The Reproductive Biology and Ecology of *Colchicum* (Colchicaceae) in southern Africa

BY

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Statement

Professor Jeremy Midgley and Professor Steven Johnson supervised this thesis. It is hereby declared that this thesis, submitted in fulfilment of requirements for the degree of Master of Science at the University of Cape Town, is the result of my own investigation, except where the work of others is acknowledged.

Ciara Kleizen

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"In the end, we will conserve only what we love, we will love only what we understand, we will understand only what we are taught."

-Baba Dioum, Senegalese conservationist

Colchicum scabromarginatum (left) and *Colchicum coloratum* subsp. *coloratum* (right)

CHAPTER 1

Introduction

Pollination syndromes

The observation that many plants have developed sets of structural and functional adaptations that encourage visitation by pollinators has led biologists to define pollination syndromes. These syndromes reflect patterns of convergent evolution and serve to generate hypotheses about potential pollinators based on a plant's morphological characteristics (Faegri and van der Pijl, 1979; Johnson and Steiner, 2000).

A floral syndrome associated with pollination by non-flying mammals (therophily) including marsupials, primates, and rodents (therefore excluding bats), has been identified (Turner, 1982; Wiens *et al.,* 1983; reviewed in Carthew and Goldingay, 1997). Plants adapted for pollination by non-flying mammals tend to have robust flowers that are dull in colour, and exude a pungent odour. Nocturnal anthesis and nectar production is another characteristic of this syndrome. In order to provide a worthwhile reward for visitors, the flowers occur in tight inflorescences, copious amounts of nectar and pollen are produced as well as concentrated, sucrose-rich nectar (Wiens *et al.*, 1983; Carthew and Goldingay, 1997). There are however, important differences within the broad "non-flying mammal pollination syndrome" that differentiate between the three main non-flying mammal groups. For example, plants pollinated by primates tend to produce brightly coloured, large flowers and produce non-pungent nectar (Carthew and Goldingay, 1997; Nilsson *et al.,* 1993; Kress *et al.*, 1994). Flowers pollinated by nocturnal arboreal mammals tend to be non-cryptic and non-geoflorous (Carthew and Goldingay, 1997); whereas flowers pollinated by nocturnal terrestrial mammals, tend to be geoflorous and cryptic (Wiens *et al.*, 1983; Carthew and Goldingay, 1997).

Johnson *et al.* (2001) used this syndrome to successfully predict pollination by small non-flying mammals in the South African lily *Massonia depressa* (Hyacinthaceae) and also predicted from floral syndromes that a similar pollination system would occur in the genus *Androcymbium* (Colchicaceae) (now included in the genus *Colchicum*, Manning *et al.*, 2007). However, apart from some breeding system studies of cultivated plants (Membrives *et al.*, 2002), there has been almost no information published on the reproductive biology of *Colchicum* species.

The study system

Species of the genus *Colchicum* (Colchicaceae) are geophytes with an annual cycle (Membrives *et al.*, 2002). There are approximately 150 species in *Colchicum* (Manning *et al.*, 2007) which are distributed through Africa and the Mediterranean (Vinnersten and Manning, 2007). This study focuses on *Colchicum scabromarginatum* and *C. coloratum* subsp. *coloratum,* which are species found in the Succulent Karoo region of South Africa that display many "therophilous" characteristics. Floral traits of these species were compared with those of a suspected bee-pollinated species, *Colchicum hantamense*, which occurs in the same region. Comparisons were made to look for characters that may e characteristic of the non-flying mammal pollination syndrome.

The Succulent Karoo biome of southern Africa extends from southern Namibia (-26°8' S, 14°56' E), southwards along the western side of the South African escarpment to the eastern border of the Western Cape Province (-34° S, 23°11' E) (Lombard *et al.*, 1999) (Fig. 1). This biome is one of twenty-five internationally recognized biodiversity hotspots. A hotspot is an area of exceptional concentration of endemic species which is experiencing loss of habitat at an accelerated rate (Myers *et al.,* 2000). This region is a sparse, succulent-rich, fire-free biome that harbours a very high number of geophytes (Cowling *et al.*, 1999); and when compared with regions that have similar semi-arid environments, the richness of the

Succulent Karoo flora is exceptional at all spatial scales (Cowling and Hilton-Taylor, 1994). The Succulent Karoo is characterized by highly predictable annual rainfall and a moderate temperature regime throughout the year (Cowling *et al.* 1999). Many plants have adapted their reproductive strategies and modes of pollination in order to increase their chances of reproducing in the short period where there is sufficient moisture to ensure successful seed set (Le Roux and Schelpe, 1997). The region has become a focus of conservation activities and there is a need to understand how activities like farming that result in habitat fragmentation and reductions in population sizes, impact on reproductive biology of endemic plant species in the region.

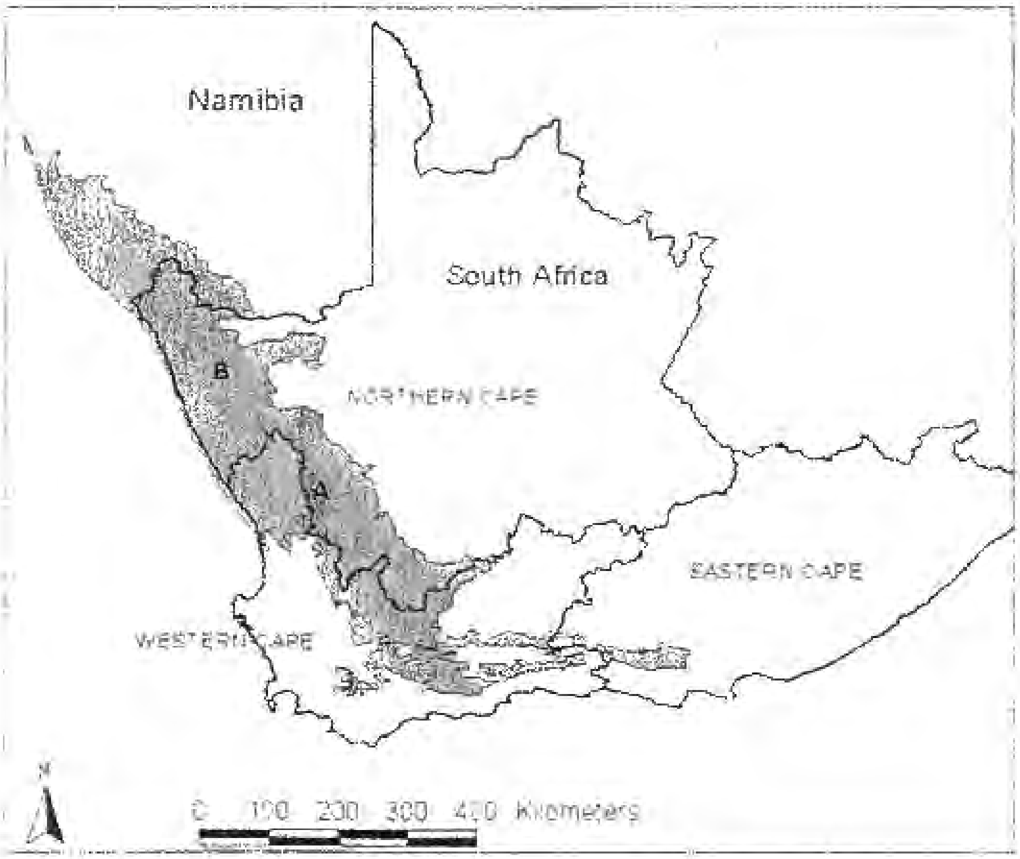

Figure 1. Map showing the boundary of the Succulent Karoo biome (shaded area) in relation to three South African provinces and Namibia (Rutherford and Westfall, 1997). A = the town Nieuwoudtville, where *Colchicum coloratum* was found. B – the town Springbok, north of which *C. scabromarginatum* was found.

Rodent pollination systems

As recently as 1979 (cf Faegri and van der Pijl, 1979), mammal pollination was considered to be quite rare, with the exception of chiropterophily (bat pollination) (Rebelo and Breytenbach, 1987). The notion that non-flying mammals might act as pollinators was first proposed by Porsch (1936). Small mammals, particularly rodents, were first proposed and shown to be pollinators of certain South African Proteaceae species some 30 years ago (Rourke and Wierns, 1977; Wiens and Rourke, 1978; Wiens *et al.*, 1983). Non-flying mammals also pollinate certain Proteaceae species in Australia, where flower products comprise an important part of the small mammals' diet (eg. Carthew, 1993; Wiens *et al.*, 1979). In South Africa, flowers pollinated by small mammals have been referred to as an unreliable and limited food source due to their short flowering seasons (Wiens *et al.*, 1983). However, Fleming and Nicolson (2002) showed that *Protea humiflora* plays an important role in the reproduction of rodent pollinators by providing food when other resources are low and the mammals energy requirements are high, especially if they are breeding (Rebelo and Breytenbach, 1987). Now, it is suspected that there are at least 100 non-flying mammal-plant mutualisms in the world (Rourke and Wiens, 1977; Carthew and Goldingay, 1997; Goldingay *et al.*, 1987).

Rodent-pollinated flowers have been reported as emitting a "yeasty" or "musky" scent to humans (Wiens *et al.*, 1983; Johnson *et al.*, 2001). Hardly any research has investigated the scent chemistry of rodent pollinated species. It has become apparent that there are certain general trends in the scent chemistry of plants pollinated by different classes of pollinators (reviewed in Dobson, 2001). Of course there are exceptions in each pollination category; however it is becoming evident that animal pollinators are acting as selective agents in determining the type of compounds found in the floral scent bouquet. Studies have been conducted to determine whether there has been convergent evolution between floral scent compounds in plants pollinated by the same class of pollinator. Bat-pollinated flowers for example, emit strong odours that are described as “fermented, garlicky, and pungent” and sulfur-containing compounds appear to be a typical component of the floral bouquet (Knudsen and Tollsten, 1995; von Helversen *et al.*, 2000). Hawkmoth-pollinated flowers, which emit “strong, sweet” scents, also share certain distinctive compound classes (oxygenated terpenoids, nitrogenous oximes) (Knudsen and Tollsten, 1993). There is a need for research on the floral scent of rodent pollinated plants as the identification of any trends in the scent chemistry would allow for a more detailed rodent pollination syndrome.

In order to understand the importance of a rodent pollinator-plant mutualism, factors that affect seed set need to be investigated. Pollen limitation is broadly defined as a reduction in fruit- or seed production from that which is physiologically possible, because of either too few visits by pollinators, or insufficient amounts of compatible pollen being transferred onto stigmas (Zimmerman and Pyke, 1988). If pollen limitation is the result of pollinator inactivity, then the factors affecting the attractiveness of plants and visitation rate of pollinators need to be explored. These factors include attractants and rewards (such as fragrances, nectar and pollen), and display (Worley *et al.,* 2000). Display can refer to the population size, the plants’ proximity to one another (population density); as well as the number of flowers on a plant, or the relative appearance of flowers on a plant or in the inflorescence (Kunin, 1997). All these factors play a role in determining how robust an animal-plant mutualism is, and how the plant species might react to a process such as habitat fragmentation.

General aims

The purpose of this study was to determine if the rodent pollination system extends to *Colchicum*, as predicted by Johnson *et al.* (2001), to test the importance of floral scent for attraction of rodent pollinators, and to investigate the factors that influence seed set in populations.

The thesis consists of three stand-alone chapters, all linked to the theme of rodent-pollination but written as separate scientific papers, and a general conclusion. In all cases I did the majority of the field-work, analyses and writing. Below I have specified my further contributions to each chapter.

Chapter 2: Pollination Systems of *Colchicum* (Colchicaceae) in Southern Africa: Evidence for Rodent Pollination.

This chapter investigates the hypothesis that *Colchicum scabromarginatum* and *C. coloratum* are rodent pollinated, and investigates certain floral traits such as the breeding systems, the effects of excluding rodents on the seed set and the daily variation in nectar volume and concentration.

I did all the field-work for this chapter (including developing methodology based on my novel observations on *C. scabromarginatum* and on the possibility of bird pollination). I dealt with submission of the paper to the journal.

Chapter 3: The scent chemistry of a rodent pollinated plant, *Colchicum scabromarginatum*, and its relevance to pollination biology.

This chapter analyses the floral scent of the rodent pollinated plant, *Colchicum scabromarginatum.* The rodents' preferences for the different chemical compounds occurring in the nectar are also tested in a Y-maze olfactometer.

I performed the field tests and collected the animals. Professor Johnson determined the scent compounds in his laboratory, from field collected samples.

Chapter 4: Natural Variation in seed set of *Colchicum coloratum*.

This chapter explores pollen limitation in *Colchicum coloratum*. The relationship between rodent abundance and population size as well as plant size is also investigated. I determined the methodology and study sites and completed all the field-work

Chapter 5: Summary

This chapter details the main findings and conclusions of this thesis.

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CHAPTER 2

Pollination Systems of *Colchicum* (Colchicaceae) in southern Africa: Evidence for rodent-pollination

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ABSTRACT

- *Background and Aims* Plants adapted for pollination by rodents tend to exhibit a distinct floral syndrome that includes dull coloured and geoflorous inflorescences and nocturnal anthesis and nectar production. On the basis of their floral traits, it was predicted that two African *Colchicum* species (C. *scabromarginatum* and *C. coloratum*) are rodent pollinated.
- *Methods* Field studies were carried out in the semiarid Succulent Karoo region of South Africa. Live trapping of rodents was conducted and pollen loads on the rodents were quantified. The daily periodicity of nectar production was determined. Selective exclusion and controlled pollination experiments were also conducted.
- *Key Results* Live-trapped rodents were found to carry large amounts of *Colchicum* pollen on the fur of their snouts, and in their faeces. Birds were occasional pollinators of flowers of *C. coloratum*. During the evening, the nectar volume and concentration increased for both species. When vertebrates were excluded from C. *scabromarginatum* and *C. coloratum* plants, there was a significant decrease in seed set compared with open control plants. On the other hand, vertebrate exclusion did not significantly affect seed production of a congener *C. hantamense* which has floral traits associated with insect pollination. Breeding system experiments revealed that both C. *scabromarginatum* and *C. coloratum* require pollinators for seed production. *Colchicum scabromarginatum* is strictly self-incompatible, whereas *C. coloratum* is partially self-compatible.
- *Conclusions* Pollination by rodents occurs in two African *Colchicum* species. One of the species, *C. scabromarginatum*, appears to depend exclusively on rodents for seed production,

while birds and autonomous selfing may contribute to seed production in *C. coloratum*. These are the first records of rodent pollination in the Colchicaceae.

Key words: convergent evolution; floral syndrome; pollination; rodents; birds; insects; *Colchicum scabromarginatum*; *Colchicum coloratum*; Succulent Karoo; southern Africa.

INTRODUCTION

The semi-arid Succulent Karoo region of South Africa is considered one of the earth's biodiversity hotspots (Mucina *et al.,* 2006). Reasons for this diversity are varied and may include pollinator driven speciation (Johnson, 2006; van der Niet *et al.*, 2006). The evolution of a rodent pollination system in a succulent Karoo geophyte, *Massonia depressa* (Hyacinthaceae), was reported by Johnson *et al.* (2001). These authors predicted that other geophytes in the same area are also rodent-pollinated. This prediction was based on apparent convergent floral morphology between flowers of these geophytes and rodent-pollinated Cape proteas (cf Rourke and Wiens, 1977; Wiens and Rourke, 1978; Wiens *et al.*, 1983). In this study, two *Colchicum* species (*C. scabromarginatum* and *C. coloratum*) from the succulent Karoo region were identified as likely candidates for a rodent pollination system on the basis of their "therophilous" floral traits.

Plants adapted for pollination by nonflying mammals (including rodents, marsupials and primates) tend to have robust flowers that are dull in colour, cup-shaped, and situated at ground level (geoflorous) (Wiens and Rourke, 1978). They also produce copious amounts of nectar and a have a stigma-nectar distance of about 10 mm (Wiens *et al.*, 1983). The time of flowering of nonflying mammal pollinated plants tends to be late winter which has been suggested to reflect the willingness of mammals to supplement their diet with nectar at this time of low food availability (Rourke and Wiens, 1977).

Many authors have remarked on the "yeasty" odour of flowers. This is assumed to be the primary cue for long distance attraction as mammals, such as rodents, have a well-developed sense of smell and the well-hidden flowers are usually pollinated at night when visual cues would not be effective (Rourke and Wiens, 1977; Rebelo and Breytenbach, 1987). Scent emission, nectar secretion and floral anthesis tend to be nocturnal, coinciding with the activity patterns of many mammals, such as most rodents (Wiens *et al.*, 1983; Johnson *et al.*, 2001).

Verification of nonflying mammal pollination is difficult because the animals' nocturnal habits largely preclude direct field-observations. Evidence must be obtained from other lines of investigation which may include examination of pollen loads on the fur and in the faeces of trapped animals (Carthew and Goldingay, 1997) and selective exclusion of vertebrates from flowers. If the latter treatment results in reduced fecundity, and birds and bats can be excluded as flower visitors using other evidence, then pollination by rodents can be inferred.

To test the hypothesis that *C. scabromarginatum* and *C. coloratum* subsp. *coloratum* are pollinated by rodents, we addressed the following research questions. 1) Do plants have a breeding system that makes them dependent on pollinator visits for seed production? 2) Are floral morphology, nectar properties and nectar secretion patterns consistent with rodent pollination? 3) Do rodents visit flowers and act as pollen vectors for the study species? 4) Does experimental exclusion of rodents result in diminished seed production?

MATERIALS AND METHODS

Study species and Study sites

All species of the genus *Androcymbium* (± 60 species) have been included within an expanded circumscription of the genus *Colchicum* (±90 species; Manning *et al.*, 2007). The expanded genus, *Colchicum,* of the family Colchicaceae is defined by its reduced or absent stem, androecial nectaries and 2-4-porate pollen (Manning *et al.*, 2007; Vinnersten and Manning, 2007). The genus *Colchicum* is widely distributed through Africa and the Mediterranean, with marked centres of diversity in winter-rainfall regions of both hemispheres (Vinnersten and Manning, 2007). The flowers of both *Colchicum scabromarginatum* (Schltr. and K. Krause) J. C. Manning and Vinn (= *Androcymbium scabromarginatum*) and *C. coloratum* J. C. (Manning and Vinn) (= *Androcymbium pulchrum*) are geoflorous and the inflorescences are robust and cup-shaped. Like rodent pollinated proteas (Wiens *et al.*, 1983), these two *Colchicum* species also flower in winter. *Colchicum scabromarginatum* has the dull-coloured floral bracts that are typical of rodent-pollinated plants (Fig. 1. B), whereas in *C. coloratum* the bracts are a reddish colour (Fig. 1. A) (Membrives *et al.*, 2002). The third species included in this study is *Colchicum hantamense* (Engl.) J.C.Manning and Vinn. This species displays insect pollination characteristics, such as white coloured floral bracts (Membrives *et al.*, 2002) and a sweet spicy scent. Honeybees frequently visit flowers of this species (Fig. 1. G; C. Kleizen and S. D. Johnson, pers. obs.). Therefore in this study, *Colchicum hantamense* was used for comparisons with an insect-pollinated congener. Voucher specimens of the three study species are deposited in the Bews Herbarium, Pietermaritzburg.

This study was carried out in the semiarid Succulent Karoo region of South Africa during August 2006 and July to September, 2007. Two large populations of *C. scabromarginatum* (>1000 plants) were located in sparse vegetation on Naries farm 30km north of Springbok. One population was on the top of a ridge (29°41'S, 17°39'E, elev. 794m) and the other occurred alongside a dam (29°41'S, 17°40'E, elev. 703 m). Several populations of *C. coloratum* were located in the vicinity of Niewoudtville. These populations were situated at the following sites: an empty plot in the centre of town (31°22'S, 019°06'E, elev. 713 m), a dolerite ridge on the farm Glen Lyon (31°25' S, 019°09' E, elev. 774m), a slope on Glen Lyon farm (31°23'S, 019°09'E, elev. 742m), alongside a river on Glen Lyon farm (31°22'S, 019°15'E, elev. 703m), the Nieuwoudtville Flower Reserve (31°21'S, 019°08'E, elev. 747m), Matjiesfontein farm (31°28'S, 019°04'E, elev. 700 m) and Hotbergfontein farm (31°22'S, 019°12'E, elev. 758m). The population of *Colchicum hantamense* that we studied was located in the Nieuwoudtville Flower Reserve (31°21'S, 019°08'E, elev. 761m).

Breeding system

To establish whether the study species depend on pollinator visits for seed production and whether or not they possess a genetic self-incompatible system, a breeding system experiment was conducted. Twenty inflorescences of both *C. scabromarginatum* and *C. coloratum* were covered by pollinator-excluded cages while the plants were in the budding phase. Once flowers had opened, three treatments were applied to each inflorescence: 1) pollinated by hand with pollen from a different plant (after which the flower was emasculated), 2) pollinated by hand with self-pollen to determine whether plants are self-compatible, and 3) unmanipulated to test for autonomous self-fertilization. At the end of the flower season, the number of seeds in one locule per flower was counted and multiplied by three to estimate the number of seeds per flower (there are three locules per flower).

Floral and nectar characteristics

In order to determine daily variation in nectar, the standing crop of nectar was measured from all six nectaries of ten different randomly selected flowers every three hours for approximately 24 hours for both *C. scabromarginatum* and *C. coloratum*. The nectar volume was measured using 100μl capillary tubes (Drummond Scientific Company, Broomall, Pennsylvania, USA) and the nectar concentration was quantified using a 0-50% field refractometer (Bellingham and Stanley, Tunbridge Wells, UK). The pattern of floral anthesis in *C. coloratum* was obtained by recording the number of open flowers on 20 inflorescences approximately every four hours for 22 hours. For *C. scabromarginatum*, *C. coloratum* and *C. hantamense*, the floral dimensions were measured from ten flowers, each sampled from different plants, and rounded off to the nearest 0.5mm.

Rodent trapping and pollen loads

On the nights of the 17, 18, 19 and 20 July, 2007, 60 sherman traps were laid out in the *Colchicum scabromarginatum* population. On the nights of the 8, 9 and 10 August 2006, 84 "gutter-pipe" traps were laid out each night amongst the *C. coloratum* inflorescences on the dolerite ridge on Glen Lyon farm. Between 6 August and 12 September 2007, 90 traps were set every evening for three consecutive nights at each of the six *C. coloratum* sites. Traps were set at dusk and laid out in lines of 15 traps, with four metres between each trap; and all traps were baited with rolled peanut butter and oats. Traps were checked in the early morning (between 0600 and 0700) and once a rodent had been captured, that trap was not re-used. Captured rodents were identified and temporarily placed in a plastic bag with a hole in one corner through which the snout of the rodent protruded. The fur just around the nose of each trapped rodent was swabbed for ten seconds with a small block of fuschin gelatine (Beattie, 1971). Each of the fuschin gelatine samples was then melted onto a slide and the number of pollen grains was counted over three scans of the length of the coverslip. Rodent faeces were collected from the traps and stored in 70% alcohol. Liquid fuschin gelatine was added to this solution, and this mixture was mounted on a slide. These samples were also examined microscopically for the presence of pollen. This would include pollen ingested directly, through feeding and indirectly through grooming (Fleming and Nicolson, 2002).

As it is virtually impossible to observe rodents in the field at night (Wiens *et al.*, 1983), we recorded the foraging behaviour of captured rodents. An individual *Aethomys namaquensis* (Namaqua rock mouse) was released into a glass tank (100cm long by 100cm wide) with a 10cm deep layer of sand containing four fresh *C. scabromarginatum* inflorescences. Fresh *Oxalis* flowers from the same area were also placed in the tank in order to determine if the rodents visit flowers selectively. In another experiment, an *Aethomys namaquensis* individual and *Gerbillurus paeba* (Hairy-footed gerbil) individual were released separately into a tank with four fresh *C. coloratum* inflorescences. Later, inflorescences of the apparently insect-pollinated species *Colchicum hantamense* were placed in the same tank. The foraging behaviour of the rodents was observed from 18h00 until 0100.

Observations during daylight were conducted for a total of ten hours in the *C. scabromarginatum* populations and a total of 20 hours in the *C. coloratum* populations. The *Colchicum hantamense* population was observed for four hours. During the field observations of *C. coloratum* in site 1, birds (sparrows, weaverbirds and starlings only) were observed to visit the flowers and feed on nectar (Fig. 1. C), with pollen visibly dusted on the feathers surrounding the beaks of the birds (Fig. 1. D). Subsequently, four hours of mist netting was conducted at site 1 in order to catch birds after a feeding bout to verify if the pollen originated from *Colchicum* flowers. Captured birds were identified and the feathers surrounding the beak of the bird were swabbed with a small block of fuschin gelatine (Beattie, 1971). Microscopic slides were created and analysed using the same procedure as from captured rodents. No birds were observed visiting *C. coloratum* inflorescences at any of the other sites.

Selective exclusion experiments

In order to investigate the importance of rodents for seed production, ten pairs of *C. scabromarginatum* plants from the ridge population at Naries, and ten pairs of *C. coloratum* plants at Glen Lyon, were selected. One plant per pair was enclosed in a wire cage with a mesh diameter of 15mm x 20 mm. This exclosure excluded rodents, but allowed insects free access to the flowers. The other plant was left unmanipulated as a control. At the end of the flowering season, the number of seeds per flower was counted. This experiment was also conducted on *C. hantamense*. Given our observations of bee visitation to flowers of *C. hantamense*, we expected selective exclusion of rodents to have no effect on seed set of this species.

Figure 1. A. Inflorescence of *Colchicum coloratum*. Scale 5 cm. B. Inflorescence of *Colchicum scabromarginatum*. Scale 5 cm. C. Cape sparrow visiting a *C. coloratum* inflorescence at site 1. Scale 10 cm. D. *C. coloratum* pollen deposited on the feathers surrounding the beak of the bird. Scale 5 cm. E and F. *Aethomys* pushes its head among *C. scabromarginatum* anthers to reach the nectar, with pollen visibly dusted on the snout of the rodent. Scale 5 cm. G. A honeybee visiting a *C. hantamense* inflorescence. Scale 5 mm.

RESULTS

Breeding system

Cross-pollination of both *C. scabromarginatum* and *C. coloratum* plants resulted in significantly higher seed set than when the plants were self-pollinated or unmanipulated (Table 1). *Colchicum scabromarginatum* plants only set seed when cross-pollinated. *Colchicum coloratum* plants set only one third as many seeds when self-pollinated as when cross-pollinated and even fewer seeds when left unmanipulated (Table 1). All *Colchicum* species are hypogynous (Meyer, 2000), and we noted that the three study species appeared to be protogynous.

Table 1. Results of controlled pollination experiments to determine the breeding system of *Colchicum scabromarginatum* and *C. coloratum*. Values are medians (range) and were analyzed using Friedman's test with plant treated as a blocking factor.

Species	N (plants)	Seeds per flower			X^2	P
		Unmanipulated	Self-pollinated	Cross-pollinated		
	20	0	0	114	27.9	<0.0001
C. scabromarginatum		(0-6)	(0-12)	(15-228)		
	20	3	18	52.5	36.8	<0.0001
C. coloratum		(0-24)	(0-72)	(6-150)		

Floral traits

Large volumes of nectar (up to 210 µL) were found in individual *C. scabromarginatum* flowers during the night. In *C. scabromarginatum* the maximum average volume of nectar (193.3 µL) was secreted at midnight, after which the average volumes declined until after midday when they started increasing again (Fig. 2A). The average concentration of *C. scabromarginatum* nectar increased steadily throughout the day, reaching a maximum of 64.8% sucrose at midnight (Fig. 2A). In *C. coloratum* the average nectar volume and concentration increased slowly throughout the day, reaching a maximum volume of 24.4 µL at midnight and maximum concentration of 28.1% sucrose at 19h00 (Fig. 2B). The sticky nectar secreted by *C. scabromarginatum* and *C. coloratum* is extremely apparent, whereas the nectar secreted by *C. hantamense* is difficult is see with the naked eye.

In the genus *Colchicum*, the nectar is secreted from androecial nectaries, which are situated at the base of the filaments (Manning *et al.*, 2007). The nectar wells up into a chamber which is an average of 5.25 mm wide by 3.7 mm deep in *C. scabromarginatum* and 3.65 mm wide by 2.65 mm deep in *C. coloratum* (Table 2). The nectar chambers of *C. scabromarginatum* and *C. colchicum* were noticeably larger, with a more sculptured structure in contrast with the significantly smaller chamber size of *C. hantamense* (2.4 mm wide by 1.55 mm deep, Table 2). The results from the floral measurements (Table 2) show that both *C. scabromarginatum* and *C. coloratum* follow the 10 mm rule of the therophilous syndrome and that the average stigma-nectar distances of both species are significantly greater than that of *C. hantamense* ($F_{(2, 27)} = 83.002$, $P = 0.000001$). The floral measurements show that the mean inflorescence diameter and the mean number of flowers per inflorescence were greater in *C. scabromarginatum* than in *C.*

coloratum and *C. hantamense* (Table 2). The uppermost bracts of *C. scabromarginatum* curve over the flowers and were also thicker and appeared less likely to tear or rip than the bracts of either *C. coloratum* or *C. hantamense.*

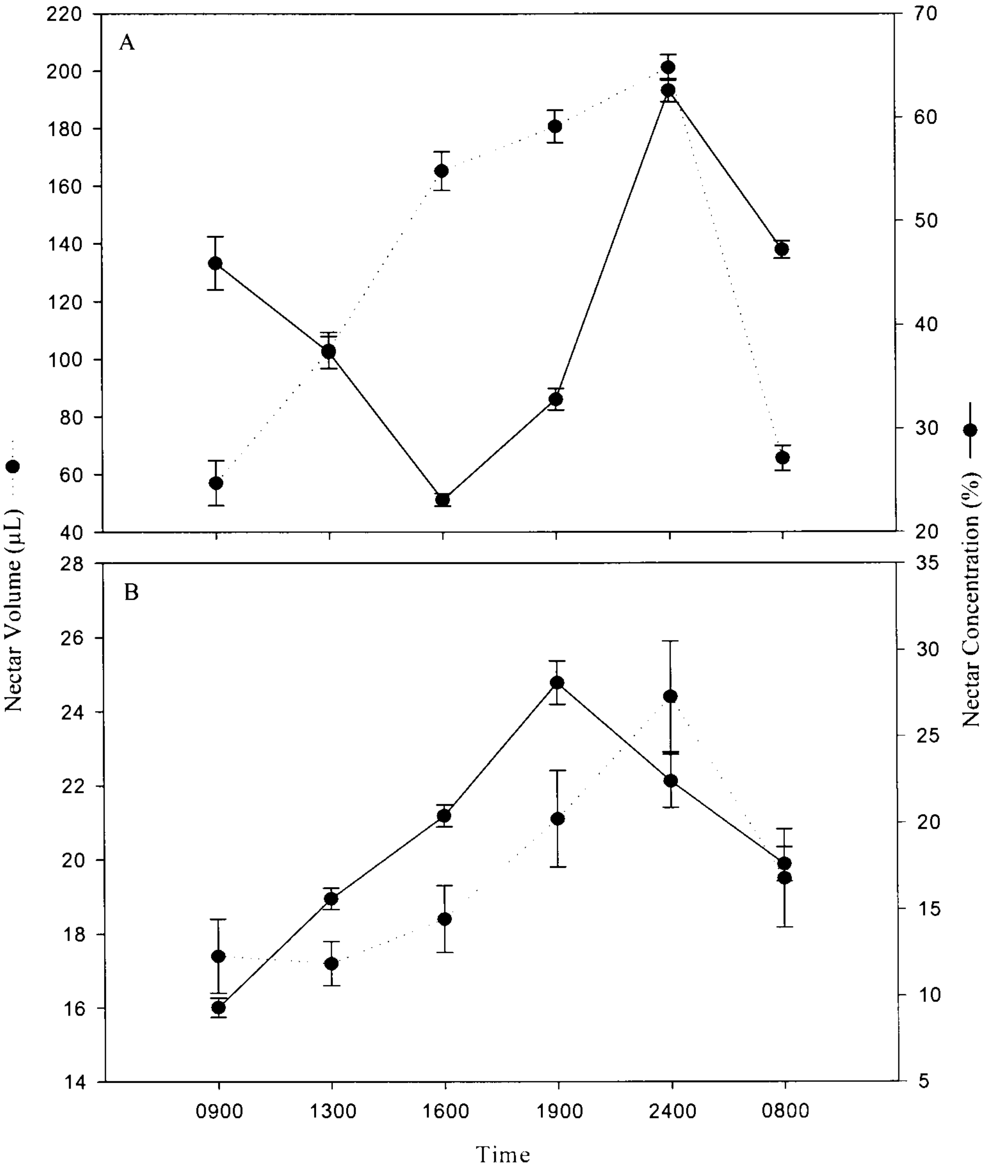

Figure 2. Daily variation in the average nectar volume and concentration per flower for *Colchicum scabromarginatum* (A) and *C. coloratum* (B). Bars represent ± 1 SE.

Table 2. Floral characteristics measured for each of the *Colchicum* species. Values are means ± SE and dissimilar letters indicate significant ($P < 0.05$) differences between the species, as determined by one-way ANOVA followed by post-hoc Tukey LSD tests ($n = 10$).

Characteristic	*C. scabromarginatum*	*C. coloratum*	*C. hantamense*
Nectar chamber width (mm)	5.25 ± 0.23^{a}	3.65 ± 0.18^{b}	2.4 ± 0.21^{c}
Nectar chamber depth (mm)	3.7 ± 0.2^{a}	2.65 ± 0.15^{b}	1.55 ± 0.12^{c}
Anther height (mm)	10.25 ± 0.29^{a}	11.7 ± 0.4^{a}	4.65 ± 0.24^{b}
Stigma height (mm)	8.95 ± 0.68^{a}	14.8 ± 1.22^{b}	7.3 ± 0.47^{a}
Stigma-nectar distance (mm)	10.2 ± 0.47^{a}	13 ± 0.61^{b}	4.6 ± 0.23^{c}
Diameter of inflorescence (cm)	14.36 ± 1.63^{a}	5.1 ± 0.38^{b}	3.46 ± 0.9^{b}
Flowers per inflorescence	11.8 ± 1.17^{a}	3.1 ± 0.23^{b}	3.3 ± 0.6^{b}

At the start of the floral anthesis experiment (08h00), there were 14 open flowers (0.7 per inflorescence) on the 20 marked *C. coloratum* inflorescences. After approximately 24 hours, there were 42 open flowers (2.1 per inflorescence) on these inflorescences. Floral anthesis took place mainly in the afternoon. In the 4 hour period between 12h00 and 16h00, 11 flowers (39%) opened. The number of flowers that opened per time period differs significantly from values expected if opening is random ($\chi 2 = 12.5$, $P < 0.05$).

Rodent trapping and pollen loads

Trapping in the *C. scabromarginatum* population resulted in the capture of eight individual rodents, all *Aethomys namaquensis* (A. Smith), commonly known as the namaqua rock mouse

(Family Muridae, subfamily Murinae) (Table 3). A total of 28 individuals representing four rodent species were captured when trapping was conducted in the *C. coloratum* populations (Table 3). Nocturnally active rodents included two murid species (Family Muridae, subfamily Murinae), *Aethomys namaquensis* (A. Smith) and *Mus minutoides* (A. Smith), the pygmy mouse; and one gerbil species (Family Muridae, subfamily Gerbillinae), *Gerbillurus paeba* (A. Smith), the hairy-footed gerbil. *Rhabdomys pumilio* (Thomas) (Family Muridae, subfamily Murinae), the Cape striped field mouse, was the only diurnal species captured. Rodents were captured amongst *C. coloratum* inflorescences at all of the six sites except for site 1. Microscopic examination of the fuschin gelatin blocks showed that *C. scabromarginatum* pollen was the only pollen present and was abundant on the snouts (mean of 154 ± 19 grains per slide) and in the faeces (mean of 21.3 ± 4.8 grains per slide) of all of the eight captured rodents from the *C. scabromarginatum* population (Table 3). *Colchicum coloratum* pollen was found on the snouts of 24 out of the 28 captured rodents from the *C. coloratum* populations (mean of 29 ± 5.8 grains per slide). Faeces from all of the 28 rodents contained *C. coloratum* pollen (mean of 214 ± 34.5 grains per slide) (Table 3). In the rodents captured amongst *C. coloratum* plants, there was only one other type of pollen (an unidentified Asteraceae) and this was very sparse.

The captive rodents all visited the respective *Colchicum* inflorescences at approximately midnight (Fig. 1 E, F). The animals moved between all the inflorescences and had pollen dusted on their snouts (Fig. 1E, F). The pollen-covered snouts of the rodents made contact with the stigmas while they lapped nectar. As the rodents lapped up the nectar, their snouts moved very rapidly and appeared to push down firmly into the flower. However, the flowers were not damaged in any way after each feeding bout. All the rodents ignored the other flowers placed in the tanks (*Oxalis* and *C. hantamense* respectively). After visiting all the flowers in the tank, the rodents spent several minutes grooming pollen from their fur.

During the field observations, no insects were observed to visit either *C. scabromarginatum* or *C. coloratum*, but bees were frequently observed to visit *C. hantamense* inflorescences. From four hours of mist netting for birds at *C. coloratum* site 1, a total of 19 birds were captured. Microscopic analysis of the fuschin gelatine samples showed that the Cape Sparrow, *Passer melanurus* (S. Muller) (Family Passeridae), the Cape weaver, *Ploceus capensis* (Linnaeus) (Family Ploceidae) and the Masked weaver, *Ploceus intermedius* (Rüppell) (Family Ploceidae) carried large amounts of *C. coloratum* pollen on their feathers (an average of 614 pollen grains, SE = 112, N = 18). The Cape glossy starling, *Lamprotornis nitens* (Rafinesque) (Family Sturnidae), however, was also captured in the mist net after feeding on *C. coloratum* nectar, yet there was no pollen found on the feathers of this bird.

Table 3. Pollen loads of the rodents captured at the different sites.

Year	Site	Rodent species	No. animals captured	No. of animals with pollen on snout (mean pollen count per slide ± SE)	No. of animals with pollen in feces (mean pollen count per slide ± SE)
C. scabromarginatum					
2007	Naries	*Aethomys namaquensis*	8	8 (154±19)	8 (21.3±4.8)
C. coloratum					
2006	Glen Lyon	*Aethomys namaquensis*	9	6 (5.5 ± 2.6)	9 (212 ± 155.6)
2007	site 1		0	-	-
	site 2	*Gerbillurus paeba*	1	1(26)	1 (155)
	site 3	*Mus minutoides*	2	2 (14 ± 0.5)	2 (125 ± 0)
		Rhabdomys pumilio	1	1 (20)	1 (185)
	site 4	*Aethomys namaquensis*	3	3 (63 ± 16.7)	3 (405 ± 159.4)
		Mus minutoides	1	1 (22)	1 (94)
	site 5	*Aethomys namaquensis*	3	3 (54 ± 8.4)	3 (411 ± 129.2)
		Rhabdomys pumilio	2	2 (43 ± 12.5)	2 (232 ± 43.5)
	site 6	*Rhabdomys pumilio*	2	2 (28 ± 11)	2 (158 ± 80)
		Gerbillurus paeba	4	3 (17 ± 7.2)	4 (166 ± 14.5)

Selective exclusion experiment

The exclusion of rodents from *C. scabromarginatum* and *C. coloratum* plants resulted in a significant decline in seed set relative to the unmanipulated controls, but this was not evident in the apparently insect-pollinated species *C. hantamense* (Fig. 3). Excluding rodents from the *C.*

scabromarginatum, *C. coloratum* and *C. hantamense* flowers reduces seed set by approximately 97%, 82% and 8% respectively (Fig 3).

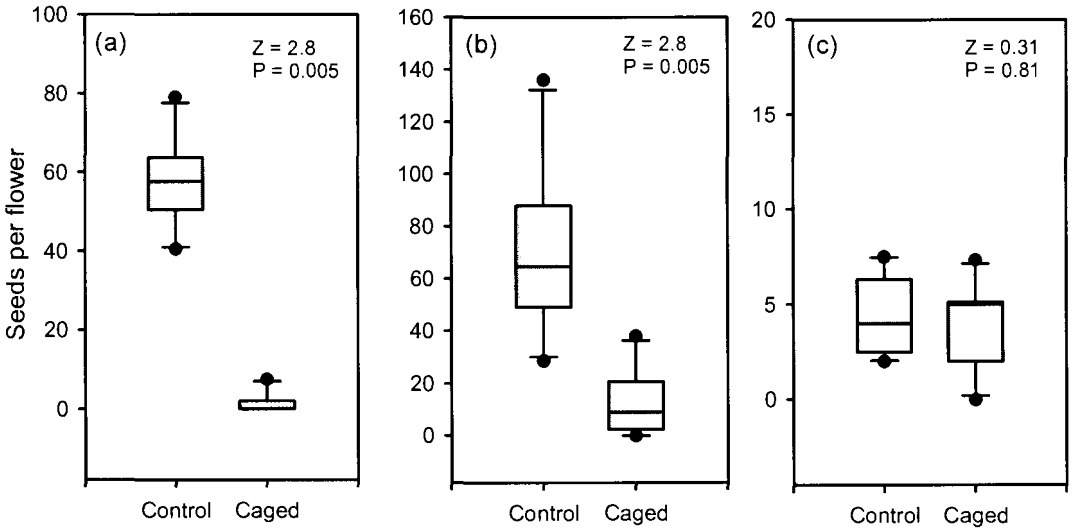

Figure 3. The effect of exclusion of vertebrates on the median number of seeds produced per flower in plants of *Colchicum scabromarginatum* (a), *C. coloratum* (b), *C. hantamense* (c). Z values were obtained from the Wilcoxon test for paired samples. Samples sizes were 13 plants per treatment group for all species.

DISCUSSION

The results of this study show that both *C. scabromarginatum* and *C. coloratum* are rodent pollinated species. Captive rodents willingly and nondestructively foraged on inflorescences of these species, but not on those of the bee-pollinated congener *C. hantamense*, and are clearly effective pollen vectors. Copious amounts of *Colchicum* pollen was deposited on the snouts of field-caught rodents as well as those in the tank experiments (Fig 1E-F). The cup like shape of the inflorescence and the distance between the nectar and stigma in *C. scabromarginatum* and *C. coloratum* (greater than 10 mm) ensures that rodent's snouts fit neatly into the flower and brush against the stigmas and anthers while it laps nectar from the nectary chambers (Fig 1E-F). The position of the uppermost bracts curved over the flowers of *C. scabromarginatum* would most likely make it more difficult for insects or birds to reach the flowers than rodents, which are able to push the bracts aside to reach the nectar.

The breeding experiment showed that both of the study species are dependent on pollinator visits for seed production - *C. scabromarginatum* is a strongly self-incompatible species, while *C. coloratum* is partially self-incompatible. The latter result is consistent with the findings of Membrives *et al.* (2002) who performed breeding system experiments on plants of *C. coloratum* that had been grown from seed and concluded that the species was "preferentially self-incompatible".

Experimental exclusion of vertebrates resulted in sharp and significant decreases in seed set in both *C. scabromarginatum* and *C. coloratum* (Fig. 3). This was consistent with the apparent complete absence of insect visitors to inflorescences of these species. Although we would also have excluded birds and bats with this technique, the effect of the treatment was almost certainly due to rodent exclusion as birds were not observed as flower visitors in the populations in which this experiment was conducted and flower feeding bats are not known from the Succulent Karoo region. These results contrast with those obtained for the insect-visited species *C. hantamense* in which vertebrate exclusion had no effect on seed set (Fig. 3). As *C. hantamense* is not capable of autogamy (Membrives *et al.*, 2002), this lack of an effect of caging indicates that the cages did not hinder access by insect pollinators.

By flowering in winter and early spring, *C. scabromarginatum* and *C. coloratum*, like therophilous *Protea* species, may provide an important energy source for rodents such as *Aethomys namaquensis* that breed in late July (Fleming and Nicolson, 2002). The primarily nocturnal anthesis and nectar secretion patterns also correlate with the nocturnal activity of rodent pollinators. The large mean standing crop of nectar in *C. scabromarginatum* and *C. coloratum* (Fig. 2) is also consistent with vertebrate pollination. By contrast, flowers of the insect-pollinated species *C. hantamense* contain very small volumes of nectar and this is secreted mainly during daylight hours (Membrives *et al.,* 2002). During the scope of this study, we were not able to investigate the measures of phenology for the three species involved. However, Membrives *et al.* (2002) investigated the reproductive biology of *C. coloratum* and *C. hantamense*, but not for *C. scabromarginatum*. They found that there were 185±20 days between emergence and senescence for *C. colchicum* and 191±13 days for *C. hantamense*, and that both species flower for approximately two months long.

How ecologically important are rodents to C. scabromarginatum *and* C. coloratum*?*

The observation of short-billed birds visiting *C. coloratum* inflorescences and carrying *Colchicum* pollen at one of our study sites indicates that this species may not have a strict dependence on rodents for pollination. The site where this was observed was the only one situated in an urban area and sparrows and other birds may be more plentiful and opportunistic at this site. However, the nocturnal nectar secretion and capture of rodents carrying *Colchicum* pollen at five out of the six sites where we studied *C. coloratum* make up a strong case for rodents being its primary pollinators. Nevertheless, we cannot exclude the possibility that birds are secondary pollinators of this species. Indeed, the reddish floral bracts of this species could be interpreted as a trait for attraction of birds. Weaverbirds are known to legitimately pollinate flowers (Botes *et al.*, 2008). However, there are no studies known by us that show sparrows pollinating flowers.

The ecological dependence of *C. scabromarginatum* on rodents is undoubtedly much higher than in *C. coloratum*. This species has extremely cryptic green inflorescences with bracts that have to be folded open to gain access to the flowers (Fig. 1B). The plants are often hidden in the vegetation or among rocks and were not seen to be visited by birds or insects. It is also strictly self-incompatible and incapable of autogamy (Table 1).

The evolution of rodent pollination in geophytes

Rodent pollination has now been found in two African geophytic lineages - Hyacinthaceae (Johnson *et al.*, 2001) and Colchicaeae (this study). There are many studies recording flower visitation and pollination by arboreal mammals (Lumer, 1980; Kress *et al.*, 1994), however the Succulent Karoo region has a rich representation of geophytes in which insect-pollinated flowers are situated close to the ground and thus pre-adapted for pollination by terrestrial rodents. Such flowers probably often receive exploratory visits by hungry rodents, and if these have mutations for traits that make rodents more effective than insects at transferring pollen, then it is not hard to imagine selection shaping flowers along the lines of a rodent pollination floral syndrome. This study, like the earlier one by Johnson *et al.* (2001), has shown that floral syndromes can be useful for generating testable hypotheses about the existence of particular pollination systems. The clade containing *C. scabromarginatum* and *C. coloratum* has two other species. Of these, *C.*

circinatum (Manning *et al.*, 2007) is possibly rodent pollinated because of its dull green colour and viscous nectar.

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CHAPTER 3

The chemistry and function of floral scent of a rodent-pollinated plant, *Colchicum scabromarginatum* (Colchicaeae)

ABSTRACT

The floral scent of the rodent pollinated geophyte, *Colchicum scabromarginatum* (Colchicaceae) is studied. Floral scent was collected with dynamic headspace extraction techniques. Coupled gas chromatography-mass spectrometry analyses of the headspace samples resulted in the identification of 47 compounds, with aliphatic esters being dominant. Experiments using captive *Aethomys namaquensis* mice in a Y-maze olfactometer showed that in the absence of any visual contact with *C. scabromarginatum* flowers, the mice were attracted to the flowers, suggesting that the floral scent is the primary attractant for rodents. In the Y-maze, mice were attracted to a combination of the three most abundant esters (hexyl acetate, ethyl butyrate and butyl acetate) over paraffin oil. However, they did not show a strong preference for any one of the three most common compounds when offered in binary choices between two different esters. Further studies will be required to determine if aliphatic esters are common compounds and attractants in the floral bouquets of rodent pollinated species.

Key words: *Colchicum scabromarginatum*, Colchicaceae, floral scent, nectar, esters, rodent pollination, southern Africa.

INTRODUCTION

Animal-mediated pollination is essential in the reproductive biology of many angiosperms and tends to be associated with floral syndromes that include cues such as floral colour, shape and size, morphology, phenology and scent, which are used by pollinators to seek, locate and visit flowers (Faegri and van der Pijl, 1979). Of these cues, floral fragrances have been the most difficult to characterize, as they are usually subjectively described (to the human nose). However, analytical methods used to investigate floral scent now allow for the exact chemical description of fragrances (reviewed in Raguso, 2001). Chemically, a floral scent bouquet may contain from one to more than 100 compounds (Levin *et al.*, 2001; Gerlach and Schill, 1991).

It is generally assumed that floral scent functions to attract animal pollinators to flowers (Faegri and van der Pijl, 1979), and that these visitors associate floral scents with a food reward (Knudsen *et al.*, 1999). In some cases, plants use scents that elicit the innate attraction of pollinators to sexual partners or brood sites (Schiestl, 1999; Ayasse, 2001). Scented nectar provides a direct and honest signal to nectar-foraging animals (Raguso, 2004). Scent compounds in nectar may, however, also have a function in defence physiology, antimicrobial activity and deterrence from predators and parasitoids (Pichersky and Gershenzon, 2002).

One of the traits that defines the non-flying mammal pollination syndrome is a "musky or yeasty" odour, which is predominantly produced at night (Turner, 1982). This is assumed to be the primary cue for long distance attraction as rodents have a well-developed sense of smell and the flowers are usually pollinated at night when visual cues would not be effective (Rourke and Wiens, 1977; Rebelo and Breytenbach, 1987). This type of system appears to be ideal for a study of floral scent and its effects on plant-pollinator relationships.

However, hardly any research has been done on the scent of rodent pollinated plants. *Colchicum scabromarginatum* (Colchicaceae) has been shown to be strictly self-incompatible and pollinated exclusively by rodents (Kleizen *et al.,* 2008). The objectives of this study are to provide a chemical description of the scent of a rodent pollinated plant (*Colchicum scabromarginatum*) and to determine if the entire scent blend as well as the individual compounds that dominate the scent blend are attractive to the pollinators.

MATERIALS AND METHODS

Study plant and study site

The geophyte *Colchicum scabromarginatum* displays many of the traits that characterize the therophilous syndrome, such as geoflorous, dull-coloured flowers which produce copious amounts of nectar and pollen, nocturnal anthesis and nectar production and flowers in tight inflorescences. The viscous, sugar-rich nectar also emits a strong odour (Kleizen *et al.*, 2008).

This study was carried out in the semiarid Succulent Karoo region of South Africa. The study site was Naries farm 30km north of Springbok (29°41'S, 17°39'E, elev. 794m where a large population of *C. scabromarginatum* (>1000 plants) occurs on a rocky hillside.

Floral scent collection and GCMS analyses

The odour samples were collected in the field during the spring of 2007. Since rodent pollinators are active mainly during the evening, odour samples were collected from 18h00 until 21h00. Scent was collected using dynamic headspace extraction methods. For each of the three samples collected, four inflorescences were enclosed in a polyacetate bag (15 cm by

15cm in size) and air was pumped from the bag at a realized flow rate of 50 ml/min through small cartridges containing the adsorbent polymer, 1mg of tenax® and 1 mg of carbotrap® activated charcoal. Control samples were taken from *C. scabromarginatum* leaves and surrounding air.

Analyses of scent composition were carried out using a Varian CP-3800 GC (Varian, Palo Alto, California) with a 30 m X 0.25 mm internal diameter (film thickness 0.25 μm) Alltech EC-WAX column coupled to a Varian 1200 quadrupole mass spectrometer in electron-impact ionization mode. In order to thermally desorb the samples from the cartridges, the following procedure was conducted. Cartridges were placed in a Varian 1079 injector equipped with a "Chromatoprobe" thermal desorbtion device (Dotterl and Jurgens, 2005). Helium at a flow rate of 1 ml min^{-1} was used as the carrier gas. The injector was held at 40 °C for 2 min with a 20:1 split, increased to 200 °C at 200 °C min-1 in splitless mode for thermal desorbtion, held for 10 min. After a 3 min hold at 40 °C, the GC oven was ramped up to 240 °C at 10 °C min-1 and held there for 12 min. Compounds were identified using Varian Workstation software with the NIST05 mass spectral library and verified, where possible, using retention times of authentic standards and published Kovats indices. Compounds present at similar abundance in the controls were considered to be contaminants and excluded from further analysis.

Y-maze olfactometer experiments

Four *Aethomys namaquensis* mice (two males and two females) were captured using 50 "gutter pipe traps" that were laid out amongst a *Colchicum scabromarginatum* population overnight. The captive rodents were then placed in separate tanks. This rodent species was recently shown to be the primary pollinator of *C. scabromarginatum* at this site (Kleizen *et al.,* 2008).

The response of the four mice to different scent stimuli was tested in a Y-maze olfactometer from 21 July until 26 July 2008. The Y-maze olfactometer is composed of three sections of clear Perspex pipe, one central tube and two tubes forming the arms of the "Y" with metal box compartments and fans fitted to their ends (Fig. 1). The fans drew air into the chamber from both ends simultaneously, sending the air and any scent cues from the two compartments down the tubes towards the mouse at c. 1000 ml/min. A mouse in the Y-maze cannot see into the compartment where a vial containing scent may be placed as it is separated by thick wire gauze. The positions of the different stimuli in each experiment were swapped periodically. During Y-maze tests, the experimenter was positioned at the top of the Y-maze so to minimize any influence on either arm of the maze. Each of the four tanks housing the mice had a trap door on one side of the tank which could be opened to place the entrance to the Y-maze into the tank. The rodents were trained and allowed to habituate inside a Y-maze olfactometer individually and sequentially, each of them in a single session of approximately three hours, until the mice moved around freely in the Y-maze and there appeared to be no preference for moving to either arm.

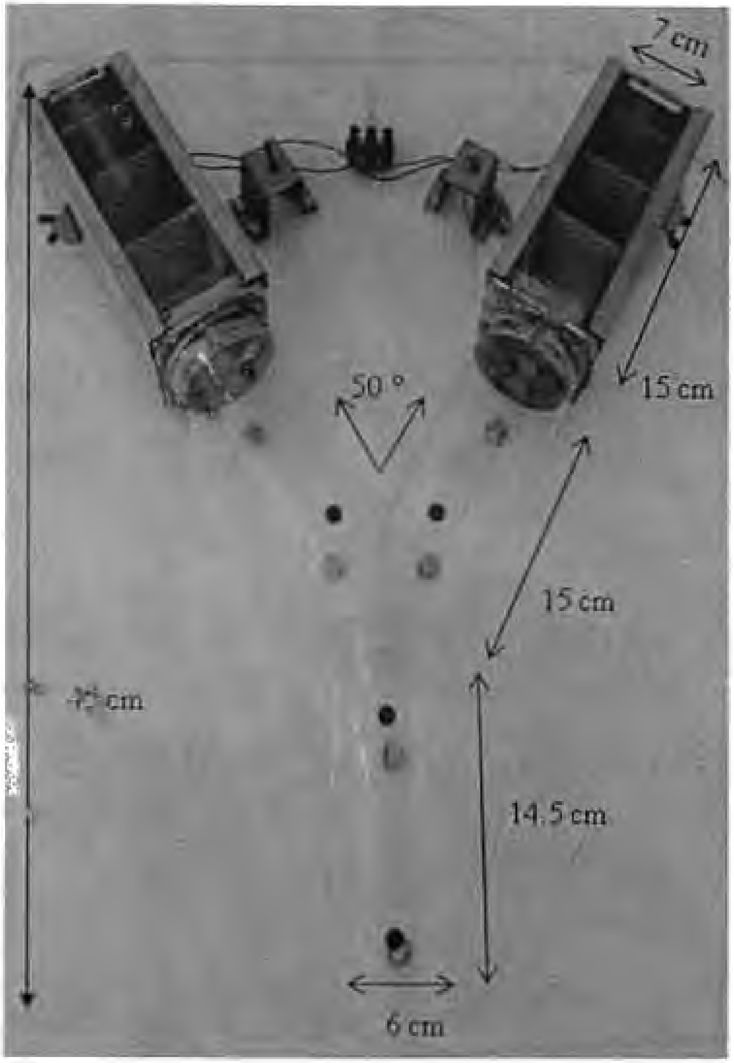

Figure 1. The dimensions of the perspex stem and arms of the Y-maze olfactometer used.

In order to determine if rodent pollinators are attracted to *Colchicum scabromarginatum* flowers based on scent alone, we placed freshly collected flowers in one chamber of the Y-maze and leaves of *C. scabromarginatum* in the opposite chamber. Each mouse completed 20 runs down the Y-maze towards one of the compartments.

To determine if mice were attracted to a blend of three of the most common esters found in the scent, mice were given a choice between a combination of equal amounts (20μL) of each of the three compounds diluted in paraffin oil (1:4000 dilution by volume) and pure paraffin oil. An observed versus expected chi-squared test was conducted in order to ensure that the mice were reacting to the chemical compounds and not solvent. Each mouse completed 15 runs down the Y-maze. Once the fans had been turned on, if a rodent failed to move from its tank into the Y-maze after two minutes, this trial was discarded. A Mann-whitney test was conducted in order to determine if the number of discarded trials differed significantly between the males and the females.

Finally, to determine if mice had a preference for any one of the three most abundant compounds (butyl acetate, ethyl butyrate and hexyl acetate), the separate compounds were diluted in paraffin oil (1:4000 dilution by volume) and tested against each other by placing 20μL of the paraffin oil solution of each compound onto filter paper. For these experiments, each mouse completed 15 runs. A choice by each rodent was recorded when the mouse moved into the compartment just before the stimuli. Type 3 Generalized estimating equation (GEE) analyses were conducted using Genmod (SAS system) to investigate the mice's preference for flowers over leaves, and each of the three compounds over the other two chemical compounds. These tests were conducted with a binomial distribution and the logit function was applied. The GEE analyses investigated the effect of the sex of the mice and the effect of the alternate compound available in the Y-maze on the preference for the compound in question.

RESULTS

Floral scent collection and GCMS analyses

In total, 62 scent compounds were detected in the *Colchicum scabromarginatum* nectar samples from which 47 have been identified (Table 1). These compounds generally fell into two compound classes, the aromatics and the terpenoids. Aliphatic esters dominated the scent samples, with ethyl butyrate (26.6%), hexyl acetate (11.8%) and butyl acetate (28.6%) being the most abundant compounds. Another abundant compound identified was the aromatic ether, anisole, which comprised 10.3% of the scent bouquet.

Table 1. Floral scent composition (%) of *Colchicum scabromarginatum.* Columns 1, 2 and 3 show the results from three replicate measures.

Class	**Compound**	**1**	**2**	**3**
Aliphatic alcohol	1-Octanol	0.00	0.13	0.17
Aliphatic alcohol	2-Octanol, (R)-	0.06	0.15	0.06
Aliphatic alcohol	3-Octanol	0.05	0.21	0.15
Aliphatic alcohol	Unknown(4-Hexen-1-ol, (Z)-)	0.04	0.05	0.05
Aliphatic aldehyde	Decanal	0.13	0.00	0.00
Aliphatic aldehyde	nonanal	0.10	0.05	0.10
Aliphatic ester	2-Butenoic acid, 3-methyl-, ethyl ester	8.54	0.78	0.70
Aliphatic ester	4-Penten-1-yl acetate	0.48	0.00	0.25
Aliphatic ester	Butyl acetate	38.11	20.47	27.10
Aliphatic ester	Butyl butyrate	0.00	0.00	0.25
Aliphatic ester	cis-3-Hexenylacetate	0.27	0.13	0.16
Aliphatic ester	Decyl acetate	0.00	0.00	0.12
Aliphatic ester	Ethyl butyrate	3.64	41.84	34.36
Aliphatic ester	Ethyl hexanoate	5.60	2.37	2.54
Aliphatic ester	Ethyl octanoate	1.28	0.18	0.03
Aliphatic ester	Hexyl acetate	16.35	12.91	6.13
Aliphatic ester	Octyl acetate	0.61	1.41	0.80
Aliphatic ketone	2-Decanone	0.06	0.12	0.11

Aliphatic ketone	2-Decanone	0.00	0.13	0.05
Aliphatic ketone	2-Heptanone	0.60	1.02	2.16
Aliphatic ketone	2-Hydroxy-3-pentanone	0.12	0.60	1.03
Aliphatic ketone	2-Nonanone	0.11	0.61	0.29
Aliphatic ketone	2-Octanone	0.76	2.46	0.85
Aliphatic ketone	2-Propanone, 1-methoxy-	0.00	0.00	1.42
Aromatic alcohol	Phenethyl alcohol	0.00	0.02	0.01
Aromatic aldehyde	Benzaldehyde	0.10	0.02	0.04
Aromatic ester	Ethyl benzoate	0.02	0.38	0.01
Aromatic ether	anisole	7.17	6.62	17.07
Aromatic ether	Anisole, o-methyl-	0.10	0.29	0.02
Irregular terpenoid	6-Methyl-5-hepten-2-one	0.23	0.22	0.19
Monoterpene	(Z)-Ocimene	0.12	0.57	0.00
Monoterpene	1,6-Octadien-3-ol, 3,7-dimethyl	0.00	0.08	0.00
Monoterpene	alpha-Pinene	6.98	0.00	0.00
Monoterpene	beta-Myrcene	6.62	0.98	0.41
Monoterpene	beta-Pinene	0.85	0.88	0.93
nitrogen compound	Trimethylpyrazine	0.05	0.14	0.17
Sesquiterpene	.(Z)-á-Farnesene	0.03	0.67	0.16
Sesquiterpene	à-Bergamotene	0.03	0.57	0.15
Sesquiterpene	á-Cubebene	0.00	0.00	0.02
Sesquiterpene	á-Sesquiphellandrene	0.00	0.44	0.14
Sesquiterpene	B-Caryophyllene	0.07	0.20	0.12
Sesquiterpene	Curcumene	0.00	0.03	0.02
Sesquiterpene	ë-Cadinene	0.00	0.01	0.01

Sesquiterpene	Germacrene D	0.04	0.03	0.00
Sesquiterpene	Unidentified sesquiterpene	0.03	0.03	0.02
Sesquiterpene	Unidentified sesquiterpene	0.03	0.08	0.00
Sesquiterpene	Unidentified sesquiterpene	0.00	0.38	0.11
Sesquiterpene	Unidentified sesquiterpene	0.00	0.02	0.02
Sesquiterpene	Unidentified sesquiterpene	0.00	0.01	0.01
Sesquiterpene	Unidentified sesquiterpene	0.00	0.12	0.04
Sesquiterpene	Unidentified sesquiterpene	0.00	0.10	0.00
Sesquiterpene	Unknown sequiterpene	0.09	0.18	0.06
Spiro compound	1,6-Dioxaspiro[4.5]decane, 2-ethyl-, (2R-trans)-	0.07	0.09	0.07
Unknown	1-Methoxycycloheptatriene	0.00	0.00	0.11
Unknown	2-Furanmethanol, acetate	0.00	0.03	0.00
Unknown	2-Heptanone, 5-methyl-	0.26	0.59	0.57
Unknown	Unknown (43,81)	0.04	0.08	0.06
Unknown	Unknown (54,82,126)	0.00	0.10	0.06
Unknown	Unknown (41,45,74)	0.00	0.00	0.02
Unknown	Unknown (41,87,97)	0.15	0.31	0.33
Unknown	Unknown (43,67)	0.00	0.12	0.13
Unknown	Unknown (43,55,98)	0.00	0.00	0.02

Responses of rodents in Y-maze olfactometer

All of the *Aethomys namaquensis* individuals exhibited a significant preference for the odour of the *Colchicum scabromarginatum* flower over the *C. scabromarginatum* leaves. However, the females showed a stronger preference (88% of trials in favour of the flowers) than then male mice (75% of the trials) ($\chi2 = 3.79$, P = 0.05).

A blend of the three common esters in the scent significantly attracted mice. Overall the mice preferred the compound blend over the paraffin oil in 71.6% of the 60 trials and there was no significant difference between the female and male mice ($\chi2 = 0.90$, $P > 0.05$) (Fig. 2).

There were more discarded trials from the male (mean of 4.2, SE = 0.25) than from the female rodents (mean of 2.3, SE = 0.26) ($Z = 3.33$, $P < 0.05$). We were not able to measure the time each rodent took to make a decision once it had entered the Y-maze olfactometer from its tank, as all movements were very quick.

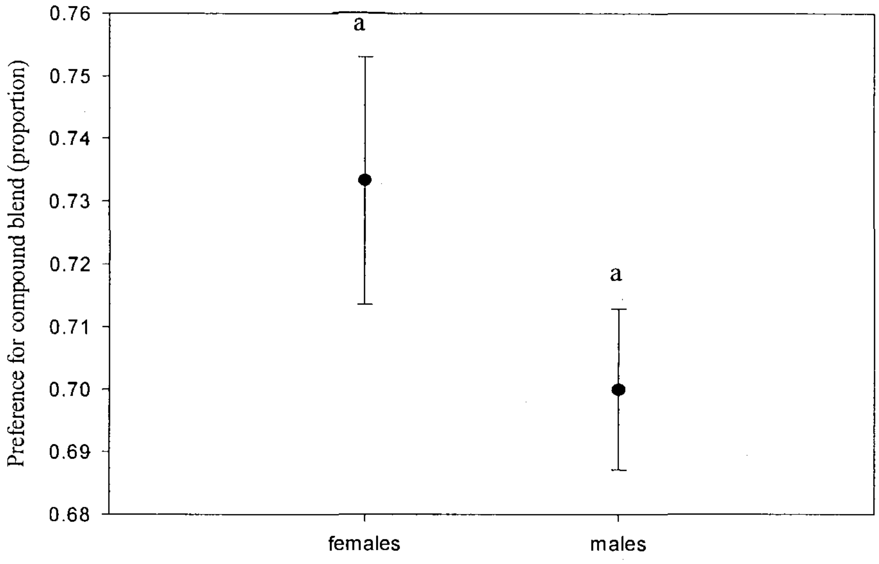

Figure 2. The proportion of trials where the male and female mice chose the blend of three compounds over paraffin oil in the Y-maze olfactometer. Bars represent ± 1 SE. Dissimilar letters indicate significant differences ($P < 0.05$).

Mice were highly attracted to the single compounds offered, entering the maze within seconds of the choice being offered. The mice's preference for ethyl butyrate was not affected by the sex of the mouse ($\chi2 = 0.09$, $P > 0.05$) nor whether the alternate compound in the Y-maze was butyl acetate or hexyl acetate ($\chi2 = 0.00$, $P > 0.05$). The overall preference for ethyl butyrate over the other compounds was 55.6%. Similarly, the preference for hexyl acetate was not affected by the sex of the mice ($\chi2 = 0.80$, $P > 0.05$) nor the alternate compound available ($\chi2 = 0.94$, $P > 0.05$). The overall preference for hexyl acetate was 46%. The females displayed no significant preference for butyl acetate over either of the other compounds. Males, however, preferred ethyl butyrate over butyl acetate ($\chi2 = 7.33$, $P < 0.05$) but preferred butyl acetate over hexyl acetate ($\chi2 = 8.24$, $P < 0.05$) (Fig. 3).

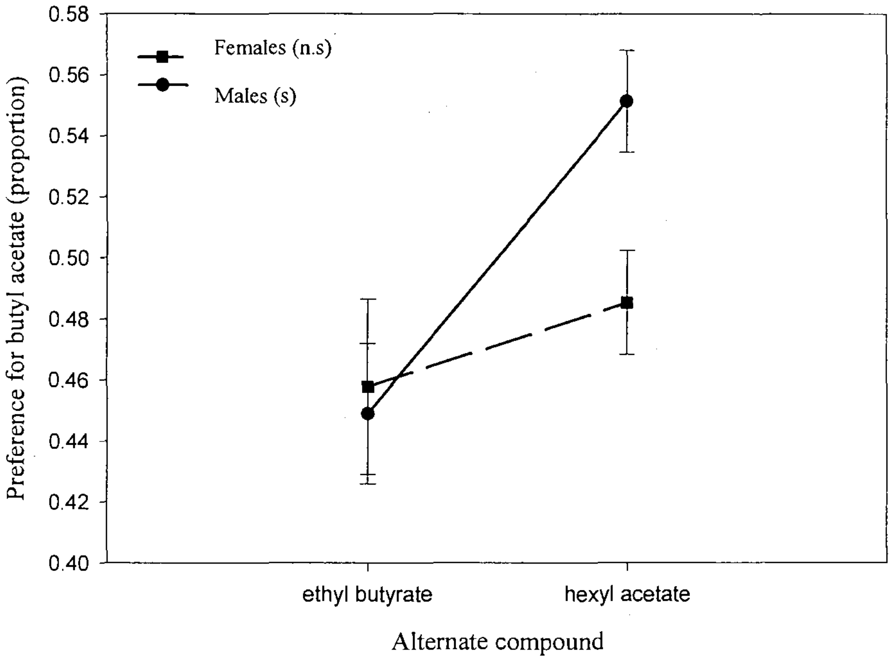

Figure 3. The preference of the male and female mice for the compound butyl acetate when the alternate compound in the Y-maze was either ethyl butyrate or hexyl acetate. Bars represent ± 1 SE. (s) and (n.s) = significant ($P < 0.05$) and non significant ($P > 0.05$) difference between the proportion of trials where the mice chose butyl acetate over the alternate compound in the Y-maze.

DISCUSSION

This study shows that the floral scent of *Colchicum scabromarginatum* is highly attractive to its rodent pollinators. In the first Y-maze olfactometer experiment, male and female

Aethomys namaquensis mice were exposed to floral odours in the absence of any tactile or visual contact. Nevertheless, all the mice preferred the odour of the *Colchicum scabromarginatum* flowers over the leaves. Our notion that olfactory cues are the only attractive signal to pollinators is supported by the fact that *C. scabromarginatum* inflorescences are dull green in colour. Furthermore, the rodents that pollinate this species are active mainly at night and would have to rely mainly on scent cues to find food.

The mice were attracted to a blend of the three most abundant esters in the floral scent over paraffin oil, suggesting that these compounds could be the basis for the attraction to the entire scent of *Colchicum scabromarginatum* flowers. The three most abundant compounds in the nectar, hexyl acetate, ethyl butyrate and butyl acetate, have been described as being reminiscent of fruits and herbs, apple, and pear respectively (Acree and Arn, 2004); which is fitting as these are food types that are often favoured by rodents.

Mice did not exhibit a strong preference for any of the three esters which were tested in pairwise choices. Since the alternate compounds in the Y-maze had no effect on the mice's preference for ethyl butyrate or hexyl acetate, we can conclude that the mice did not prefer ethyl butyrate or hexyl acetate over the other compounds in the Y-maze. A significant preference was observed by the male mice when the preference for butyl acetate was investigated. However, since this was only one occurrence of a significant preference between the compounds out of all the Y-maze experiments, we can only conclude that overall, there appears to be no clear preference for any one of the chemical compounds by the mice.

Results from studies on microtine rodents suggest that for males, the use of space is mainly affected by the spatio-temporal distribution of receptive mates, whereas for females, it is mainly affected by food distribution (Ims, 1987; Ostfeld, 1985). Therefore, a reason for the greater preference for flowers in the females than the males as well as the higher number of discarded trials in the male mice might be because they were not as interested in foraging for food as the females. The females' top priority was most likely searching for food, so they were more responsive to food stimuli. i.e. the odour of the *C. scabromarginatum* nectar or its chemical compounds.

This study has shown that esters are the most abundant compounds in the scented nectar of *Colchicum scabromarginatum*, and that rodents are attracted to the scent of the flowers. Additional scent analyses of rodent pollinated plants as well as rodent responses to various compounds are needed in order to determine if there are any common trends in the scent chemistry of rodent pollinated plants. In future, perhaps the scent chemistry of plants could be useful in predicting other species that may be rodent pollinated.

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CHAPTER 4

Variation in seed set amongst populations of a rodent pollinated geophyte, *Colchicum coloratum*

ABSTRACT

The farming activities in the town of Nieuwoudtville in the Succulent Karoo region of South Africa has lead to the confinement of many plant and animal species to fragmented patches. The geophyte *Colchicum coloratum* subsp. *coloratum* (Colchicum) was found to grow in five patches of variable sizes in and around Nieuwoudtville. This species is dependent on rodent visitation for seed production. The influence of variation in population size and plant size on seed set was investigated as well as whether there is pollen limitation in this species. A pollen-supplementation experiment revealed that there is pollen limitation in *C. coloratum*, and that much of the natural seed set could be the result of pollinator-mediated selfing. The five populations appeared to have different rodent abundances. However, population size did not have an effect on seed set, nor the abundance of rodents in the area. This suggests that the mutualism between *C. colchicum* and its rodent pollinators is robust, and that habitat fragmentation in Nieuwoudtville has not yet affected the seed production of this geophyte.

Keywords: *Colchicum coloratum* subsp. *coloratum*; Colchicaceae; pollen limitation; seed set; population size; plant size; habitat fragmentation; rodent pollination; Succulent Karoo.

INTRODUCTION

The pervasive nature of habitat fragmentation makes it one of the most important factors threatening the survival of plant and animal species (Saunders *et al.*, 1991). In order to predict what the effects of habitat fragmentation will be on a particular plant species, the factors that determine and effect the plants' seed set need to be investigated. There are a variety of factors that may limit seed production in natural populations, such as the availability of suitable pollinators and pollen (pollen limitation), the availability of resources (resource limitation), herbivory or predation of plant structures and unfavourable environmental conditions (Vaughton and Ramsey, 1995).

Habitat fragmentation can have disruptive effects on plant-pollinator mutualisms (Lamont *et al.,* 1993; Aizen and Feinsinger, 1994a; 1994b; Ågren, 1996; Murren, 2002). Small plant populations are expected to be less attractive to pollinators than large populations (Sih and Baltus, 1987), which could result in certain plant populations not being visited by pollinators. There are however, some plant-pollinator mutualisms that appear robust against habitat fragmentation, with pollinators mediating gene flow between fragments (Dick, 2001; White *et al.*, 2002). In a particular plant population, flowers are distributed at a number of levels; flowers may be clustered into inflorescences, inflorescences may be clustered on a single plant, and then the individual plants may be clustered close together or far apart. A pollinator must make decisions concerning how best to exploit the plants and the associated rewards (Goulson, 2000). Several studies have shown that large floral displays are "optimal" (Lack, 1982; Bell, 1985; Queller, 1985). Plants with many flowers have also shown to attract more pollinators which do visit flowers for longer (Klinkhamer *et al.*, 1989; Klinkhamer and de Jong, 1990).

The isolation and reduction in the size of plant populations are also common consequences of habitat fragmentation, which in turn can reduce the likelihood of pollination (Ågren, 1996; Morgan, 1999). Many studies have shown that foragers spend shorter time and visit fewer flowers in small patches (Kinkhamer *et al.,* 1989; Klinkhamer and de Jong, 1990; Brody and Mitchell, 1997). This may result in lower seed set as a consequence of a reduction in the quantity and quality of available pollen, which constitutes an ecological Allee effect and genetic Allee effects respectively (Ellstrand and Elam, 1993).

Colchicum coloratum subsp. coloratum, found in the Succulent Karoo region of South Africa, grows in populations of varied sizes, separated by farmlands. This species has been shown to be dependent on rodents for seed set (Kleizen *et al.*, 2008). In order to determine how habitat fragmentation is affecting *C. coloratum*, I investigated variation in seed set amongst different populations of this species. The following questions: 1) Is there pollen limitation in *C. coloratum* populations with different rodent abundances? 2) Is seed set positively related to the size of the population? and (3) Are there any correlations between seed set and population size or plant size?

MATERIALS AND METHODS

Study species and study sites

Colchicum coloratum J. C. Manning and Vinnersten (Colchicaceae) is a geophyte with an annual vegetative cycle (Membrives *et al.,* 2002), that flowers in winter with geoflorous and robust inflorescences. Membrives *et al.* (2002) performed breeding system experiments on cultivated plants of *C. coloratum* that had been grown from seed and concluded that the species was "preferentially self-incompatible". This breeding system was confirmed by field experiments conducted by Kleizen *et al.* (2008). Self-fertilization can occur, but only by the means of a pollinator moving pollen between the flowers of an inflorescence. Therefore, this species does depend on its rodent pollinators for seed production.

This study was carried out in the semiarid Succulent Karoo region of South Africa, during August and September of 2007. Based on availability, five populations were chosen in the vicinity of the town of Nieuwoudtville. These populations were situated at the following sites: a slope on Glen Lyon farm (31°23'S, 019°09'E, elev. 742m), alongside a river on Glen Lyon farm (31°22'S, 019°15'E, elev. 703m), the Nieuwoudtville Flower Reserve (31°21'S, 019°08'E, elev. 747m), Matjiesfontein farm (31°28'S, 019°04'E, elev. 700 m) and Hotbergfontein farm (31°22'S, 019°12'E, elev. 758m). All of the populations occurred as a small cluster of *C. coloratum* plants within a sea of natural vegetation, and they were all on flat ground. All five populations were noticeably different in terms of population size and inflorescence size.

Natural seed set and pollen limitation

To determine if seed production was limited by insufficient pollination, a pollen supplementation experiment was conducted. At each population, ten pairs consisting of two closely situated *C. coloratum* plants (maximum 4 m apart) were randomly selected. In each pair, one plant received supplemental pollen and the other was marked as a control, and was left untreated. To accomplish pollen supplementation, pollen-laden anthers were collected from plants growing 4-6m from the experimental plant, and were brushed over the stigmas of the experimental plants, so to saturate them with pollen. At the end of the flowering season, seed set was assessed by counting the number of seeds per flower from all of the 20 inflorescences.

Earlier, Kleizen *et al.* (2008) conducted a breeding system experiment, which indicated the approximate numbers of seeds per flower when flowers were selfed, outcrossed or left unmanipulated. When this experiment was conducted, all the stigmas were saturated with pollen in order to ensure that the seed set was the result of pollen quality and not pollen quantity. The mean number of seeds per flower that resulted from the unmanipulated, self-pollinated, and cross-pollinated treatments were rounded off, and used as an indication of whether the plants in the present study were selfed, crossed or whether neither had occurred. In the present study, we therefore assume that if there are less than five seeds per flower, there has been no pollination activity. If there are more than five seeds and less than 50 seeds per flower, we assume self pollination has occurred; and if there are more than 50 seeds per flower, we assume that cross-pollination has taken place.

Rodent trapping

The total number of rodent individuals captured at each of the *C. coloratum* populations was taken as a measure of rodent abundance at each population. To test whether the rodent abundance varied noticeably between different populations of *C. coloratum*, live trapping was conducted. A total of 90 Sherman traps were set every evening for three consecutive evenings at each of the five *C. coloratum* populations. Traps were set at dusk and laid out in lines of 15 traps, with four metres between each trap; and all traps were baited with peanut butter and rolled oats. Traps were checked in the early morning (between 0600 and 0700) and the species of captured rodents identified. The weather conditions during the trapping at each population were very similar.

Differences between populations

In order to establish whether the population size and/or plant size affect seed set, the length and breadth of ten randomly chosen inflorescences was measured (in order to calculate inflorescence area) as well as the number of flowers per inflorescence at each of the five sites. The density of *C. coloratum* plants was also measured by counting the number of plants growing in four 5 x 5 m randomly chosen quadrats per population. The area of each of the five populations of *C. coloratum* plants was also measured (a measure of population size). Due to the fact that only five populations were included in this study, simple one-factor regression analyses were conducted on the data to search for any trends.

RESULTS

Pollen limitation in Colchicum coloratum *populations*

Supplemental hand pollination increased mean seed set by 57%. Seed set was significantly higher in the inflorescences that received supplemental pollen than the control inflorescences in all of the five populations (Fig. 1).

From the assumptions made based on the breeding system experiment, we assume that 64% of the seed set following the supplemental pollen treatment is the result of crossing; and 36% of the seed set was the result of selfing. On the other hand, of the 50 control inflorescences, we assume that 80% of the seeds set were the result of self-pollination, 12%, the result of cross-pollination and 8% of the inflorescences set less than 5 seeds, therefore we assumed that no visitation took place.

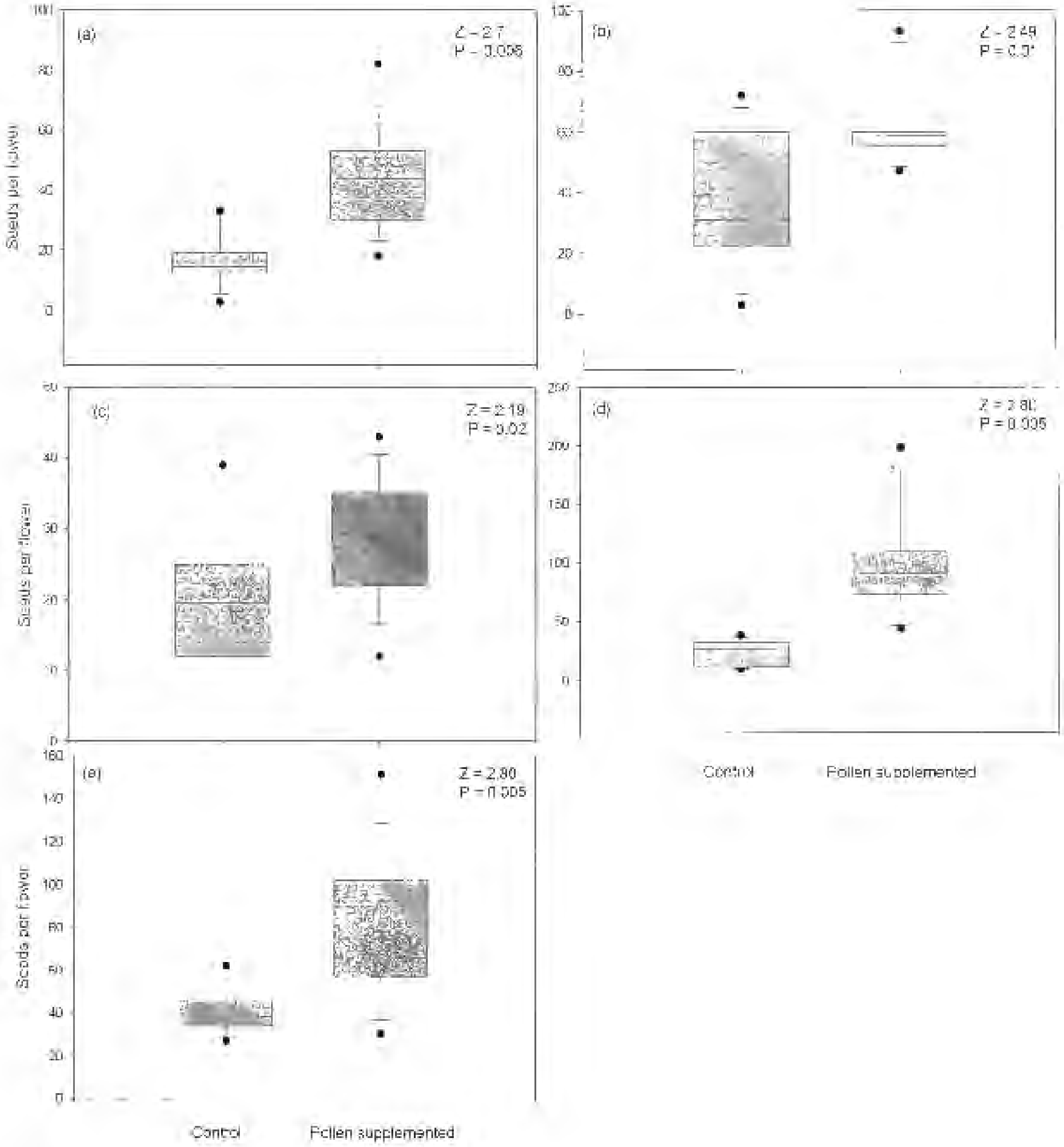

Figure 1. The median number of seeds produced by the flowers supplemented with pollen and the control flowers in the *Colchicum coloratum* population on the slope at Glen Lyon farm (a), alongside a river at Glen Lyon farm (b), the Nieuwoudtville Flower Reserve (c), Matjiesfontein Farm (d) and Hotbergfontein farm (e). Z values were obtained from the Wilcoxon test for paired samples. Samples sizes were 10 plants per treatment group, with a p-level < 0.05 considered significant.

Rodent abundance and Colchicum coloratum *characteristics*

From all five populations, a total of 19 individuals were captured, representing four rodent species: two murid species (Family Muridae, subfamily Murinae), *Aethomys namaquensis* (A. Smith) and *Mus minutoides* (A. Smith); one gerbil species (Family Muridae, subfamily Gerbillinae), *Gerbillurus paeba* (A. Smith); and one diurnal species, *Rhabdomys pumilio* (Thomas) (Family Muridae, subfamily Murinae). Rodents were most abundant at the population on Hotbergfontein farm with the capture of 6 individuals over the three trapping nights; whereas the least number of rodents were captured at the *C. coloratum* population on a slope on Glen Lyon (1 individual) (Table 1).

Table 1. Numbers and species of rodents captured at each of the *Colchicum coloratum* populations.

Site	Total no. rodents captured	Rodent species
slope, Glen Lyon	1	*Gerbillurus paeba (1)*
Alongside river,Glen Lyon	3	*Mus minutoides (2)* *Rhabdomys pumilio (1)*
Flower Reserve	4	*Aethomys namaquensis (3)* *Mus minutoides (1)*
Matjiesfontein	5	*Aethomys namaquensis (3)* *Rhabdomys pumilio (2)*
Hotbergfontein	6	*Rhabdomys pumilio (2)* *Gerbillurus paeba (4)*

There was no relationship between plant population size and mean seed set (Pearson's r = 0.0095, N = 6, P = 0.9858). Rodent abundance was however, positively correlated with the mean inflorescence size (area) (Fig. 2a) and the mean number of flowers per inflorescence (Fig. 2b) at each of the *C. coloratum* populations (Pearson's r = 0.913, N = 5, P = 0.021; and, Pearson's r = 0.951, N = 5, P = 0.012 respectively). Rodent abundance was not affected by either the size of the *C. coloratum* population (Pearson's r = 0.671, N = 5, P = 0.214) (Fig. 3a), nor the plant density (Pearson's r = 0.226, N = 5, P = 0.714) (Fig. 3b).

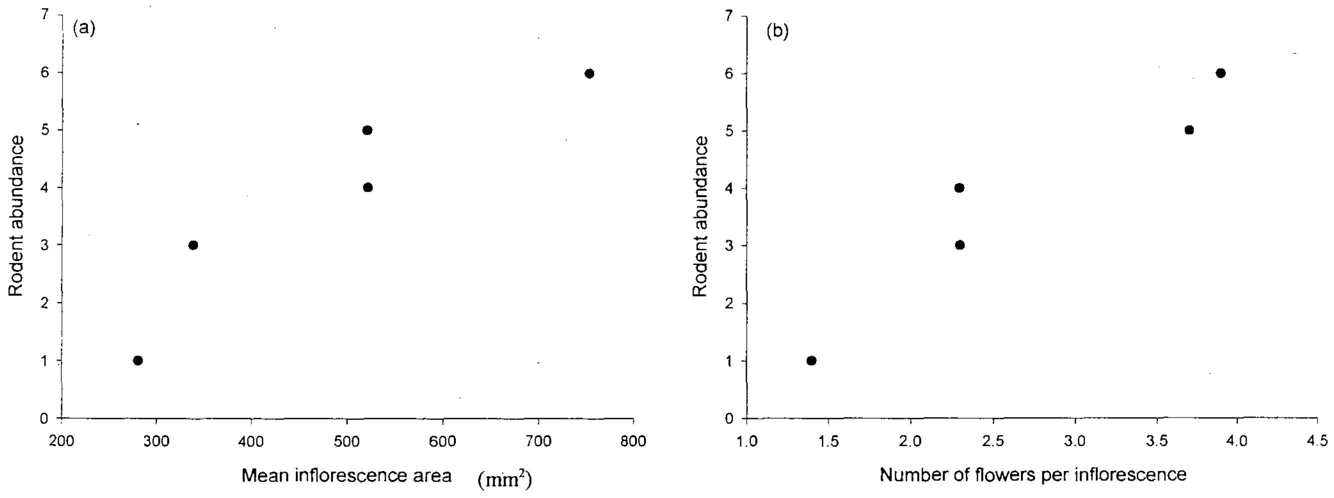

Figure 2. Relationship between rodent abundance and mean inflorescence area (mm^2) (a), and the number of flowers per inflorescence of *C. coloratum* (b).

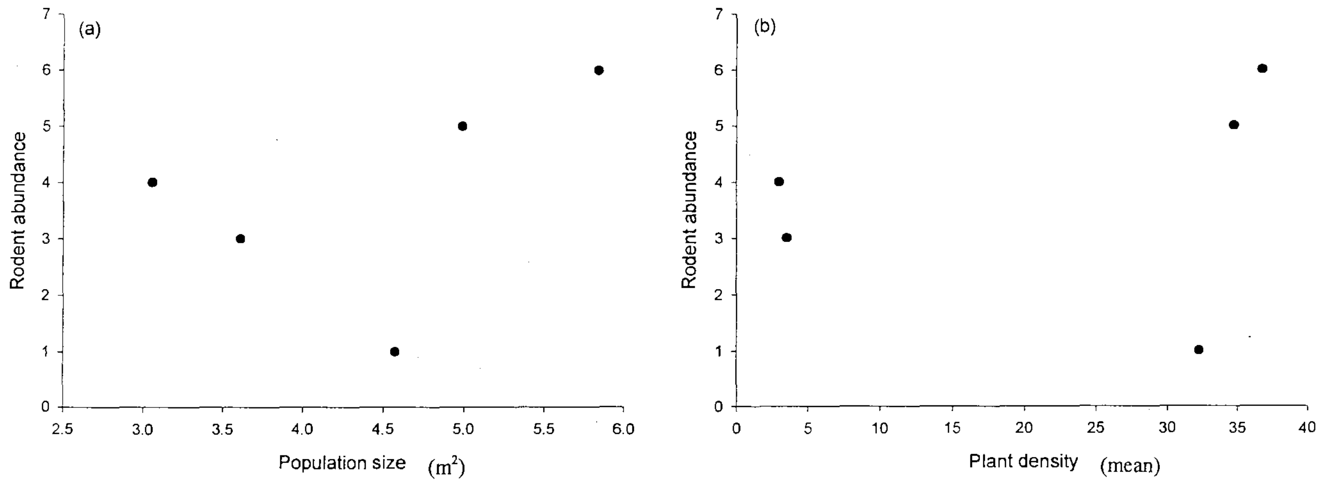

Figure 3. Relationship between rodent abundance and population size (m^2) of *C. coloratum* (a), and the plant density (mean number of plants growing in the four 5 x 5m quadrats) (b).

DISCUSSION

Pollen limitation

A previous study on *C. coloratum* demonstrated that autonomous self-pollination is limited and that seed production depends strongly on pollinators (Kleizen *et al.*, 2008). In all of the five populations included in this study there was a significantly lower mean seed set in the control inflorescences than in the inflorescences that received cross supplemental pollen; which indicates that in these populations, plants are pollen limited. Pollen limitation is either the result of flowers receiving too few visits by pollinators, or pollinator behaviour that results in insufficient amounts of compatible pollen being deposited on stigmas (Vaughton and Ramsey, 1995). Kleizen *et al.* (2008) showed that when plants were left untreated, hardly any seeds were set. Therefore the high degree of selfing that was seen in the control plants of the pollen-supplementation experiment must be the result of rodent visitation. Thus pollen limitation in populations of *Colchicum coloratum* is probably the result of the high degree of pollinator-mediated geitonogamous self-pollination. Because self-fertilization can occur, these *C. coloratum* plants are able to achieve the potential benefits of outcrossing while at the same time lessening the impacts of pollen limitation (Vaughton and Ramsey, 1995).

Since small mammals constitute the first link in the food chain of many carnivores and raptors (Lima and Dill, 1990), predator avoidance plays an important role in rodents' search for food. Therefore, from a rodent's perspective, it is safer to lap up the nectar in all the flowers in one inflorescence (allowing for geitonogamous self-pollination) before it moves to another inflorescence as it will be more vulnerable to predation while running in between plants. Kleizen *et al.* (2008) observed captive individuals of *Aethomys namaquensis* and *Gerbillurus paeba,* feeding on nectar from *C. coloratum* inflorescences. Both individuals moved between all the flowers of one inflorescence before moving to another inflorescence. Therefore, cross-pollination is likely to only occur when a rodent moves from the last flower of one inflorescence to the first flower it visits on another inflorescence, thus explaining the observed high degree of selfing.

Effects of different population parameters

The Allee effect is broadly defined as a low per capita rate of increase of small populations relative to larger ones (Stephens *et al.,* 1999). The lack of any relationship between *C. coloratum* population size and seed set in *C. coloratum* suggests that there are no Allee effects in the studied populations. Even a population with a low rodent abundance produced a similar mean number of seeds as did the populations with greater rodent abundances (Fig. 1). This suggests that even small rodent populations are sufficient to pollinate the plants in an area.

Rodent abundance was positively correlated with inflorescence size and the number of flowers per inflorescence. If it is assumed that the amount of nectar in an inflorescence is positively correlated with inflorescence size; then a likely explanation for this is that the larger, more conspicuous, and more rewarding an inflorescence, the more rodents venture out into the field to access the nectar of *C. coloratum*. The size of the *C. coloratum* population and the density of the plants had no effect on rodent abundance. This is probably because a rodent is likely to visit a few, large inflorescences that are positioned close to its burrow or a position of shelter. A rodent will not be able to take advantage of a large population of inflorescences and move long distances in fear of predators. Sih and Baltus (1987) showed that in larger sized patches of catnip, visitation rates were higher for honey bees and bumblebees, but lower for solitary bees. This indicates that population size has different effects on different pollinators. Although there are many studies showing the positive correlation between plant density and pollination success (Silander, 1978; Kunin, 1992, 1993; Allison, 1990; House, 1993), until now, no study has looked at the effects on a rodent-pollinated system.

Conclusions

Our study shows that pollen limitation in natural populations of *C. coloratum* is probably the result of reduced pollen quality (i.e. self pollen) rather than the result of low pollinator visitation, because stigmas were all fully covered with pollen. Rodent abundances had no effect on seed set, therefore even when there were small rodent populations, *Colchicum coloratum* was adequately pollinated. Rodent abundance was positively correlated with inflorescence size and number of flowers per inflorescence, but not with *C. coloratum* population size or plant density. Therefore, it seems as though display on an individual plant level is more efficient at attracting rodents than on a population level. Despite the *C. coloratum* occurring in patches separated by farming activities, it was shown that all the populations had similar seed production. This indicates that the mutualism between *C. coloratum* and its rodent pollinators appears to be robust, and that at present, this species is resilient to habitat fragmentation.

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CHAPTER 5

Summary

The main aim of this study was to determine whether *Colchicum scabromarginatum* and *C. coloratum* are rodent pollinated species. In addition, I examined other aspects of the reproductive biology of these species, such as the breeding system, the effects of excluding rodents from *C. scabromarginatum* and *C. coloratum* plants, and the nectar secretion patterns. Having successfully determined that they are rodent-pollinated, I examined two further aspects related to rodent-pollination in these species. Firstly, I investigated the chemistry of the scented nectar of *C. scabromarginatum* and its attractiveness to rodent pollinators. Lastly I investigated pollen limitation and the natural variation in seed set amongst populations of *C. coloratum*, since populations of *C. coloratum* were noticeably different in terms of population size and plant size. In particular I was interested whether this mutualism between geophyte and rodents is intact at all sites.

My results indicate that *Colchicum scabromarginatum* and *C. coloratum* are the first species in the family Colchicaceae to be recorded as being rodent pollinated. This extends the rodent pollination system to two African geophytic lineages – Hyacinthaceae (Johnson *et al.*, 2001) and Colchicaceae (present study). The Succulent Karoo region has a rich representation of geophytes in which insect-pollinated flowers are situated close to the ground and thus pre-adapted for pollination by terrestrial rodents. Such flowers probably often receive exploratory visits by hungry rodents, and if these have mutations for traits that make rodents more effective than insects at transferring pollen, then it is not hard to imagine selection shaping flowers along the lines of a rodent pollination floral syndrome. This study, like the earlier one by Johnson *et al.* (2001), has shown that floral syndromes can be useful for generating testable hypotheses about the existence of particular pollination systems. The clade containing *C. scabromarginatum* and *C. coloratum* has two other species. Of these, I suggest that *C. circinatum* is possibly rodent pollinated because of its dull green colour and viscous nectar.

Colchicum scabromarginatum is strongly self-incompatible, therefore depends on rodents exclusively for seed production. *Colchicum coloratum* also depends on rodent visitation. However, this species is partially self-compatible, thus autonomous selfing and opportunistic visitation by birds may also contribute to seed production. Nectar volume and concentration increases in the evenings to coincide with nocturnal activity patterns of the rodent pollinators. The exclusion of rodents from *C. scabromarginatum* and *C. coloratum* results in a significant decrease in seed set. Vertebrate exclusion, however, does not affect the seed set in the related species *C. hantamense*, which has floral traits associated with insect pollination.

Aethomys namaquensis mice, the most common pollinators, are able to assess the presence of *Colchicum scabromarginatum* flowers on the basis of olfactory cues alone. The floral scent contains at least 62 chemical compounds, which fall into three main classes, aliphatics, aromatics and terpenoids. Aliphatic esters, reminiscent of fruit and herbs, dominate the scent. Mice preferred a mixture of the most abundant esters, butyl acetate, ethyl butyrate and hexyl acetate, over paraffin oil. They did not exhibit strong preferences when offered in binary choices between two different esters. Additional scent analyses of rodent pollinated plants as well as rodent responses to various compounds are needed in order to determine if there are any common trends in the scent chemistry of rodent pollinated plants. In future, perhaps the scent chemistry of plants could be useful in predicting other species that may be rodent pollinated.

There is pollen limitation in natural populations of *C. coloratum*. This may be due to the lack of sufficient quantity of pollen or, alternatively, the result of pollinator-mediated selfing which results in reduced seed set. Since stigmas were saturated with pollen, the pollen limitation is most likely explained by pollinator-mediated selfing. Because some self-fertilization can occur, *C. coloratum* plants are able to achieve the potential benefits of outcrossing while at the same time lessening the impacts of pollen limitation. The rodent abundance in a population of *C. coloratum* does not have an effect of seed set, therefore even small rodent populations are efficient for pollination. In terms of attracting rodent visitation, inflorescence size appeared to be more important than population size. Despite the *C. coloratum* occurring in patches separated by farming activities, it was shown that all the populations had similar seed production. This indicates that the mutualism between *C. coloratum* and its rodent pollinators appears to be robust, and that at present, this species is resilient to habitat fragmentation.

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