peringueyi*. Floral oil, which is offered as a reward, is secreted by epithelial elaiophores on the tip of the lip appendage. The orchids share a set of floral features including pungent scent, yellow-green flowers and a September peak in flowering. The same floral features occur in an additional eight co-occurring orchid species in which pollination by *R. peringueyi* is predicted, or confirmed by other studies. Thus, the *R. peringueyi* pollination guild consists of about 13 species. The members of the guild reduce interspecific reproductive interference by differences in pollinarium length, or by the use of exclusive pollinarium attachment sites on the body of the bee. The members of the guild were found to vary greatly in their capacity for vegetative reproduction, which is predicted to translate into a range of responses to the variation in the abundance of *R. peringueyi*.

The relationships between oil-secreting flowers and the oil-collecting bees which pollinate them are among the most specialized mutualistic animal-plant interactions (Buchmann, 1987). In the temperate regions of Southern Africa, the oil-collecting bee fauna comprises 24 species in the genus *Rediviva* (Melittidae)(Whitehead & Steiner, 2001). *Rediviva* has two distinct centers of endemism: 9 species occur in the high altitude summer-rainfall grasslands of the Drakensberg range in the north-east of South Africa; and 15 species occur in the winter-rainfall shrublands and succulent deserts around the Cape of Africa (Whitehead & Steiner, 2001).

In the degree of specialization and the complexity of pollination mechanisms, floral relations of the genus *Rediviva* are likely to rival the better known euglossine bee pollination system of the New World (Williams, 1982). In contrast with the euglossine pollination system, where orchids offer scent as a reward for male bees, plants in the *Rediviva* pollination system offer oil to female bees, who probably use it mainly as a larval food (Vinson et al., 1997; Vogel, 1974) and possibly also in nest construction (Cane et al., 1983). Female *Rediviva* collect oil primarily from two groups of plants, the Scrophulariaceae (tribes Hemimerideae and Bowkerieae) and the Orchidaceae (subtribe Coryciinae)(Manning & Brothers, 1986; Steiner, 1989; Steiner & Whitehead, 1996; Vogel, 1984). The recent discovery of oil-secretion in *Tritoniopsis parviflora* (Iridaceae) brings the estimated number of oil-secreting South African species likely to be pollinated by *Rediviva* to about 140 in 14 genera (Manning & Goldblatt, 2002). The geographical distribution of these oil-secreting species matches that of *Rediviva* with a Drakensberg and a Cape center of endemism. The Cape center is somewhat richer in both plant and bee species.

The *Rediviva* of the Cape center are known to use at least 12 genera of oil-secreting host plants (Whitehead & Steiner, 2001). Studies of the pollination biology of some of these species have thus far revealed fascinating biology. The long-spurred *Diascia whiteheadii* and *D. tanyceras* (Scrophulariaceae) are pollinated by the exceptionally long-legged *R. longimanus* and *R. emdeorum* respectively (Vogel, 1984; Whitehead et al., 1984). The smallest *Rediviva*, *R. albifasciata*, is the only pollinator of *Colpias mollis* (Scrophulariaceae) -- other co-occurring *Rediviva* species are excluded by the narrow entrance to the flowers (Steiner & Whitehead, 2002). In mid summer, the flowers of the bizarre rheophyte *Ixianthes retzioides* (Scrophulariaceae) are pollinated solely by *R. gigas*, the only *Rediviva* species which is active after spring (Steiner & Whitehead, 1996). *R. gigas* is also the pollinator of *Tritoniopsis parviflora* (Iridaceae), from which it obtains both nectar and oil (Manning & Goldblatt, 2002). In addition, the precise mechanism by which two Cape orchids (genus *Disperis*) are pollinated by oil-collecting *Rediviva* has been described (Steiner, 1989).

The initial aim of this study was to describe the pollination biology of *Pterygodium catholicum* (Orchidaceae). From there the study grew to include several other oil-secreting orchids which flowered in association with *Pterygodium catholicum* (Orchidaceae) in lowland vegetation at the Cape during Spring. The foundation for this work has been laid by meticulous systematic studies of oil-secreting orchids (Kurzweil et al., 1991; Linder & Kurzweil, 1999) and oil-collecting bees (Whitehead & Steiner, 2001).

Methods

Study period, species and sites

Between August and October of the years 1997 to 2002 I studied the pollination biology of oil-secreting orchids at 16 study sites located in a 8 250 km^2 study area bound by Darling, Tulbagh, Botrivier and Cape Point. Study sites are listed in the text where relevant. The principal study species were *Pterygodium alatum*, *P. cafrum*, *P. catholicum*, *P. volucris* and *Corycium orobanchoides* (all Orchidaceae, subtribe Coryciinae). Less detailed observations (mainly morphological) where made on the following rare species: *Disperis villosa, D. bolusiana, D. cucullatum, D. circumflexa, D. capensis, P. inversum, P. cruciferum*. Scrophulariaceae (*Hemimeris racemosa* and *Diascia spp.*) were the only other oil-secreting plant species which flowered contemporaneously with *P. catholicum* at the study sites. *Hemimeris racemosa* was common at several sites; *Diascia spp.* were common only at Malmesbury.

Floral phenology, advertisement and reward

At each site, the first and last date of flowering was recorded. Flowers or inflorescences were collected, dissected, described and photographed. Floral parts are named according to Kurzweil and Linder (1991). Voucher specimens were pressed or preserved in FAA and placed in the Bolus Herbarium, University of Cape Town. To determine which floral parts were responsible for scent secretion, floral parts of *P. catholicum* were separated and placed in vials and the strength of the scent compared subjectively.

The Sudan IV test was used to confirm the presence of floral oil in the study species. The crystals dissolve in oil, staining all the liquid red; the crystals do not dissolve in aqueous solutions, which remain unstained. Crystals were sprinkled onto the liquid film which was visible under magnification on some floral parts.

Breeding systems

To determine whether *P. catholicum* is capable of autonomous self pollination, insects were excluded by enclosing budding plants in wire cages over which fine gauze bags were stretched (Darling Hills: pollinator excluded *N* = 54 flowers, 16 plants; unmanipulated *N* = 341 flowers, 66 plants). *P. alatum* plants were similarly enclosed at Malmesbury (pollinator excluded *N* = 42 flowers, 12 plants; unmanipulated *N* = 238 flowers, 36 plants).

To determine whether *P. catholicum* is self-compatible, hand-pollination experiments were conducted at Rondebosch Common using similarly enclosed plants. Pollinaria were collected using a bent dissecting needle and dabbed onto the stigma, which could be accessed only by freeing the tip of the lip appendage from the dorsal sepal (self pollination *N* = 31 flowers; cross pollination *N* = 32 flowers). Capsule set was recorded, rather than seed set, because seeds are very small and numerous and are released from the capsules soon after maturation.

Vegetative reproduction and habitat preferences

To investigate the possibility of vegetative reproduction, I excavated the entire root system of five unpollinated *P. catholicum* plants in sandy soil at Houhoek at the end of the growing season (October). The root system was washed and separated on metal mesh. The roots were straightened and the distance between parent and daughter tubers was measured and the condition and size of the tubers was recorded. One plant of *P. volucris* was similarly excavated at Botrivier. For the other study species, the spatial distribution and growth form of plants was noted. The habitat of the study species was observed, photographed and described.

Pollination observations

I observed pollinator behaviour on 33 days between mid August and mid October. Pollinator visitation rate was quantified for *P. catholicum* and *P. alatum* by observing ~10 m^2 patches of the focal plant species for 15 min intervals from a distance of 2 m. Visitation rate was calculated as visits per flower per hour. It was possible to distinguish *R. peringueyi* from other *Rediviva* species with reasonable certainty on the basis of its larger size and dull, non-reflective thorax. Quantitative observations were restricted to warm (>20 °C), windless hours between 10:30 and 15:30.

Pollinators were netted at 15 of the study sites, sexed, identified according to Whitehead and Steiner (2001), and examined microscopically for the presence of pollinaria. Pollinaria were identified to species level by comparison with reference collections from the study sites. Bee body parts were named according to Michener (1944).

Results

Floral phenology, advertisement and reward

Leaves are present during the wet winter and spring. All aboveground parts are absent during the dry summer. Flowering in the study species was roughly synchronous, started in mid August, peaked in September (spring) and ended at the end of October. *P. alatum* was the first to flower and *P. inversum* the last. In the absence of pollination *P. catholicum* inflorescences remained in flower for about four weeks with each flower lasting about three weeks. Flowering for all of the study species was most prolific in the first spring following a fire in the preceding summer. In old vegetation most plants produced only leaves.

The study species are all pale yellowish-green in colour (Fig. 5.1). Roughly circular areas of small magenta spots occur on the perianth segments immediately above the oil-secreting region in three species. In *P. catholicum* the spots are located on the apex of the petals, in *P. volucris* they are on the dorsal sepal and in *Corycium orobanchoides*, the lateral petals have magenta tips (Fig. 5.1). After pollination, the flowers of *P. catholicum* turn red in some populations. All the study species emit a pungent scent reminiscent of citrus and soap. In *P. catholicum* the scent is secreted primarily by the petals, but also by the lip and lip appendage.

The presence of floral oil was confirmed in all the principal study species (*P. alatum*, *P. cafrum*, *P. catholicum*, *P. volucris* and *Corycium orobanchoides*). The oil is visible as a film on the tip of the lip appendage. The lip appendage is a large upright outgrowth of the lip (Fig. 5.2). Its variously sculpted tip is usually tucked into the deepest recess of the hood formed by the petals and sepals (Fig. 5.1). In *P. alatum*, *P. cafrum* and *P. catholicum* the oil is concentrated in twin depressions on the abaxial

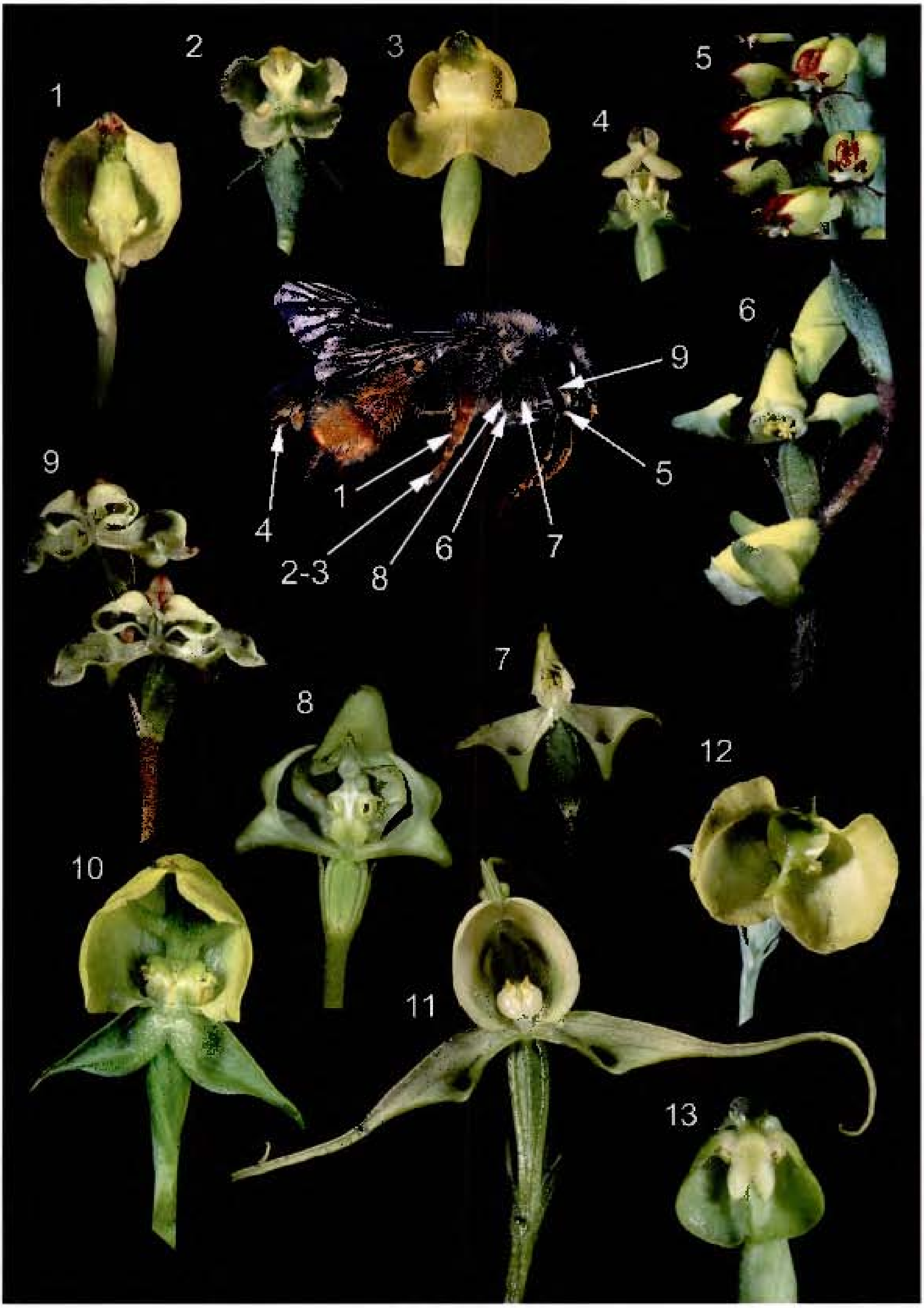

Fig. 5.1 The *R. peringueyi* pollination guild. **Centre,** the oil-collecting bee *Rediviva peringueyi* with arrows indicating pollinarium attachment sites of orchid species. **1,** *Pterygodium catholicum*. **2,** *P. alatum*. **3,** *P. cafrum*. **4,** *P. volucris*. **5,** *Corycium orobanchoides*. **6,** *Disperis villosa*. **7,** *D. bolusiana* subsp. *bolusiana*. **8,** *D. cucullatum*. **9,** *D. circumflexa* subsp. *circumflexa*. **10,** *P. cruciferum*. **11,** *D. capensis*. **12,** *P. platypetalum*. **13,** *P. inversum*. Attachment sites 6-9 after Steiner (1989). Pollination by *R. peringueyi* is confirmed in 1-7 and predicted in 8-13 on the basis of floral morphology. *R. peringueyi* 4X life size, orchids 2X life size. Photographs 5-12 provided by Bill Liltved.

Fig. 5.2 Variation in lip morphology in *Pterygodium*. **1**, *P. catholicum* **2**, *P. alatum* **3**, *P. cafrum* **4**, *P. volucris* **5**, *P. cruciferum* **6**, *P. inversum*. L, lip; LA, lip appendage; S stigma; T, anther thecum; V, Viscidium. Scale bars, 5 mm.

(front) surface of the tip of the lip appendage (Fig. 5.2). In *P. volucris* the oil pools in an apical depression in the tip of the lip (Fig. 5.2). In *Corycium orobanchoides* most of the surface of the lip appendage is oily.

In the principal study species the oil-secreting region was found to be smooth, and in this respect differs from the oil-secreting regions in *Disperis* which are covered in glandular oil-secreting trichomes (Manning & Linder, 1992; Steiner, 1989). Thus, the elaiophores of the *Pterygodium* and *Corycium* species included in the study were found to be epithelial rather than trichome elaiophores *sensu* Vogel (1974).

Breeding systems

In *P. catholicum* pollinator exclusion reduced capsule production to 0%, whereas 98 − 1 % of control flowers produced seed capsules. Similarly in *P. alatum* pollinator exclusion reduced capsule production to 0%, whereas 68 ± 5 % of control flowers produced seed capsules. Although there is no mechanism for autonomous self-pollination, *P. catholicum* flowers do produce seed capsules after being hand-pollinated with their own pollen (80 ± 10 % capsule production if cross-pollinated by hand; 76 ± 11 % capsule production if self-pollinated by hand).

Vegetative reproduction and habitat preferences

At three sites (Tygerberg, Botrivier and Paarl) small populations of *P. inversum* were found to consist of discrete, widely separated individuals with no evidence of vegetative reproduction. At two of these sites, the dry inflorescence from the previous year occurred alongside the current inflorescence (Fig. 5.3a). In all the other orchid species studied, the leaves and inflorescences were found to occur in clumps, suggesting vegetative reproduction (Fig. 5.3). The size and density of clumps differed between species. *Disperis circumflexa*, *D. cucullatum*, *D. bolusiana*, *D. capensis* and *Pterygodium cafrum* were found to form small dense colonies (0.5-1 m in diameter, 5-15 ramets). *P. alatum*, *Corycium orobanchoides* and *D. villosa* formed larger and denser colonies (1-2 m, 20-100 ramets). *P. catholicum* and *P. cruciferum* formed very large colonies (3 m to > 10 m in diameter, 100-500 ramets), with sparse and dense areas (Fig. 5.3).

Fig. 5.3 Growth forms in the *R. peringueyi* pollination guild. **a**, *P. inversum* flowering plant and dry inflorescence from previous year, height 25 cm (Botrivier). **b**, *P. caffrum*, height 16 cm (Paarlberg). **c**, *Corycium orobanchoides*, height 33 cm (Churchaven). **d**, *P. catholicum*, height 14 cm (Chapman's Peak). **e**, *P. volucris*, distance between primary tuber and daughter tuber 3 cm. **f**, *P. volucris* leaves, length 9 cm.

Fig. 5.4 Vegetative reproduction in *Pterygodium catholicum* **a,** Two excavated *P. catholicum* inflorescences, one in full flower (left) and one wilted (right). Scale bar = 3 cm. **b,** Detail of "a". P, swollen primary tuber of flowering inflorescence; R, shriveled primary tuber of wilted inflorescence; D, daughter tubers on the ends of ephemeral stolonoid roots of wilted inflorescence. Scale bar, 1 cm. Photography Bill Liltved.

Excavation confirmed vegetative reproduction in *P. volucris* and *P. catholicum* (Fig. 5.3e & Fig. 5.4). Vegetative reproduction was found to occur by the production of daughter tubers at the tips of ephemeral stolonoid roots. One ramet of *P. catholicum* with unwilted flowers had a swollen primary tuber (length = 7.5 mm), and one small daughter tuber (3.1 mm). Four older ramets of *P. catholicum* with wilted unpollinated flowers where found to have shriveled primary tubers (average length 4.8 mm, range 4 – 6.1 mm), and 3 – 5 spherical daughter tubers (average diameter = 6.1 mm, range = 4.5 – 9 mm). The stolonoid connecting roots averaged 104 mm (range 52-155 mm). All ramets lacked a replacement tuber, which normally occurs immediately adjacent to the primary tuber in ground orchids (Dixon, 1991; Linder & Kurzweil, 1999).

The study species occurred in the highest concentrations at low altitude (< 500 m) on clay soils. Within these areas they were most common on south-facing slopes or the edges of seepage areas. At several sites all of the study species occurred together and in association with the oil-secreting *Hemimeris racemosa* (Scrophulariaceae). Populations of the species often abutted, and replaced each other in a predictable sequence along a moisture gradient. The sequence started in water-logged soil (*P. cruciferum*), followed by seepage margins and south-facing slopes (*P. catholicum, D. villosa, H. racemosa, P. cafrum*, and *P. volucris*), and continued to hill crests and north facing slopes (*P. alatum* and *P. inversum*). *C. orobanchoides* also occupied the dry end of the sequence but on sandier soil (at the bases of clay hills). *C. orobanchoides* and *P. catholicum* occupied the broadest range of habitats.

Flower visitors, visitation rate and pollen loads

I captured insects on the flowers of *Pterygodium alatum, P. cafrum, P. catholicum* and *Corycium orobanchoides* at six sites. All captured insects were identified as female *R. peringueyi* (Table 5.1). In addition to the pollen of *Hemimeris racemosa*, captured *R. peringueyi* were found to carry the pollinaria of *Pterygodium alatum, P. cafrum, P. catholicum, P. volucris* and *Corycium orobanchoides* attached to precise parts of their bodies via sticky viscidia (Table 5.2, Fig. 5.1). The maximum number of pollinarium species carried by an individual bee was 3 and the maximum number of pollinaria carried was 14.

Table 5.1. Captured *Rediviva peringueyi* and their orchid pollen loads.

Plant species observed	*R. peringueyi* individuals captured	Total number and identity of pollinaria attached to captured *R. peringueyi*				
		tholicum	*atum*	*frum*	*lucris*	*obanchoides*
Pterygodium catholicum	7	35	3	4	6	0
Pterygodium alatum	4	0	18	0	0	0
Pterygodium cafrum	3	0	0	3	1	0
Corycium orobanchoides	1	0	0	0	0	3
Hemimeris racemosa	8	0	3	0	0	0

Table 5.2 Pollen and pollinarium attachment sites on captured individuals of female *Rediviva peringueyi.*

Plant species	Number of attached pollinaria observed	Viscidium attachment site on female *Rediviva peringueyi*
Pterygodium alatum	24	Dorsal surface of distitarsus (tarsomere 5) of middle legs
Pterygodium cafrum	7	Dorsal surface of distitarsus (tarsomere 5) of middle legs
Pterygodium catholicum	35	Posterior edge of distal half of basitarsus (tarsomere 1) of middle legs
Pterygodium volucris	7	Ventral surface of the last abdominal segment
Corycium orobanchoides	3	Ventral surface of distal half of tibia of front legs
Disperis villosa	1	Trochanter of front legs*
Disperis bolusiana spp bolusiana	1	Femur of front legs*
Hemimeris racemosa	--	Ventral surface of thorax

*Steiner (1989)

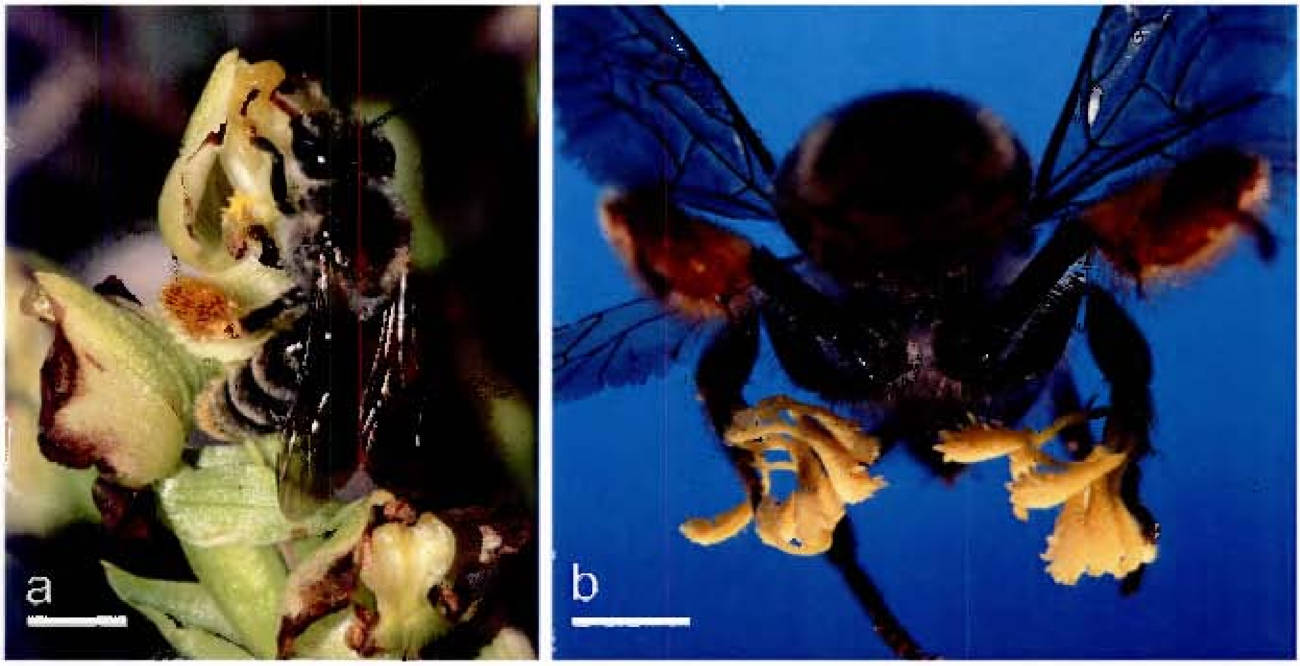

Fig. 5.5 *Rediviva peringueyi* pollination mechanism. a, *R. peringueyi* collecting floral oil from the apex of the lip appendage of *Pterygodium alatum* with a rapid rubbing motion of her front feet. While collecting oil, the bee hangs onto the lip appendage with her middle feet, onto which the pollinaria (visible) become attached. Scale bar = 3 mm. b, Several pollinaria of *P. catholicum* attached precisely to the basitarsi of the middle legs of *R. peringueyi* via the sticky viscidia. b, Scale bar = 1 mm.

R. peringueyi individuals were also dusted with loose pollen grains of several nectariferous plants. The scopae of the hind legs were usually filled with a mixture of collected pollen and copious oil.

At most sites the rate of *R. peringueyi* visitation to orchids was too low to be accurately censused. The highest orchid visitation rates occurred at Romansrivier (0.05 visits per *P. catholicum* flower per hour) and at the Darling Wildflower Reserve (0.46 visits per *P. catholicum* flower per hour; 0.40 visits per *P. alatum* flower per hour). Visits to orchids lasted 2-5 seconds. At Darling *R. peringueyi* was observed to visit *P. catholicum*, *P. alatum* and *Hemimeris racemosa* (Scrophulariaceae) in a single foraging bout. At this site visitation rate of *R. peringueyi* to *H. racemosa* was roughly equal to that recorded at the orchids (0.4 visits per flower per hour). The short tongue length of *R. peringueyi*, was reflected in its choice of nectar plants: *Moraea gawleri*, *Moraea tricolor* and *Oxalis obtusa.*

Pollinator behaviour in relation to floral morphology

Female *Rediviva peringueyi* were observed to collect floral oil from the shallow depressions in the apex of the lip appendage of *Pterygodium alatum* with a rubbing motion of their front tarsi (Fig. 5.5). The tarsus of the left front leg was inserted into the right-hand depression, and simultaneously the tarsus of the right front leg was inserted into the left hand depression. While collecting oil with their front feet the bees were observed to hang onto the flower, which is tilted slightly downwards. The tarsi of the hind legs gripped the margins of the lip, while the middle legs fixed the bee in a hanging position. The tarsal hooks of the middle legs hooked onto holdfasts at the base of the lip appendage. In this position, the tarsi slotted into channels formed between the rostellum arms and the lip appendage. The viscidia (pollinarium attachment discs) lie against the outer walls of these channels and face inwards towards the lip appendage, such that they became attached to the dorsal surfaces of the tarsi (Fig. 5.2). In *P. alatum*, the viscidia are set ~0.5 mm back from the holdfasts so that they coincide with the position of the distitarsus (tarsal segment 5). The pollinaria are extracted from the thecae (anther sacs) when the bee withdraws from the flower. Free from the flower, and attached to the bee, the caudicle (narrow portion of the pollinarium), was observed to bend through ~150° to bring the tip of the distal end of the pollinarium around to the posterior edge of the tarsus. The flexing occurs within 1-3 seconds.

In *P. alatum*, the stigma is located close to the viscidia on the tips of the rostellum arms (Fig. 5.2). The short distance between the viscidium and the stigma corresponds with the unusually short caudicles of *P. alatum*. When a pollinarium was brought into contact with the stigma, a proportion of the massulae were observed to adhere to the stigma and several were broken free when the pollinarium was withdrawn.

The functional morphology of *P. cafrum* is very similar to that of *P. alatum*. The viscidia are also located ~0.5 mm from the holdfast onto which the tarsal hooks latch, and thus also become attached to the dorsal surface of the distitarsus. The main, functional difference between the species is in the length of the pollinaria (caudicles), which in *P. cafrum* are twice as long as in *P. alatum* (Fig. 5.2). The distance between the viscidium and the stigma is of corresponding length. In *P. cafrum* the stigma is located beyond the reach of *P. alatum* pollinaria on the adaxial side of the lip appendage. When withdrawn from the thecae, the pollinaria of *P. cafrum* underwent the same flexing that was observed in *P. alatum*.

In *P. catholicum* pollinator behaviour was observed to be similar to that described for *P. alatum*. The visiting bee is largely hidden inside the galeum (hood) formed by the petals and dorsal sepal (Fig. 5.1). The galeum presumably limits illegitimate lateral and dorsal access to the oil-secreting region. An important difference with the previous two species is the fact that the viscidia are set ~2.5 mm back from the holdfast and lie in the bottom of the channel at the base of the lip appendage (Fig. 5.2). Thus located, the viscidia attach to the narrow posterior surface of the distal half of the basitarsus (tarsal segment 1). The stigma faces upwards and is adaxial to the lip appendage. The caudicles are correspondingly long, but were not observed to undergo flexing when withdrawn from the thecae.

I was not able to make detailed observations of pollinator behaviour on *P. volucris*. The unusual site to which pollinaria were attached on captured bees (ventral surface of last abdominal segment) suggests the following scenario. In contrast to the previous three species, dorsal access into the galeum is made possible by a circular aperture between the tips of the petals and the dorsal sepal (Fig. 5.1). The bee inserts its front legs through this aperture and reaches for oil which pools in the cup at the tip of the lip appendage (Fig. 5.2). She holds on by hugging around the outside of the petals with her middle legs, which slot into the narrow constriction near the top of the galeum. The hind tarsi fit into short channels, which are located on either side of the viscidia and are formed between tooth-like callosities on the lateral lip lobes (Fig. 5.1). In this position, the ventral surface of the last abdominal segment makes contact with the viscidia which face outwards and towards the underside of the bee. The callosities, which do not occur in the other species considered here, presumably ensure the exact placement of the hind tarsi, and hence the abdomen.

In *Corycium orobanchoides* the oil-secreting lip appendage is housed in a deeply conical galeum, which is too narrow to accommodate the bee (Fig. 5.1). Visiting bees were observed to remain outside the flower and reached for the oil in the back of the galeum by inserting their front legs into the narrow channels that run alongside the lip appendage. The viscidia are located along the length of the channels ~5 mm anterior to the tips of the lip appendage. Thus when the tarsi of the front legs are in contact with the tip of the lip appendage, the large viscidia became attached to the ventral surface of the distal half of tibia of the front legs. Details were not discernable, but it seems likely that the bees were holding on by hooking the tarsal hooks of the middle legs onto the small lateral projections of the lip. The hind legs secure a purchase on neighbouring flowers in the densely packed inflorescence. As in other spotted species (*H. racemosa*, *P. catholicum* & *P. volucris*), the magenta spots on the tips of each of the two petals coincide with the position of the left and right compound eye when the bee is in the oil-collecting position.

Observation of pollinator behaviour suggested that an apparently important functional measurement is the distance between the holdfast (where the tarsal hooks of the middle legs attach) and the oil-secreting region. The bee has to span this distance in order to obtain the reward. In *P. alatum* and *P. cafrum* the oil secreting region (tip of the lip appendage) is located ~5mm distant from the holdfast, and in *P. catholicum* and *C. orobanchoides* holdfast and oil are located ~7.5 mm apart.

Discussion

R. peringueyi *pollination mutualisms*

Female oil-collecting bees belonging to the species *Rediviva peringueyi* were found to be the sole pollinators of five orchids: *Pterygodium catholicum*, *P. alatum*, *P. cafrum*, *P. volucris* and *Corycium orobanchoides* (Fig. 5.1). A previous study adds *Disperis villosa* (Orchidaceae) to the list of species which are apparently only pollinated by *R. peringueyi* (Steiner, 1989). All have the center of their distribution in West Coast Renosterveld vegetation (Low & Rebelo, 1996) on the coastal plain at the Cape of Africa.

The plants were found to offer oil as a reward, which female bees were seen to collect with a rubbing motion of the front tarsi. Tarsal segments 2-5 of the front legs of *R. peringueyi* are coated in absorbent, plumose hairs (Whitehead & Steiner, 2001). Floral oil is not known to be used as an adult food by oil-collecting bees, but is collected by female bees, who, alone, are involved in brood care (Cane et al., 1983; Vinson et al., 1997; Vogel, 1974). The fact that only female bees were caught on the flowers of the study species confirms the finding that oil is the only reward.

Various morphological contrivances of the orchid flowers ensure that *R. peringueyi* is unable to collect the reward without making contact with the sticky viscidia, which become attached to precise, species specific parts of the body of the bee (Fig. 5.1). Bees were seen to fly between orchid inflorescences with pollinaria attached. Captured bees were carrying up to 16 pollinaria belonging up to three species of orchids (Table 5.1). Bees were observed to alight on flowers in a position that would result in contact between the pollinaria and the stigma (Fig. 5.5). The exclusion of insects from the flowers of *P. catholicum* and *P. alatum* reduced seed set from high levels in control plants to zero. Thus, sexual reproduction in these orchids is dependent on *R. peringueyi.*

The study species apparently reduce interspecific reproductive interference by the use of mutually exclusive pollinarium attachment sites on the body of the bee (Fig. 5.1). Only two species, *P. cafrum* and *P. alatum*, were found to use the same pollinarium attachment site. Interspecific pollen transfer between these two species is avoided by differences in the length of the pollinaria. The pollinaria of *P. cafrum* are twice as long as those of *P. alatum*, and the stigma is correspondingly distantly located.

The subtribe Coryciinae are characterized by a large and uniquely complex lip appendage, which has been referred to as "the most bizarre floral structure in the orchids" (Kurzweil et al., 1991) (Fig. 5.2). Observations of pollinator behaviour have elucidated the function of this organ. The appendage secretes the reward (oil), from epithelial elaiophores at its tip, and in some species of *Pterygodium* also provides the holdfast onto which the middle legs of the bee hook. Thus, the lip appendage forms the central functional axis of the flower. The diversity of pollen attachment sites (Fig. 5.1) is directly reflected in the structural diversity of lip appendages (Fig. 5.2).

The R. peringueyi pollination guild and floral syndrome

The six plant species (*P. alatum, P. cafrum, P. catholicum, P. volucris, Corycium orobanchoides* and *Disperis villosa*), which have been shown to be exclusively pollinated by *R. peringueyi* share the following floral features (Fig. 5.1): oil-secretion; pungent citrus-soapy scent; pale yellowish-green flowers; flowering period 15 August to 25 October (peak in mid to late September); distance between oil-secreting region and holdfast (for middle legs) 5-7.5 mm; absence of black markings. Three of the species (*P. catholicum, P. volucris* and *Corycium orobanchoides*) share the presence of small magenta spots which coincide with the position of the eyes of *R. peringueyi* when in the oil-collecting position.[2] Similarity among the plant species pollinated by *R. peringueyi* probably results both from common ancestry and convergent pollinator-driven selection.

The following rare species in the subtribe Coryciinae, also fit the above syndrome of floral features (Fig. 5.1): *Pterygodium inversum, P. cruciferum, P. platypetalum, Disperis cucullatum, D. bolusiana* subsp. *bolusiana, D. circumflexa* subsp. *circumflexa* and a cream-coloured form of *D. capensis*[3]. Pollination by *R. peringueyi* is predicted in these species. A fourteenth species, *P. connivens* also fits the syndrome, but the connivent sepals probably limit access by bees to the interior of

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[2] This feature is not exclusive to the *R. peringueyi pollination* guild, but also occurs in some other *Rediviva* pollinated orchids and in *Hemimeris racemosa.*

[3] Oil secretion has not been confirmed for the cream-coloured form of *D. capensis.*

the flower, and the presence of an "abnormal gynostemium"(Kurzweil et al., 1991) suggests the possibility of autonomous self-pollination.

Pollination guilds – groups of plants that share a common pollinator and a syndrome of common floral features – have been described in other Cape pollination systems (Johnson, 1994b; Johnson et al., 2001; Manning & Goldblatt, 1996, 1997), but not within the *Rediviva* system. The usefulness of pollination syndromes has been questioned largely on the basis of the observation that many pollination systems are generalist (Ollerton, 1998; Waser et al., 1996). However, in specialized pollination systems, where the fauna of available pollinators is limited and relatively well known, pollination syndromes are a useful predictive tool (Johnson et al., 2001; Johnson & Steiner, 2000; Ollerton, 1998).

R. peringueyi visits a number of oil-producing species in the genera *Hemimeris* and *Diascia* (Whitehead & Steiner, 2001). These Scrophulariaceae are also visited by a range of other *Rediviva* species (Chapter 4)(Whitehead & Steiner, 2001). It seems unlikely that *R. peringueyi* in particular has played a significant role in the evolution of their floral features, which are quite distinct from those of the *R. peringueyi* pollination guild in its strict sense.

Pterygodium hallii is excluded from the *R. peringueyi* pollination guild, for the same reason. *R. peringueyi* probably plays a role in the pollination of *P. hallii* in isolated populations which occur on the West Coast Lowlands. However, the distribution of *P. hallii* is centered along the western margins of the Karoo outside the distribution range of *R. peringueyi*. The floral characteristic of this species (particularly the presence of large black markings) probably reflect co-evolution with *R. macgregori*.

The R. peringueyi *pollination guild in the context of the greater* Rediviva *pollination system*

The distribution of the *R. peringueyi* guild is centered in the West Coast Lowland, which extends northwards from the Cape Peninsula for about 150 km and is about 60 km wide. At 12 mm, *R. peringueyi* is the largest of six *Rediviva* species which commonly occur on these coastal lowlands. Specialized floral advertisement and morphology apparently prevents the smaller, sympatric *Rediviva* from accessing the rewards provided by the members of the *R. peringueyi* guild.

The scentless, bright yellow flowers of *Hemimeris racemosa* attract the full range of *Rediviva* species as well as several species of beetle (Chapter 4). In contrast the members of the *R. peringueyi* pollination guild have inconspicuous pale yellowish-green flowers, and a pungent scent, which presumably acts as a major attractant for *R. peringueyi*. In *Corycium orobanchoides* 3,5-dimethoxy toluene has been found to be an important component of this unusual scent (Manning & Goldblatt, 2002).

Morphological specialization of the members of the *R. peringueyi* pollination guild might prevent smaller bee species from accessing their oil. In these species, the oil-secreting tip of the lip appendage is distantly located from the holdfast, which is at the base of the lip appendage. *R. peringueyi* were observed to hold onto the holdfasts with their middle feet while collecting oil with their front feet. Morphometrics suggests that the smaller *Rediviva* would be unable to span the distance between holdfast and reward with ease. In this study, smaller *Rediviva* species were captured and observed only on sympatric short-spurred Scrophulariaceae, in which floral oil is more readily available. Observations of the foraging behaviour of oil-collecting bees on *H. racemosa* suggest that small size and the presence of oil-collecting hairs on the middle legs (absent in *R. peringueyi*) are possible adaptations for oil-collection from short-spurred Scrophulariaceae.

Two additional species of *Rediviva* (*R. longimanus* and *R. micheneri*) would, on the basis of their morphology, be able to access the reward offered by the members of the *R. peringueyi* pollination guild. These are exceptionally long-legged bees, which specialize in the collection of floral oil from the long-spurred *Diascia spp.* (Whitehead et al., 1984; Whitehead & Steiner, 2001). These two *Rediviva* enter the West Coastal Lowlands at their northern fringe, and its is possible that they play a role in the pollination of some of the species in the *R. peringueyi* guild in this area.[4]

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[4] Steiner (1989) has captured a specimen of *R. longimanus* with a pollinarium of *Disperis circumflexa*. No locality information is provided, but it seems likely that the pollinarium belongs to *D. circumflexa* subsp. *aemula*, which differs in several respects from the members of the *R. peringueyi* pollination guild.

Conclusions and predictions

The group of plant species in which pollination by *R. peringueyi* is proven or predicted form a relatively discrete compartment inside the greater *Rediviva* pollination system. In the following chapters I explore the causes and the consequences of the loss of *R. peringueyi* from this pollination compartment. Short term effects of the loss of *R. peringueyi* are measured as effects on seed set. Longer term responses are reflected in patterns of plant genetic diversity, species composition and ultimately infraspecific and infra-generic morphological variation. Key reproductive features which can be expected to determine responses to the loss of *R. peringueyi* have been explored in this and the previous chapter. These include: 1) the degree of pollinator specificity, 2) the degree to which seed set is dependent on pollination and 3) the degree to which population persistence depends on seeds (Bond, 1994).

6 Natural and anthropogenic sources of variation in pollination by an oil-collecting bee, *Rediviva peringueyi*

Twenty seven established conservation areas were assessed for their ability to conserve a specialized pollination mutualism between an oil-collecting bee, *Rediviva peringueyi,* and an oil-secreting orchid, *Pterygodium catholicum.* Pollination rate and hence capsule set varied among conservation areas from 98% to zero. Key variables that significantly influenced the ability of nature reserves to conserve the orchid-bee mutualism were: 1) soil type 2) successional stage of the vegetation and 3) the interaction between reserve size and the nature of the surrounding land-use. Pollination failure occurs in nature reserves with sandy soil, and in small conservation areas (4 – 385 ha) in an urban matrix. Temporal patterns of variability were linked to the successional stage of the vegetation. Interspecific correlation of variation in capsule set, suggest that the results obtained here can be extrapolated to other members of the *R. peringueyi* pollination guild.

The ecological consequences of the fragmentation of once continuous habitats into isolated remnants is a global biodiversity concern . One important consequence of habitat fragmentation, which has received relatively little attention, is the potential erosion of biodiversity through the breakdown of pollination mutualisms. Studies to date have detected lower levels of pollination in very small fragments (< 1ha) of natural vegetation (Aizen & Feinsinger, 1994; Cunningham, 2000b; Donaldson et al., 2002; Jennersten, 1988; Lennartsson, 2002; Lindberg & Olesen, 2001; Robertson et al., 1999). Are similar declines occurring at a larger geographical scale, relevant for established conservation areas?

This study focuses on the specialized pollination mutualisms between an oil-collecting bee, *Rediviva peringueyi*, and the oil-secreting orchid, *Pterygodium catholicum*, which was described in Chapter 5. The study area is the lowlands of the Cape of Africa, a global hotspot of biodiversity (Goldblatt & Manning, 2002). Over most of the lowlands, natural habitats have been reduced to small remnants by agricultural and urban expansion (Heijnis et al., 1999; Maze & Rebelo, 1999; McDowell & Moll, 1992). Conservation areas of varying size have been established, or are being planned (Heijnis et al., 1999; von Hase et al., 2004), to conserve the endemic lowland flora. I selected 27 of the established conservation areas and asked: Does the current reserve network adequately conserve the *R. peringueyi-P. catholicum* pollination mutualism?

The main dependent variable used in this study is capsule set in *P. catholicum*. Capsule set in *P. catholicum* can provide a good indication of pollination rate by *R. peringueyi* only if other environmental factors, such as the availability of growth resources (e.g. nutrients and water), do not limit capsule set. Hence, the first portion of this study is devoted to establishing whether capsule set provides a good indication of pollination rate. In the second portion of the study, I examine the relationship between variation in capsule set across the landscape and a range of predictor variables. The predictor variables included both natural and anthropogenic features of the landscape. Lastly, I test whether the pattern of variation in reproductive success (capsule set) in *P. catholicum* is matched by other plant species that are pollinated by the same oil-collecting bee, thus allowing generalization of the conclusions reached in this study.

The study addressed the following specific questions:

- Is seed capsule production limited by pollinator availability, or by the availability of other resources?
- How does seed capsule production vary across the conservation landscape in relation to:
    - size of reserve
    - matrix in which the reserve is located
    - soil type
    - successional stage of the vegetation
- Can the results of this analysis be extrapolated to other plant species which are pollinated by the same species of oil-collecting bee?

Methods

Study species

The principal study species, *Pterygodium catholicum*, is the most common orchid in the Cape flora. Plants flower profusely in the first few years after fire, but produce only leaves in older vegetation. Inflorescences are approximately 15 cm in height and have a median of 3 flowers (range, 1-12). The flowers secrete oil as a rewards and are pollinated exclusively by the oil-collecting bee *Rediviva peringueyi*. The pollen packets (pollinaria) of *P. catholicum* become attached to the middle legs of the bee, while it collects oil from the flower with its front legs. After pollination, the flower produces a capsule filled with microscopic, wind-dispersed seeds. Although *P. catholicum* is incapable of autogamy, plants were found to be self-compatible (Chapter 5).

P. catholicum shares its pollinator (*R. peringueyi*) with about 20 other oil-secreting plants that flower in the lowlands of the Cape from August to September (Chapter 4 & 5). Three of these, *Pterygodium alatum*, *Corycium orobanchoides* (Orchidaceae) and *Hemimeris racemosa* (Scrophulariaceae) were included in components of this study.

Study sites

I selected 27 areas of natural vegetation within a 12 500 km^2 study area located in the West Coast Lowlands (Fig. 6.1)(Appendix 6.1). Most of the selected areas are proclaimed nature reserves aimed at the conservation of the endemic lowland flora. There is a long history of intensive land-use in the study area. Thus, the conservation areas have been isolated from each other by transformed area for between 50 and 150 years (Joubert & Moll, 1992; McDowell & Moll, 1992).

Dependent variable: capsule set

The main dependent variable used in this study to detect the breakdown of pollination is capsule set. I recorded capsule set in *P. catholicum*, *P. alatum* and *C. orobanchoides* by marking flowering plants with adhesive jewelry tags and returning

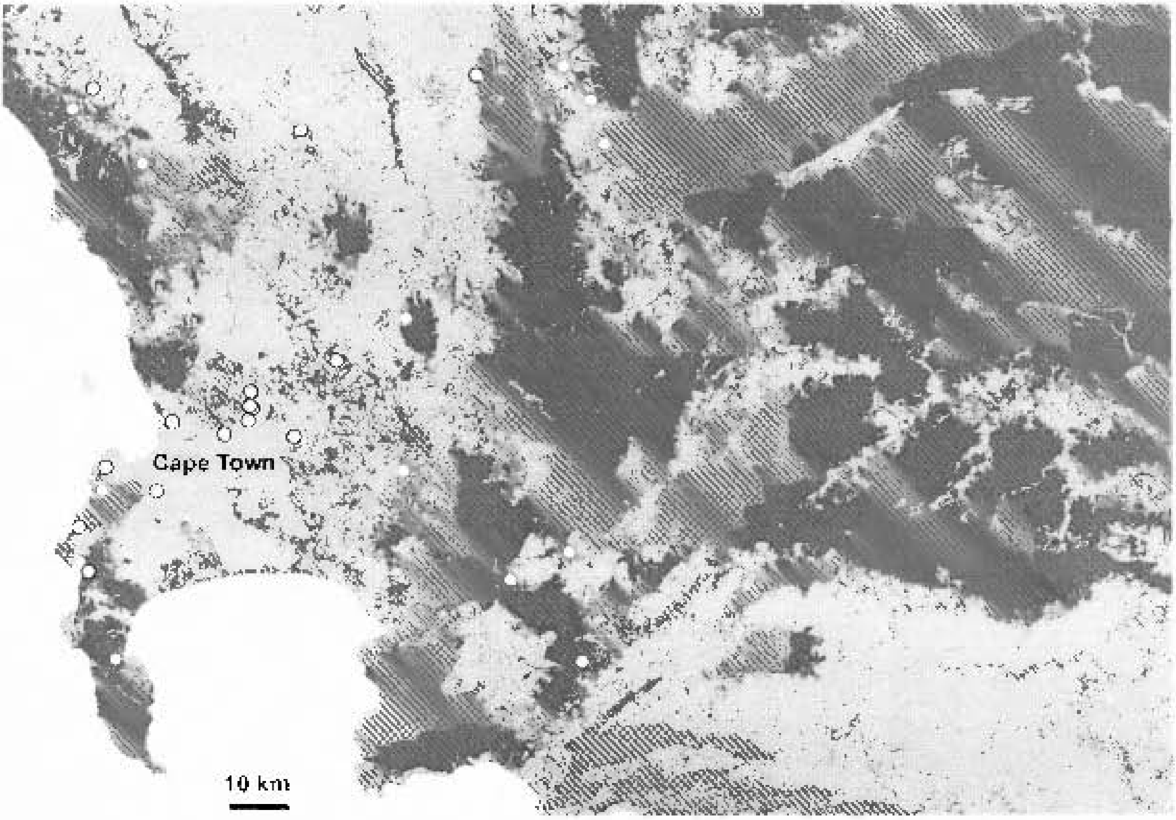

Fig. 6.1 Location of study sites at the Cape of Africa. Dark gray indicates areas of natural vegetation; light gray indicates areas which have been transformed by urbanization or agriculture.

after flowering to determine the proportion of flowers that had produced seed capsules. Long after flowering the withered, dry flowers still remain attached to the inflorescence regardless of whether or not they produce seed capsules. Successful seed set is indicated by a swollen ovary (a capsule), which dehisces along longitudinal slits. Aborted ovaries are thin, shriveled and twisted and contain masses of unfilled seeds still attached to the three parietal placentas. Swollen and dehiscing capsules were opened and checked under 10 X magnification for the presence of filled seeds. The seed coat in the orchids in this study is translucent, allowing easy distinction between filled and unfilled seeds. Filled seeds are spindle shaped, swollen in the middle and have dark brown contents. Unfilled seeds are narrow, are not swollen in the middle, and have light coloured contents. I recorded capsule set, rather than seed set, because seeds are minute and numerous and are released from the capsules soon after maturation. In *P. catholicum* and *P. alatum* all flowers on sample inflorescences were check. In *C. orobanchoides* underdeveloped, apical flowers were excluded.

For the principal study species, *P. catholicum*, the median number of sample years at each of 27 sites was 2 (range 1-4). The median sample size was 122 flowers (range, 13-799). In total, capsule production was recorded for 10 101 flowers on 2 791 plants (Appendix 6.1). The two secondary study species were less common and were sampled less intensively than *P. catholicum*. Each was sampled once only at each of 12 (*C. orobanchoides*) and 11 (*P. alatum*) sites. For *P. alatum* the median sample size was 238 flowers (range, 58-738). In total, capsule production was recorded for 3 247 flowers on 578 *P. alatum* plants. For *C. orobanchoides* the median sample size was 160 flowers (range, 20-890). In total, capsule production was recorded for 2 980 flowers on 225 *C. orobanchoides* plants.

Proximate determinants of capsule set in *P. catholicum*

The availability of resources, such as nutrients and water, can potentially influence capsule set in *P. catholicum*, and this would lead to a weak relationship between capsule set and pollination. To determine the strength of the relationship between capsule production and pollination, I quantified the pollination rate at each of the 10 conservation areas and related it to capsule set. Pollination rate was quantified in two ways:

1) Field-collected orchid flowers were checked to determine whether the pollinaria had been removed and whether there were pollen massulae adhering to the stigma. Wilted inflorescences were collected at the end of the flowering season and preserved in 96% ethanol before microscopic dissection (40X). I recorded the presence or absence, rather that number, of massulae on the stigma since one massula is sufficient to fertilize all the ovules (S. D. Johnson pers. com.). Empty, but undamaged, pollinarium sacs, indicated pollinarium removal by a pollinator. Occasional pollinaria removed by pollen-feeding insects can be identified (and excluded), because herbivorous insects cause damage to the membranous pollinarium sac and typically eat the pollen, but not the viscidium (pollinarium attachment disc) or caudicle (arm). Pollination rates were determined at 15 study sites. Median sample size was 115 flowers (range, 35-241). In total, pollination rates were determined for 1 795 flowers on 436 plants. Pollination rates were related to capsule set data obtained during the same sample year.

2) Censuses of pollinator visitation rate were conducted at six of the sites by observing ~5 m^2 patches of oil-secreting *Hemimeris racemosa* (Scrophulariaceae) plants for two 15 min intervals from a distance of 2 m. *P. catholicum* density is too low to allow practical censusing of *R. peringueyi* visitation rates to its flowers. Four *Rediviva* species visited the flowers of *H. racemosa* at the study sites. However, it was possible to distinguish *R. peringueyi*, the sole pollinator of *P. catholicum* from other *Rediviva* species with reasonable certainty on the basis of its larger size and dull, non-reflective thorax. Visitation rate observations were restricted to warm (>20 °C), windless hours between 10:30 and 15:30. Visitation rate was calculated as visits per flower per hour. Visitation rates were related to capsule set data obtained during the same sample year.

I experimentally tested the relationship between pollination rate and seed set in two ways. 1) At Rondebosch Common, a site with low capsule set, I supplemented natural pollination by hand-pollinating all the flowers on 21 plants. Pollinaria were collected using a bent dissecting needle and dabbed onto the stigma, which could be accessed only by freeing the tip of the lip appendage from the dorsal sepal. A further

82 control plants received only natural pollination. 2) On the Darling Hills, at a site with high capsule set, I excluded pollinators from 16 plants with fine gauze bags stretched over wire frames. A further 66 control plants were left open to receive natural pollination.

Capsule set in *P. catholicum* in relation to environmental factors

Generalized linear models were used to test the effect of four environmental variables on capsule set in *P. catholicum*. The explanatory variables were:

1) *Size of reserve*. The size of the 27 remnants of natural vegetation (reserves) was determined from a fine scale digital map of natural remnants using geographic information system software (Arcview 3.2 & ArcInfo 8.2, Environmental Systems Research Institute Inc.). The map was produced from LANDSAT7 imagery, ground-truthed and manually refined as described in Lloyd *et al.* (1999) and von Hase *et al.* (2004). The boundaries of reserves were drawn to include any areas of untransformed land, which were continuous with the formally proclaimed conservation area. Conservation areas were then classified according to size. Large conservation areas ranged from 2 579 ha to 155 247 ha (median = 15 805 ha, n = 13); small conservation areas range from 4 ha to 609 ha (median = 30 ha, n = 14). Reserve size [large; small] was a categorical fixed factor.

2) *Matrix in which the reserve is located.* Conservation areas were located either in a rural matrix, consisting mostly of plowed fields used for crop or livestock production, or in an urban matrix within the Cape Town Metropolitan area. Matrix type [urban; rural] was a categorical fixed factor.

3) *Soil type*. In a simplified view, the Cape consists of a mosaic of two fundamentally different soil types: coarse-grained, sandy soils, poor in essential plant nutrients, and finer grained clay soils richer in nutrients (Goldblatt & Manning, 2002). The clay soils generally occur at low altitude (< 500 m) on coastal plateaus and valley bottoms, which alternate with sandstone (quartzitic) mountain ranges. Soil type [clay; sand] was a categorical fixed factor.

4) *Successional stage of the vegetation.* The number of years since fire occurred was included as a potentially important variable on the basis of the observation that

flowering in *P. catholicum* and the other members of the *R. peringueyi* guild peaks in early post-burn vegetation. For recently burnt sites the time since the last fire was known; for sites that were not burnt recently, the time since the last fire was estimated from the condition of the vegetation. The post-burn age of the vegetation was a continuous fixed variable ranging from 1 to 13 (median 3).

Plants from one conservation area were pooled, so that the total number of flowers sampled at a site was the number of "trials", and the number of capsules produced was the number of "successes". As a first step, generalized linear models (GLM) with quasi-binomial error structure were fitted in the statistical software "R" version 1.6.0 (Venables & Ripley, 2002). Starting from the full model, which included all possible interaction terms between the four explanatory variables, the minimal adequate GLM was obtained by sequential removal of non-significant model terms (analysis of deviance, F-tests, $p > 0.05$). The selected minimum adequate GLM contained all main effects of the four variables plus the interaction between *Matrix* and *Size*.

The minimum adequate GLM included pseudo-replication because some sites were sampled repeatedly in successive years. At the final stage of analysis, pseudo-replication was avoided by including *Site* as a random effect in a generalized linear mixed model (GLMM)(function glmmPQL; penalized quasi likelihood approach; random effects of site on intercept and slope).

Extrapolation of results to other members of the *R. peringueyi* pollination guild

To test the generality of the conclusions of the first part of this study, I correlated capsule set in *P. catholicum* with capsule set in two other oil-secreting species, *P. alatum* and *C. orobanchoides*, which are also pollinated by *R. peringueyi*. Average capsule set at each site was determined and related to average capsule set in *P. catholicum* at the same site in the same year. Before statistical testing, data were arcsine transformed to normalize the error distribution.

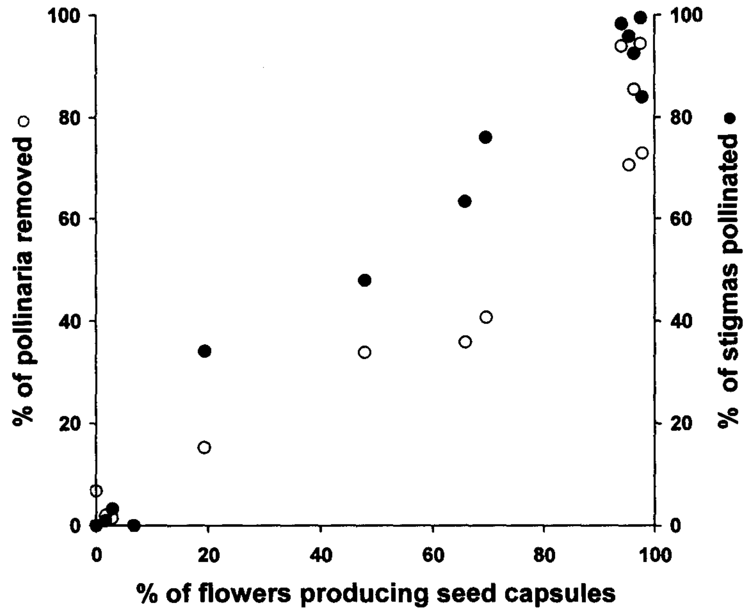

Fig. 6.2 Relationship across 15 sites between the percentage of *Pterygodium catholicum* flowers producing seed capsules and two measures of pollination success: the percentage of pollinaria removed by pollinators ($r = 0.97$, $P < 0.0001$, $N = 15$, open markers); the percentage of stigmas with adhered pollen ($r = 0.99$, $P < 0.0001$, $N = 15$, filled markers).

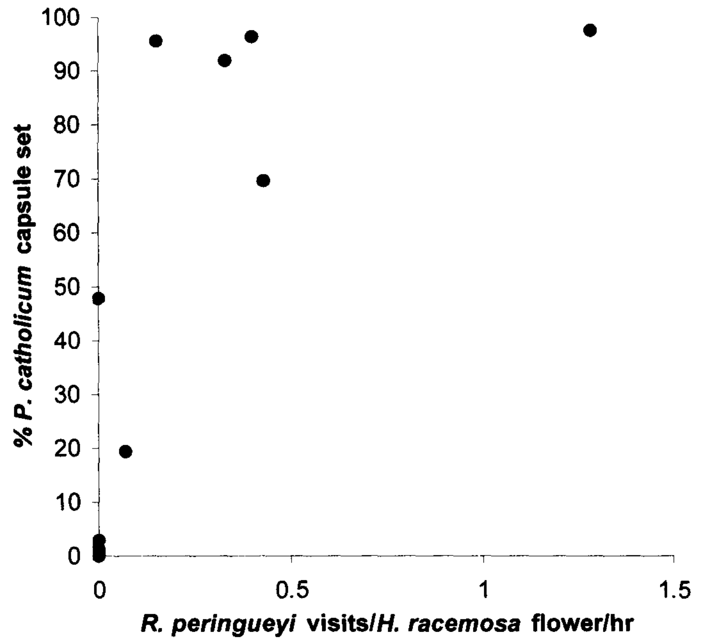

Fig. 6.3 Relationship between capsule set in *Pterygodium catholicum* and visitation rate by the oil-collecting bee *Rediviva peringueyi*. Visitation rate was determined at the flowers of *H. racemosa*, an abundant, sympatric, oil-secreting host plant of *R. peringueyi* (Spearman $r = 0.86$, $N = 11$, $p = 0.0007$).

Results

Capsule set

Seed capsule production in *Pterygodium catholicum* varied among conservation areas from 0 % to 98 % (median 18 %, mean 38 %)(Appendix 6.1).

Proximate determinants of variation in capsule set in *P. catholicum*

A strong relationship was found between capsule set and two measures of pollination rate (pollinarium removal and pollen receipt)(Fig. 6.2). This result was supported by pollinator censuses. At sites where *Rediviva peringueyi* was absent, capsule set was low, where *R. peringueyi* occurred capsule set was high (Fig. 6.3).

Experimentation confirmed a causal relationship between pollination and capsule set. At a site with low capsule set, supplemental hand pollination increased capsule set from 0% in control plants to 78 ± 7%. At a site with high capsule set, the exclusion of pollinators with gauze bags reduced capsule production to 0%, whereas 98 ± 1 % of control flowers produced seed capsules. The relationship between pollination and capsule set was not modified by herbivores. Little or no evidence of herbivory or seed predation was observed.

Capsule set in *P. catholicum* in relation to environmental factors

Two sources of natural variability in the landscape significantly affected capsule set (Table 6.1). These were soil type [clay; sand], and the successional stage of the vegetation [years since fire, range 1-13]. Capsule set decreased with increasing age of the vegetation and was lower on sandy soils (Table 6.1, Fig. 6.4).

Two anthropogenic factors, namely the size of the conservation area [large; small] and the matrix [urban; rural] in which it is located did not have a significant effect on capsule set when considered separately, but the interaction between these two factors did have a significant effect (Table 6.1). Capsule set was significantly lower in small nature reserves if these were located in an urban matrix (Fig. 6.4).

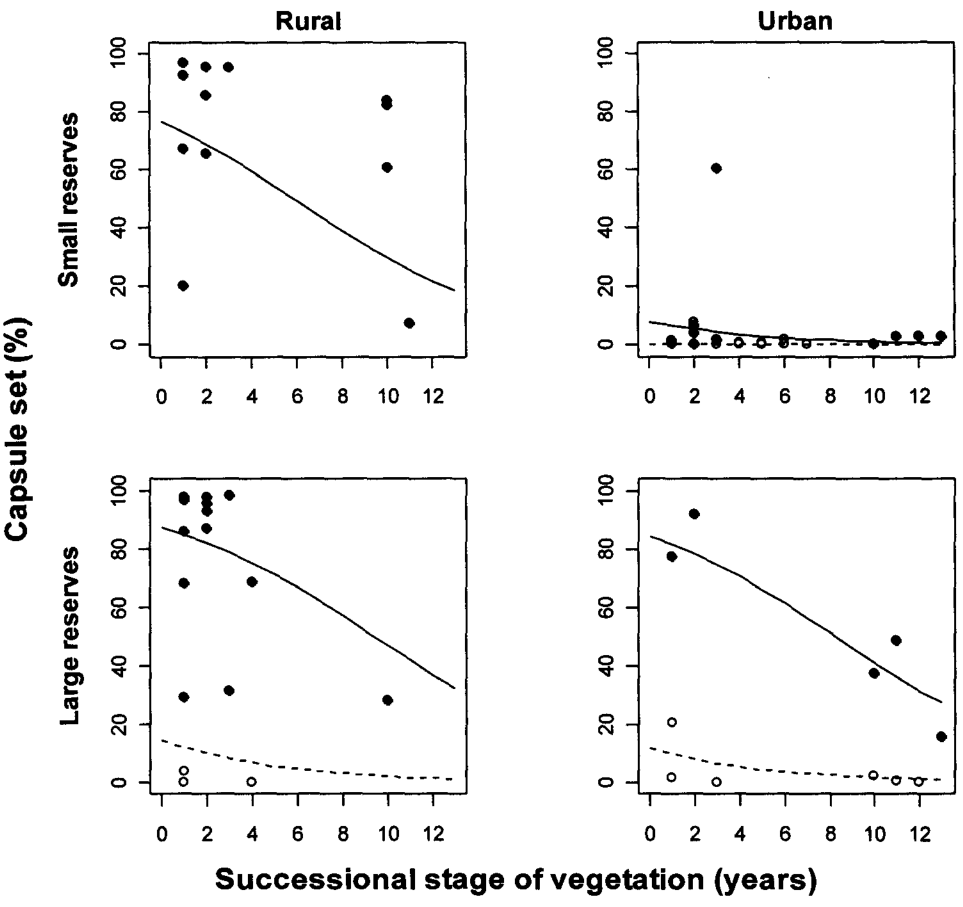

Fig. 6.4 Capsule set in *Pterygodium catholicum* in relation to the interaction between four explanatory variables. Open markers are sites located on sandy soils; filled markers are sites located on clay soils. Lines are predictions generated by a generalized linear mixed model (Table 6.1). Dashed lines indicate predictions for sandy sites; solid lines indicate predictions for clay sites.

Table 6.1 Significance tests for effect of reserve characteristics on seed set in *Pterygodium catholicum* by means of a generalized linear mixed model.

Explanatory variable*	Value†	S.E.	DF	t-value	p-value
Intercept	1.93	0.46	32	4.24	0.0002
SOIL (sand; clay)	-3.72	1.00	22	-3.71	0.0012
YEARS SINCE FIRE	-0.21	0.08	32	-2.50	0.0176
MATRIX (urban; rural)	-0.24	0.77	22	-0.31	0.7569
SIZE (small; large)	-0.73	0.59	22	-1.24	0.2285
MATRIX * SIZE	-3.42	1.23	22	-2.78	0.0108

*Underlined variables are in the intercept

†Values are on the logit scale

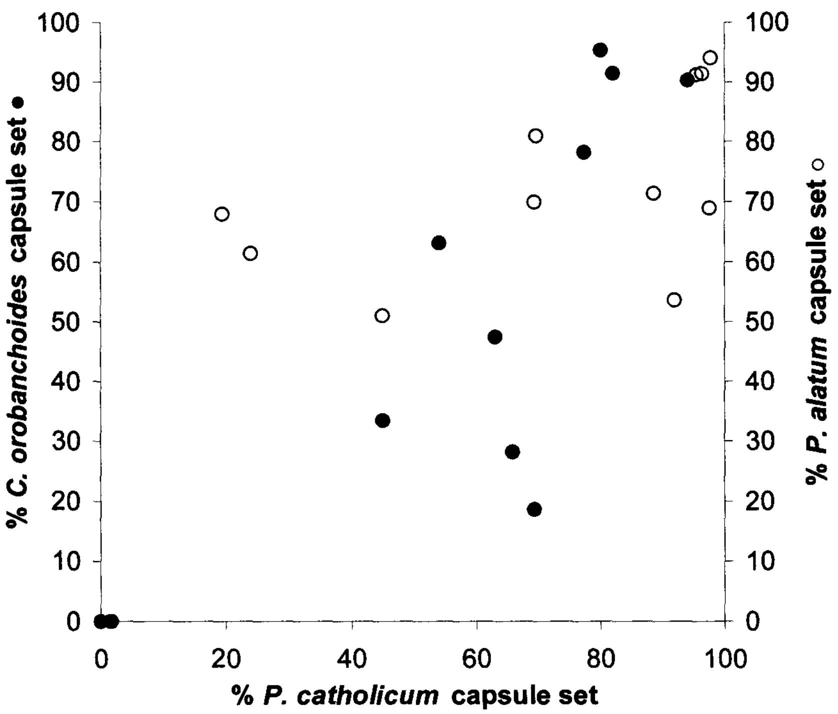

Fig. 6.5 Relationship between capsule set in three orchids which co-occur across a range of sites. Seed capsule formation in all three species is dependent on pollination by the oil-collecting bee *Rediviva peringueyi*. Filled markers, *Corycium orobanchoides*; open markers *Pterygodium alatum*.

Extrapolation of results to other members of the pollination guild

Capsule set in *Corycium orobanchoides* varied among sites from 0 % to 95 % (median 47 %). Across 12 sites, the pattern of spatial variation in capsule set in *C. orobanchoides* matched the pattern of variability in capsule set in *P. catholicum* ($r = 0.904, p < 0.001$)(Fig. 6.5).

Capsule set in *Pterygodium alatum* varied among sites from 51 % to 94 % (median 70 %). At sites where *P. catholicum* experienced very low levels of capsule set, *P. alatum* was absent. Capsule set in *P. alatum* was weakly correlated with capsule set in *P. catholicum* across the range of sites where the two species occurred together ($r = 0.56, N = 11, p = 0.076$)(Fig. 6.5).

Discussion

Proximate determinants of capsule set in P. catholicum

The percentage of *Pterygodium catholicum* flowers that produced seed capsules varied among sites from near 100% to zero. There are three common proximate sources of variation in seed set in plants: 1) variation in the availability of growth resources (e.g. Corbet, 1998; Zimmerman & Pyke, 1988), 2) variation in the availability of genetic resources (Charpentier et al., 2000; Dorken & Eckert, 2001; Warburton et al., 2000; Wilcock & Jennings, 1999), and 3) variation in pollination rate (e.g. Rathcke, 2000; Steiner & Whitehead, 1996). In the case of *P. catholicum* correlative and experimental data lend support to the latter explanation.

Variation in two measures of pollination rate (the percentage of stigmas that have received pollen and the percentage of pollinaria that have been removed) explained almost all of the observed variation in capsule set in *P. catholicum*, suggesting that factors other than pollination played a relatively small role (Fig. 6.2).

The existence of a causal relationship between pollination and capsule set is confirmed by experimental results. Supplementation of natural pollination with hand pollination, increased capsule set at a site with low pollination rate from zero to 78 %. This result suggests that capsule set failure in *P. catholicum* does not result from a lack of resources for growth (e.g. water and nutrients). Locally available pollen was used in the pollen-supplementation experiment, suggesting that sufficient genetic resources (good quality pollen) existed locally. In fact, in Chapter 5 I presented results which suggest that *P. catholicum* is self-compatible. At a site with high pollination rate, the exclusion of pollinators with gauze bags reduced capsule production from 98 % to 0%. This result demonstrates the importance of pollinators in ensuring high levels of capsule set. The role of *Rediviva peringueyi*, in particular, is evident from pollinator censuses. At sites where *R. peringueyi* was absent, capsule set was low; where *R. peringueyi* occurred, capsule set was high (Fig. 6.3).

In summary, capsule set in *P. catholicum* is a direct reflection of the pollination activity of *R. peringueyi*. Capsule set fails due to low levels of pollination by *R. peringueyi*. High levels of capsule set indicate high levels of pollination by this oil-collecting bee.

Natural and anthropogenic sources of variation in capsule set

Natural features of the landscape were the best predictors of the pattern of variation in sexual reproductive success (capsule set) in *P. catholicum*. Capsule set decreased significantly with increasing post-burn age of the vegetation and was very low or zero on sandy soils under all circumstances (Table 6.1, Fig. 6.4).

Anthropogenic impacts on pollination were detectable against this uneven background. The size of the conservation area and the surrounding landuse (matrix) interacted to produce a significant effect (Table 6.1). Capsule set was significantly lower in small nature reserves if these were located in an urban matrix (Fig. 6.4). This result suggests that anthropogenic impacts on pollination are occurring at the scale of proclaimed conservation areas, and that many additional conserved areas may become threatened as landuse in surrounding areas intensifies towards monoculture or urbanization. In this study, Signal Hill (385 ha) is the largest urban conservation area, located on clay soil, in which apparent anthropogenic pollination collapse was detected.

What determines the observed patterns of variation in pollination by R. peringueyi?

This study does not identify the mechanisms by which the dependent variables (soil type; successional stage; reserve size and matrix) act on pollination rate in *P. catholicum.* However, some likely scenarios can be suggested.

In small populations, and in areas with low densities of flowering plants, pollination rates may be low because isolated plants are less likely to be discovered by pollinators, small populations are less attractive to pollinators and low densities of plants increase the cost of foraging (e.g. Agren, 1996; Fritz & Nilsson, 1994; Groom, 1998; Lamont et al., 1993; Mustajarvi et al., 2001). Successional stage is a major determinant of variation in population size and density in the *R. peringueyi* pollination community. Flowering peaks in the first Spring after a fire and declines dramatically in subsequent years. Thus, the decline in pollination observed in late successional vegetation (Fig. 6.4 & Table 6.1) is likely to result from a decline in flower density and number, which in turn is likely to affect bee abundance (Steffan-Dewenter & Tscharntke, 2001). Soil type may act similarly. Sandy soils at the Cape have lower nutrient status than clay soils and support a lower abundance and diversity of oil-secreting plant species in the *R. peringueyi* guild. The abundance of oil sources drops to particularly low levels in late successional vegetation on sandy soil.

The independent variables used in this study may act directly on the abundance of *R. peringueyi* rather than through the agency of plant density. *R. peringueyi* abundance in sandy area may be limited by the availability of nesting sites in unconsolidated sand and by lower temperatures, which prevail in sandy areas because these occur either in close proximity to the sea or at high altitude. Similar factors have elsewhere been found to play an important role in determining local patterns of bee abundance (Corbet et al., 1993; Gess & Gess, 1993; Gess, 1996, 2002; Liow et al., 2001; Saure, 1996; Vinson et al., 1993; Westrich, 1996). Direct effects on bee abundance might explain the lack of pollination in large populations of *P. catholicum* on sandy soil. For example, pollination rate was very low in early successional vegetation at Houhoek and Chapman's Peak despite the large size of the conservation areas and estimated population sizes were 3000 and 5000 flowering ramets respectively.

Land-use practices in surrounding areas may impact on pollination in conservation areas by determining the permeability of the matrix to the movement of pollinators. Extensive, nectar-less areas may sever migration corridors between conservation areas, while a biodiverse rural matrix may not present a significant barrier to the movement of *R. peringueyi* (Marlin & LaBerge, 2001; McIntyre & Hostetler, 2001). These anthropogenic habitat fragmentation effects seems to be the most plausible explanation for the absence of *R. peringueyi* from early post-burn vegetation in small urban reserves such as Signal Hill and in the De Villiers Road Green Belt, despite estimated population sizes of 700 and 800 flowering ramets of *P. catholicum*.

Extrapolation to other members of the R. peringueyi *pollination guild*

P. catholicum is one member of a guild of 14 orchid species which apparently depend only on *R. peringueyi* for pollination throughout all, or most, of their range (Chapter 5). The existence of a strong correlation between variation in capsule set in *P. catholicum* and variation in capsule set in another member of the guild, *Corycium orobanchoides*, suggests that a common underlying cause (i.e. the pattern of variation in *R. peringueyi* abundance across the landscape) determines sexual reproductive success in both species (Fig. 6.5).

In another member of the guild, *Pterygodium alatum* capsule set was high in all monitored populations (Fig. 6.5). At sites where *P. catholicum* and *C. orobanchoides* experienced low levels of capsule set, *P. alatum* was absent. This result begs questions regarding the factors which determine the relative ability of the members of the *R. peringueyi* pollination guild to persists in the absence of pollination. In Chapter 8 I attempt to address this question.

Anthropogenic impacts on oil-collecting bees compared with scent-collecting bees

The *Rediviva* pollination system finds comparison in the better known euglossine bee pollination system of the Neotropics. The males of three genera of euglossine bees (Apidae: Euglossini) are the pollinators of 35 genera of orchids from which they collect floral fragrances with brushes on their front legs (Williams, 1982). The fragrances are probably used for attracting mates. Approximately half of the orchids in the euglossine pollination system are pollinated by only one bee species, and half are pollinated by 2 to 12 species (Williams, 1982). The euglossine system has the advantage that males are readily attracted to artificial scent baits or unmanned traps containing scent baits. Compared with female *Rediviva* bees, which collect oil as a larval food, male euglossine bees collect small volumes of orchid secretions and visit relatively few flowers.

Janzen's (1970) observations on the importance of euglossine bees as long-distance pollen vectors, in part led him to hypothesize that anthropogenic landscape transformation may threaten biodiversity through the "insidious" loss of plant-pollinator interactions (Janzen, 1974). These predictions were borne out by a study conducted at the famous artificial forest fragmentation study near Manaus (Powell & Powell, 1987). In this experiment, 1 ha , 10 ha and 100 ha forest patches were isolated from continuous forest by about 100 m of clearcut. For some, but not all, euglossine species visitation rates at artificial baits declined after fragmentation and was correlated with fragment size. Whether these declines were sufficient to affect pollination remains an open question. Five years later fragmentation effects on euglossine abundance could be detected only in the 1 ha fragments (Becker et al., 1991). By this stage forest in the clearcut areas had partially regenerated.

The picture of euglossine bees as long-distance pollinators, which are little affected by small-scale habitat fragmentation is supported by later studies. *Eulaema cingulata* readily crossed 100 to 500 m of open water to effect equal pollination on small islands in the Panama canal (Murren, 2002). In the highly fragmented Atlantic forests of Brazil euglossine bee community composition did not differ between bait stations set in mature forest, disturbed forest and fragments (200, 156 & 14 ha), which were isolated from mainlands by about 1 km of pasture. Subsequent studies with marked bees confirmed regular long-distance movement (1.7 km)(Tonhasca et al., 2003) resulting in a homogeneous pollinator landscape (Tonhasca et al., 2002), quite different from that observed in this study.

Conclusion

This study showed that considerable variability in seed capsule set in a guild of oil-secreting orchids was determined by variable abundance of a single pollinating bee species. The presence of the pollinator, and therefore of seed capsules, varied depending on soil type and the successional stage of the vegetation. Once this natural variation has been accounted for, there remained a clear signal of pollination failure due to pollinator loss in small conservation areas surrounded by human-altered landscape.

Appendix 6.1 Variation among sites in capsule set in *Pterygodium catholicum* in relation to soil type, size of the conservation area, matrix in which it is located and sample year.

Soil type	Size	Matrix	Site	Year	Capsule set	*N* (flowers)
clay	large	rural	Bobejaanberg	1998	0.82	28
			Loufontein	1999	0.69	85
			Paarlberg	2000	0.95	220
				2001	0.83	122
			Romansrivier	1998	0.98	799
				1999	0.94	85
				2000	0.36	54
				2001	0.68	186
			Darling Hills	2000	0.98	341
				2001	0.98	192
			Teewaterskloofdam	1999	0.89	116
			Voëlvleidam	2001	0.29	316
		urban	Tygerberg	1998	0.45	854
				1999	0.48	189
				2001	0.52	431
	small	rural	16km S of Tulbagh	2000	0.66	23
				2001	0.08	82
			Darling Renosterveld Reserve	1998	0.80	17
				1999	0.96	443
				2000	0.86	28
			Joostenberg	1999	0.92	269
				2000	0.94	126
				2001	0.90	44
			Malmesbury	2000	0.19	401
				2001	0.63	251
			Tulbagh	2000	0.70	92
			Uitkamp Wetland	2001	0.77	31
		urban	Brackenfell	1999	0.54	25
			DeVilliers Rd Durbanville	2000	0.00	390
			Durbanville Nature Reserve	1998	0.00	61
				1999	0.03	112
				2000	0.03	72
				2001	0.02	144
			Duthie Nature Reserve	2001	0.05	284
			Signal Hill	1998	0.03	654
				1999	0.00	79
				2000	0.02	126
sand	large	rural	Houhoek	2001	0.00	254
			Red Hill	2000	0.03	104
				2001	0.00	54
		urban	Chapman's Peak	1998	0.24	258
				2000	0.00	61
			Pipe track	1998	0.02	50
				1999	0.01	151
				2001	0.00	25
			Suikerbossie	2000	0.01	75
	small	urban	Durbanville Racecourse	2000	0.07	269
				2001	0.02	184
			Milnerton Racecourse	1998	0.00	67
				1999	0.00	13
				2000	0.01	122
				2001	0.02	58
			Rondebosch Common	1998	0.01	131
				1999	0.00	122
				2000	0.00	223
				2001	0.00	108

7 Reconstruction of a historical pollination landscape confirms the disruption of pollination in small conservation areas

Widespread reports of low pollination rates suggest a recent anthropogenic decline in pollination that could threaten natural and agricultural ecosystems. Nevertheless, unequivocal evidence for a decline in pollination has remained elusive because it was not possible to determine historical pollination rates. Here we reconstruct a historical pollination landscape by detecting the genetic echo of past pollination, and by determining historical pollination rates from rehydrated herbarium specimens. The results suggest that substantial spatial variation in pollination rates existed historically in relation to soil type differences. However, a mismatch between the reconstructed and the contemporary pattern of spatial variation in pollination, identifies recent anthropogenic impacts which overlie the natural patterns of variability: plant-pollinator mutualisms have recently collapsed in some small conservation areas.

There is concern that widespread reports of low pollination rates might represent a recent decline in pollination caused by diverse anthropogenic impacts on pollination (Allen-Wardell et al., 1998; Buchmann & Nabhan, 1996; Cane & Tepedino, 2001; Kearns et al., 1998; Kremen & Ricketts, 2000; Renner, 1998). However, the lack of historical, base-line data on pollination rates has hampered the assessment of the magnitude of human impact on pollination (Roubik, 2001; Thomson, 2001).

In small scale studies, seed set or pollination rate data from less impacted areas has been used as a baseline for comparison with nearby impacted areas (Aizen & Feinsinger, 1994; Donaldson et al., 2002; Jennersten, 1988; Murren, 2002; Robertson et al., 1999). However, at a larger geographical scale, which is more meaningful for conservation planning, between-area comparisons of pollination rate are likely to be confounded by the natural pattern of spatial variation in pollination (Campbell, 1987; Dorken & Eckert, 2001; Johnson & Bond, 1992; Lipow et al., 2002; Steiner & Whitehead, 1996).

In Chapter 6 I presented evidence of low pollination rates in a number of small conservation areas in the Cape Town Metropolitan Area. Do these low pollination rates represent a recent anthropogenic decline, or a pre-existing condition related to some underlying natural cause which prevails in the Cape Town area?

To identify recent declines in pollination we reconstruct the historical pattern of spatial variation in pollination strength by detecting the genetic echo of past pollination. At one site we test molecular evidence by determining historical pollination rates from rehydrated herbarium specimens.

Methods

Study species

The orchid, *Pterygodium catholicum*, is a common member of a large guild of oil-secreting plants that flower at the Cape from August to September and are specialized for pollination by female oil-collecting bees belonging to the species *Rediviva peringueyi* (Chapter 5). The pollen packets (pollinaria) of *P. catholicum* become attached to the distal half of the basitarsus of the middle legs, while the bee collects oil from the flower with its front legs (Chapter 5). After pollination, the flower produces a capsule filled with microscopic, wind-dispersed seeds. Seed production and hence sexual reproduction is dependent on pollination (Chapter 6).

In addition to sexual reproduction via seeds, *P. catholicum* is capable of asexual, vegetative reproduction. At the end of the growing season, daughter tubers are produced at the tips of ephemeral stolonoid roots which measure ~9.5 cm in length (Chapter 5). Daughter tubers produce their first leaf at the onset of the next growing season. The production of daughter tubers is likely to occur primarily during the favourable growing conditions that prevail during the first few years after a fire.

Study sites

The study area was the highly fragmented lowlands of the Cape of Africa, as defined in Chapter 6. Within the 12 500 km^2 study area, we selected ten proclaimed conservation areas for detailed study (Fig. 7.1). These ten sites are a subset of the 27 sites used in the Chapter 6. The ten conservation areas were selected to reflect a range of sizes, matrix characteristics [urban; rural] and soil types [clay; sand] (Table 7.1).

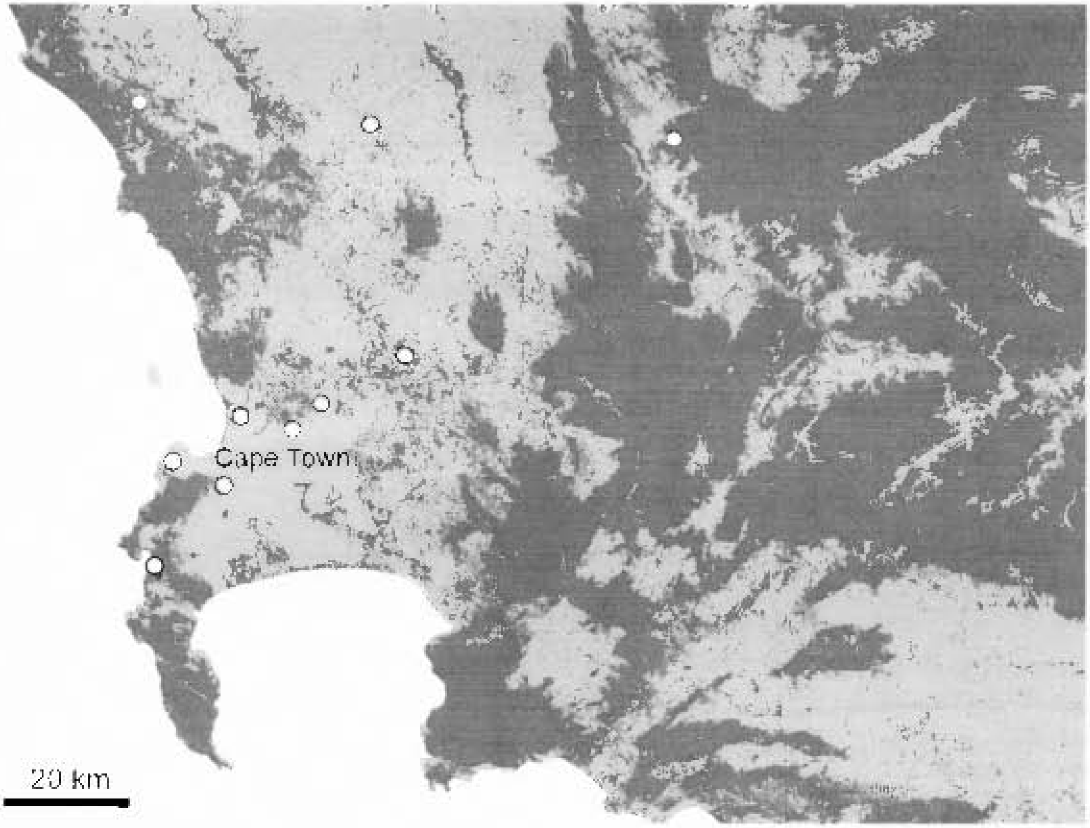

Fig. 7.1 Location of study sites at the Cape of Africa. Dark gray indicates areas of natural vegetation; light gray indicate areas which have been transformed by urbanization or agriculture.

Contemporary patterns of variation in sexual reproduction

We studied the patterns of spatial and temporal variation in capsule set in *P. catholicum* among the ten conservation areas by returning to marked plants after flowering. The number of sample years at each site ranged from 2 to 4 (median 3). Samples consisted of a median of 40 plants (range 12-238). In total, capsule production was recorded for 6 433 flowers on 1 760 plants. We recorded capsule set, rather than seed set, because seeds are minute and numerous and are released from the capsules soon after maturation. Swollen capsules were opened and checked under magnification for the presence of filled seeds.

Variation in genetic diversity across the landscape

We used DNA fingerprinting (ISSR) to determine the level of genetic diversity of *P. catholicum* populations in the ten conservation areas. At each site nine ramets were sampled from 9 x 9 m plots so that each ramet was 4.5 m from its nearest neighbour. By sampling at the 4.5 m scale we avoided sampling recent clonal reproduction, because daughter tubers are produced at a distance of about 10 cm from the parent plant (Chapter 5). Genetic diversity was calculated as the mean Euclidean distance between the DNA fingerprints of the ramets sampled at each site.

DNA fingerprints (genotype-specific banding patterns) were obtained by extracting DNA from silica-dried leaf material using the DNeasyTM Plant Mini Kit (Qiagen Ltd., UK). PCR reactions comprised 20 ng of template DNA and 1 u of taq polymerase (Roche Molecular Biochemicals) in 20 µl of 1.5 mM $MgCl_2$, 1 x PCR buffer, and 4 mM dNTPs with 6 µM of each primer. Thirty cycles of 1 min at 94°C; 2 min at primer annealing temperature; 30 s at 72°C were followed by a 5 min extension at 72°C. Annealing was performed 2°C–5°C above the calculated melting temperature, Tm °C = (4 x GC) + (2 x AT). PCR products were separated using pre-cast polyacrylamide gels (Clean Gel 48S, Pharmacia Biotech) and the Multiphor II (Pharmacia Biotech) flatbed system. Products were visualised using silver staining. Every sample was screened using five primers showing high levels of polymorphism amenable to scoring by eye: [GA]8-C, [CA]8-G, [AC]8-YG, CT-[GA]8-GTG, and GG-[CA]8-ATC. For each population all major (intense) bands were scored. Additional minor bands were only scored if it was possible to assess the band position for all samples within a population. Bands were scored as present (1) or absent (0), and both polymorphic and monomorphic amplicons were scored. Reproducibility was demonstrated by repeat PCRs using two of the primers for two independent extractions of one sample.

Herbarium pollination study

Herbarium records offer a potential tool for exploring historical trends in pollination rates. Ideally, one would like an area from which plants have been sampled repeatedly for many years. One site, Signal Hill, served the purpose.

In order to determined contemporary pollination rates on Signal Hill we collected wilted inflorescences at the end of the flowering season and preserved them in 96% ethanol. We examined the lower-most flower for the presence of pollinaria using a 40x magnification dissecting microscope. Either one or two empty, but undamaged, pollinarium sacs, indicated a pollinator visit. Occasional pollinaria removed by pollen-feeding insects can be identified (and excluded), because herbivorous insects cause damage to the membranous pollinarium sac and typically eat the pollen, but not the viscidium (pollinarium attachment disc) or caudicle (arm). Pollination rates were determined for 43 plants in 1998 and for 27 plants in 2001.

Contemporary pollination rates were compared with historical pollination rates determined from herbarium specimens collected on Signal Hill between 1883 and 1939. After 1940, Cape Town expanded rapidly and all potential habitats for *R. peringueyi* in areas near Signal Hill were converted to urban areas. We removed the oldest (lowermost) flower from pressed plants, avoiding those bearing many buds. The flowers were rehydrated in warm water containing Tween 20 wetting agent, microscopically examined as describe above, re-dried and replaced. The pollinaria of *P. catholicum* are very unlikely to be dislodged during the collecting or pressing of specimens, because the viscidia can only be accessed by inserting a fine object (e.g. tarsus of *Rediviva* bee or dissecting needle) into the narrow groove between the lip appendage and the rostellum arm (Chapter 5). In total, 22 herbarium specimens were examined from the following Herbaria: K, BM, BOL, NBG, SAM, GUTHRIE (Appendix 7.1).

Results and discussion

Contemporary pattern of variation in sexual reproduction

The level of seed capsule production, and hence sexual reproduction, varied among the 10 conservation areas from zero to 98%. The rate of capsule production was high in five rural reserves (four large and one small); in contrast, capsule production was zero or near zero in four small urban reserves (Table 7.1). For each of these four urban reserves, fertility was zero in at least one sample year. Capsule production was also low on Chapman's Peak, a large conservation area located on sandy soil. Temporal variation in capsule production across the four sample years was small relative to spatial variation (Table 7.1).

The historical pollination landscape

The failure of pollination is cause for concern if it represents a change from historical conditions attributable to anthropogenic effects. However, for many plant species, pollination failure is a normal condition, resulting not from recent, anthropogenic environmental degradation, but instead from a variety of pre-existing natural causes, which prevail in parts, or in all, of the distribution range (Campbell, 1987; Dorken & Eckert, 2001; Eriksson, 1996; Johnson & Bond, 1992; Wilcock & Neiland, 2002). To distinguish between pre-existing natural variability in pollination and recent anthropogenic influences we reconstructed the historical pollination landscape. As a first approach, we used patterns of genetic diversity as an indication of past pollination.

Mode of reproduction has a strong influence on genetic diversity. Reliance on clonal vegetative reproduction as a means of persistence in the absence of sexual reproduction results in lowered genetic diversity (Cook, 1983; Eckert & Barrett, 1993; Harper, 1977; Williams, 1975). In contrast, when there is sexual reproduction, genetic diversity is maintained (Eckert & Barrett, 1993; Harper, 1977; Williams, 1975). Thus, stable spatial patterns of variation in the ratio of sexual to asexual reproduction will be reflected in the distribution of genetic diversity across the landscape. Areas of high pollinator abundance and frequent sexual reproduction will be characterized by high genetic diversity; areas of natural pollinator scarcity will have low genetic diversity.

Table 7.1 Sexual reproductive success of *Pterygodium catholicum* in 10 nature reserves.

Reserve Name	Size (ha)*	Surrounding landscape[†]	% of flowers producing seed capsules ± SE	N (sample years)
Rondebosch Common	39	Urban	0.2 ± 0.2	4[‡]
Milnerton Racecourse	42	Urban	0.6 ± 0.4	4
Signal Hill	385	Urban	1.6 ± 0.9	3[‡]
Durbanville Reserve	6	Urban	1.9 ± 0.7	4
Chapman's Peak	5055	Urban and sea	13.2 ± 10.7	2[‡]
Malmesbury	29	Rural	41.2 ± 21.8	2[‡]
Tygerberg Reserve	2579	Rural and urban	52.2 ± 2.5	3
Hexrivier	>100,000	Rural	74.4 ± 14.0	4[‡]
Joostenberg Reserve	2602	Rural	92.0 ± 1.2	3[‡]
Darling Hills	>100,000	Rural	97.8 ± 0.2	2[‡]

* Reserve sizes were determined from digitised satellite images and include all untransformed land contiguous with the formally proclaimed conservation area.

[†] Reserves were in an urban setting (within metropolitan Cape Town) or a rural setting (surrounded by ploughed fields).

‡ Indicates sites burnt during the sample period. Fires are a source of temporal variation in capsule production because flowering in *P. catholicum* is stimulated by fire (Chapter 5 & 6).

This genetic imprint will be slow to respond to recent perturbations if genetic individuals are long lived. For example, when pollinators are lost from populations of long-lived plants, and sexual reproduction ceases, genetic diversity will start to decline; but, a legacy of high genetic diversity will remain for many years (Ellstrand & Roose, 1987). We used this property of genetic inertia in *P. catholicum* populations to reconstruct the historical *R. peringueyi* pollination landscape.

We found that genetic diversity in *P. catholicum* differed greatly among the ten conservation areas, suggesting a historical landscape that included areas of high pollination and sexual reproduction, as well as areas with less frequent sexual reproduction. The distribution pattern of genetic diversity appeared to match stable, underlying features of the landscape, which have the potential to influence the natural patterns of abundance of *R. peringueyi*. Three sites with low genetic diversity occurred on sandy soils, while seven sites with high genetic diversity occurred on clay soils (Fig. 7.2).

Comparison of the historical and contemporary pattern of spatial variation in reproductive success

Comparison of the distribution of genetic diversity (an indication of historical levels of pollination) with current levels of sexual reproduction allows the identification of recent declines in pollination (Fig. 7.2). Currently low levels of pollination at Durbanville Nature Reserve and Signal Hill (a portion of the Cape Peninsula National Park) can not account for the high levels of genetic diversity which exist at these sites.

The mismatch between genetic diversity and current levels of sexual reproduction suggests a recent decline in the abundance of *R. peringueyi* in these two small urban reserves (6 ha and 385 ha respectively). In contrast, the currently low level of pollination in three conservation areas located in sandy areas do not indicate a recent decline, because the low levels of genetic diversity at these sites suggest a long history of rare sexual reproduction (Fig. 7.2).

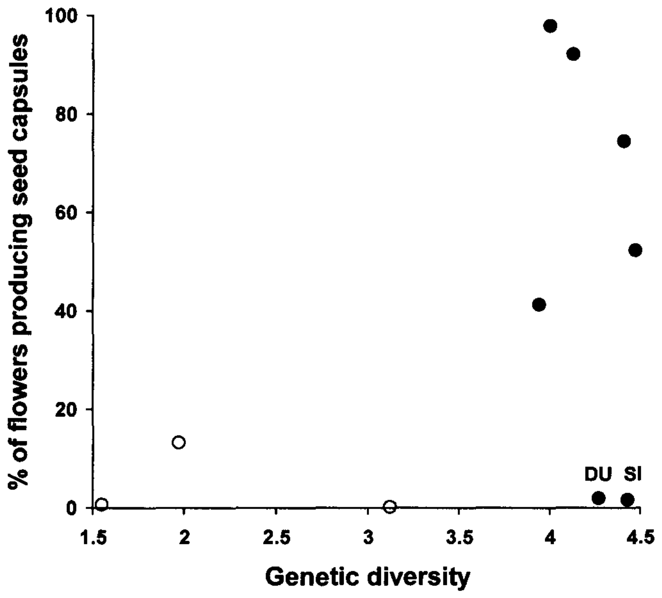

Fig. 7.2 Relationship between genetic diversity (a reflection of historical pollination rates) and seed capsule production (a reflection of contemporary pollination rates) among ten conservation areas. Open markers indicate conservation areas located on sand; filled markers are conservation areas on clay. Low rates of sexual reproduction in sandy areas are in keeping with low levels of genetic diversity in sandy areas, suggesting that pollinator scarcity on sand represents a long-term stable condition. High levels of genetic diversity on clay suggests historically high pollination rates in these areas, which have recently declined to low levels in two small, urban nature reserves, Durbanville (DU) and Signal Hill (SI). Genetic diversity is the mean Euclidean distance between ISSR fingerprints of ramets sampled at the site (Appendix 2). The percentage of flowers producing seed capsules is the mean over a 2-4 year period.

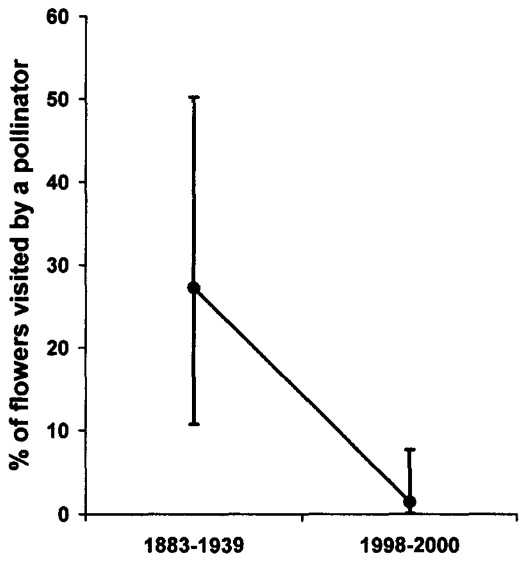

Fig. 7.3 Change in pollination rate over time. Herbarium specimens of the orchid, *P. catholicum*, collected at Signal Hill, preserve a historical record of pollination that is much higher than contemporary pollination rates at the same site. The contemporary rate falls outside the 95% limits (bars) of the binomially distributed historical visitation rate, indicating that the change over time is statistically significant.

Confirmation of recent pollinator decline using herbarium specimens

The historical record of pollination preserved in old herbarium specimens confirms a recent decline in pollination on Signal Hill, a high priority conservation area (Cowling et al., 1996a; Joubert & Moll, 1992; Pauw & Johnson, 1999). The absence of one or both pollinaria from herbarium specimens of *P. catholicum* indicates a pollinator visit. The estimate of historical pollination rates obtained in this way is conservative, because collectors choose the freshest flowers from a population, whereas wilted flowers are more likely to have been visited. Nevertheless, herbarium specimens from Signal Hill preserve a historical record of pollination rates that are significantly higher than those observed today (Fig. 7.3). Higher pollination rates in the past are consistent with the high levels of genetic diversity at the site at present.

In conclusion, reproductive failure in areas of sandy soil is probably an ancient phenomenon caused by the naturally low abundance of *R. peringueyi* in these areas. Reproductive failure in small, urban conservation areas on clay soil indicates a recent anthropogenic decline in pollination.

Appendix 7.1 List of *Pterygodium catholicum* specimens used in the herbarium pollination study.

Herbarium	Collector	Sheet number	Year
Kew	F. Wilms	3658	1883
British Museum	F. Wilms	3658	1883
British Museum	R. Schlechter	1339	1892
British Museum	R. Schlechter	1339	1892
Kew	R. Schlechter	1339	1892
Kew	R. Schlechter	1339	1892
Kew	R. Schlechter	1339	1892
Kew	R. Schlechter	1339	1892
British Museum	A. H. Wooley	501	1895
Bolus	A. H. Wooley	501	1895
Bolus	McCloughlin	29	1911
Bolus	McCloughlin	29	1911
Guthrie	E. Kensit	888	1913
Bolus	M. H. Giffen	69668	1923
Bolus	M. H. Giffen	69668	1923
Compton	P. Bond	113	1938
Compton	W. F. Barker	354	1938
Compton	W. F. Barker	354	1938
Compton	W. F. Barker	354	1938
Compton	P. Bond	113	1939
Compton	P. Bond	113	1939
Compton	P. Bond	113	1939

8 Pollination matters: pollinators determine community composition and drive floral evolution in oil-secreting plants

Pollination rate in a community of oil-secreting plant species was found to be associated with the kinds of plant species present in the community. Species composition followed a predictable sequence. Species with the highest demographic dependence on pollination occurred only where the pollination rate was high. Pollination "hotspots" and areas of high community diversity occurred on clay soils; pollination "cold-spots" and low community diversity occurred on sandy soils and in small, urban conservation areas in a highly transformed matrix. Species with low demographic dependence on pollination were found to compensate for the absence of a specialist pollinator (*Rediviva peringueyi)* by relying on alternative pollinators, vegetative reproduction and self-pollination. Repeated patterns of infraspecific morphological variation suggest an alternative to extinction: the evolution of floral traits that decrease dependence on *R. peringueyi.*

The worldwide decline of pollinators has highlighted the importance of determining the role of mutualistic relationships in organizing biological communities (Kearns et al., 1998). While several studies report lower pollination rates in areas of anthropogenic disturbance, few consider the ultimate consequence of plant reproductive failure: extinction or evolution.

In Chapters 6 & 7 I presented data which suggests that the oil-collecting bee *Rediviva peringueyi* is absent from small conservation areas located in highly transformed landscapes. Does the loss of this pollinator matter to the community of oil-secreting plants? Bond (1994) has suggested that the future risk of plant extinction due to pollination failure can be assessed by considering three plant features: 1) degree of dependence on pollinators for seed set, 2) degree of pollinator specificity and 3) degree of dependence on seeds for population persistence. These considerations can be combined in an index of vulnerability (*VI*) by taking products as follows:

$VI = BS \times PS \times SD$

Here BS stands for breeding system, PS for pollinator specificity and SD for demographic dependence on seeds. Plant species are ranked for each term from 0 to 1. For example, an autogamous, wind-pollinated annual with no seed bank has a $VI = 0 \times 0 \times 1 = 0$. In contrast, a dioecious plant with one specialized pollinator and a lifespan of several years has a $VI = 1 \times 1 \times 0.2 = 0.2$.

Bond's vulnerability index can be phrased as a hypothesis about the role of pollinator dependence, pollinator specificity and seed dependence in determining plant population responses to pollinator loss. Are species with high indices of vulnerability absent from the oil-secreting plant communities in areas of low *R. peringueyi* abundance? If the answer is yes, the implication is that the oil-collecting bee plays a role in shaping the plant community and that the anthropogenic loss of this pollinator has triggered plant extinction.

The alternative to plant extinction following pollinator loss is persistence by reliance on self pollination, alternative pollinators, vegetative reproduction or individual longevity. What is the long-term future of these populations? Johnson (1996) suggests that selection for more efficient pollination systems in a pollinator-limited context, is the primary driving force behind floral evolution in the Cape flora. In the long-term, does the loss of *R. peringueyi* result in the evolution of new floral features which promote alternative means of pollination?

Methods

Study sites and species

I selected 35 areas of natural vegetation within a 13 000 km^2 study area located in the Cape Lowlands. Most of the selected areas are proclaimed nature reserves aimed at the conservation of the endemic lowland flora. The study species are oil-secreting plants which are known to be visited by the oil-collecting bee *R. peringueyi*. This community is described in Chapter 4 and Chapter 5. The presence of at least one flowering member of the *R. peringueyi* pollination community[5] was the criterion for the selection of sites.

Assessing vulnerability to the loss of *R. peringueyi*

Bond's vulnerability index ($VI = BS \times PS \times SD$)(Bond, 1994) was calculated to assess the potential impact of pollinator loss on the members of the *R. peringueyi* pollination community. The index terms (*BS*, *PS* & SD) were estimated on the basis of field studies (Chapter 4 & Chapter 5). I used 1 – (pollinator excluded capsule set/open pollination capsule set) as a measure of reproductive dependence on pollinators [*BS*], and 1/(number of pollinators) as a measure of pollinator specificity [*PS*]. Demographic dependence on seeds [*SD*] was calculated as 1/maximum age of individuals. The age in an individual includes time spent as a dormant seed. In clonal Orchidaceae the maximum age of individuals was roughly estimated by dividing the radius of the largest clonal colony by the yearly growth increment (distance between parent and daughter tuber). As a measure of community vulnerability [*CV*], I used the vulnerability index [*VI*] of the most vulnerable species in the community.

Geographical variation in the composition of the *R. peringueyi* community

At each of the 35 sites the composition of the *R. peringueyi* pollination community was determined by thorough searching. Species were identified according to Linder and Kurzweil (1999) and Goldblatt and Manning (2000). The median number of sample years at each site was 2 (range 1-4). Almost all sites could be censused in an early post-burn stage when the members of the *R. peringueyi* pollination community flower most prolifically.

Pollinator abundance and the vulnerability of the plant community

To test the prediction that vulnerable species will be absent from the community of oil-secreting plants in areas of *R. peringueyi* poverty, I related community vulnerability indices [*CV*] to capsule set in *P. catholicum*. Capsule set in *P. catholicum*, is an indication of *R. peringueyi* abundance and community wide

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[5] The "*R. peringueyi* pollination community" includes plant species, such as *Hemimeris racemosa*, which have more than one pollinator. In this respect the "*R. peringueyi* pollination community" differs from the less inclusive "*R. peringueyi* pollination guild" as defined in Chapter 5.

pollination levels (Chapter 6).

P. catholicum was a common member of the *R. peringueyi* pollination community at 26 of the 35 sites. At these sites, capsule set was determined by returning to marked plants after flowering. The median number of sample years at each was 2 (range 1-4). The median sample size was 122 flowers (range, 13-799). In total, capsule production was recorded for 9 754 flowers on 2 702 plants. Swollen capsules were opened and checked under magnification for the presence of filled seeds.

As a second approach, I directly related natural and anthropogenic features of conservation areas to community vulnerability indices. In Chapter 6, significant determinants of pollinator abundance in conservation areas were found to include soil type [clay; sand], size of the conservation area [large; small] and the matrix [urban; rural] in which it is located. In this study, sites were classified using these same criteria.

Patterns of infraspecific morphological variation in the *R. peringueyi* pollination guild

I studied infraspecific variation in floral features of the study species to test the prediction that pollinator deprived populations of the *R. peringueyi* pollination guild will exhibit floral features that decrease demographic dependence on *R. peringueyi*. Flowering plants were described, measured, photographed, and pressed. The flowering time of each population was noted. Infraspecific patterns of variation in floral morphology and flowering time were related to the distribution pattern of *R. peringueyi*.

Results

Assessing vulnerability to the loss of *R. peringueyi*

The plant species in the *R. peringueyi* pollination community varied greatly in their predicted vulnerability to pollinator loss (Table 8.1). At one extreme are the autogamous Scrophulariaceae in which seed set is independent of cross-pollination ($VI = 0$). At the other extreme is *Pterygodium inversum*, which is pollinated only by

Table 8.1 Vulnerability indices and their derivation for the members of the *R. peringueyi* pollination community.

Species	Breeding system [*BS*]	Pollinator specificity [*PS*]	Seed dependence [*SD*]	Vulnerability Index [*VI* = *BS* x *PS* x *SD*]
Hemimeris racemosa (monomorphic)	0	0.20	0.17	0
Pterygodium connivens	0	1	0.01	0
Diascia elongata	0	0.17	0.17	0
Hemimeris sabulosa	0	0.20	0.17	0
Diascia diffusa	0	0.17	0.17	0
Pterygodium catholicum	1	1	0.01	0.01
Pterygodium cruciferum	1	1	0.01	0.01
Corycium orobanchoides	1	1	0.02	0.02
Disperis villosa	1	1	0.02	0.02
Hemimeris racemosa (dimorphic)	0.80	0.20	0.17	0.03
Pterygodium alatum	1	1	0.04	0.04
Disperis capensis (cream form)	1	1	0.07	0.07
Pterygodium cafrum	1	1	0.07	0.07
Pterygodium volucris	1	1	0.07	0.07
Disperis bolusiana	1	1	0.08	0.08
Disperis circumflexa	1	1	0.10	0.10
Disperis cucullatum	1	1	0.10	0.10
Pterygodium inversum	1	1	0.17	0.17

Table 8.2 Average and maximum community vulnerability indices and community diversity in relation to soil-type, size of conservation area and matrix in which it is located. The *R. peringueyi* community was surveyed at a total of 35 sites.

Soil	Size classification	Matrix	Avg. (Max.) no. of plant species in *R. peringueyi* community	Avg. (Max.) community vulnerability index	No. of sites surveyed
clay	large	rural	4.71 (7)	0.10 (0.17)	7
		urban	7.00 (7)	0.17 (0.17)	1
	small	rural	3.57 (6)	0.04 (0.07)	7
		urban	2.40 (4)	0.02 (0.03)	5
sand	large	rural	2.00 (3)	0.01 (0.03)	6
		urban	2.00 (3)	0.02 (0.04)	5
	small	urban	2.75 (4)	0.03 (0.07)	4

R. peringueyi, requires pollination for seed set, and is totally dependent on seeds for population growth and short term persistence (*VI* = 0.17).

Geographical variation in the composition of the *R. peringueyi* pollination community

The number of species in the *R. peringueyi* pollination community varied across 35 study sites from a maximum of seven species to a minimum of one species. These communities varied from invulnerable to pollinator loss (*CV* = 0) to vulnerable (*CV* = 0.17).

Pollinator loss and plant community change

The decline in the number of plant species present in the *R. peringueyi* pollination community paralleled decreasing levels of capsule set in *Pterygodium catholicum* (Fig. 8.1). Species with vulnerable life histories were absent from areas with low capsule set (Fig. 8.2). Rich, communities, which included vulnerable species, occurred in rural conservation areas if these were located on clay substrates. Depauperate communities, made up of invulnerable species, occurred on sandy substrates and in small, urban conservation areas on clay substrates (Table 8.2; Appendix 8.1). Small conservation areas in a rural setting contained significantly more vulnerable species than similar-sized conservation areas in an urban setting (Rural median $CV = 0.04$; $N = 7$; Urban median $CV = 0.02$; $N = 5$; Mann-Whitney U-test, $Z = 2.089, p = 0.037$).

Patterns of infraspecific morphological variation in the *R. peringueyi* pollination guild

Four of the study species exhibited pronounced intra-specific variation in floral features across the clay/sand soil-type boundary. In each of these cases, populations on sandy soil exhibited floral features that can be interpreted as adaptations which lower demographic dependence on *R. peringueyi* (Table 8.3).

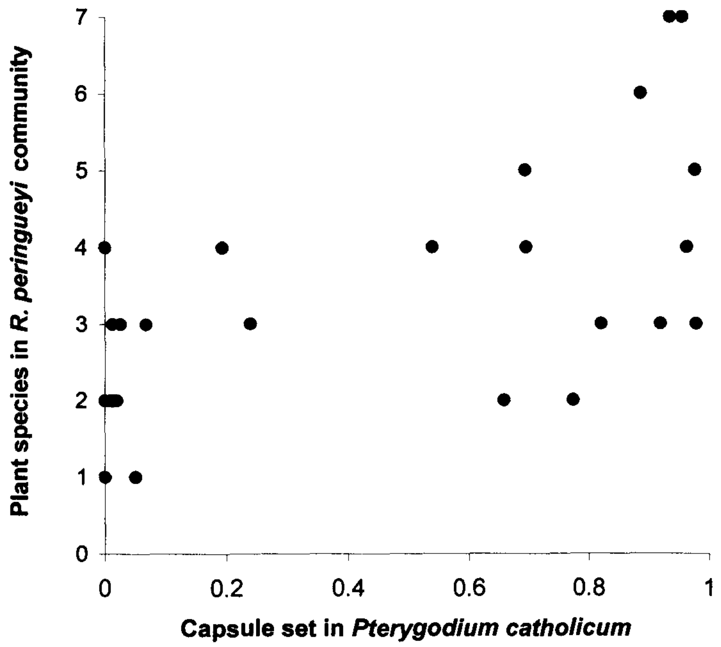

Fig. 8.1 Relationship between the number of oil-secreting plant species in the *R. peringueyi* pollination community and capsule set (proportion of flowers producing seed capsules) in *Pterygodium catholicum*, an oil-secreting orchid which is pollinated by *R. peringueyi* (Spearman $r = 0.62$, $N = 26$, $p = 0.0007$).

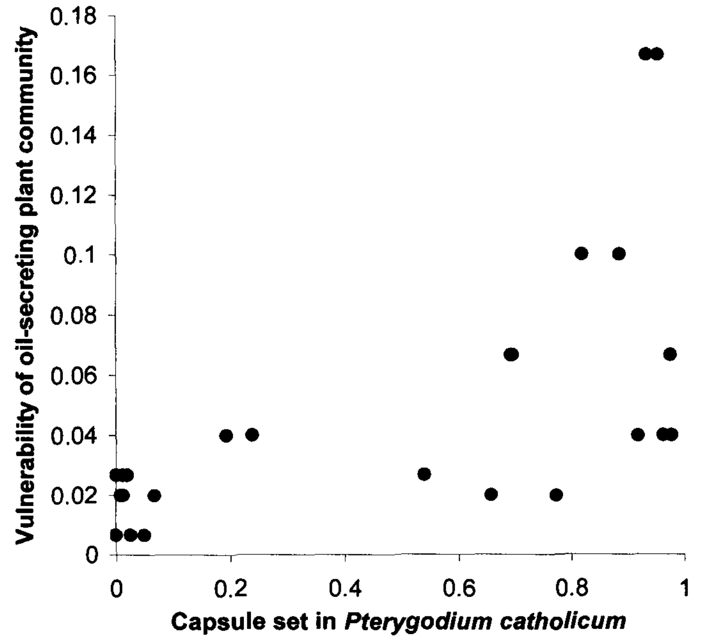

Fig. 8.2 Relationship between the community vulnerability index (*CV*) and capsule set (proportion of flowers producing seed capsules) in *Pterygodium catholicum*, an oil-secreting orchid which is pollinated by *R. peringueyi* (Spearman $r = 0.75$, $N = 26$, $p < 0.0001$).

Discussion

Pollinator abundance and plant community composition

The abundance of *R. peringueyi* and the composition of the *R. peringueyi* pollination community were found to vary in unison across the landscape. Depauperate communities of oil-secreting plants occurred where pollination rates were low; diverse communities coincided with pollination "hotspots" (Fig. 8.1). Bond's vulnerability index successfully predicted plant community composition. Depauperate plant communities were not a random subset of the oil-secreting community. Instead, they were composed of species that possessed vegetative or floral features that reduced demographic dependence on pollination by *R. peringueyi* (Table 8.1; Fig. 8.2).

Depauperate communities in pollination "cold-spots" are apparently reliant on features which compensate for the absence of *R. peringueyi.* The ubiquitous *Pterygodium catholicum*, for example, has low demographic dependence on seeds [*SD*], because it is capable of clonal vegetative reproduction by the formation of extra bulbils on root tips (Chapter 5). Some clones, formed in this way, measured more than 10 m in diameter. In long-term stable *R. peringueyi* pollination "cold-spots" the rate of sexual reproduction was very low or zero. In these pollination "cold-spots" populations were found to have lower levels of genetic diversity, suggesting greater dependence on clonal vegetative reproduction (Chapter 7).

Hemimeris racemosa (Scrophulariaceae), was the second most ubiquitous species recorded in this study. In this annual species, reproductive compensation occurs through relaxed pollinator specificity [*PS*]. Pollinator censuses conducted at flowering populations of the typical form of *H. racemosa* revealed the absence of *Rediviva peringueyi* from 9 of 16 conservation areas. At 7 of these 9 *R. peringueyi* pollination "cold-spots", smaller *Rediviva* species (*R. bicava, R. intermixta* and *R. parva*) still occurred, and were providing pollination services to *H. racemosa* (Chapter 4). At only two *H. racemosa* sites did the *Rediviva* pollination system fail entirely. Bright coloration and accessible rewards are factors that contribute to the relative lack of pollinator specificity in this species.

Table 8.3 Infraspecific floral variation in relation to soil-type in species belonging to the *R. peringueyi* pollination guild.

Species	Soil	Floral features	Flowering	Probable pollinator
Pterygodium catholicum	Clay	pale yellowish-green; acrid scent; synanthus	September	*R. peringueyi*
	Sand	bright lemon yellow; sweet scent; hysteranthus*	November	*R. gigas*
Pterygodium cafrum	Clay	pale yellowish-green	September	*R. peringueyi*
	Sand	bright lemon-yellow	November	*R. gigas*
Hemimeris racemosa	Clay	stigma and anthers separated; many large flowers	September	*Rediviva spp.*
	Sand	stigma and anthers in contact; few, small flowers	August	*autonomous self-pollination*
Disperis capensis	Clay	cream; oil-secreting (?)	September	*R. peringueyi*
	Sand	purple; no reward; modeled on *Polygala bracteolata*	August	*Xylocopa spp.* †

*This distinct form has not been included under the name *P. catholicum* in this thesis and is always referred to as "the late-flowering form of *P. catholicum*". Not all *P. catholicum* populations on sandy substrates are of this type.

†Johnson (1994a)

A capacity for reproductive compensation in breeding systems [*BS*] further reduces vulnerability in *H. racemosa* (Table 8.1). Despite the complete absence of *R. peringueyi* at two populations of the typical form of *H. racemosa*, capsule set did not decline below 26 % (Chapter 4). A similar level of capsule set was observed at some sites when pollinators were excluded with gauze bags, suggesting that a large proportion of capsule set in the absence of *Rediviva spp.* was attributable to autogamous self-pollination.

In summary, the *R. peringueyi* pollination community in pollination "cold-spots" consists only of invulnerable species [low *VI*]. Some evidence suggests that, persistence in these areas is dependent on mechanisms which compensate for the absence of *R. peringueyi.*

Landscape features in relation to pollination and community composition

Pollination "hotspots", and areas of high community vulnerability and diversity, occurred on clay soil. Pollination "cold-spots", and depauperate, invulnerable communities of oil-secreting plants occurred on sandy substrates (Table 8.2; Appendix 8.1). What is the mechanism that relates soil type to pollination and plant community composition?

Soil type commonly limits plant distribution at the Cape (Cowling & Lamont, 1998; Goldblatt & Manning, 2002; Linder, 1985). Differences in the growth environment may exclude a subset of the *R. peringueyi* pollination community from sandy areas, resulting in less diverse communities and lower population densities. However, the observation that communities in sandy areas were composed of species with low pollination vulnerability (Fig. 8.2), can not be explained by differences in the growth environment. This pattern suggests that plant species composition reflects the spatial pattern of variation in the pollination environment rather than variation in the growth environment. Possible mechanisms by which soil type determines the pollination environment have been discussed in Chapter 5.

Anthropogenic impacts on pollinator abundance and plant community composition were detectable against the uneven background of naturally occurring spatial variation. Pollination "cold-spots" and depauperate plant communities, composed only of species that are demographically invulnerable to pollinator loss, occurred in small, urban nature reserves on clay substrates (Table 8.2; Appendix 8.1). This result suggests the anthropogenic extirpation of pollination dependent plant species from the plant community in established conservation areas.

The finding that variation in the abundance of *R. peringueyi* determined the species composition of the community of oil-secreting plants is surprising in the context of the current understanding of the demographic importance of pollination. Pollination mutualisms are generally considered to be unimportant in determining plant population growth rates, and hence plant distributions, because pollination often does not enhance seed set, and an increase in seed set seldom results in population growth (Addicott, 1986). To the best of my knowledge, only one study has demonstrated that pollination is important for plant population persistence. In small meadows isolated by juniper shrubs, experimentally introduced populations of a grassland herb experienced low population viability due to inbreeding depression and reduced seed production, both caused by pollinator deficit (Lennartsson, 2002).

Density dependent mortality due to the lack of sufficient suitable establishment sites is a common reason for the lack of a strong relationship between pollination and population growth (Crawley, 1990; Harper, 1977). In orchid populations in particular, the availability of suitable microhabitats, rather than the availability of seeds, is thought to limit population growth (Calvo, 1993; Dixon, 1991; Kull, 1998). Consider that a single orchid capsule releases thousands of wind-dispersed seeds (e.g. Willems & Melser, 1998). Calvo (1993) has hypothesized that a weak link between seed set and recruitment explains the apparent persistence of orchid populations despite characteristically low capsule set (Ackerman & Montalvo, 1990; Calvo & Horvitz, 1990; Johnson & Bond, 1997; Neiland & Wilcock, 1998; Primack & Hall, 1990; Willems & Melser, 1998; Zimmerman & Aide, 1989).

While low pollination rates are the normal condition in many orchid species, the magnitude of spatial variation in capsule set (89% to 0 %) encountered in this study, is unusual, and possibly important in explaining the result obtained here. Also important is the observation that seed set was zero in successive years at some sites. The complete failure of seed set will lead to population decline, even if recruitment is normally density dependent, limited by available sites or by processes which act on other life history stages. Invulnerability to complete seed set failure can only be guaranteed by individual longevity.

Evolutionary consequences of pollinator loss

Stable spatial patterns of natural variation in pollinator occurrence provide an opportunity to examine the long-term consequences of pollinator loss. In Chapter 7 I found that populations of *P. catholicum* in long-term stable pollination "cold-spots" (sandy areas) had low levels of genetic diversity, which seem likely to result from the prevalence of clonal vegetative reproduction and the lack of sexual reproduction. What is the long-term future of these populations?

Table 8.3 presents a preliminary summary of the patterns of infraspecific floral variation in four of the members of the *R. peringueyi* pollination guild. I interpret these patterns as representing three different evolutionary solutions to the lack of *R. peringueyi* in sandy areas. Firstly, the loss of herkogamy rescues populations of *H. racemosa* in sandy areas, by allowing autonomous self-pollination and thus freeing these populations from demographic dependence on *R. peringueyi* (Chapter 4). Secondly, pollinator shift from *R. peringueyi* to *R. gigas*, the only oil-collecting bee which occurs in the mountainous, sandy area of the Cape, is an alternative solution. *R. gigas* is active during Nov/Dec, when it collects oil from several bright-yellow-flowered plant species (Manning & Goldblatt, 2002; Steiner, 1998; Steiner & Whitehead, 1996; Whitehead & Steiner, 1993, 2001). The bright, yellow flowers and Nov/Dec flowering time of some populations of *P. catholicum* and *P. cafrum* in sandy areas, suggest local adaptation to pollination by *R. gigas*. The similarity of these species to other members of the *R. gigas* pollination guild is particularly striking when they flower interspersed. Thirdly, pollination by deceit of carpenter bees (*Xylocopa*), is associated with the loss of oil-secretion in the purple-flowered form of *Disperis capensis*, which occurs commonly in sandy areas at the Cape (Johnson, 1994a; Pauw & Johnson, 1999). Flowering peaks from late-July to early-August, which is when novice carpenter bees emerge. The purple form of *D. capensis* does not flower in association with any other oil-secreting plants. In contrast, on clay soil, a cream-coloured form of *D. capensis* flowers in September in association with the members of the *R. peringueyi* pollination guild. In the examples discussed here, ancestral populations may have occurred on either soil type.

Population differentiation becomes speciation when populations become sufficiently distinct to warrant taxonomic recognition. Thus, the cleistogamous and presumably autogamous *P. connivens* has been described as a separate species, sister to *P. cruciferum* (Linder & Kurzweil, 1999). *P. connivens* is known from one population, which occurs on sandy soil at Cape Point. *P. cruciferum* occurs on clay soils and is probably pollinated by *R. peringueyi* (Chapter 5). Similarly, the sand-loving *Hemimeris sabulosa* differs from the clay-loving *H. racemosa* in lacking stylar polymorphism and herkogamy. This pattern of differentiation at the species level repeats the pattern of differentiation observed at the infraspecific level within *H. racemosa* (Chapter 4).

Speciation across the pollination environment boundary (Goldblatt et al., 2000; Goldblatt et al., 1995, 1998, 2001; Johnson, 1996; Johnson et al., 1998; Johnson & Steiner, 1997; Manning & Linder, 1992; Rebelo et al., 1985; Steiner, 1998) and speciation across the growth environment boundary (Cowling & Holmes, 1992; Cowling & Lamont, 1998; Cowling & Lombard, 2002; Cowling et al., 1996b; Cowling et al., 1990; Cowling et al., 1994; Goldblatt & Manning, 2002; Linder, 1985) are often presented as alternative explanations for the extraordinary species richness at the Cape. These two proposed mechanism may operate in unison where pollination boundaries coincide with soil-type (or growth environment) boundaries. Pollinator shifts may facilitate rapid vegetative adaptation to novel growth environments by allowing genetic isolation of adjacent populations.

Conclusion

On the basis of the results presented in this chapter, I suggest that pollination matters in a community of oil-secreting plants. The species composition of the community was correlated with the abundance of the pollinator, an oil-collecting bee. The loss of this pollinator due to anthropogenic habitat fragmentation may have led to the extirpation of a vulnerable subset of species, which have high demographic dependence on pollination by the oil-collecting bee. In the long-term, patterns of geographical variation in the abundance of the oil-collecting bee appear to drive floral evolution in the community of oil-secreting plants.

Appendix 8.1 Community vulnerability indices and community diversity at 35 sites in relation to soil-type, size of conservation area and matrix in which it is located.

Soil	Size	Matrix	Site	Community vulnerability index [*CV*]	No. of plant species in *R. peringueyi* community
			[illegible]	[illegible]	[illegible]
			Loufontein	0.07	5
			Paarl	0.17	7
			Romansrivier	0.04	3
			Smallpad	0.07	5
			Teewaterskloofdam	0.10	6
		urban	Tygerberg	0.17	7
	small	rural	16km S of Tulbagh	0.02	2
			Contreberg	0.04	6
			Darling Renosterveld Reserve	0.04	4
			Joostenberg	0.04	3
			Malmesbury burn site	0.04	4
			Tulbagh	0.07	4
			Uitkamp Wetland	0.02	2
		urban	Brackenfell	0.03	4
			DeVilliers Rd Durbanville	0.01	1
			Durbanville Nature Reserve	0.03	4
			Duthie Nature Reserve	0.01	1
			Signal Hill	0.02	2
sand	large	rural	Cape Point	0.03	3
			Churchhaven	0.02	2
			Greyton	0.00	1
			Houhoek	0.01	1
			Red Hill	0.01	3
			Rondeberg	0.02	2
		urban	Chapman's Peak (Houtbaai)	0.04	3
			Chapman's Peak (Monkey valley)	0.03	1
			Glencairn	0.00	1
			Pipe track	0.03	2
			Suikerbossie	0.03	3
	small	urban	Durbanville Racecourse	0.02	3
			Milnerton Racecourse	0.01	2
			Rondebosch Common	0.02	2
			Rondevlei	0.07	4

Conclusion: How pollination webs unravel

Pollination communities

In this thesis I have described two pollination communities: the sunbird pollination community, of which *Brunsvigia orientalis* is a typical member; and the oil-collecting bee pollination community, of which *Pterygodium catholicum* is a typical member. Each community consists of a group of plants which share a single pollinator, or a small group of related pollinators. Specialized floral traits and narrow flower preferences together define the limits of these communities. The pollinators (*Nectarinia* sunbirds and *Rediviva* bees) maintain the integrity of the community by acting as mobile links (sensu Gilbert, 1981).

Natural patterns of variation in pollination

I found that the natural pollination landscape was a mosaic of "hotspots" and "cold-spots". "Hotspots" of sunbird pollination occurred in the mesic south-east. Here, *Brunsvigia orientalis* was a member of a large pollination community which included many sunbird-pollinated plant species and two or three sunbird species. Cold-spots of sunbird pollination occurred in the dry north-west. Here only the smallest sunbird species was found to occur and *B. orientalis* flowers provided the only nectar for sunbirds. "Hotspots" of oil-collecting bee pollination, occurred on relatively nutrient-rich clay soils. Here, *Pterygodium catholicum* is a member of a diverse community of synchronously flowering oil-secreting plants. The abundance of *R. peringueyi* is high, as are pollination rates and the rate of sexual reproduction via seed in all the plants in the community. Pollination "cold-spots" were found to occur in nutrient-poor, sandy areas. Here *P. catholicum* occurred in isolation or in association with a small subset of the *R. peringueyi* pollination community. In these reduced communities, pollination rate and the rate of sexual reproduction via seed was low or zero.

Sexless and seedless plant populations in pollination "cold-spots" may have originated from, and may be maintained by, seed dispersal from pollination "hotspots". In this scenario, the high dispersability of the Orchidaceae is an important factor in setting up patterns of spatial variability in pollination, by allowing frequent dispersal beyond the limits of pollinator distribution.

Patterns of short-term temporal variation of large amplitude were found to overlie the stable spatial patterns of variation in pollination described above. Ten-year cyclical fluctuations in pollination were linked to the cycle of post-fire succession. In early successional vegetation pollination rates were low in the sunbird pollination community and high in the oil-collecting bee pollination community. In late successional vegetation the reverse situation occurred.

Anthropogenic impacts

Anthropogenic impacts on pollination could be detected against this uneven background of spatial and temporal variation. Pollination in the sunbird pollination community and the oil-collecting bee pollination community failed or was significantly depressed in small conservation areas located in a highly transformed matrix. True declines only occurred where anthropogenic impacts (e.g. habitat fragmentation) coincided with natural pollination hotspots. Thus, the anthropogenic effect is to increase the number of pollination "cold-spots" by reducing "hotspots" to "cold-spots".

Anthropogenic "cold-spots" superficially resemble natural "cold-spots", and this makes it difficult to identify anthropogenic declines in pollination. For example, both anthropogenic and natural "cold-spots" lack large bodied pollinators (*Nectarinia famosa* and *Rediviva peringueyi*), while smaller members of the same genera were still present. Anthropogenic cold-spots, however, differ from natural cold-spots in being recent.

Plant species in both natural and anthropogenic pollination cold-spots were not a random subset of the pollination community. Instead, they were composed of species that possessed vegetative or floral traits that reduced vulnerability to pollinator loss. These traits included relatively generalized pollination systems, a capacity for self-pollination and the ability to reproduce vegetatively. The occurrence of only invulnerable species in anthropogenic "cold-spots" suggests the recent extirpation of pollination dependent plant species following pollinator loss.

Long-term consequences of pollinator loss

Natural pollination "cold-spots" provide insight of the potential long-term consequences of anthropogenic pollinator loss. For example, in *Pterygodium catholicum*, the occurrence of low levels of genetic variability in natural pollination cold-spots suggests that the loss of plant genetic diversity will be one consequence of the anthropogenic decline in pollinators, as plants become increasingly reliant on vegetative reproduction and self-pollination.

In the very long-term, evolution may ameliorate the effects of pollinator loss, if plants have sufficient time to evolve floral traits that decrease dependence on the absent pollinator. For example, where *B. orientalis* occurred in natural pollination cold-spots, smaller flowers reflected the loss of large sunbirds from the pollination community. Similarly, in natural "cold-spots" of oil-collecting bee pollination, populations of oil-secreting plant species displayed a range of novel floral features that apparently promote self-pollination, carpenter bee pollination or pollination by other species of oil-collecting bees. Thus, two processes, pollinator driven extinction and the evolution of decreased demographic dependence on specialized pollinators may together account for the rarity of plant species which are dependent on specialized pollinators for population persistence.

The loss of genetic diversity, and ultimately the evolution of novel floral features, are long-term consequences of pollinator loss which are not yet reflected in anthropogenic pollination "cold-spots". This property of genetic and morphological inertia can be used to identify recent human-caused declines in pollination by allowing anthropogenic pollination cold-spots to be distinguished from pre-existing natural pollination cold-spots. For example, the occurrence of high levels of genetic diversity in small, urban conservation areas indicated that oil-collecting bees have recently been lost from these areas. Similarly, the occurrence of large-flowered *Brunsvigia orientalis* plants in small conservation areas indicated that large sunbirds occurred there prior to the recent urbanization of the lowlands of the Cape.

A metaphor

The spatial pattern of variation in pollination systems across a human impacted landscape at the Cape can be compared with a woven fabric in which the threads are individuals belonging to different species. The weft of vulnerable species (large-bodied pollinators and plants with high demographic dependence on pollination) is woven through the warp of less vulnerable species. Adverse environmental conditions (a lack of nutrients, high fire frequencies, drought and habitat fragmentation) wear away the weft of vulnerable species to create threadbare holes, where only the stronger warp of invulnerable species remains. Warp threads are themselves worn thin by the gradual loss of genetic diversity. Where holes appear it is difficult to determine which thread breaks first, rather, there is a simultaneous loss of the integrity of the fabric. Stronger warp threads extend beyond the edges of the fabric to form a frayed margin. Here, some strands are picked up and woven into the fabric of adjacent pollination systems.

Conserving pollination

How can the loss of pollinators, and the plants which depend on them, be prevented? The results of this study provide a few guidelines. First, is the finding that unexpectedly large conservation areas are needed in order to conserve certain specialized plant-pollinator interactions. In the *Nectarinia* pollination community declines in pollination occurred if conservation areas were smaller that 1113 ha in size. In the *R. peringueyi* pollination community declines in pollination occurred in conservation areas smaller that 385 ha.

The observation that declines in pollination occurred in small conservation areas only if they were surrounded by areas of intensive land-use (urban areas), points to the importance of considering the relationship between connectivity and matrix characteristics in conservation planning. For example, relatively biodiverse agricultural landscapes are apparently permeable to the movement of oil-collecting bees, while extensively transformed landscapes (urban areas and maybe also monocultures) seem to act as a barrier to the movement of pollinators. Thus, the major future impact on biodiversity in the lowlands of the Cape, where virtually all arable areas have already been plowed, may be the loss of connectivity between unplowed remnants due to the intensification of agricultural practices in the matrix. The conservation of plant-pollinator interaction in proclaimed conservation areas may benefit from legislation aimed at maintaining biodiversity in surrounding agricultural areas.

The appropriate design of a system of conservation areas is the first step towards pollination conservation. However, natural patchiness in pollinator occurrence complicates conservation planning. For example, the proximity of Table Mountain (a large, sandy conservation area) does not ameliorate the fragmentation-related decline of *R. peringueyi* on Signal Hill (a small conservation area with clay soils), because this oil-collecting bee is naturally absent from sandy areas. These natural patterns of variability in pollinator abundance are difficult to determine directly. In this study, soil-type, the post-fire age of the vegetation and aridity gradients were found to be useful proxies of pollinator abundance. Such proxies may be incorporated into algorithms aimed at identifying priority areas for conservation.

In established conservation areas, management practices, in particular burning regimes, can have a major impact on pollination. For example, in this study, oil-collecting bees were found to be common in early post-burn successional vegetation; while sunbirds were common in mature vegetation. "Block burning" is the only way of catering simultaneously for such diverse requirements.

References

Ackerman, J.D. & Montalvo, A.M. (1990) Short- and long-term limitations to fruit production in a tropical orchid. *Ecology*, **71**, 263-272.

Addicott, J.F. (1986). On the population consequences of mutualism. In *Community Ecology* (eds D. J. & T.J. Case), pp. 425-436. Harper and Row, New York.

Agren, J. (1996) Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology*, **77**, 1779-1790.

Aizen, M.A. & Feinsinger, P. (1994) Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. *Ecology*, **75**, 330-351.

Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S., Cane, J., Cox, P.A., Dalton, V., Feinsinger, P., Ingram, M., Inouye, D., Jones, C.E., Kennedy, K., Kevan, P., Koopowitz, H., Medellin, R., Medellin-Morales, S., Nabhan, G.P., Pavlik, B., Tepedino, V., Torchio, P., & Walker, S. (1998) The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology*, **12**, 8-17.

Baker, H.G. (1955) Self-compatibility and establishment after "long-distance" dispersal. *Evolution*, **9**, 347-368.

Baker, H.G. (1966) The evolution, functioning and breakdown of heteromorphic incompatibility systems. I. The Plumbaginaceae. *Evolution*, **20**, 349-368.

Barrett, S.C.H. (1989) The dissolution of a complex genetic polymorphism: the evolution of self-fertilization in tristylous *Eichhornia paniculata* (Pontederiaceae). *Evolution*, **43**, 1398-1416.

Barrett, S.C.H., ed. (1992) *Evolution and function of heterostyly*. Springer Verlag, Berlin.

Barrett, S.C.H., Cole, W.W., Arroyo, J., Cruzan, M.B., & Lloyd, D.G. (1997) Sexual polymorphisms in *Narcissus triandrus* (Amaryllidaceae): Is this species tristylous? *Heredity*, **78**, 135-145.

Barrett, S.C.H. & Harder, L.D. (1996) Ecology and evolution of plant mating. *Trends in Ecology & Evolution*, **11**, A73-A79.

Barrett, S.C.H., Jesson, L.K., & Baker, A.M. (2000) The evolution and function of stylar polymorphisms in flowering plants. *Annals of Botany*, **85**, 253-265.

Barrett, S.C.H. & Shore, J.S. (1987) Variation and evolution of breeding systems in the *Turnera ulmifolia* L. complex (Turneraceae). *Evolution*, **41**, 340-354.

Bascompte, J., Jordano, P., Melian, C.J., & Olesen, J.M. (2003) The nested assembly of plant-animal mutualistic networks. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 9383-9387.

Bawa, K.S. (1990) Plant-pollinator interactions in tropical rain forests. *Annual Review of Ecology and Systematics*, **21**, 399-422.

Beattie, A.J. (1971) A technique for the study of insect-borne pollen. *Pan-Pacific Entomologist*, **47**, 82.

Becker, P., Moure, J.S., & Peralta, F.J.A. (1991) More About Euglossine Bees in Amazonian Forest Fragments. *Biotropica*, **23**, 586-591.

Bolten, A.B. & Feinsinger, P. (1978) Why do hummingbird flowers secrete dilute nectar. *Biotropica*, **10**, 307-309.

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

Bond, W.J. & Van Wilgen, B.W. (1996) *Fire and plants* Chapman & Hall, London.

Bruna, E.M. & Kress, W.J. (2002) Habitat fragmentation and the demographic structure of an Amazonian understory herb (*Heliconia acuminata*). *Conservation Biology*, **16**, 1256-1266.

Buchmann, S.L. (1987) The ecology of oil flowers and their bees. *Annual Review of Ecology and Systematics*, **18**, 343-369.

Buchmann, S.L. & Nabhan, G.P. (1996) *The Forgotten Pollinators* Island Press, Washington.

Calvo, R.N. (1993) Evolutionary demography of orchids - intensity and frequency of pollination and the cost of fruiting. *Ecology*, **74**, 1033-1042.

Calvo, R.N. & Horvitz, C.C. (1990) Pollinator limitation, cost of reproduction, and fitness in plants: a transition matrix demographic approach. *American Naturalist*, **136**, 499-516.

Campbell, D.R. (1987) Interpopulational variation in fruit production: the role of pollination-limitation in the Olympic Mountains. *American Journal of Botany*, **74**, 269-273.

Cane, J.H., Eickwort, G.C., Wesley, F.R., & Speilholz, J. (1983) Foraging, grooming and mate-seeking behaviors of *Macropis nuda* (Hymenoptera, Melittidae) and use of *Lysimachia* (Primulacaea) oils in larval provisions and cell linings. *American Midland Naturalist*, **110**, 257-264.

Cane, J.H. & Tepedino, V.J. (2001) Causes and extent of declines among native North American invertebrate pollinators: detection, evidence, and consequences. *Conservation Ecology*, **5**, 1.

Carpenter, F.L. (1983). Pollination energetics in avian communities: simple concepts and complex realities. In *Handbook of experimental pollination biology* (eds C.E. Jones & R.J. Little), pp. 215-234. Scientific and Academic Editions, New York.

Charlesworth, B. & Charlesworth, D. (1979) A model for the evolution of distyly. *American Naturalist*, **114**, 467-498.

Charpentier, A., Grillas, P., & Thompson, J.D. (2000) The effects of population size limitation on fecundity in mosaic populations of the clonal macrophyte *Scirpus maritimus* (Cyperaceae). *American Journal of Botany*, **87**, 502-507.

Cook, R.E. (1983) Clonal plant populations. *American Scientist*, **71**, 244-253.

Corbet, S.A. (1998) Fruit and seed production in relation to pollination and resources in bluebell, *Hyacinthoides non-scripta. Oecologia*, **114**, 349-360.

Corbet, S.A. (2000) Conserving compartments in pollination webs. *Conservation Biology*, **14**, 1229-1231.

Corbet, S.A., Fussell, M., Ake, R., Fraser, A., Gunson, C., Savage, A., & Smith, K. (1993) Temperature and the Pollinating Activity of Social Bees. *Ecological Entomology*, **18**, 17-30.

Cowling, R.M. & Holmes, P.M. (1992) Endemism and speciation in a lowland flora from the Cape Floristic Region. *Biological Journal of the Linnean Society*, **47**, 367-383.

Cowling, R.M. & Lamont, B.B. (1998) On the nature of Gondwanan species flocks: Diversity of Proteaceae in Mediterranean south-western Australia and South Africa. *Australian Journal of Botany*, **46**, 335-355.

Cowling, R.M. & Lombard, A.T. (2002) Heterogeneity, speciation/extinction history and climate: explaining regional plant diversity patterns in the Cape Floristic Region. *Diversity and Distributions*, **8**, 163-179.

Cowling, R.M., Macdonald, I.A.W., & Simmons, M.T. (1996a) The Cape Peninsula, South Africa: Physiographical, biological and historical background to an extraordinary hot-spot of biodiversity. *Biodiversity and Conservation*, **5**, 527-550.

Cowling, R.M., Rundel, P.W., Lamont, B.B., Arroyo, M.K., & Arianoutsou, M. (1996b) Plant diversity in Mediterranean-climate regions. *Trends in Ecology & Evolution*, **11**, 362-366.

Cowling, R.M., Straker, C.J., & Deignan, M.T. (1990) Does microsymbiont-host specificity determine plant species turnover and speciation in Gondwanan shrublands? A hypothesis. *South African Journal of Science*, **86**, 118-120.

Cowling, R.M., Witkowski, E.T.F., Milewski, A.V., & Newbey, K.R. (1994) Taxonomic, edaphic and biological aspects of narrow plant endemism on matched sites in mediterranean South-Africa and Australia. *Journal of Biogeography*, **21**, 651-664.

Cox, P.A. (1983) Extinction of the Hawaiian avifauna resulted in a change of pollinators for the ieie, *Freycinetia arborea*. *Oikos*, **41**, 195-199.

Crawley, M.J. (1990) The population dynamics of plants. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **330**, 125-140.

Cunningham, S.A. (2000a) Effects of habitat fragmentation on the reproductive ecology of four plant species in mallee woodland. *Conservation Biology*, **14**, 758-768.

Cunningham, S.A. (2000b) Depressed pollination in habitat fragments causes low fruit set. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **267**, 1149-1152.

Darwin, C. (1877) *The different forms of flowers on plants of the same species* Murray, London.

Debinski, D.M. & Holt, R.D. (2000) A survey and overview of habitat fragmentation experiments. *Conservation Biology*, **14**, 342-355.

Dixon, K. (1991). Seeder/clonal concepts in western Australian orchids. In *Population ecology of terrestrial orchids* (eds T.C.E. Wells & J.H. Willems), pp. 111-123. SPB Academic Publishing, The Hague.

Donaldson, J., Nanni, I., Zachariades, C., Kemper, J., & Thompson, J.D. (2002) Effects of habitat fragmentation on pollinator diversity and plant reproductive success in renosterveld shrublands of South Africa. *Conservation Biology*, **16**, 1267-1276.

Dorken, M.E. & Eckert, C.G. (2001) Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). *Journal of Ecology*, **89**, 339-350.

Dyer, R.A. (1950) A review of the genus *Brunsvigia*. *Plant Life*, **6**, 63-83.

Dyer, R.A. (1951) A review of the genus *Brunsvigia* Heist. *Plant Life*, **7**, 45-64.

Eckert, C.G. & Barrett, S.C.H. (1992) Stochastic loss of style morphs from populations of tristylous *Lythrum salicaria* and *Decodon verticillatus* (Lythraceae). *Evolution*, **46**, 1014-1029.

Eckert, C.G. & Barrett, S.C.H. (1993) Clonal reproduction and patterns of genotypic diversity in *Decodon verticillatus* (Lythraceae). *American Journal of Botany*, **80**, 1175-1182.

Ellstrand, N.C. & Roose, M.L. (1987) Patterns of genotypic diversity in clonal plant species. *American Journal of Botany*, **74**, 123-131.

England, P.R., Beynon, F., Ayre, D.J., & Whelan, R.J. (2001) A molecular genetic assessment of mating-system variation in a naturally bird-pollinated shrub: Contributions from birds and introduced honeybees. *Conservation Biology*, **15**, 1645-1655.

Eriksson, O. (1996) Regional dynamics of plants: a review of evidence for remnant, source-sink and metapopulations. *Oikos*, **77**, 248-258.

Ernst, A. (1955) Self-fertility in monomorphic primulas. *Genetica*, **27**, 91-148.

Faegri, K. & Pijl, L.v.d. (1979) *The principles of pollination ecology* Pergamon, Oxford.

Feinsinger, P. (1976) Organization of a tropical guild of nectarivorous birds. *Ecological Monographs*, **46**, 257-291.

Feinsinger, P., Wolfe, J.A., & Swarm, L.A. (1982) Island Ecology - Reduced hummingbird diversity and the pollination biology of plants, Trinidad and Tobago, West-Indies. *Ecology*, **63**, 494-506.

Fisher, R.A. (1941) The theoretical consequences of polyploid inheritance for the mid style form in *Lythrum salicaria*. *Annals of Eugenics*, **11**, 31-38.

Ford, H.A. (1981) Territorial behaviour in an Australian nectar-feeding bird. *Australian Journal of Ecology*, **6**, 131-134.

Frazer, M. & McMahon, L. (1994) *Between two Shores* David Philip, Cape Town.

Fritz, A.L. & Nilsson, L.A. (1994) How Pollinator-Mediated Mating Varies with Population-Size in Plants. *Oecologia*, **100**, 451-462.

Galen, C. (1996) Rates of floral evolution: Adaptation to bumblebee pollination in an alpine wildflower, *Polemonium viscosum*. *Evolution*, **50**, 120-125.

Gess, F.W. & Gess, S.K. (1993). Effects of increasing land utilization on species representation and diversity of aculeate wasps and bees in the semi-arid areas of southern Africa. In *Hymenoptera and Biodiversity* (eds J. LaSalle & I.D. Gauld), pp. 83-113. CAB International, Wallingford.

Gess, S.K. (1996) *The Pollen Wasps: ecology and natural history of the Masarinae* Harvard University Press, Cambridge (Mass.).

Gess, S.K. (2002) The bees' needs. *Veld & Flora*, **88**, 114-116.

Gibbs, D. (2003) The reestablishment of an extinct *Erica* (*Erica verticillata*) into the wild. In Fynbos Forum Conference Abstracts, pp. 22. NRF, Hartenbos.

Gilbert, L.E. (1981). Food web organization and the conservation of neotropical diversity. In *Conservation Biology: An Evolutionary Ecological Perspective* (eds M.E. Soule & B.A. Wilcox), pp. 11-33. Sinauer, Sunderland.

Goldblatt, P., Bernhardt, P., & Manning, J.C. (2000) Adaptive radiation of pollination mechanisms in *Ixia* (Iridaceae : Crocoideae). *Annals of the Missouri Botanical Garden*, **87**, 564-577.

Goldblatt, P. & Manning, J. (2000) *Cape Plants: a conspectus of the Cape Flora of South Africa* National Botanical Institute of South Africa and MBG Press, Cape Town.

Goldblatt, P. & Manning, J.C. (2002) Plant diversity of the Cape Region of southern Africa. *Annals of the Missouri Botanical Garden*, **89**, 281-302.

Goldblatt, P., Manning, J.C., & Bernhardt, P. (1995) Pollination Biology of *Lapeirousia* Subgenus *Lapeirousia* (Iridaceae) in Southern Africa - Floral Divergence and Adaptation for Long-Tongued Fly Pollination. *Annals of the Missouri Botanical Garden*, **82**, 517-534.

Goldblatt, P., Manning, J.C., & Bernhardt, P. (1998) Adaptive radiation of bee-pollinated *Gladiolus* species (Iridaceae) in southern Africa. *Annals of the Missouri Botanical Garden*, **85**, 492-517.

Goldblatt, P., Manning, J.C., & Bernhardt, P. (2001) Radiation of pollination systems in *Gladiolus* (Iridaceae : Crocoideae) in southern Africa. *Annals of the Missouri Botanical Garden*, **88**, 713-734.

Goldblatt, P., Savolainen, V., Porteous, O., Sostaric, I., Powell, M., Reeves, G., Manning, J.C., Barraclough, T.G., & Chase, M.W. (2002) Radiation in the Cape flora and the phylogeny of peacock irises *Moraea* (Iridaceae) based on four plastid DNA regions. *Molecular Phylogenetics and Evolution*, **25**, 341-360.

Grant, A.L. (1938) A monograph of the genus *Hemimeris*. *Annals of the Missouri Botanical Garden*, **25**, 435-453.

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

Groom, M.J. (1998) Allee effects limit population viability of an annual plant. *American Naturalist*, **151**, 487-496.

Hanks, K. (2003) Beyond boundaries: communities benefit from Cape Flats biodiversity. *African Wildlife*, **57**, 46-48.

Hansen, D.M., Olesen, J.M., & Jones, C.G. (2002) Trees, birds and bees in Mauritius: exploitative competition between introduced honey bees and endemic nectarivorous birds? *Journal of Biogeography*, **29**, 721-734.

Harper, J.L. (1977) *Population Biology of Plants* Academic Press, London.

Harrison, J.A., Allan, D.G., Underhill, L.G., Herremans, M., Tree, A.J., Parker, V., & Brown, C.J., eds. (1997a) *The atlas of southern African birds*. Vol. 2: Passerines. BirdLife South Africa, Johannesburg.

Harrison, J.A., Allan, D.G., Underhill, L.G., Herremans, M., Tree, A.J., Parker, V., & Brown, C.J., eds. (1997b) *The atlas of southern African birds*. Vol. 1: Non-passerines. BirdLife South Africa, Johannesburg.

Heijnis, C.E., Lombard, A.T., Cowling, R.M., & Desmet, P.G. (1999) Picking up the pieces: a biosphere reserve framework for a fragmented landscape - the coastal lowlands of the Western Cape, South Africa. *Biodiversity and Conservation*, **8**, 471-496.

Hobbs, R.J. & Yates, C.J. (2003) Impacts of ecosystem fragmentation on plant populations: generalising the idiosyncratic. *Australian Journal of Botany*, **51**, 471-488.

Huryn, V.M.B. (1997) Ecological impacts of introduced honey bees. *Quarterly Review of Biology*, **72**, 275-297.

Husband, B.C. & Barrett, S.C.H. (1992) Genetic Drift and the Maintenance of the Style Length Polymorphism in Tristylous Populations of Eichhornia-Paniculata (Pontederiaceae). *Heredity*, **69**, 440-449.

Inoue, K. (1993) Evolution of mutualism in plant pollinator interactions on islands. *Journal of Biosciences*, **18**, 525-536.

Janzen, D.H. (1970) Euglossine bees as long-distance pollinators of tropical plants. *Science*, **171**, 203-205.

Janzen, D.H. (1974) The deflowering of Central America. *Natural History*, **83**, 49-53.

Jennersten, O. (1988) Pollination in *Dianthus deltoides* (Caryophyllaceae) - effects of habitat fragmentation on visitation and seed set. *Conservation Biology*, **2**, 359-366.

Jesson, L.K. & Barrett, S.C.H. (2002a) Enantiostyly in *Wachendorfia* (Haemodoraceae): The influence of reproductive systems on the maintenance of the polymorphism. *American Journal of Botany*, **89**, 253-262.

Jesson, L.K. & Barrett, S.C.H. (2002b) Enantiostyly: Solving the puzzle of mirror-image flowers. *Nature*, **417**, 707-707.

Johnson, S.D. (1994a) Preliminary observation on the pollination of *Disperis capensis* (Orchidaceae). *South African Orchid Journal*, **25**.

Johnson, S.D. (1994b). Red flowers and butterfly pollination in the fynbos of South Africa. In *Plant-animal interactions in Mediterranean-type ecosystems* (eds M. Arianoutsou & R.H. Groves). Kluwer Academic Press, Dordrecht.

Johnson, S.D. (1996) Pollination, adaptation and speciation models in the Cape flora of South Africa. *Taxon*, **45**, 59-66.

Johnson, S.D. & Bond, W.J. (1992) Habitat dependent pollination success in a Cape orchid. *Oecologia*, **91**, 455-456.

Johnson, S.D. & Bond, W.J. (1997) Evidence for widespread pollen limitation of fruiting success in Cape wildflowers. *Oecologia*, **109**, 530-534.

Johnson, S.D., Linder, H.P., & Steiner, K.E. (1998) Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). *American Journal of Botany*, **85**, 402-411.

Johnson, S.D., Pauw, A., & Midgley, J. (2001) Rodent pollination in the African lily *Massonia depressa* (Hyacinthaceae). *American Journal of Botany*, **88**, 1768-1773.

Johnson, S.D. & Steiner, K.E. (1997) Long-tongued fly pollination and evolution of floral spur length in the *Disa draconis* complex (Orchidaceae). *Evolution*, **51**, 45-53.

Johnson, S.D. & Steiner, K.E. (2000) Generalization versus specialization in plant pollination systems. *Trends in Ecology & Evolution*, **15**, 140-143.

Joubert, C. & Moll, E.J. (1992) A phytosociological study of Signal Hill, Cape-Town, utilizing both perennial and ephemeral species. *Bothalia*, **22**, 255-282.

Kearns; C.A. & Inouye, D.S. (1997) Pollinators, flowering plants, and conservation biology - Much remains to be learned about pollinators and plants. *Bioscience*, **47**, 297-307.

Kearns, C.A., Inouye, D.W., & Waser, N.M. (1998) Endangered mutualisms: The conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics*, **29**, 83-112.

Keast, J.A. (1968) Seasonal movements in the Australian honeyeaters (Meliphagidae) and their ecological significance. *Emu*, **67**, 159-210.

Keighery, G.J. (1982). Bird pollinated plants in Western Australia and their breeding systems. In *Pollination and evolution* (eds J.A. Armstrong, A.H. Powell & A.J. Richards). Royal Botanic Gardens, Sidney.

Kelly, D., Ladley, J.J., Robertson, A.W., & Norton, D.A. (2000). Limited forest fragmentation improves reproduction in the declining New Zealand mistletoe *Peraxilla tetrapetala* (Loranthaceae). In *Genetics, demography and viability of fragmented populations* (eds A.G. Young & G.M. Clarke), pp. 241-251. Cambridge University Press, Cambridge.

Kemper, J., Cowling, R.M., Richardson, D.M., Forsyth, G.G., & McKelly, D.H. (2000) Landscape fragmentation in South Coast Renosterveld, South Africa, in relation to rainfall and topography. *Austral Ecology*, **25**, 179-186.

Kremen, C. & Ricketts, T. (2000) Global perspectives on pollination disruptions. *Conservation Biology*, **14**, 1226-1228.

Kress, W.J. (1985). Pollination and reproductive biology of *Heliconia*. In *The botany and natural history of Panama* (eds W.G. D'Arcy & M.D. Correa A.), Vol. 10, pp. 267-270. Missouri Botanical Garden, St. Louis.

Kull, T. (1998) Fruit-set and recruitment in populations of *Cypripedium calceolus* L. in Estonia. *Botanical Journal of the Linnean Society*, **126**, 27-38.

Kurzweil, H., Linder, H.P., & Chesselet, P. (1991) The Phylogeny and Evolution of the *Pterygodium-Corycium* Complex (Coryciinae, Orchidaceae). *Plant Systematics and Evolution*, **175**, 161-223.

Lamont, B.B., Klinkhamer, P.G.L., & Witkowski, E.T.F. (1993) Population fragmentation may reduce fertility to zero in *Banksia goodii* - a demonstration of the Allee effect. *Oecologia*, **94**, 446-450.

le Maitre, D.C. & Midgley, J.J. (1992). Plant reproductive ecology. In *The ecology of fynbos: nutrients, fire and diversity* (ed R.M. Cowling). Oxford University Press, Cape Town.

Lennartsson, T. (2002) Extinction thresholds and disrupted plant-pollinator interactions in fragmented plant populations. *Ecology*, **83**, 3060-3072.

Lindberg, A.B. & Olesen, J.M. (2001) The fragility of extreme specialization: *Passiflora mixta* and its pollinating hummingbird *Ensifera ensifera. Journal of Tropical Ecology*, **17**, 323-329.

Linder, H.P. (1985). Gene flow, speciation, and species diversity patterns in a species-rich area: the Cape Flora. In *Species and speciation.* (ed E.S. Vrba), Vol. 4, pp. 53-57. Transvaal Museum Monograph, Pretoria.

Linder, H.P. & Kurzweil, H. (1999) *Orchids of Southern Africa* A. A. Balkema, Rotterdam.

Liow, L.H., Sodhi, N.S., & Elmqvist, T. (2001) Bee diversity along a disturbance gradient in tropical lowland forests of south-east Asia. *Journal of Applied Ecology*, **38**, 180-192.

Lipow, S.R., Bernhardt, P., & Vance, N. (2002) Comparative rates of pollination and fruit set in widely separated populations of a rare orchid (Cypripedium fasciculatum). *International Journal of Plant Sciences*, **163**, 775-782.

Lloyd, J.W., van den Berg, E.C., & van Wyk, W. (1999). *CAPE Project: The mapping of threats to biodiversity in the Cape Floristic Region with the aid of remote sensing and geographic information systems.*, Rep. No. GW/ A1999/ 54. Agricultural Research Council, Institute for Soil, Climate and Water, Pretoria.

Low, A.B. & Rebelo, A.G., eds. (1996) *Vegetation of South Africa, Lesotho and Swaziland.* Department of Environmental Affairs & Tourism, Pretoria.

Luyt, R. & Johnson, S.D. (2001) Hawkmoth pollination of the African epiphytic orchid *Mystacidium venosum*, with special reference to flower and pollen longevity. *Plant Systematics and Evolution*, **228**, 49-62.

Manning, J. & Goldblatt, P. (2002) The pollination of *Tritoniopsis parviflora* (Iridaceae) by the oil-collecting bee *Rediviva gigas* (Hymenoptera : Melittidae): the first record of oil-secretion in African Iridaceae. *South African Journal of Botany*, **68**, 171-176.

Manning, J.C. & Brothers, D.J. (1986) Floral relations of four species of *Rediviva* in Natal (Hymenoptera: Apoidea: Melittidae). *Journal of the Entomological Society of Southern Africa*, **40**, 107-114.

Manning, J.C. & Goldblatt, P. (1996) The *Prosoeca peringueyi* (Diptera: Nemestrinidae) pollination guild in southern Africa: Long-tongued flies and their tubular flowers. *Annals of the Missouri Botanical Garden*, **83**, 67-86.

Manning, J.C. & Goldblatt, P. (1997) The *Moegistorhynchus longirostris* (Diptera: Nemestrinidae) pollination guild: Long-tubed flowers and a specialized long-proboscid fly pollination system in southern Africa. *Plant Systematics and Evolution*, **206**, 51-69.

Manning, J.C. & Linder, H.P. (1992) Pollinators and evolution in *Disperis* (Orchidaceae), or why are there so many species. *South African Journal of Science*, **88**, 38-49.

Marlin, J.C. & LaBerge, W.E. (2001) The native bee fauna of Carlinville, Illinois, revisited after 75 years: a case for persistence. *Conservation Ecology*, **5**, U91-U116.

Maze, K.E. & Rebelo, A.G. (1999) *Core flora conservation areas on the Cape Flats* Botanical Society of South Africa, Cape Town.

McDowell, C. & Moll, E. (1992) The influence of agriculture on the decline of West Coast Renosterveld, South Western Cape, South Africa. *Journal of Environmental Management*, **35**, 173-192.

McIntyre, N.E. & Hostetler, M.E. (2001) Effects of urban land use on pollinator (Hymenoptera : Apoidea) communities in a desert metropolis. *Basic and Applied Ecology*, **2**, 209-218.

Michener, C.D. (1944) Comparative external morphology, phylogeny, and classification of the bees (Hymenoptera). *Bulletin of the American Museum of Natural History*, **82**, 158-326.

Milton, S.J., Bond, W.J., Du Plessis, M.A., Gibbs, D., Hilton-Taylor, C., Linder, H.P., Raitt, L., Wood, J., & Donaldson, J.S. (1999) A protocol for plant conservation by translocation in threatened lowland Fynbos. *Conservation Biology*, **13**, 735-743.

Murren, C.J. (2002) Effects of habitat fragmentation on pollination: pollinators, pollinia viability and reproductive success. *Journal of Ecology*, **90**, 100-107.

Mustajarvi, K., Siikamaki, P., Rytkonen, S., & Lammi, A. (2001) Consequences of plant population size and density for plant- pollinator interactions and plant performance. *Journal of Ecology*, **89**, 80-87.

Neiland, M.R.M. & Wilcock, C.C. (1998) Fruit set, nectar reward, and rarity in the Orchidaceae. *American Journal of Botany*, **85**, 1657-1671.

Nilsson, L.A., Rabakonandrianina, E., Razananaivo, R., & Randriamanindry, J.J. (1992) Long pollinia on eyes - hawk-moth pollination of *Cynorkis uniflora* Lindley (Orchidaceae) in Madagascar. *Botanical Journal of the Linnean Society*, **109**, 145-160.

Ollerton, J. (1998) Pollination - Sunbird surprise for syndromes. *Nature*, **394**, 726-727.

Ornduff, R. (1972) The breakdown of trimorphic incompatibility in *Oxalis* section *Corniculatae*. *Evolution*, **25**, 52-65.

Ornduff, R. (1974) Heterostyly in South African flowering plants: a conspectus. *South African Journal of Botany*, **40**, 169-187.

Paton, D.C. (1993) Honeybees in the Australian Environment. *Bioscience*, **43**, 95-103.

Paton, D.C. (1997) Honeybees and the disruption of plant-pollinator systems in Australia. *Victorian Naturalist*, **114**, 23-29.

Paton, D.C. (2000) Disruption of bird-plant pollination systems in southern Australia. *Conservation Biology*, **14**, 1232-1234.

Pauw, A. (1998) Pollen transfer on birds' tongues. *Nature*, **394**, 731-732.

Pauw, A. (2001) Aliens, flames and flowers: a report-back on the Cape fires. *African Wildlife*, **55**, 20-25.

Pauw, A. & Johnson, S.D. (1999) *Table Mountain: a natural history* Fernwood Press, Cape Town.

Powell, A.H. & Powell, G.V.N. (1987) Population-dynamics of male euglossine bees in Amazonian forest fragments. *Biotropica*, **19**, 176-179.

Primack, R.B. & Hall, P. (1990) Costs of Reproduction in the pink lady's slipper orchid - a 4-year experimental study. *American Naturalist*, **136**, 638-656.

Rathcke, B.J. (2000) Hurricane causes resource and pollination limitation of fruit set in a bird-pollinated shrub. *Ecology*, **81**, 1951-1958.

Reader's Digest Association South Africa (1984) *Reader's Digest Atlas of Southern Africa* Reader's Digest Association South Africa, Cape Town.

Rebelo, A.G. (1987). Bird pollination in the Cape Flora. In *A preliminary synthesis of pollination biology in the Cape flora* (ed A.G. Rebelo), pp. 83-108. CSIR, Pretoria.

Rebelo, A.G., Siegfried, W.R., & Oliver, E.G.H. (1985) Pollination syndromes of *Erica* species in the southwestern Cape. *South African Journal of Botany*, **51**, 270-280.

Renner, S.S. (1998) Effects of habitat fragmentation on plant pollinator interactions in the tropics. In The 37th symposium of the British Ecological Society (eds D.M. Newbey & H.H.T. Prins), pp. 339-360. Blackwell Science, London.

Robertson, A.W., Kelly, D., Ladley, J.J., & Sparrow, A.D. (1999) Effects of pollinator loss on endemic New Zealand mistletoes (Loranthaceae). *Conservation Biology*, **13**, 499-508.

Roubik, D.W. (1996). Measuring the meaning of honey bees. In *The conservation of bees* (eds A. Matheson, S.L. Buchmann, C. O'Toole, P. Westrich & I.H. Williams). Academic Press, London.

Roubik, D.W. (2001) Ups and downs in pollinator populations: When is there a decline? *Conservation Ecology*, **5**, U27-U55.

Saunders, D.A., Hobbs, R.J., & Margules, C.R. (1991) Biological Consequences of Ecosystem Fragmentation - a Review. *Conservation Biology*, **5**, 18-32.

Saure, C. (1996). Urban habitats for bees: the example of the city of Berlin. In *The Conservation of Bees* (eds A. Matheson, S.L. Buchmann, C. O'toole, P. Westrich & I.H. Williams).

Siegel, S. & Castellan, N.J. (1988) *Nonparametric statistics for the behavioral sciences*, 2nd edn. McGraw-Hill Book Company, New York.

Skead, C.J. (1967) *The Sunbirds of southern Africa, also the Sugarbirds, the White-eyes and the Spotted Creeper* A. A. Balkema, Cape Town.

Smith, T.B., Freed, L.A., Lepson, J.K., & Carothers, J.H. (1995) Evolutionary Consequences of Extinctions in Populations of a Hawaiian Honeycreeper. *Conservation Biology*, **9**, 107-113.

Sokal, R.R. & Rohlf, F.J. (1995) *Biometry*, 3rd edn. W. R. Freeman and Company, New York.

Steffan-Dewenter, I., Munzenberg, U., & Tscharntke, T. (2001) Pollination, seed set and seed predation on a landscape scale. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **268**, 1685-1690.

Steffan-Dewenter, I. & Tscharntke, T. (2001) Succession of bee communities on fallows. *Ecography*, **24**, 83-93.

Steiner, K.E. (1987). Breeding systems in the Cape flora. In *A preliminary synthesis of pollination biology in the Cape flora* (ed A.G. Rebelo), Vol. 141, pp. 22-51. CSIR, Pretoria.

Steiner, K.E. (1989) The pollination of *Disperis* (Orchidaceae) by oil-collecting bees in southern Africa. *Lindleyana*, **4**, 164-183.

Steiner, K.E. (1998) The evolution of beetle pollination in a South African orchid. *American Journal of Botany*, **85**, 1180-1193.

Steiner, K.E. & Whitehead, V.B. (1988) The association between oil-producing flowers and oil-collecting bees in the Drakensberg of southern Africa. **25**, 259-277.

Steiner, K.E. & Whitehead, V.B. (1996) The consequences of specialization for pollination in a rare South African shrub, *Ixianthes retzioides* (Scrophulariaceae). *Plant Systematics and Evolution*, **201**, 131-138.

Steiner, K.E. & Whitehead, V.B. (2002) Oil secretion and the pollination of *Colpias mollis* (Scrophulariaceae). *Plant Systematics and Evolution*, **235**, 53-66.

Stiles, F.G. (1981) Geographical aspects of bird-flower coevolution, with particular reference to Central America. *Annals of the Missouri Botanical Garden*, **68**, 323-351.

Stouffer, P.C. & Bierregaard, R.O. (1995) Effects of forest fragmentation on understory hummingbirds in Amazonian Brazil. *Conservation Biology*, **9**, 1085-1094.

Thomson, J.D. (2001) Using pollination deficits to infer pollinator declines: Can theory guide us? *Conservation Ecology*, **5**, art. no.-6.

Tonhasca, A., Albuquerque, G.S., & Blackmer, J.L. (2003) Dispersal of euglossine bees between fragments of the Brazilian Atlantic Forest. *Journal of Tropical Ecology*, **19**, 99-102.

Tonhasca, A., Blackmer, J.L., & Albuquerque, G.S. (2002) Within-habitat heterogeneity of euglossine bee populations: a re-evaluation of the evidence. *Journal of Tropical Ecology*, **18**, 929-933.

Turner, I.M. (1996) Species loss in fragments of tropical rain forest: A review of the evidence. *Journal of Applied Ecology*, **33**, 200-209.

Van Wilgen, B.W. (1981) Some effects of fire frequency on fynbos plant community composition and structure at Jonkershoek, Stellenbosch. *South African Forestry Journal*, **118**, 42-55.

Vaughton, G. (1996) Pollination disruption by European honeybees in the Australian bird-pollinated shrub Grevillea barklyana (Proteaceae). *Plant Systematics and Evolution*, **200**, 89-100.

Venables, W.N. & Ripley, B.D. (2002) *Modern Applied Statistics with S*, 4 th edn. Springer-Verlag, Heidelberg.

Vinson, S.B., Frankie, G.W., & Barthell, J. (1993). Threats to the diversity of solitary bees in a neotropical dry forest in Central America. In *Hymenoptera and Biodiversity* (eds J. LaSalle & I.D. Gauld), pp. 53-81. CAB International, Wallingford.

Vinson, S.B., Williams, H.J., Frankie, G.W., & Shrum, G. (1997) Floral lipid chemistry of *Byrsonima crassifolia* (Malpigheaceae) and a use of floral lipids by *Centris* bees (Hymenoptera: Apidae). *Biotropica*, **29**, 76-83.

Vogel, S. (1954) Blütenbiologische Typen als Elemente der Sippengliederung: dargestellt anhand der Flora Südafrikas. *Botanische Studien*, **1**, 1-338.

Vogel, S. (1974) Ölblumen und ölsammelnde Bienen. *Trop. Subtrop. Pflanzenwelt*, **7**, 285-547.

Vogel, S. (1984) The *Diascia* flower and its bee-and-oil-based symbiosis in southern Africa. *Acta Botanica Neerlandica*, **33**, 509-518.

von Hase, A., Rouget, M., Maze, K., & Helme, N. (2004). *A fine-scale conservation plan for Cape Lowlands Renosterveld: technical report*, Rep. No. CCU 2/03. Botanical Society of South Africa, Cape Town.

Warburton, C.L., James, E.A., Fripp, Y.J., Trueman, S.J., & Wallace, H.M. (2000) Clonality and sexual reproductive failure in remnant populations of *Santalum lanceolatum* (Santalaceae). *Biological Conservation*, **96**, 45-54.

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

Waser, N.M. & Real, L.A. (1979) Effective mutualism between sequentially flowering plant species. *Nature*, **281**, 670-672.

Washitani, I. (1996) Predicted genetic consequences of strong fertility selection due to pollinator loss in an isolated population of *Primula sieboldii*. *Conservation Biology*, **10**, 59-64.

Washitani, I., Osawa, R., Namai, H., & Niwa, M. (1994) Patterns of female fertility in heterostylous *Primula sieboldii* under severe pollinator limitation. *Journal of Ecology*, **82**, 571-579.

Westerkamp, C. (1990) Bird flowers: hovering vs. perching exploitation. *Botanica Acta*, **103**, 366-371.