Pollination Ecology of Mesembs

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Abstract

Mesembryanthemaceae is one of the main plant families in the Succulent Karoo biome of South Africa. While its pollination ecology still remains largely unstudied, the system is thought to be overall generalised. This study sought to verify whether Mesemb species flowering during September and October in Vrolijkheit Nature Reserve have generalised or specialised pollination interactions. The determinants of insect choice to a particular plant species were investigated and possible mechanisms adopted by different plant species to prevent interspecific pollen transfer were explored. The system was found to have a generalisation level of 24.7%, indicating that one of four possible interactions actually took place. A relatively high degree of overlap in insect visitors was found between the three white-flowered species (*Mesembryanthemum longistylum*, *Phyllobolus grossus* and *P. splendens*). Colour was one of the main determinants of insect choice, whereby *Drosanthemum speciosum*, the only red-flowered species in the study showed the highest degree of specialisation. Both scent and nectar production were relatively important in attracting flower visitors. Seasonality in flowering appeared to be a very important mechanism used to reduce overlap in insect visitors, especially among intrageneric species and those that had flowers of the same colour. Daily patterns in scent and nectar production also appeared to play a role in lowering pollinator-sharing. Given the general floral structure of most Mesemb flowers, these mechanisms are likely to be very important in contributing to species reproductive isolation and the low occurrence of hybrids recorded in natural conditions.

Introduction

Mesembryanthemaceae is one of three main succulent families in the world with Cactaceae and Crassulaceae, comprising approximately 2000 species each (Ihlenfeldt, 1994). More than 90% of Mesemb species, however, occur in the Succulent Karoo Region of southern Africa, an area that covers about 200,000km^2 (Ihlenfeldt, 1994). This region is characterised by great habitat heterogeneity and reliable winter-rainfall (Cowling & Hilton-Taylor, 1999). The high predictability in precipitation causes the majority of plants to invest in reproduction and produce large amounts of seeds (Cowling *et al.*, 1999). As most plants are obligate outcrossers and rely on the recruitment of seedlings for persistence, pollinator attraction is essential and results in mass multi-species displays, especially in late autumn and spring (Cowling *et al.*, 1999). The flora in the Succulent Karoo comprises mainly Aizoaceae (among which are Mesembs), Asteraceae, Poaceae, Iridaceae and Geraniaceae, which show very high levels of endemism. 40.3% of species are endemic, while at generic level, endemism is 9.2% (Cowling & Hilton-Taylor, 1999). In Mesembs, species and genus density is extremely high, with 53 genera or subgenera found in a degree square (Ihlenfeldt, 1994). Also the fauna of the Succulent Karoo shows a high degree of endemism, particularly among certain insect groups, such as monkey beetles (Hopliini), which have their centre of species richness in this region (Vernon, 1999). Hymenoptera is also characterised by high levels of species richness, which is often correlated to those in the flora, particularly the flowering annuals (Vernon, 1999). Specialisation in the pollination systems of certain plants and adaptive radiation with their pollinators has been documented in the Succulent Karoo, for example in the pollination of *Diascia* and *Hemimeris* by the oil-collecting *Rediviva* bees (Whitehead & Steiner, 1985) or in pollination of Iridaceae by monkey beetles (Goldblatt *et al.*, 1998). A number of genera of colletid bees and masarid wasps also tend to show specialised relationships with members of the Mesembryanthemaceae, which again seem to have contributed to each others' evolution (Vernon, 1999).

As the Succulent Karoo is a winter-rainfall desert that was created 5Mya, and members of Mesembryanthemaceae were first identified in the pollen records dating back to the end of the Pleistocene (Desmet *et al.*, 1998), many have wondered how this family could have speciated at such a rapid rate. Johnson (1996) indicated that in the Cape flora certain plant families have radiated in their vegetative characters, reflecting an adaptation to the physical environment; others have speciated according to their floral morphologies, through a pollinator-driven process. In Mesembs there is an unrivalled variety of life forms, ranging from annuals to stem succulents with deciduous leaves, from forms that spend all or part of their life-cycle sunken into the ground to small trees that reach three meters in height (Ihlenfeldt, 1994). The phenomenon of neoteny has been recognised as an important process in the evolution and speciation of members of this family, causing much variation in vegetative characters, such as in the genus *Argyroderma* (Hartmann, 1975) and members of *Mitrophyllinae* (Ihlenfeldt, 1975). In addition to plant-size and leaf form, species have also differentiated according to investment strategy, choosing between withstanding droughts as adults or survival through seeds, taking advantage of periods of above-average rainfall (Ihlenfeldt, 1994). All these adaptations are responses to environmental pressure and physical gradients in geology, geomorphology, temperature, humidity level, soil pH and ion content, which characterise the Succulent Karoo (Ihlenfeldt, 1994).

On the contrary, floral structure does not show much variation: most genera have dish to bowl-shaped flowers with many large showy petals (Ihlenfeldt, 1994), suggesting pollinator-driven speciation to be absent in this group of plants. Monkey beetles, however, appear to have caused adaptive radiation in geophytes, but as these pollinators favour flattened, radially symmetrical flowers, convergent evolution still results in conservative floral morphology (Goldblatt *et al.*, 1998). Although there are very few studies that have looked at pollination ecology of Mesembs (but see Struck, 1992; 1994), there is a belief that the system is overall generalised (Ihlenfeldt, 1994), as one would expect given the general floral structure. Some instances of more specialised interactions have been recognised in certain genera, for example in the pollination of certain pale-coloured Mesembs by masarid wasps, which tend to be their most common and reliable visitor (Gess & Gess, 1989). Also in *Erepsia* and *Conophytum* different species have evolved mechanisms to attract a more limited suite of pollinators, ranging from lepidopterans in some to bombyliids in others (Liede *et al.*, 1991). Struck (1994), on the other hand, in his study on insect visitors to Mesembs and Asteraceae found that Hymenoptera were the most frequent order of insect visitors, followed by bombyliid bee flies (Diptera) and monkey beetles (Coleoptera: Hopliinae). Some night-flowering species that were moth and hawkmoth-pollinated were also recognised, although no flower visitors were encountered on these during the study (Struck, 1994). Struck's conclusion was that the system was generalised, with insects showing opportunistic behaviour, which varied according to climatic fluctuations (mainly in rainfall) from year to year.

From the above observations (generalised pollination, unspecialised floral structure), one could speculate that competition for pollinators, hybridisation of different species and stigma clogging with foreign pollen is relatively common. On the contrary, there are very few recorded instances of

Mesemb hybridisation in nature (Hammer & Liede, 1990; Liede *et al.*, 1991; Ihlenfeldt, 1994). It is not entirely clear at this point if this is due to a shortage of studies (incorporating also genetic analyses) looking at this aspect of Mesemb biology, if it is a consequence of the poor taxonomic resolution that still affects several genera (Chesselet *et al.*, 2002), or if there is in fact a mechanism that prevents hybridisation from occurring. In *Conophytum*, for example, although there are several species growing sympatrically, the occurrence of hybrids is very rare (Liede *et al.*, 1991). Liede *et al.* (1991) found only one hybrid out of 500 parent plants for five species growing sympatrically at four sites, although insects were seen moving between the different species, which were only set apart on the basis of flower colour. While in artificial conditions there have been some successful creations of hybrids, even some of these are too delicate to survive in a natural habitat (Hammer & Liede, 1990). On the other hand if Mesembs were suffering from reproductive interference or interspecific pollen flow one would expect to find character displacement in the flowers (provided these differences in floral characters were advantageous to coexisting species: Armbruster, 1985). If it is true that hybrids are rarer than expected, and reproductive interference by different species (i.e. stigma clogging) is also relatively uncommon (seen in the extensive radiation and high diversity this family has achieved in a relatively short period of evolutionary time: Smith *et al.*, 1998), a number of questions are bound to follow. How do species maintain their genetic integrity? How do they manage to be fertilised by pollen from individuals of their own species and not of others? How has reproductive isolation been achieved in this family?

Reproductive isolating mechanisms can exist at the pre-mating or post-mating level. The former includes temporal, ethological and mechanical isolation (Levin, 1971) and is the stage where pollinators play the most important role. At the post-mating level autogamy and incompatibility occur prezygotically, while hybrid inviability, sterility, breakdown or floral isolation act at the post-zygotic stage (Levin, 1971). The purpose of this study is to investigate several aspects of Mesemb pollination ecology, to see whether Struck's (1994) overall observation of a generalised pollination system within the Succulent Karoo holds true for Mesembs or whether the system is more specialised than hypothesised thus far. The research aims to examine the pollinators' role in contributing to the plant's reproductive isolation, so that if there are certain trends involving different pollinators visiting different plant species, the basis underlying the partitioning of the pollinator assemblage is investigated. I would hypothesise that species have maintained their separate identities by developing relatively specialised interactions with their pollinators, to avoid cross-pollination with different species. Yet their floral structure does not seem supportive of specialisation. Ollerton (1996) observed that although many flowers are specialised in their floral traits, they are still visited by a suite of different pollinators, an apparent paradox. I am interested in seeing whether the converse is true: although Mesembs appear to be generalised in their floral structure, is their pollination system as generalised as one would expect?

Potential ways in which interspecific pollen transfers can be reduced include the following:

- Pollinator specialisation
    1. floral morphology
    2. flower colour
- Separation in time
    3. diurnal cycle
    4. flowering season

Pollinator specialisation

1. Floral morphology

According to Hartmann (1991), flower-shape in Mesembs (Figure 1) is important in both attracting different pollinators and in positioning pollen on different parts of an insect's body, so as to prevent it from reaching the stigmas of members of species that have a different shape. Stamen-carpet, recess and some central-cone flowers appear to be mainly bee-pollinated; tubular and central-cone flowers are psycophilous and phalaenophilous; and there are small, nocturnal shallow flowers that also seem to attract moths (Hartmann, 1991). Regarding pollen positioning on the insect, in stamen-carpet flowers this is sternotribic, i.e. pollen is placed on the insect's sternum. In small central-cone flowers as insects insert their probosces into the cone their head gets covered with pollen. In large central-cone flowers, on the other hand, pollination can be nototribic (pollen carried on insect's back), peritribic (pollen covers insect's body as it searches for nectar inside the flower's cone) and pleurotribic (insect gets pollen on its sides as it enters the inner part of stamens). In recess flowers pollination is also peritribic. Some Mesembs are also believed to be wind-pollinated and therefore produce large amounts of dry pollen that can also be used as a second option in the case of scarcity of pollinators (Hartmann, 1991).

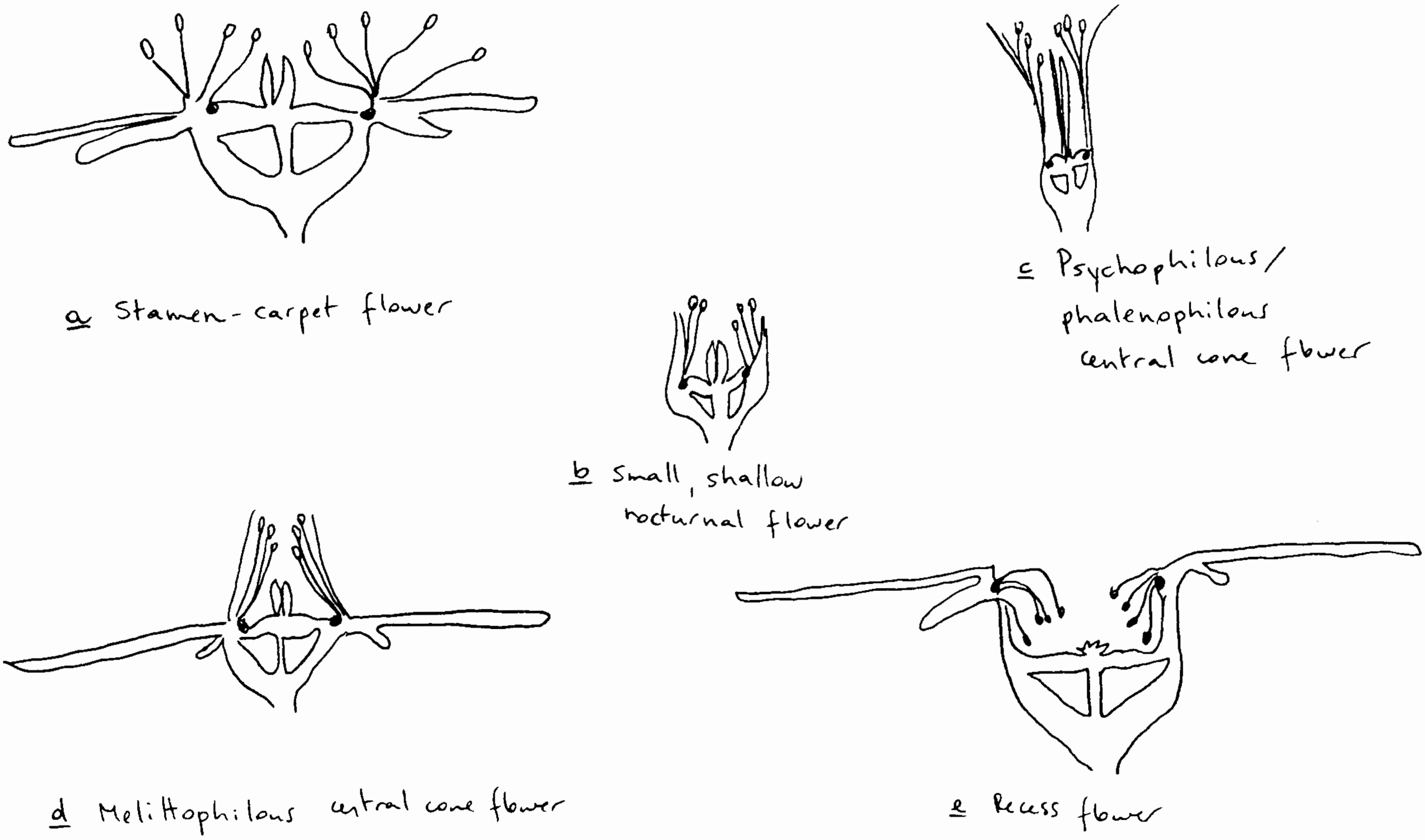


Figure 1: Mesemb flower-types according to Hartmann (1991).

Differential production of nectar and pollen by different taxa may also be a device to partition the pollinator assemblage. For example, butterflies and moths tend to be nectar collectors, while most bee species collect pollen as larval food (Kearns & Inouye, 1993). Different plant species may thus be favoured by different insect guilds. Struck (1995) observed that co-occurring species may also diversify the floral reward they provide: for example ruschioid Mesembs attract a wide suite of pollinators with copious amounts of pollen, while other species tend to attract more specialised pollinators (oligophilic spp.) by offering only nectar. Nectary structure (continuous vs. segmented ring) and shape (flat, sunken or raised), which varies according to different genera has also been suggested by Chesselet *et al.* (2002) to play a role in the pollination biology of Mesembs, although no details are provided regarding the mechanism.

2. Flower colour

According to Hartmann (1991), different-coloured flowers provide specific cues to different pollinators, with yellow, purple and white flowers mainly attracting bees; white-greenish and pale yellows favouring moths; and reds, whites and blues attracting butterflies. In order to avoid excessive sharing of pollinators between species within a genus, one would expect a high degree of intrageneric colour variation, especially in sympatric species. In genera like *Cephalophyllum*, *Drosanthemum* or *Dorotheanthus* this is seen, with flowers ranging from red to pink, yellow, orange, purple and white (Hartmann, 2002). Other genera, however, do not support this trend, and have species with mainly pale salmon, creamy and yellow flowers across the genus (e.g. *Sceletium*, *Mesembryanthemum*, *Prenia*) or pink-purple flowers (e.g. *Ruschia, Antimima*) (Hartmann, 2002). The question is then to look at whether species of similar colours share their ranges or whether they prevent cross-pollination by having different geographic ranges. Ihlenfeldt (1994) mentioned that intrageneric species that occur sympatrically are generally those to be most distantly related. In the Worcester Robertson Karoo, sympatric species are found to have flowers of similar colour. However, if one looks at their flowering season, numerous genera indicate that species with same-coloured flowers have staggered flowering peaks over the year to possibly create minimum overlap. For example *Ruschia* spp. in the Robertson area indicate that *R. intrusa* (purple-pink) flowers in June-July, followed by *R. caroli* (pink) in August-September, and by *R. multiflora* (white) and *R. approximata* (pink) that flower in October-December and November respectively. In other genera (e.g. *Phyllobolus*), however, no such pattern is found, and different species have flowers of similar colour with overlapping flowering periods.

Separation in time

3. Diurnal cycle

Staggering flower-opening times throughout the day and possibly night can also prevent pollinators from cross-pollinating flowers of different species. This pattern is seen in Mesembs, with flowers from different species opening at any time between the morning and noon, the afternoon, twilight and late evening, with differential releases of scent throughout the day (Smith *et al*., 1998). In *Conophytum*, for instance there are flowers that are open between dusk and dawn, those open throughout the day, from 10a.m. to 5p.m., and those that open for a few hours late in the afternoon (Liede *et al*., 1991). Also in *Aridaria* spp. the partitioning of the pollinator assemblage is achieved through differential flower opening. Although the four species in the genus share the same blooming season and have flowers of similar colours (white, with pale pink), *A. brevicarpa* opens at noon and closes at dusk, *A. vespertina* opens later in the afternoon and closes at dusk, *A. serotina* and *A. noctiflora* open at dusk, but the former closes in the late evening, while the latter closes in the morning (Hartmann, 2002). In order to avoid contamination with foreign pollen it pays different species to open at different times of the day. The question is then to see the degree of overlap in opening times between sympatric species and also whether different pollinators act at different times of the day. Fluctuations in the peak of insect activity and visitation on a particular plant species during the day have been observed in the Knersvlakte, with insects moving between species as their flowers begin to open (Allan Ellis, pers. comm.).

4. Flowering season

Flowering time during the year could also be significant, as this family indeed shows that species and genera may begin flowering in any one season and proceed for any length of time between one month and year-round (Smith *et al.*, 1998). This may be a mechanism used by intrageneric species to prevent, to a certain degree, overlapping of pollinators and thus pollen. Struck (1992) for example showed that in Goegab Nature Reserve the four *Ruschia* species present showed relatively little overlap in their flowering period over this three-year study, and all varied according to yearly precipitation. *R. elineata* had its yearly flower presentation maximum between June and July, in *R. cymosa* it was at the beginning of October, while *R. viridifolia* generally had a shorter flowering season, with the peak seen between start and mid-October. *R. robusta*, on the other hand, flowered late in October. In Paulshoek, staggered flowering time over the year also seems to be the mechanism whereby cross-pollination between different Mesemb taxa is prevented (Tim Hoffman, pers. comm.) and it could also be a method to prevent hybridisation from occurring.

My study

The aims of the research are

- to check which insect species pollinate which Mesemb species
- to assess which floral characteristics determine insect choice
- to assess in which way plant species prevent interspecific pollen transfer

Jordano (1987) states that "understanding how the number and strength of interactions are distributed among the species pairs is basic for analysing the evolution of mutualisms in a community". Building a pollination network showing all interactions between plant and insect species allows the calculation of connectance of the system (Jordano, 1987). This value, which shows the proportion of all possible interactions in the system that actually take place, indicates the level of generalisation of the system and thus the degree of reliance of different plant species on insect species and vice versa. The connectance value is very widely used in food webs, such as outlined by Yodzis (1980, in Jordano, 1987), but has recently also been very useful in community studies of pollination systems (Elberling & Olesen, 1999). The ways plant and animal species interact lie at the basis of an understanding of co-evolutionary change; by examining the connectance and the frequency distributions of mutual dependence one can understand constraints on coevolution (Jordano, 1987) and implications for conservation.

The results of this study will therefore not only contribute to an understanding of the potential implications of pollinator-driven speciation in this extraordinary plant family, but will also have significant conservation implications. In the Cape Floral Kingdom a number of very specialised mutualisms between certain plant species and a sparse and unusual pollinator fauna have been documented (e.g. long-tongued flies and *Lapeyrousia*, or the butterfly *Meneris tulbaghia* with *Disa* and several others, many of which probably still need to be discovered) (Bond, 1994). Similar studies show similar specialised mutualistic interactions for certain plant and insect species in the arid Karoo (e.g. oil-collecting bees and *Diascia*, *Parafidelia* bees and *Tribulus* and certain monkey beetles on Asteraceae spp: Vernon, 1999), although no conclusive results exist for Mesembs. As Mesembryanthemaceae is the main plant group present in the Succulent Karoo, and it appears highly vulnerable to further habitat change and destruction (Smith *et al.*, 1998), it is essential to understand its pollination ecology. In addition to seeing the direct effects habitat fragmentation has on the distribution of the different plant species, it is increasingly important to see the effect it may have on the loss of insect taxa, and consequently on the plants themselves. Insects in some areas are highly endangered by agriculture because of widespread herbicide and pesticide use, as well as the loss of nesting areas, and plants on which to lay eggs and on which larvae may feed due to changes in land-use and grazing too (Kearns *et al.*, 1998). It is these types of extinctions that are caused by failures in mutualistic systems that are cryptic in nature and often tend to be overlooked. Pollination is not only important for its aesthetic and ethical value, it has in fact been estimated that its total economic value amounts to US$112 billion per year (Kearns *et al.*, 1998). There are therefore a number of reasons that make it worthwhile to study the pollination system of this plant family.

Methods

Study Site

The fieldwork was undertaken in the Vrolijkheid Nature Reserve, situated in the Breede River Valley (Figure 2), approximately 15km south of Robertson in the south-western Cape region of South Africa. The vegetation of this region is known as the arid Robertson Karoo, part of the Worcester Robertson Karoo, which mainly comprises succulent species of the Aizoaceae and Crassulaceae, members of the Asteraceae and Iridaceae, as well as shrubs and small trees including the milk bush *Euphorbia mauritanica* and the sweet-thorn *Acacia karroo* (Cape Nature Conservation, 2001). Mountain renosterveld is seen on the upper ridges of the reserve. Being part of the Succulent Karoo biome, this region experiences predominantly winter rainfall, associated with westerly fronts and cut-off lows (Desmet & Cowling, 1999a). The mean annual precipitation varies between 150 and 300mm in Vrolijkheid, with two maxima experienced in April and June, while temperatures range from 2°C as the winter minimum, to even 42°C in the hottest summer days (Cape Nature Conservation, 2001). The altitude varies between approximately 200 and 600m in Vrolijkheid, with the highest peak reaching 635m. The mountains form part of the Cape Fold Belt range, which was formed during the Permo-Triassic and belongs to the Karoo and Cape Supergroups (Watkeys, 1999). The soils are derived from Malmesbury Shales and are shallow and pedologically young, ranging from sandy loam to sandy clays (Watkeys, 1999). Quartz intrusions are often found in rocky habitats on the top of hills, which contribute to high habitat diversity. The flowering peak of the 30 dominant species, which comprise 90% of the vegetation cover, occurs between August and October, and during this period spectacular displays of Mesembs can be seen on the hills in Vrolijkheid (Cape Nature Conservation, 2001).

Fieldwork

A preliminary visit was made on 15th August to assess the number of flowering Mesemb species present in Vrolijkheid and to collect a herbarium specimen of each plant, which was pressed and later identified to species level. Three subsequent visits were made on the 8th-9th and 17th-19th September, and 7th -9th October, each consisting of two full days of work. Visits were timed to coincide, when possible, with sunny weather conditions. I collected information on:

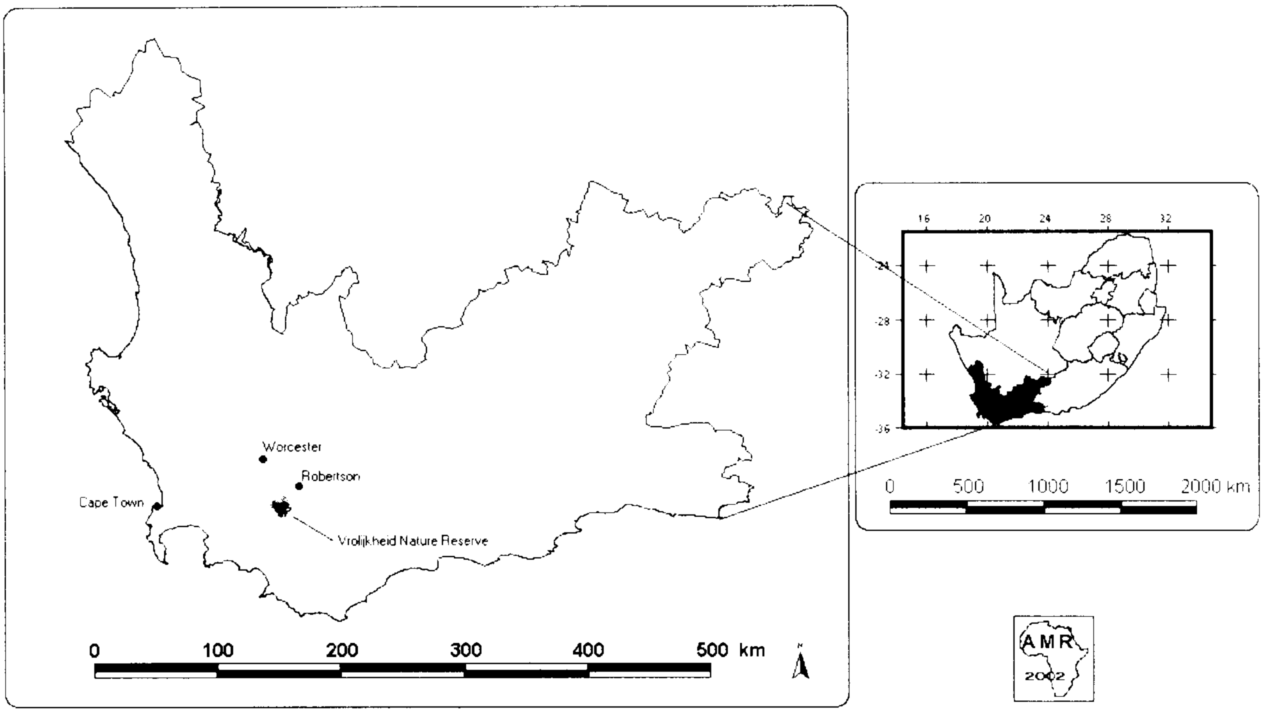


Figure 2: Map of the Western Cape in relation to the rest of South Africa (see inset), showing the location of Vrolijkheit Nature Reserve, where the fieldwork was undertaken.

1. Floral traits potentially influencing pollinator specialisation
2. Timing of floral display
3. Pollinator visits

1. Floral traits potentially influencing pollinator specialisation

During each of the three visits, I examined a number of floral traits that might influence pollinator attraction.

- To obtain an idea of the size of the display and potential attractiveness of different plant species to pollinators I estimated the proportion of buds that had already flowered on 15 plants per species, which had been chosen in the random quarter style. Measurements on flower depth (taken from the top of the anthers to the base of the calyx), width (i.e. diameter of flower) and width excluding petals were taken of three flowers on the first five of the above 15 plants. At a focal plant I assessed plant abundance, flower colour, presence of nectar lines, sexual system, dichogamy and flower functional type, based on Hartmann's (1991) categories.

- During the final field visit (8th and 9th October) nectar volume was measured for five flowers of three of the study species (*Phyllobolus grossus, P. splendens* and *Mesembryanthemum longistylum*). Nectaries in *Malephora latipetala*, *Drosanthemum barkerae* and *D. Speciosum*, also flowering at this time, were too small to allow any collection of nectar. Nectar was extracted by means of a 2μL capillary tube. Scent was assessed for the above six species by smelling 15 flowers per species and deciding whether the scent was intense (class 3), medium (class 2) or absent (class 1). Both scent and nectar volume measurements were undertaken every 1.5 hours from 10a.m. one day and 8.30 a.m. the following until 5.30 p.m.

2. Timing of floral display

- To get an idea of flowering season for each species five transects were walked randomly with respect to flowering during each visit. Each transect was 40m long, and every 2m the presence of flowering Mesemb species was recorded, in a 2X2m quadrat on one side of the transect.
- To determine opening time of a flower, in relation to both cloudy conditions and warm sunny days, I recorded temperature and wind-speed hourly between 8:30a.m. and 5:30p.m. Wind direction and cloud cover were also noted, the latter by dividing the sky into 8 parts and estimating how many of these were covered by clouds.

In order to assess whether different species avoid competition for pollinators and stigma clogging by differentiating on the basis of opening times, I recorded the relative percentage of plants with open flowers for each species every hour. I chose 10 plants per species at random, and established whether the majority of flowers on that plant were open or closed, in order to calculate the percentage of plants with open flowers.

3. Pollinator visits

- Throughout the day I made observations on pollinator visits to different species flowering. This included a number of focals made on an individual of a certain plant species for 15minutes, during which the plant was observed and all visitors to it were recorded. One specimen of each insect species was collected and identified to family level using available taxonomic keys. Type of insect activity was recorded (e.g. collection of food such as pollen or nectar, mating, perching ground etc.), and the numbers of flowers visited by each insect was recorded. By counting the total number of flowers on the plant it was possible to calculate visitation rates (both number of visits and of visitors per flower per hour) and see how this differed between different plant and insect species. Focals were made throughout the day, rotating between the flowering species.

- Observations on insect activity were made and visitors to plants were either collected or recorded throughout the day by walking around the study area, to see the change in activity with regard to time, the number of species that had open flowers, and weather conditions. Both methods of observation (focals and community-wide observations) were used with the aim of understanding how generalised or specialised the system is.

Data analysis

All plant (P) and animal (A) species were listed on the two axes of the matrix (M) beginning from those which had the greatest number of interactions to those with the fewest. The interaction between each plant and animal species was given as the average number of visits per flower per hour made by a certain insect species on a plant species throughout the study period. Different meloid, domestid and mordelid beetle species were lumped in one group, and thrips were ignored for the purpose of the study, given they were not seen to play a significant role in the transfer of pollen.

Connectance (C) was calculated by:

$$C = \frac{100 \times I}{M}$$

where I: no. of interactions observed

M: size of the matrix

$M = A \times P$

A canonical correspondence analysis (CCA) was used as an ordination method to split the insect species on the basis of flower colour. A CCA is a multivariate direct gradient analysis technique that displays an ordination, whereby the axes are constrained by linear combinations of environmental variables (Ter Braak, 1988). Total number of visitors of a particular insect species to a plant species during a focal were used and all insects which made less than five visits and all plant species that had less than 10 observations made on them were eliminated. The first two axes (that were the strongest) were graphed with the Canoco (Version 4.02) programme.

A cluster analysis of the six plant species that had more than 10 focals made on them (*Phyllobolus grossus*, *P. splendens*, *Drosanthemum barkerae*, *D. speciosum*, *Mesembryanthemum longistylum* and *Malephora latipetala*) was made using Statistica (Version 6.0). Total number of visitors of a

Table 1: Average (+/- S.D.) number of visits per flower per hour by different insect species to different flower species over the entire study period. The total number of 15min observations made on each plant species is given in brackets in the first column. Plant and insect species are ordered from those with the highest number of interactions to those with fewest.

Plant species (n observations)	Meloidae spp	Domestidae spp	Bombyliidae sp. 1	Apis mellifera	Satyridae sp. 1	Bombyliidae sp. 2	Mellitidae sp. 1	Mordelidae sp. 1	Melyridae sp. 1	Lepithrix sp. 1	Masaridae sp. 1	Tabanidae sp. 1
Phyllobolus splendens (19)	0.907 (1.618)	0.213 (0)		0.237 (0.229)	0.061 (0.027)	0.082 (0.036)	0.178 (0)	0.889 (0)	0.02 (0)			0.016 (0.006)
Drosanthemum barkerae (20)	0.102 (0.106)	0.013 (0)	0.074 (0.047)	0.16 (0)			0.169 (0.163)				0.072 (0.056)	
Mesembryanthemum longistylum (16)	0.276 (0.238)	0.213 (0)	0.127 (0.104)	0.437 (0.532)	0.089 (0.097)	0.047 (0.025)				0.025 (0)	0.151 (0.025)	
Malephora latipetala (14)	0.343 (0)	0.8 (0)	0.32 (0)			0.101 (0)		0.04 (0)				0.1 (0)
Phyllobolus grossus (13)			0.308 (0)	0.129 (0)	0.08 (0)				0.24 (0)	0.308 (0)		
Leipoldtia schultzei (6)	0.16 (0)	0.121 (0)					0.05 (0)	0.08 (0)				
Drosanthemum speciosum (21)	1.014 (1.434)		1.273 (1.357)									
Delosperma pageanum (2)		1.2 (0)										
Sceletium varians (3)	3.429 (0)	4 (0)										
Totals	6.231 (3.396)	6.56 (0)	2.102 (1.508)	0.963 (0.761)	0.23 (0.124)	0.23 (0.061)	0.397 (0.163)	1.009 (0)	0.26 (0)	0.333 (0)	0.223 (0.081)	0.116 (0.006)

Table 2: Connectance of the matrix including all plant and insect species

Connectance = 100 * I / M	C: connectance
M = A * P	M: size of matrix
A = 27	A: total no. of animals
P = 9	P: total no. of plants
M = 243; I = 60	I: total no. of observed interactions
Connectance = 24.7%	

Table 3: Connectance of the matrix excluding D. pageanum and S. varians

Connectance = 100 * I / M	C: connectance
M = A * P	M: size of matrix
A = 27	A: total no. of animals
P = 7	P: total no. of plants
M = 189; I = 56	I: total no. of observed interactions
Connectance = 29.6%	

Empididae sp. 1	Collettidae sp. 1	Megachilidae sp. 1	Pachycnema sp. 1	Heterochelus sp. 1	Eumenidae sp. 1	Anthophoridae sp. 1	Bombyliidae sp. 3	Lycaenidae sp. 1	Sarcophagidae sp. 1	Unidentified wasp	Unidentified fly 1	Unidentified fly 2	Small unidentified fly 3	Small unidentified brown beetle	**Totals**
0.56 (0)						0.046 (0.063)								0.16 (0)	3.369 (1.979)
0.026 (0.015)	0.466 (0.628)				0.013 (0)				0.091 (0.088)						1.457 (1.103)
												0.084 (0)			1.449 (1.021)
	0.933 (0.667)						0.343 (0)				0.02 (0)				3 (0.667)
		0.196 (0.144)													1.261 (0.144)
								0.044 (0)					0.027 (0)		0.482 (0)
			0.411 (0.227)	0.225 (0.134)											2.923 (3.152)
										0.114 (0)					1.314 (0)
															7.429 (0)
0.586 (0.015)	1.399 (1.295)	0.196 (0.144)	0.411 (0.227)	0.225 (0.134)	0.013 (0)	0.046 (0.063)	0.343 (0)	0.044 (0)	0.091 (0.088)	0.114 (0)	0.02 (0)	0.084 (0)	0.027 (0)	0.16 (0)	22.413 (8.066)

particular insect species to a plant species were used, after excluding meloids, domestids and species that were unidentified. The data were graphed with the city-block (Manhattan) distances as distance measure.

Results

The network in Table 1 indicates the number and strength of interactions between different plant and insect species. The connectance of the matrix is approximately 25% (Table 2), indicating that one quarter of all potential interactions between species actually takes place. If *Sceletium varians* and *Delosperma pageanum* are removed from the matrix, given very few observations were made on these species, the generalisation level of the matrix increases to nearly 30% (Table 3). The system shows a relatively high degree of specialisation, especially from the insects' point of view, but there are also a number of visitors that are seen on numerous plants.

Meloid and domestid beetles visited the greatest proportion of plants, and recorded the overall highest visitation rates: the two families combined comprised 57% of the total visitation of the whole system. As the majority of individuals observed were permanent flower visitors and were not seen travelling between flowers or plants, the matrix needs to be looked at also excluding these pollinators, that are most likely to play a minor role compared to stronger fliers such as honeybees or bee-flies. Excluding meloids and domestids, bombyliid sp. 1 and colletid bees were the insects with the highest number of visits per flower per hour, comprising 21.8 and 14.5% respectively of the total interactions in the system.

Among the plants, *Drosanthemum speciosum* was the one to show the highest specialisation among the species that were observed over more than ten focals, with a bombyliid responsible for 43% of visitation, and monkey beetles and meloids comprising the rest. *Phyllobolus splendens*, on the other hand, was visited by the largest number of insects and was also one of the species to have the overall second highest visitation rate. Once again mordelids, meloids and domestids visited high numbers of flowers (permanently). In their absence, empidids, honeybees and melittids were the most significant visitors.

The majority of other plants were also visited by relatively great numbers of insects.

In terms of overlap of insect visitors between different plant species, figure 3 shows that the two *Drosanthemum* species were relatively well isolated from the other species. *Phyllobolus grossus* and *Mesembryanthemum longistylum* were the two species to have the greatest overlap in insect visitors, followed by *P. splendens*. *Malephora latipetala* grouped relatively closely with the white-flowered species.

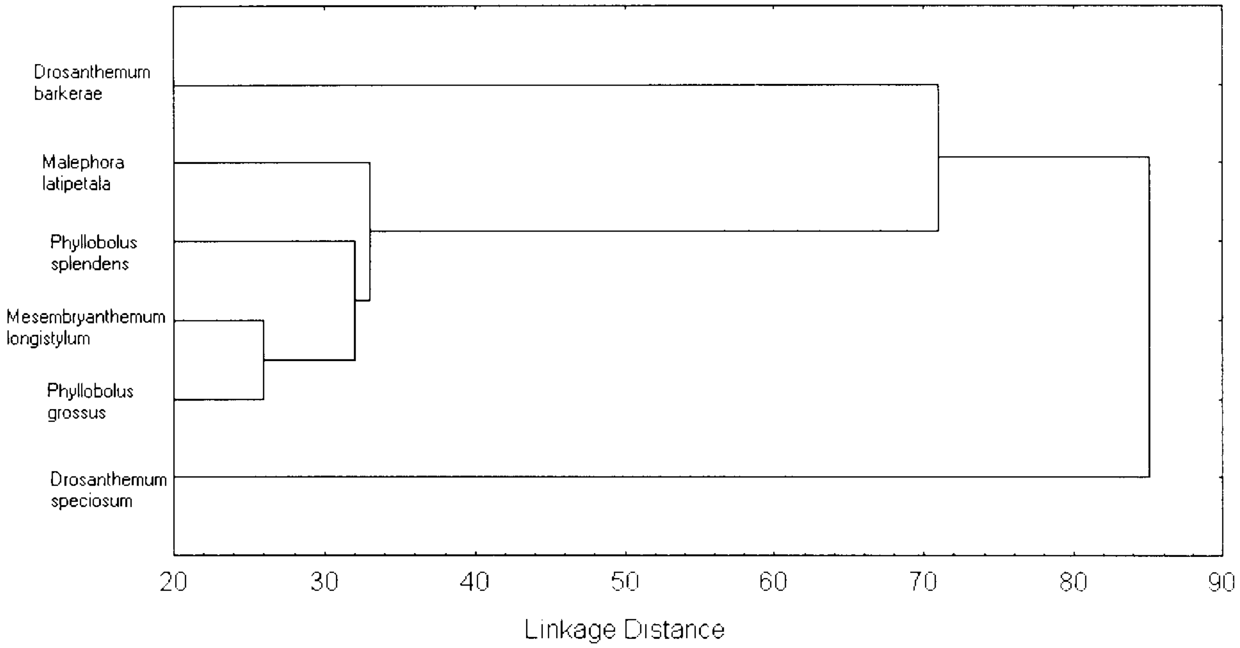


Figure 3: Cluster analysis for six Mesemb species on the basis of their insect visitors.

In table 4, a large number indicates a relatively low degree of overlap in insect visitors between different plant species. Therefore, once again, *D. speciosum* showed the least sharing of insects with the other species, followed by *D. barkerae*, which shared the most insects with *M. latipetala*. The three white-flowered species showed the highest degree of insect overlap, especially between *P. grossus* and *M. longistylum*, and *M. longistylum* and *P. splendens*.

Table 4: Proximity matrix indicating the merging distances between six Mesemb species

	Drosanthemum barkerae	*Drosanthemum speciosum*	*Malephora latipetala*	*Phyllobolus splendens*	*Mesembryanthemum longistylum*	*Phyllobolus grossus*
Drosanthemum barkerae	0	147	71	96	80	92
Drosanthemum speciosum	147	0	92	109	85	89
Malephora latipetala	71	92	0	47	33	33
Phyllobolus splendens	96	109	47	0	32	44
Mesembryanthemum longistylum	80	85	33	32	0	26
Phyllobolus grossus	92	89	33	44	26	0

1. Floral traits potentially influencing pollinator specialisation

Table 5 shows floral characteristics of the different plant species. Three out of nine species were pink, four were creamy-white, one was yellow and one red. Colour appeared to play a role in the choice of a number of insects (Figure 4), although insects visiting pink and yellow flowers grouped very closely in the ordination.

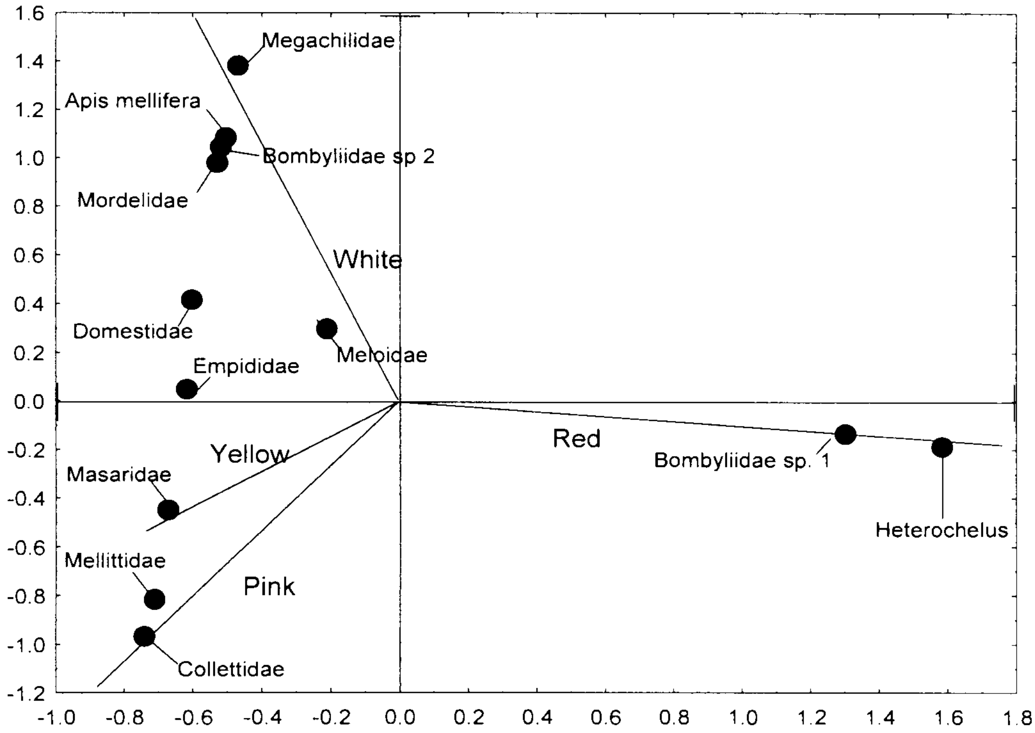


Figure 4: Canonical Correspondence Analysis (CCA) of insect species constrained according to flower colour.

Table 5: Plant attributes of the 9 study species. Flower depth, width and width excluding petals is given in mm.

Species	Flower colours	Nectar lines	Flower scent	Sexual system	Dichogamy	Flower functional type	Flower depth		Flower width		Flower width excl petals	
							Mean	SD	Mean	SD	Mean	SD
Delosperma pageanum	Purple	N	Y	Hermaphrodite	Protandry	Central cone (mellitophilous)	6.87	1.46	20.27	3.35	4.6	0.63
Drosanthemum barkerae	Pink	N	Y	Hermaphrodite	Homogamy	Stamen carpet	6.77	2.90	25.93	3.66	6.92	2.31
Drosanthemum speciosum	Red	N	Y	Hermaphrodite	Protandry	Stamen carpet	11.00	2.00	36.92	8.60	10.17	1.18
Leipoldtia schultzei	Pink-purple	Y	Y	Hermaphrodite	Protandry	Central cone (mellitophilous)	7.48	1.41	23.05	2.93	6.60	1.60
Malephora latipetala	Yellow	N	Y	Hermaphrodite	Protandry	Stamen carpet	8.53	1.61	32.87	6.70	6.95	1.32
Mesembryanthemum longistylum	White	N	Y	Hermaphrodite	Protandry	Central cone (psychophilous)	11	1.77	25.07	2.69	3.87	1.13
Phyllobolus grossus	Cream, yellow	N	Y	Hemraphrodite	Protandry	Central cone (psychophilous)	16.68	2.10	29.95	4.12	4.88	1.09
Phyllobolus splendens	White	N	Y	Hermaphrodite	Protandry	Central cone (psychophilous)	14.98	1.89	33.33	5.22	6.75	2.31
Sceletium varians	Cream, yellow	N	Y	Hemraphrodite	Protandry	Central cone (psychophilous)	12.67	2.26	47.47	8.59	9.6	1.8

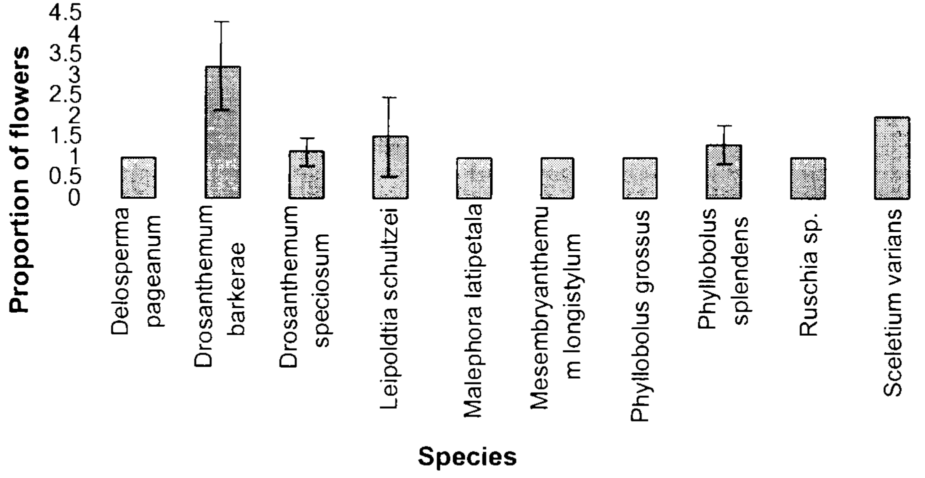


Figure 5: Mean floral presentation (±S.D.), i.e. proportion of the plant comprising flowers vs. vegetative parts, of Mesemb spp. over the study period. Class 1: 0-25% of plant comprises flowers; class 2: 25-50%; class 3: 50-75% and class 4: 75-100%.

Drosanthemum barkerae showed the highest investment in production of flowers with large displays (Figure 5). *Sceletium varians*, with its large flowers, also showed relatively high investment, but most other species did not produce either such high quantities of flowers or of such large size, relative to the plant's vegetative parts.

2. Timing of floral display

Flowering season

Graphs a-g in Figure 6 show the frequency of flowering plants of the different pink-flowered Mesembs that were in bloom during each field visit. The peaks in flowering frequency of these species in the landscape are staggered over the study-period and follow each other sequentially. A similar pattern is seen in the white-flowered species (Figure 6j-n), with *Sceletium* most abundant initially, while the two *Mesembryanthemum* spp. and *Phyllobolus grossus* are still to reach their peak. *Drosanthemum speciosum* was the most abundant species throughout the study period and had the longest flowering season, while *Malephora*'s peak was reached in the middle of the study.

Table 6 shows the phenological status of flowering of the different Mesemb species, in terms of the proportion of buds on a plant that had flowered. These values indicate that as the flowering season proceeded the plants shifted from being full of buds to full of flowers, to full of fruits and therefore potentially lost their attractiveness to pollinators. This process, like the frequency of flowering plants was staggered for the different species. A high standard deviation indicates that the flowering peak of a plant is not uniform within the population.

Changes in the abundance of different insect species in the focals were observed between the middle and final field visits (Figure 7). On the 17th-September, bombyliid sp. 1 and the honeybee *Apis mellifera* comprised half of the total pollinator assemblage. Their relative abundance decreased on the following field visit and although bombyliid sp. 1 remained the most abundant insect, colletids comprised approximately the same proportion of the total observed. More species were observed on the second visit with each comprising similar proportions of the total. A number of species observed on the second trip disappeared (e.g. tabanids, melittids and lycaenids) by the third visit while new ones were observed.

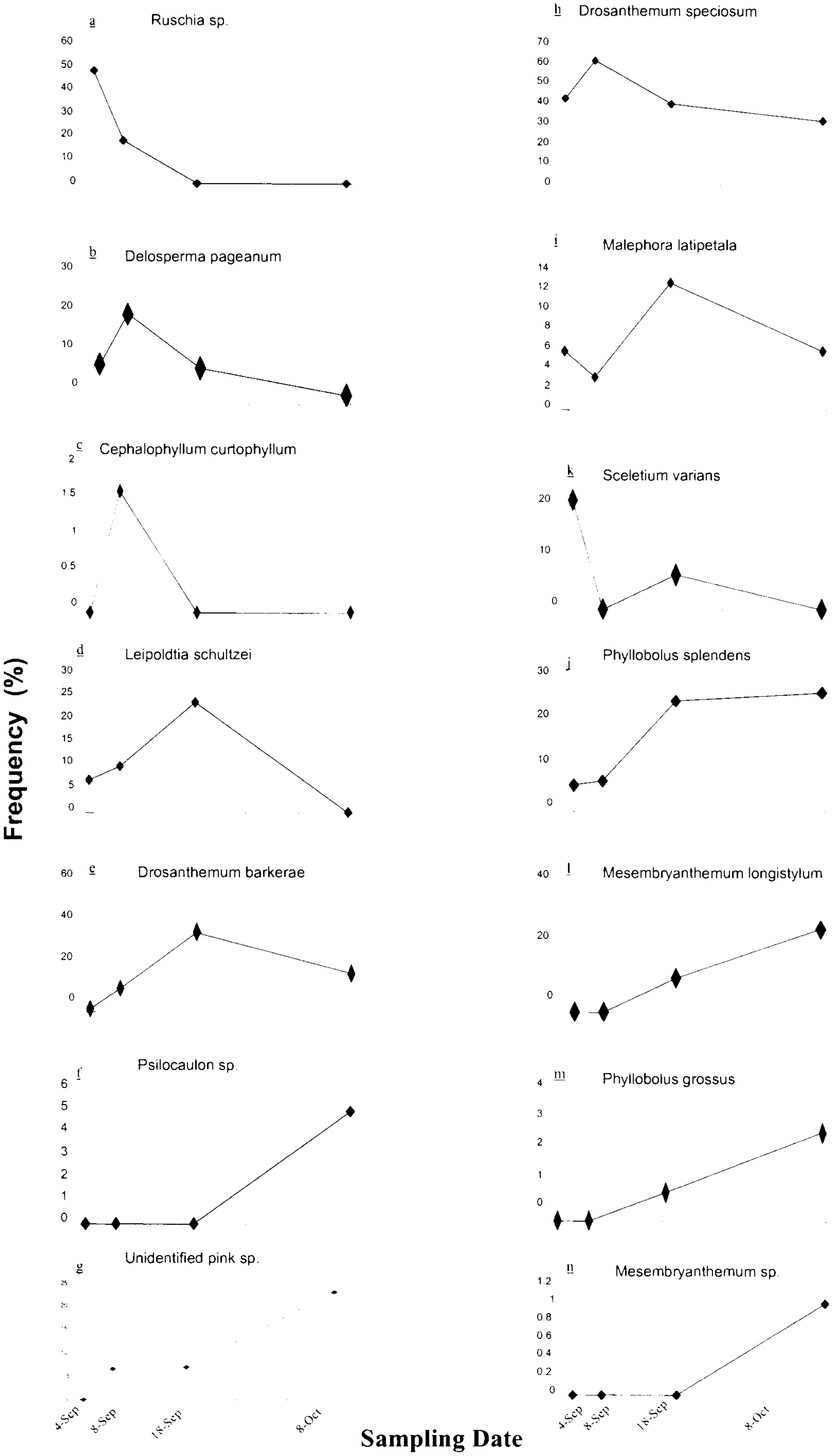


Figure 6: Frequency of different Mesemb spp flowering in transects taken 4th, 8th, and 18th-Sep and 8th-Oct. Please note the differences in scale in the y-axis. Figures a-g = pink-flowered; figures j-n = white-flowered; figure h = red-flowered and figure i = yellow-flowered.

Among these were megachilids, monkey beetles and satyrid butterflies, although their absence on the 17th-September appears related to the fact they were not observed in focals rather than in the landscape.

Table 6: Average (± S.D.) phenological status of flowering Mesemb species, according to the proportion of buds that had flowered, where class 1: 0-20%; class 2: 20-40%; class 3: 40-60%; class 4: 60-80% and class 5: 80-100%. NF means that the species was not flowering. N/A means that the information was not collected on that sampling date.

	08-Sep	**18-Sep**	**08-Oct**
Delosperma pageanum	5 (0)	NF	NF
Leipoldtia schultzei	3.67 (1.35)	5 (0)	NF
Sceletium varians	N/A	5 (0)	NF
Malephora latipetala	3.67 (0.49)	3.87 (0.64)	5 (0)
Drosanthemum barkerae	4.2 (1.52)	3.87 (1.25)	5 (0)
Drosanthemum speciosum	1.73 (0.96)	2.53 (0.52)	5 (0)
Phyllobolus splendens	1.2 (0.41)	2.6 (0.51)	4.93 (0.26)
Phyllobolus grossus	NF	1 (0)	1.2 (0.56)
Mesembryanthemum longistylum	NF	NF	1.6 (0.83)

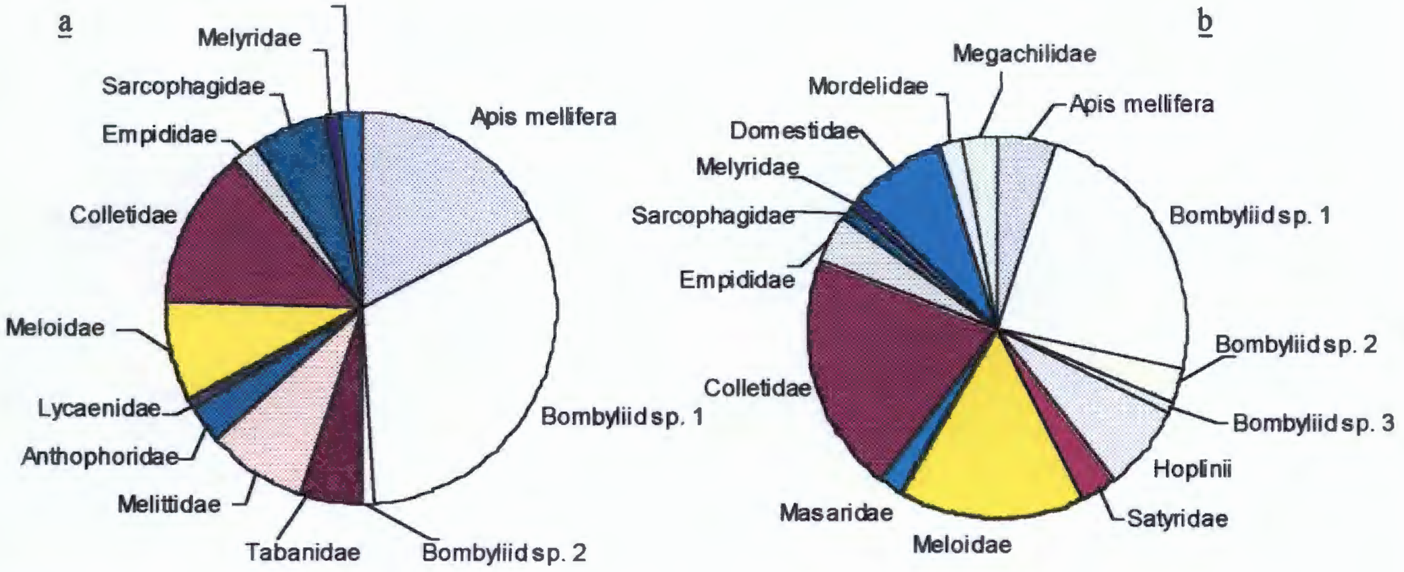


Figure 7: Relative proportions of insect taxa observed on all Mesemb spp during 15-minute focals over the 17th-19th-September (a) and 7th-9th October (b).

Figure 8 shows the species on which bombyliid sp. 1 was encountered over the study period. Whereas on the first and second visit it occurred only on *D. speciosum*, on the third visit it was seen on five different species, although *D. speciosum* remained the main one to be visited.

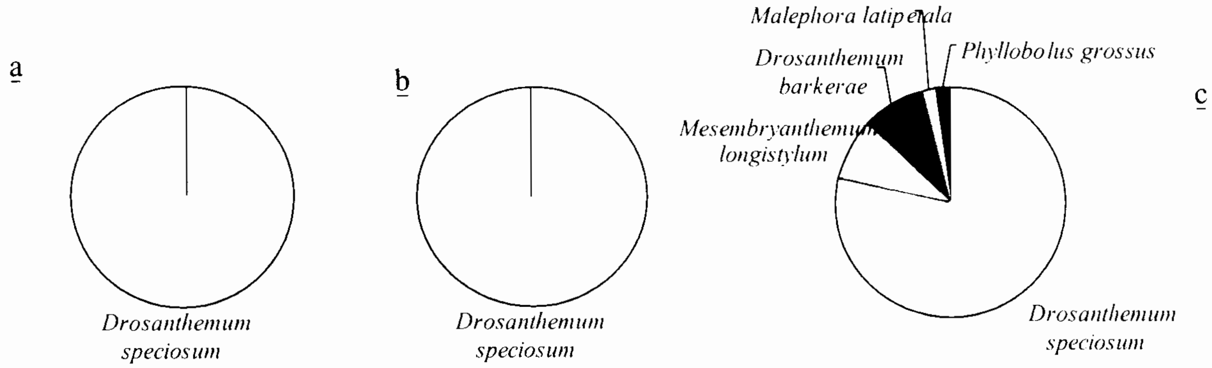


Figure 8: Mesemb species which bombyliid sp. 1 was observed visiting on 8th-9th September (a), 17th-19th September (b) and 7th-9th October (c).

Diurnal cycle

All the studied species had flowers that opened in the morning, though at different times, and closed in the evening, except for flowers of *Sceletium varians* and *Phyllobolus splendens* that were also seen open in the evening. By noon, all flowers were open at their maximum. During the day variations in scent and nectar production were noticed for all species for which these measurements were taken. These data, combined with variations in insect activity throughout the day are illustrated in Figures 9 to 20. The following observations can be made for each species:

- *Phyllobolus splendens* had a minor peak of activity in the morning and a more pronounced one in the afternoon (Figure 9). Scent was strongest in the morning, while a larger volume of nectar was collected in the afternoon, although nectar fluctuated widely between different measurements (Figure 10).
- The peak in insect activity in *Drosanthemum barkerae* seemed related to the maximum daily production of scent, which occurred in the late morning and early afternoon (Figures 11-12). Colletids were most active in the morning and at noon, while later visits were made by a wider variety of insects.

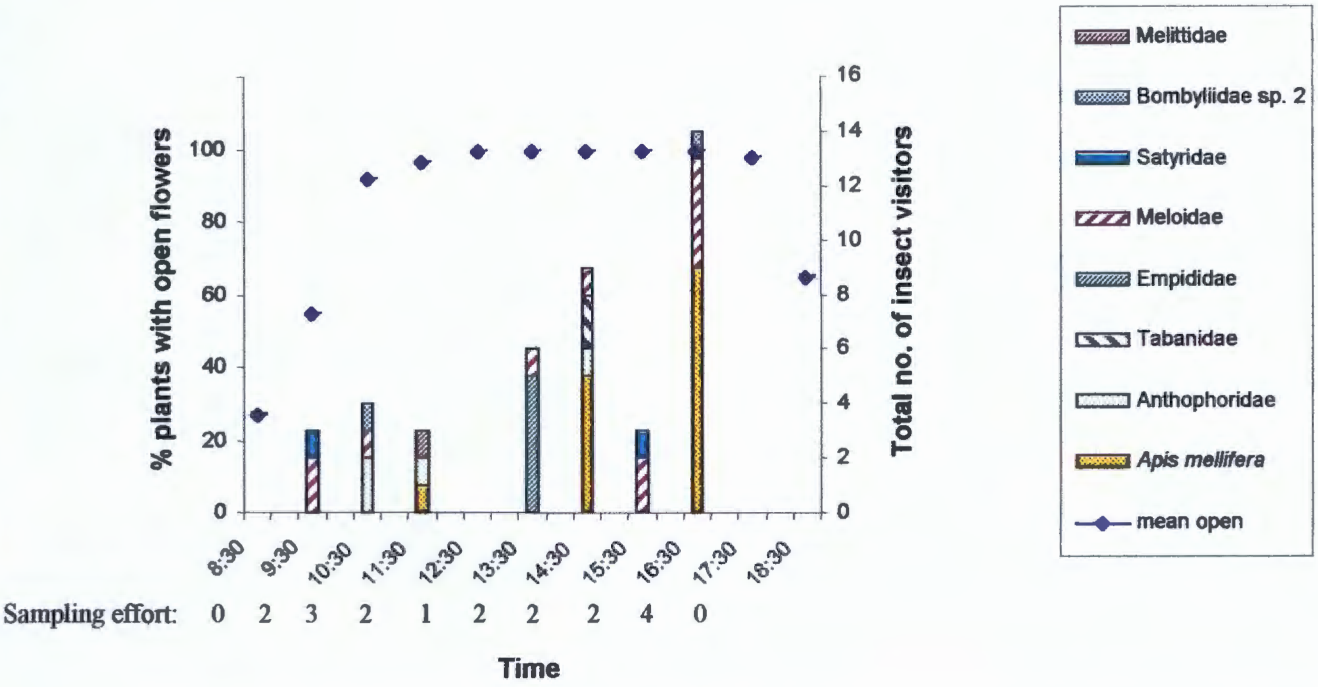


Figure 9: Average proportion of plants with open flowers (points) and total number of insect visitors observed on *Phyllobolus splendens* during 15-minute focals over entire study period (columns). Sampling effort refers to the number of focals made during each hour.

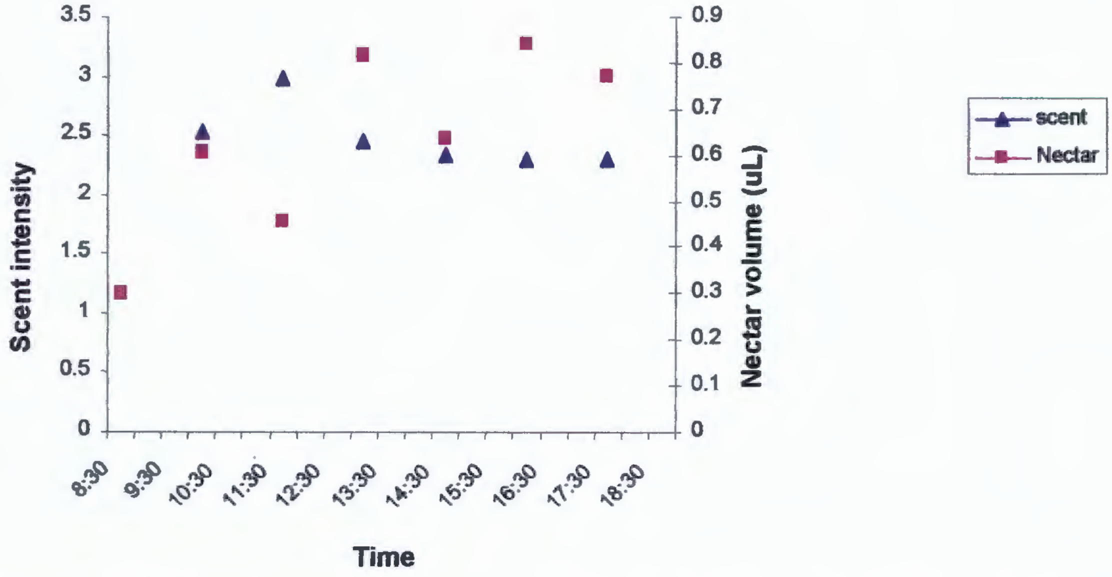
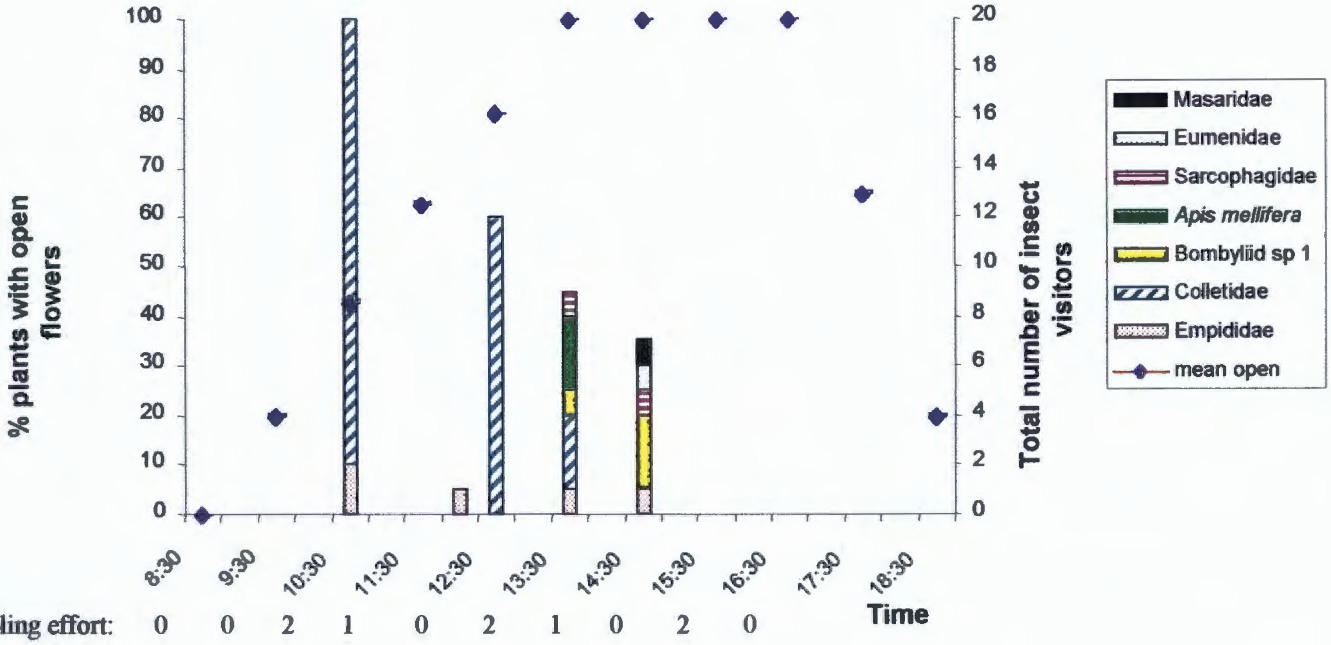


Figure 10: Average scent intensity and nectar volume measured on 8th and 9th-October in *Phyllobolus splendens*. For the scent, class 1: intense; class 2: medium and class 3: no scent.



Sampling effort: 0 0 2 1 0 2 1 0 2 0

Figure 11: Average proportion of plants with open flowers (points) and total number of insect visitors observed on *Drosanthemum barkerae* during 15-minute focals on the 8th-October (columns). Sampling effort refers to the number of focals made during each hour.

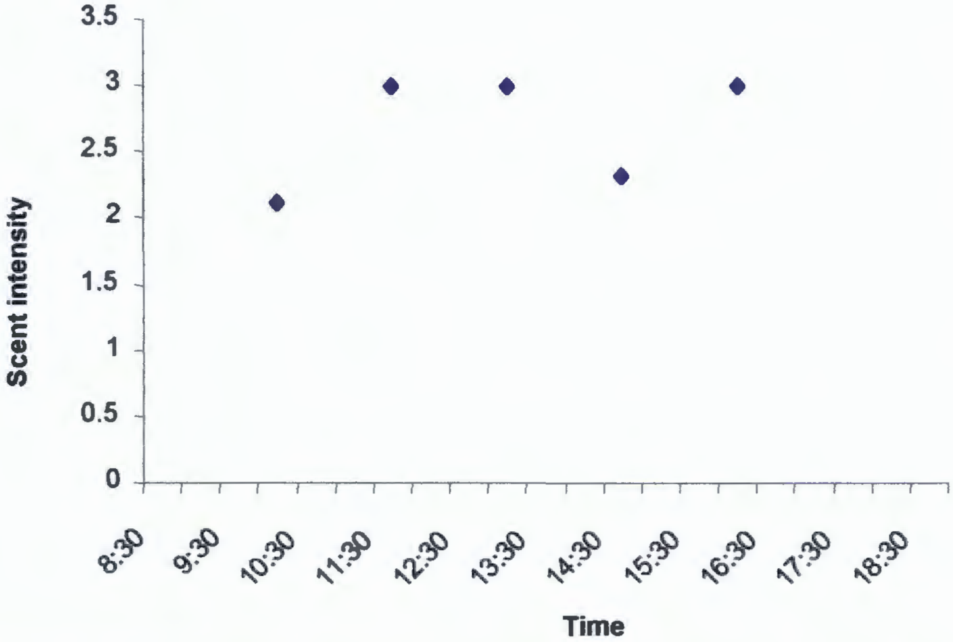
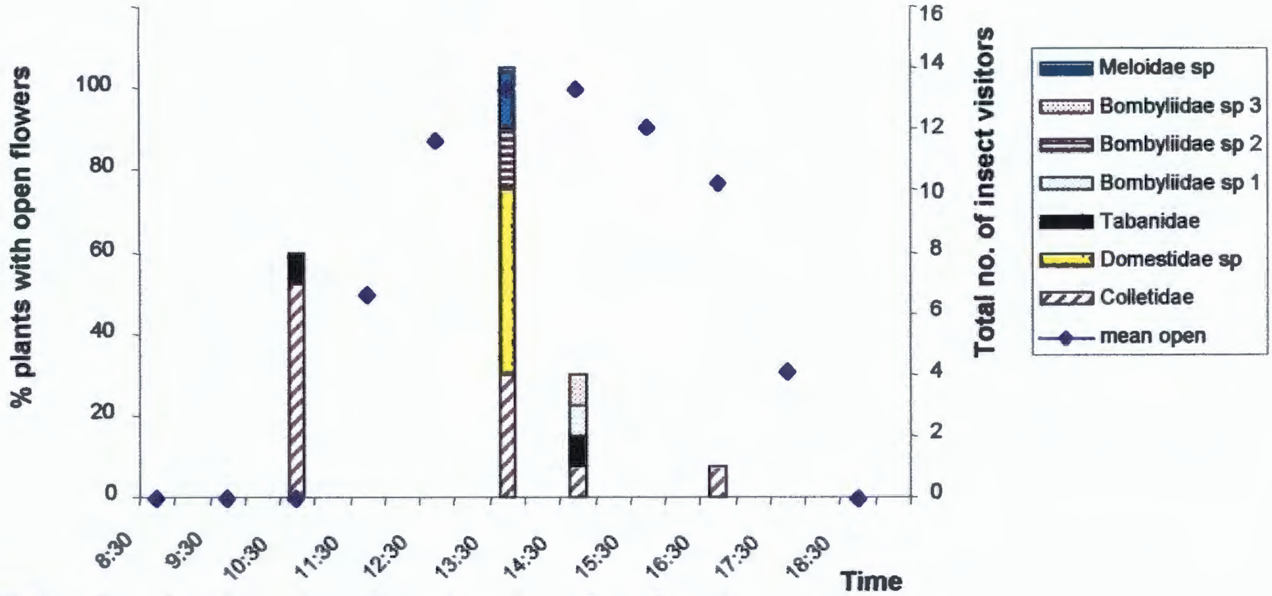


Figure 12: Average scent intensity measured on 8th October on *Drosanthemum barkerae*. Class 1: intense scent; class 2: medium and class 3: no scent.



Sampling effort: 0 0 1 1 0 4 2 0 2 0

Figure 13: Average proportion of plants with open flowers (points) and total number of insect visitors observed on *Malephora latipetala* during 15-minute focals over entire study period (columns). Sampling effort refers to the number of focals made during each hour.

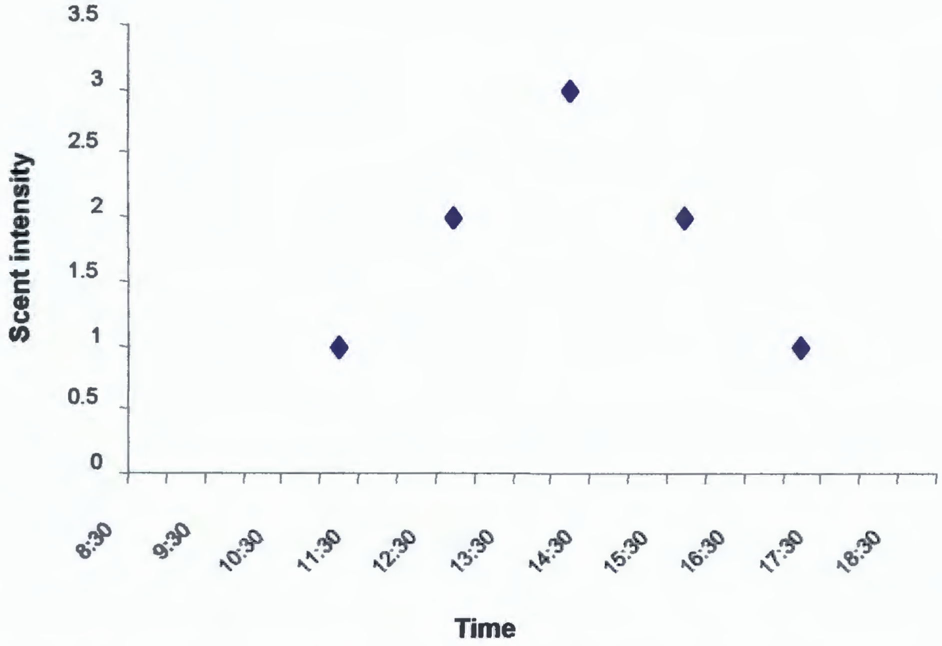


Figure 14: Average scent intensity measured on 8th October on *Malephora latipetala*. Class 1: intense scent; class 2: medium and class 3: no scent.

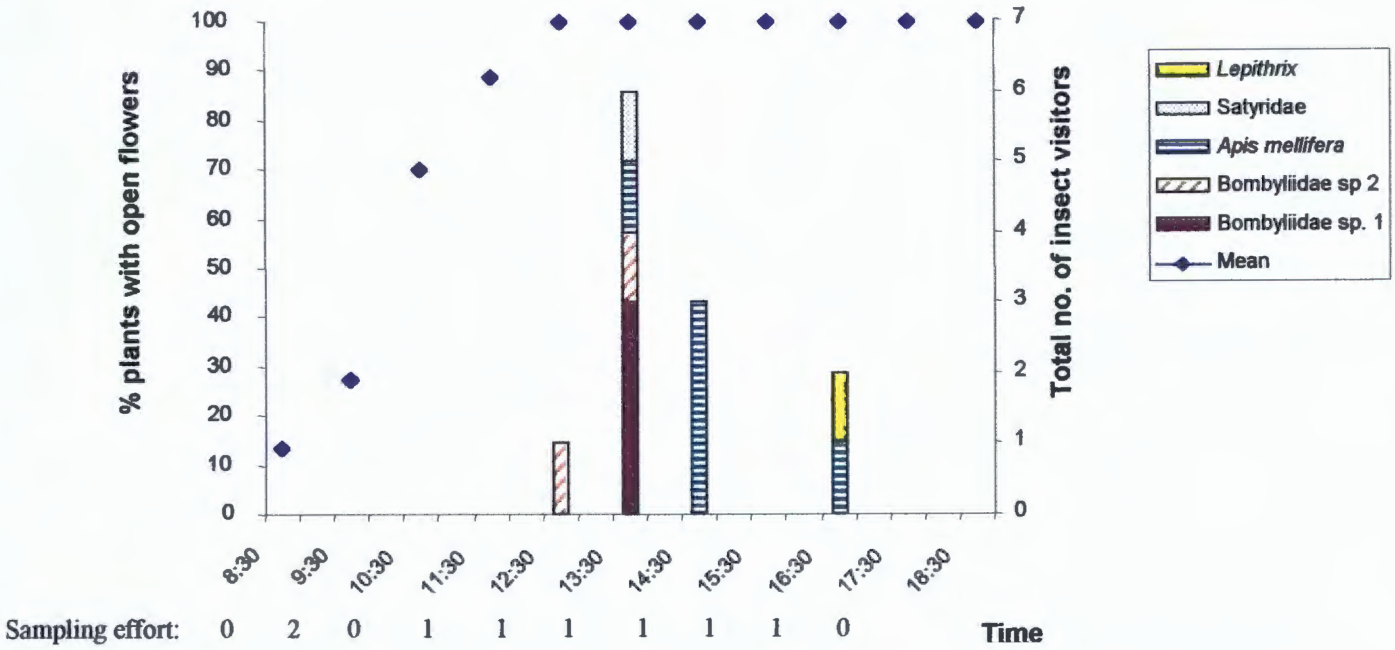


Figure 15: Average proportion of plants with open flowers (points) and total number of insect visitors observed on *Mesembryanthemum longistylum* during 15-minute focals on the 8th-October (columns). Sampling effort refers to the number of focals made during each hour.

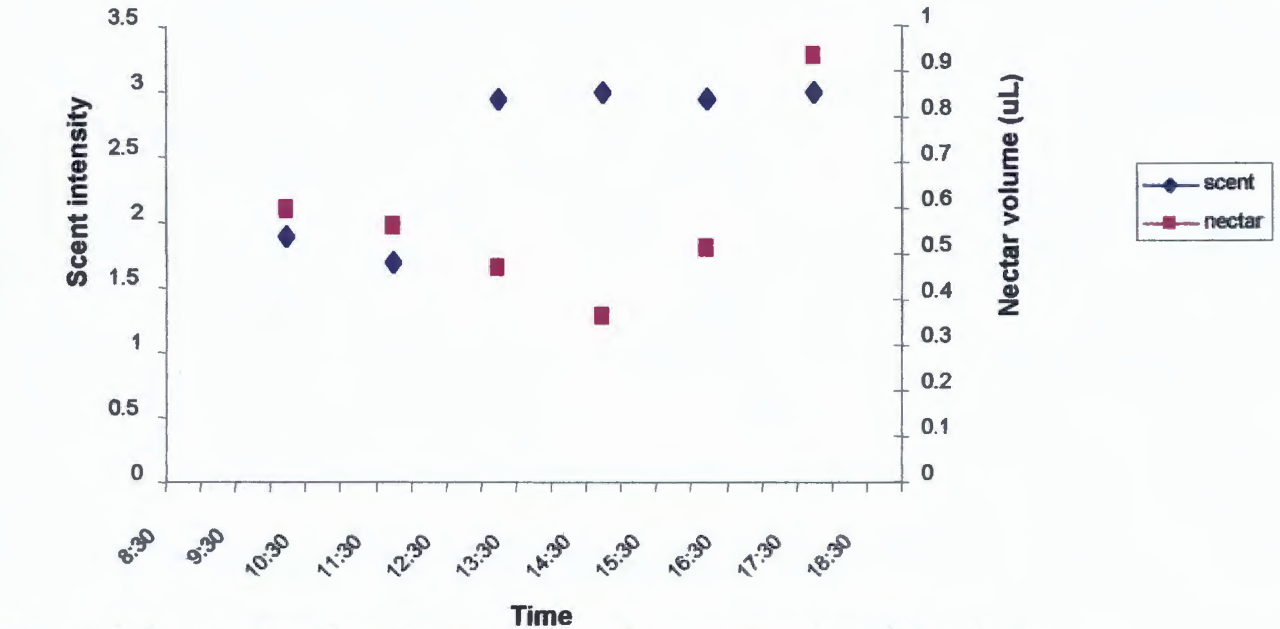
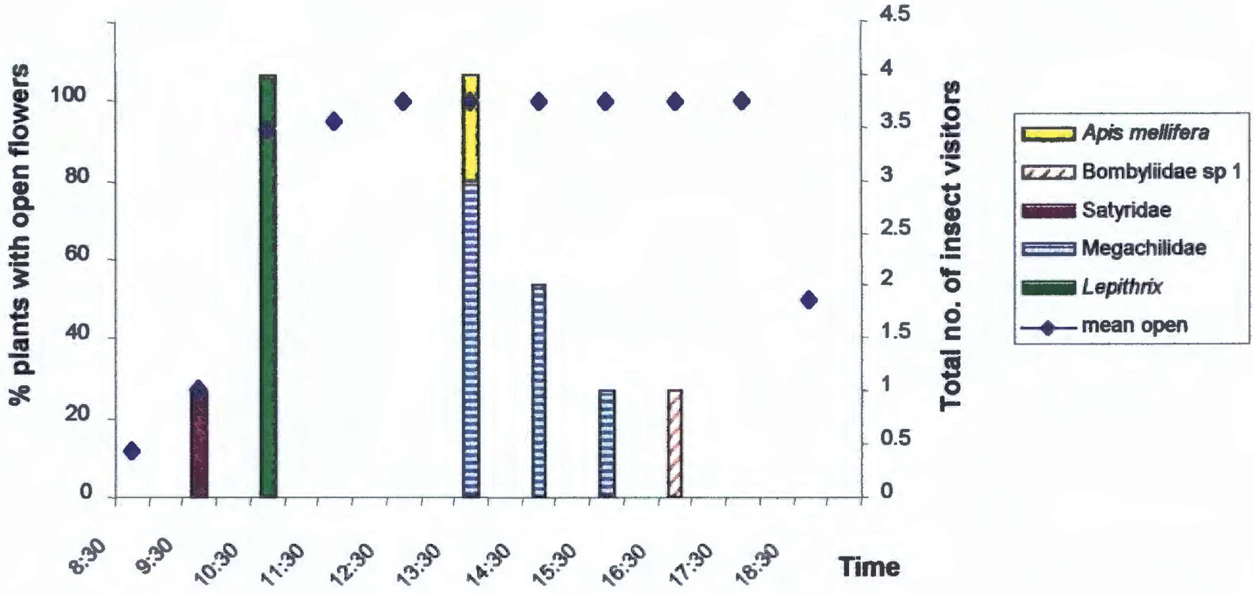


Figure 16: Average scent intensity and nectar volume measured on 8th October in *Mesembryanthemum longistylum*. For the scent, class 1: intense; class 2: medium and class 3: no scent.



Sampling effort: 0 1 2 1 0 3 2 2 2 0

Figure 17: Average proportion of plants with open flowers (points) and total number of insect visitors observed on *Phyllobolus grossus* during 15-minute focals on the 8th and 9th -October (columns). Sampling effort refers to the number of focals made during each hour.

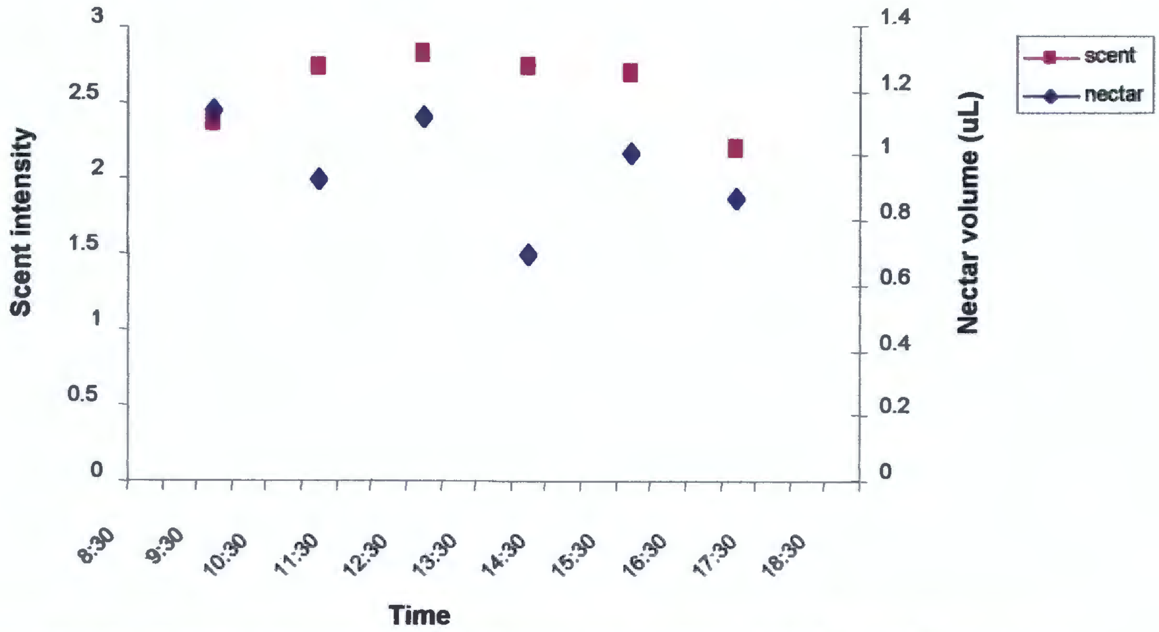


Figure 18: Average scent intensity and nectar volume measured on 8th and 9th-October in *Phyllobolus grossus*. For the scent, class 1: intense; class 2: medium and class 3: no scent.

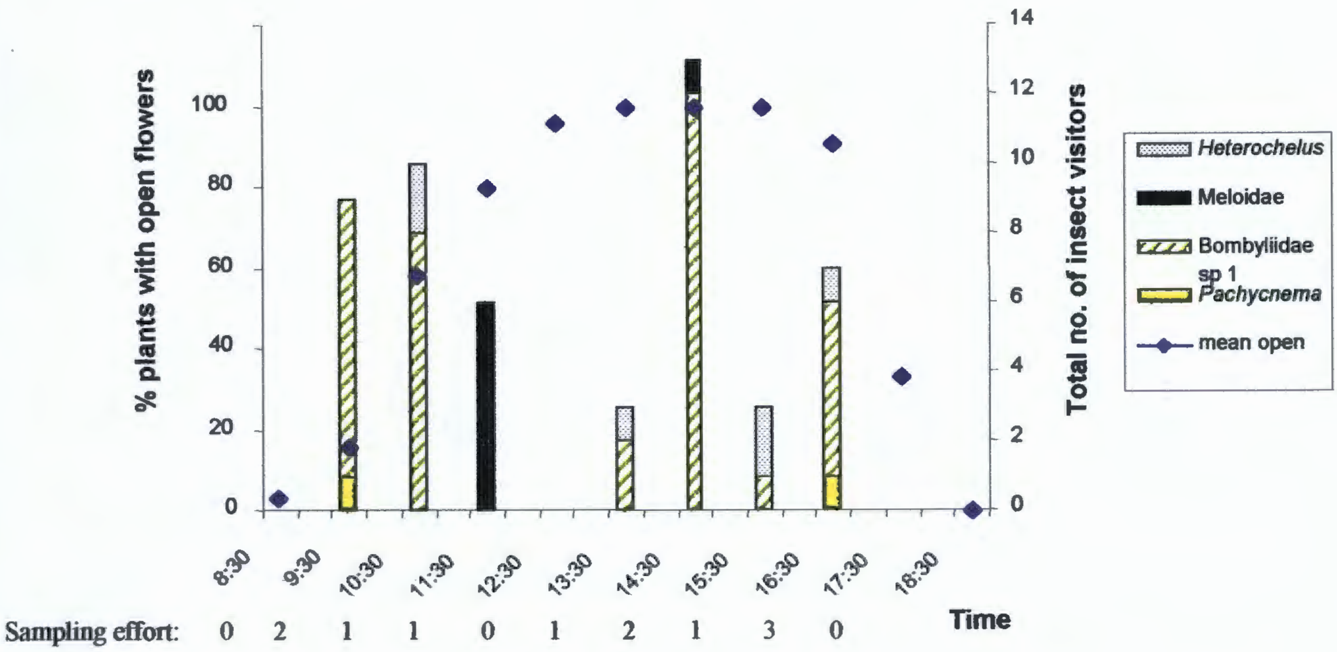


Figure 19: Average proportion of plants with open flowers (points) and total number of insect visitors observed on *Drosanthemum speciosum* during 15-minute focals on the 8th and 9th - October (columns). Sampling effort refers to the number of focals made during each hour.

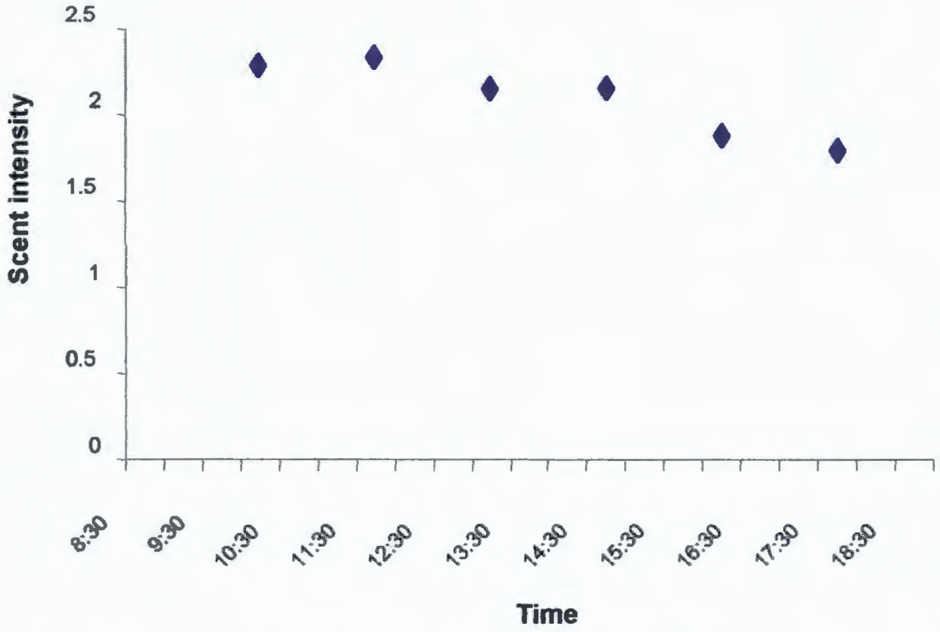


Figure 20: Average scent intensity measured on 8th and 9th-October in *Drosanthemum speciosum*. Class 1: intense scent; class 2: medium and class 3: no scent.

- In *Malephora latipetala* the peak in scent was produced at the time all flowers were open. The daily time in which all flowers were open in this species was relatively brief compared to the other species in the study. A large proportion of the insect activity occurred before many of the flowers opened, mainly by colletids. The peak in activity however occurred around 2p.m. when the scent was at its maximum (Figures 13-14).
- *Mesembryanthemum longistylum* also showed close correspondence between its peak in visitation rates and scent production. Most insects visited after 1:30p.m. irrespective of which taxon they belonged to. Nectar volume, on the contrary, fell after 1.30p.m. and was highest in the early morning and early evening (Figures 15-16).
- *Phyllobolus grossus* was visited throughout the day except over the hottest hours at midday. The morning visitors were mainly monkey beetles and the satyrid butterfly, while in the afternoon the visitors comprised hymenoptera and bee-flies. Scent production was highest in the middle of the day, while nectar volume fluctuated throughout the day and was highest in the morning (Figures 17-18).
- *Drosanthemum speciosum* received visits throughout the day, mainly by bombyliidae sp. 1 and scent decreased throughout the day (Figures 19-20).

Discussion

There has been much controversy in the literature regarding the previously widespread belief that plant-pollinator interactions tend towards specialisation (Ollerton, 1996; Waser *et al.*, 1996; Waser, 1998; Johnson & Steiner, 2000). Recent research is showing that generalisation appears to be the rule rather than the exception, with pollination syndromes showing *trends* rather than *laws* and with much overlap in pollinator preference among different plant species (Waser *et al.*, 1996).

The plant-visitor network produced for the nine Mesemb species indicated the system was relatively generalised for the plants, which were often visited by a wide suite of insects, while many of the insects showed more specialised visits. The connectance was approximately 25%, meaning that one of four possible interactions actually took place. When *Delosperma pageanum* and *Sceletium varians* were excluded from the matrix, the generalisation level increased to 30%. This is in line with Jordano's (1987) observation that as the number of species increases, there generally is a 1.5 increase in the number of interactions in a pollination system and the connectance decreases. Nevertheless, our system was more generalised than a Mediterranean shrubland, for instance, which in a study by Herrera had a connectance of 10.2% (Jordano, 1987). No such studies have been performed for the Succulent Karoo or the Cape Floral Kingdom, but given that most pollination studies in Fynbos have looked at specialised mutualisms (e.g. oil-collecting bees and orchids, the butterfly *Meneris tulbaghia* and geophytes) the matrix would at the moment be biased towards specialisation. The only study which produced an interaction network in South Africa was that for a plant-seed disperser system in a coastal dune forest, which also had approximately 25% connectance (Jordano, 1987). A greater number of species was however looked at (16 plants and 35 animals), and 143 interactions were observed. Jordano (1987) indicated that pollination systems tend to show higher specificity than seed disperser ones, so that for the same number of species a lower connectance is generally recorded in pollination systems, as our comparison illustrates.

Our system therefore showed intermediate generalisation, which still remains relatively low for a system that has overall been described as generalised, with most flowers admitting a wide range of pollinators (Ihlenfeldt, 1994). In Goegab Nature Reserve, Struck (1992) found that 16 of 18 insect species visited between one and three Mesemb species (out of a total of seven), while only two insects visited more than three plants. Also in our case, 85% of insects visited between one and three species, showing relatively little generalisation. A number of instances of pollinator overlap were however seen, especially among the white-flowered *M. longistylum*, *P. grossus* and *P. splendens*. Given that sharing of pollinators could contribute to interspecific pollen transfer, I would first like to establish which floral characteristics influenced these insects' choice and secondly how plants attempt to reduce overlap in insect visitors.

Floral traits potentially influencing pollinator specialisation

Colour appeared to play an important role in determining an insect's choice to visit different plant species, as both ordination and cluster analysis showed, indicating that the species with the highest degree of overlap were those of the same colour. The greatest discrimination in the ordination was between *D. speciosum* (red-flowered) and all other species, with bombyliid sp. 1 and the monkey beetle *Heterochelus* showing specific visits to this plant. Picker & Midgley (1996) observed that monkey beetles tend to prefer red and orange flowers followed by yellow and white and, specifically, *Heterochelus* was also seen by them to visit red flowers. Pink and yellow flowers grouped closely on the ordination, probably due to the fact that colletids visited *D. barkerae* and *M. latipetala* frequently and in high numbers. Also in the cluster analysis *D. barkerae* showed the highest overlap in insect visitors with *M. latipetala*. Both these species had stamen-carpet flowers and no nectar, features that could have also influenced colletids' choice, as they are pollen-collectors (Picker & Griffiths, 2002). Struck (1994) also mostly observed these bees on Mesemb and Asteraceae flowers. As yellow is a neutral colour in pollination biology (Picker & Midgley, 1996), this could perhaps explain its positioning close to the centre of the ordination.

Bombyliid sp. 2, the honeybee and satyrids all appeared to prefer white-flowered species. The depth of the flower and presence of scent and nectar, in addition to a pale colour, also seemed important in the satyrid's choice of flowers. *P. splendens*, *M. longistylum* and *P. grossus* were among the species to have the deepest flowers, which were of the psychophilous central-cone type. It is likely that *Sceletium varians*, with its pale, deep, scented-flowers also fits the phalenophilous and psychophilous pollination syndrome, but as only two focals were made on this species, no butterflies were observed.

Production of nectar and scent were found, in many cases, to be important determinants of visitor activity, especially with regards to diurnal fluctuations. Often a peak in scent production was reflected in an increase in visits by insects. This trend was evident in *D. barkerae*, *M. latipetala* and *M. longistylum* with a late morning, a lunchtime and early afternoon peak respectively, in both scent and insect activity (Figures 11-16). Nectar production appeared to play a role in attracting insects in *P. splendens*, where insect activity throughout the day showed some relation to nectar volume.

As a result of competition for pollinators, caused by plant dependence on reproduction and out-crossing, Cowling *et al.* (1999) suggested that large floral displays are a mechanism used as advertising to attract insects. In our system, the size of the floral display could have played a role in increasing pollinator visits in *D. barkerae*, which was visited by ten different insect species, with relatively high visitation rates. The display was not always important, however, as *M. longistylum* was also visited by ten insect species, although flowers comprised less than one quarter of the plant's surface. Although Thomson (1991, in Struck, 1994) observed a positive correlation between flower numbers and attractiveness to pollinators, a large floral display may also be advantageous in increasing the chance that pollen from one's own species is the first to reach the stigmas rather than foreign pollen (Hammer & Liede, 1990). This has important implications in reducing reproductive interference, such as stigma clogging and possibly hybridisation.

To summarise, the main determinants of insect choice appeared to be colour, especially in isolating *D. speciosum* from the other species, and the production of scent and nectar. Floral display seemed to play a minor role, as well as size of the flowers, that could not be easily correlated to an insect's presence on a plant. In the studied system, the species to be most at risk of interspecific pollen transfer therefore seem to be those of the same colour, especially if they belong to the same genus and share pollinators, as their pollen may be similar and therefore interfere in the reproductive process. *Phyllobolus splendens* and *P. grossus* could therefore suffer from stigma clogging and possibly hybridisation, as the matrix showed they shared three pollinators. These two species still had lower overlap than *P. grossus* and *M. longistylum*, however, which shared four pollinators. *Drosanthemum speciosum* and *D. barkerae* could also be at risk, as they were visited by one common insect species. The relative importance of the shared pollinators however differed between the plant species, where for example the bombyliid sp. 1 that was shared in *Drosanthemum* was responsible for 82% of visits to *D. speciosum*, but less than 5% to *D. barkerae*. Other mechanisms that the different species may be using to prevent contamination and reduce competition for pollinators also need to be explored.

Temporal isolation: seasonality

Seasonality in the flowering peaks of different species in the landscape appears to be one of the main mechanisms employed by Mesembs to reduce interspecific pollen transfers. Although the majority of species flower in spring for a limited number of months, different species may flower throughout the year, with temporal separation in flowering often seen in sympatric species (Smith *et al.*, 1998). Furthermore, colour appears to play a very important role in influencing the time of flowering of co-occurring species. Figures 20 and 21 illustrate the situation of *Ruschia* spp in Robertson and of *Antimima* in Clanwilliam, showing that the flowering peaks of species of the same or similar colour succeed each other in the landscape. In the four *Ruschia* spp found in Robertson, *R. intrusa* has purple-pink flowers, *R. caroli* has pink ones, *R. multiflora* white and *R. approximata* is also pink-flowered (Figure 21). The only two species to flower at approximately the same time are thus those that have different-coloured flowers. In *Antimima*, most species have pink-purple flowers and separation in flowering season also occurs in sympatric species, as in *Ruschia* (Figure 22). Furthermore the flowering season of most *Antimima* species is generally of only one month, reducing overlap even further.

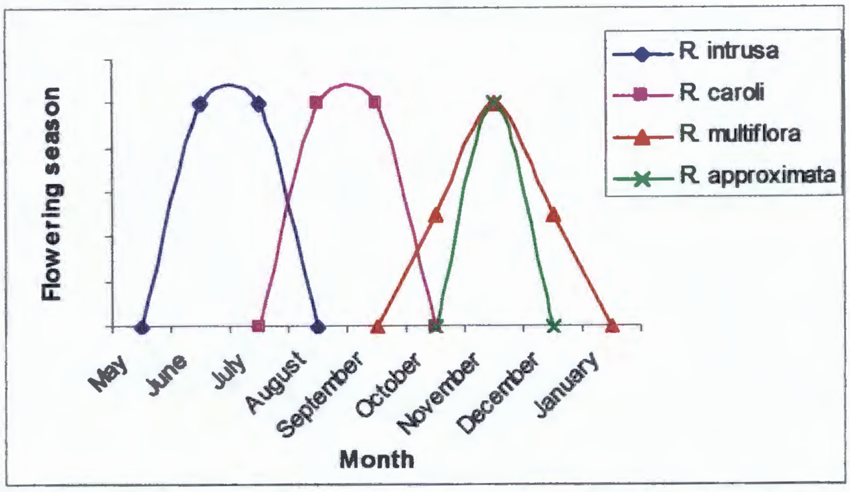


Figure 21: Flowering peaks of *Ruschia* spp found in Robertson (based on Goldblatt & Manning, 2000).

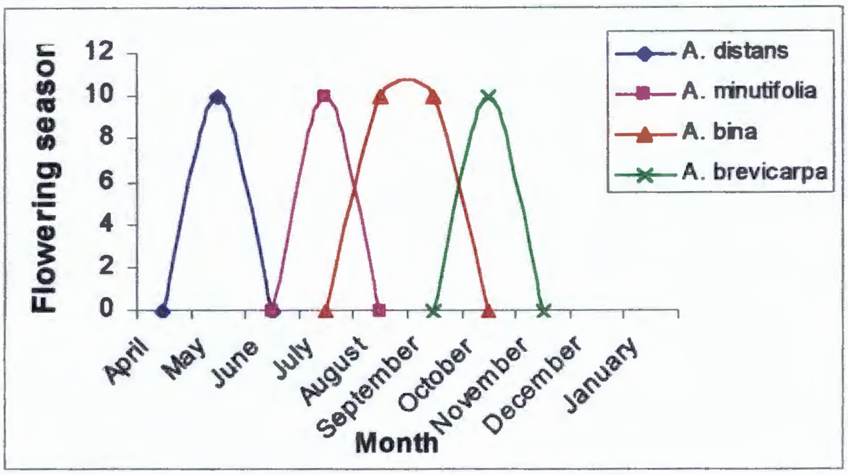


Figure 22: Flowering peaks of *Antimima* spp found in Clanwilliam (based on Goldblatt & Manning, 2000).

In terms of flowering time of intra-generic species in our system, the two *Phyllobolus* and *Mesembryanthemum* spp seemed to reach their flowering peaks at slightly different times, supporting the above trend. Also Struck (1992) observed sequential blooming periods in Namaqualand for *Ruschia*, but also for species other than Mesembs, such as *Euphorbia* and *Hermannia* spp. As our study showed that sharing of pollinators at times appeared to be related to colour of flowers of host plant, differences in flowering peak could be a way to avoid competition for pollinators and reproductive interference. It is also possible that this is a mechanism used to keep shared pollinators in an area by providing resources for a longer time period (Struck, 1992).

The species in Vrolijkheit appeared to also have sequential blooming periods, although on a much shorter scale. Many of the pink-flowered species showed a one or two-week interval between their main peak in abundance in the landscape, which could provide the necessary amount of isolation required to prevent excessive sharing of pollinators. In Paulshoek, the peak of insect activity on a particular plant species has often been noted in the first few days of the flowering season of that species, when most of the plants have fresh flowers. As time proceeds, although most plants still have numerous flowers and look attractive (to the human eye), insect activity appears to be nearly non-existent (C. Mayer, pers. comm.). In the white-flowered species in Vrolijkheit, a temporal separation in flowering was also noted and this could have been a mechanism to reduce part of the visitor overlap to different species. For instance the bombyliid sp. 2 that was found on both *P. splendens* and *M. longistylum* seemed to be prevented from causing reproductive interference by seasonality. The two plant species showed different peaks in abundance in the landscape over time: *P. splendens* was widely distributed on the second visit, and although still abundant on the final visit, showed by its floral display that its flowering season was ending (floral display of 4.93). *M. longistylum*, on the other hand, was still to reach its peak (with a value of 1.6 on the 8th Oct) and therefore the two species appeared to avoid competition for the same pollinator in this way. It is likely that the early flowering of *P. splendens*, compared to *P. grossus* and *M. longistylum* may have contributed to the relatively low pollinator overlap seen between this species and the other two (Figure 3). Three of *P. splendens*' visitors (carpenter bees, melittid bees and tabanids) were only observed during the second visit but not the third (Figure 7). Having a flowering peak at an earlier stage than the congeneric *P. grossus* may therefore be a mechanism used by *P. splendens* to isolate itself and reduce overlap in visitors.

The relatively short duration of the flowering peaks of the white and pink-flowered species seems also to provide evidence that competition for pollinators and interference is reduced in species that share the same colour. *Drosanthemum speciosum*, the only red species, on the other hand, showed the longest flowering season and dominated the landscape throughout the study period, perhaps showing it was at little risk of competition, as the ordination indeed showed that colour was important in isolating it. Its flowering display was however poor on the last field visit, when most plants had mainly fruits and relatively few flowers. This paucity of floral resources was reflected in the insect activity. Whereas during the first two field visits bombyliid sp. 1 was observed uniquely on *D. speciosum*, on the 7th-9th October it was seen on a number of other plant species, suggesting the reward offered by *D. speciosum* was not sufficient anymore. As bombyliid sp. 1 was the most abundant species observed throughout the study period it is possible that its large population required vast amounts of food that could not be provided by only one species by the end of its flowering season. Also in solitary bees involved in specialised mutualisms it has been noticed that they resort to other flowers when their floral resource becomes in short supply (Waser *et al.*, 1996). Struck (1994) also found that bombyliids were abundant in the landscape, and were among the few insects that would withstand hot or windy weather. This could also explain the fact we observed such large proportions of them, because while an increase in wind or clouds caused an almost immediate slowdown in insect activity, bombyliids generally persisted.

Temporal isolation: diurnal cycle

A number of examples in Mesembs illustrate very clear patterns in diurnal separation of flowering time. For example in *Aridaria*, as mentioned in the introduction, flower opening seems to play a role in the partitioning of the pollinator assemblage, whereby *A. brevicarpa* opens at noon, *A. vespertina* opens later in the afternoon, and *A. serotina* and *A. noctiflora* open at dusk, but close at different times of the night (Hartmann, 2002). In the system in Vrolijkheid all species overlapped in their flowering times with most flowers open by noon and closing at different times of the afternoon, except for *P. splendens* and *S. varians* that were observed with open flowers also in the evening. The pale colour of these flowers, the central-cone shape, relatively strong scent and the patterns of nectar production in *P. splendens* all suggest that moth pollination in the evening could also be important. Nectar volume increased in the evening in *P. splendens*, while in *M. longistylum* it was relatively high in the morning, decreased throughout the day and increased again by evening, again suggesting phalenophilous pollination. The cycles in scent and nectar production throughout the day appear to be an important mechanism in creating a peak in insect activity on a particular plant species at a particular time of day. This could again be a way to reduce interspecific pollen transfer in those species that have pollinator overlap. For example, as colletids were abundant and frequent visitors to both *M. latipetala* and *D. barkerae*, it is possible that these two species developed different peaks in scent production to reduce overlap. Colletids mainly visited *D. barkerae* from 11:30 a.m., while after 1 p.m. they were mainly seen on *M. latipetala*, corresponding to the respective peaks in scent production of these species. *P. splendens*, *P. grossus* and *M. longistylum* also had different daily patterns of nectar and scent production, that could have also been an attempt to reduce pollinator overlap. For example *P. splendens* and *M. longistylum* shared relatively few pollinators (Table 4), and had a morning and afternoon peak in scent production respectively.

Honeybee visits, however, may have caused interspecific pollen deposition on flowers of *M. longistylum*, *P. grossus*, *P. splendens* and *D. barkerae*. At 2 p.m., for example, honeybees were noticed on all four species, and especially in the case of the two *Phyllobolus* spp. this may result in reproductive interference. Struck (1994) observed that due to their social organisation, honeybees tend to forage on a variety of host plants, yet they do express a certain degree of fidelity to pollen sources at the individual level. Therefore, it is possible that although honeybees were seen visiting many plant species, individuals specialised on a particular host plant species. Nevertheless, honeybees are often a problem in natural conditions, as they displace native pollinators, such as solitary bees and tend to be indiscriminate pollinators (Kearns *et al.*, 1998).

Significance of the results

Observing the landscape and referring to the literature, one realises that Mesembs are a very successful plant family, that dominates the vegetation of the Succulent Karoo and which has undergone extensive diversification, producing nearly 1700 species in a short period of evolutionary time (Chesselet *et al.*, 2002). The main lines along which diversification has occurred in this family, however, seem to be in terms of vegetative structure of the plants and very little in terms of their floral features. Neoteny, specialisation and speciation along habitat gradients, which change over very small distances in the Karoo, appear to have been the main factors that have allowed such diversification in vegetative characters, plant size and investment strategies (Hartmann, 1975; Ihlenfeldt, 1975; Ihlenfeldt, 1994; Desmet & Cowling, 1999b). On the contrary, floral structure appears rather uniform in the majority of species, with colour often the main character that shows any variation, although even this, in only a number of genera.

When studying the pollination ecology of this family, one can begin to speculate on possible causes of low levels of variation in floral characters. Firstly, despite the mass multi-species displays of flowers, one remains struck by the scarcity of insect activity in the landscape, even in fine weather, and by the very low visitation rates that tend to be recorded (Struck, 1995; this study and C. Mayer, pers. comm.). Furthermore, during the study period of one month, there were approximately ten days of sunny, calm weather, while cloudy conditions, wind or rain persisted for the rest of the time. These weather conditions are however not generally expected in the months of September and October, indicating this may have been an abnormal year. Nonetheless, although the Succulent Karoo is characterised by reliable winter rainfall (Desmet & Cowling, 1999a), weather patterns can still have a degree of unpredictability, such as in this year. A number of workers in Namaqualand have noticed that adverse weather conditions interrupt most insect activity and often cause flowers to close, due to a drop in temperature, increase in cloud cover or wind (Struck, 1992; A. Ellis, pers. comm.). As many species only have a flowering period of one month, their ability to be pollinated and set seed in such a short period of time seems rather exceptional. A likely explanation is that visits by pollinators are very effective, so that although visitation is low, it is sufficient to result in successful seed-set, seen in the abundance of seedlings in the landscape. Nonetheless, the activity on flowers does not seem to be sufficient to cause pollinator-mediated selection on floral traits.

For pollinators to play a role in host plant diversification, selection must be strong enough to drive evolutionary change (Galen, 1996). In Fynbos, Johnson & Bond (1997) however argue that low insect density is an agent selecting for high floral divergence, seen in the diversification of flowers in certain genera like *Disa* (Johnson *et al.*, 1998). In Mesembs, however, selection may be weak due to the opportunistic behaviour adopted by insects, promoted by the short flowering season and adverse weather conditions that reduce the blooming period even further. Another reason that can prevent selection on floral traits is the fluctuations in visitation rates and visitor composition caused by variations in yearly precipitation, such as in Namaqualand (Struck, 1994). With a mosaic of pollinators that varies spatially and temporally, it seems difficult for plants to be subject to sufficient pressure to drive changes in floral traits. Furthermore, floral visitors vary widely in the strength and direction of selection on flower morphology, so that in a generalised system different pollinators, that have different morphologies and preferences, will provide opposing forces to which the plant must respond, thus most often providing no selective force (Ollerton, 1996). Other times, selection may be operating, but it is not clear to demonstrate, for example in the radiation of geophytes as a result of monkey beetles, due to their preference for dish-shaped flowers, that can be easily accessed by a suite of flower visitors (Goldblatt *et al.*, 1998).

Therefore it is possible that the conservative floral structure seen across the family is a result of insufficient selection caused by insects. We would however expect to see the extensive radiation undergone by insects in the Succulent Karoo to be reflected among the plants. Malaise traps and coloured pan-traps indicate that in Namaqualand and the Karoo there is very high insect diversity, especially among the Hymenoptera (S. van Noordt, pers. comm) and in monkey beetles, which comprise 1000 species in 90 genera (J. Colville, pers. comm.). Part of this radiation is reflected in a number of specialised relationships with plants, such as in oil-collecting bees and monkey beetles associated with geophytes, but further research is required to investigate pollinator-driven speciation in this environment.

Possible limitations in the study and scope for future research

The most important comment with regards to the data is that this study looked at plant visitors as opposed to plant pollinators. It would have been very useful to look at pollen loads of the insects to be able to distinguish between those that were effective pollinators and those that visited the flowers either as pollen or nectar robbers or without carrying significant amounts of pollen. This is a common problem in pollination studies, which often fail to consider that different pollinators may differ in their ability to pollinate, and thus in enabling a plant to set seed (Ollerton, 1996). The study also only looked at a snapshot in time of the system, effectively covering only six days of observations. Ideally, it should be undertaken over the whole year, as this would give a good indication of the degree of sharing of pollinators between plant species and the importance of seasonality in isolating species, particularly those of the same colour. There were numerous pink-flowered species in Vrolijkheid, but only *D. barkerae* was observed for an amount of time sufficient to be able to make comments on its visitors.

Concluding remarks

The pollination system in Vrolijkheid showed that there was a certain degree of pollinator overlap between different plant species, although some specialised relationships were seen (e.g. in *D. speciosum*). Generalisation has been explained in terms of the fact that insects and plants need to be opportunistic in an environment which shows fluctuations in precipitation that are responsible for variations in the abundance of therophytes and geophytes (Struck, 1994). Insects are therefore thought to follow a demand and supply law, responding to changes in floral display and availability of different plants (Struck, 1995). Seasonality in the flowering period of the species appears to be a very important mechanism that can prevent excessive reproductive interference, competition for pollinators and possibly hybridisation, as also recognised by Hammer & Liede (1990). Post-zygotic methods that reduce the rate of hybridisation are however also likely to be very important in Mesembs. Numerous instances of pollen refusal, seed die-off, and offspring inviability, among others, have been documented for Mesembs, which can explain the scarcity of hybrids observed in natural conditions (Hammer & Liede, 1990). Genetic studies on Mesemb species are however required in order to find out whether hybridisation is as rare as the literature describes it to be, or whether hybrids are also found under natural conditions.

It is important to stress that pollination studies in both Fynbos and Succulent Karoo have mainly focused on specialised interactions thus far. This study is therefore an invitation to begin to look at pollination from a wider angle, studying communities, and the way plant and insect species interact on a large scale. Johnson & Bond (1997) suggested that plants in the Cape Floral Kingdom suffer from pollinator scarcity. No studies have however sought to investigate this suggestion, which has furthermore often been unfoundedly extended to the Karoo. High diversification in floral characters and species radiation exists in a number of genera in both Karoo and Fynbos, such as in *Erica*, geophyte and orchid genera (Cowling, 1992). Is it possible that common driving variables act in both biomes, contributing to their world-renowned species richness? Could pollination be one of these factors? It is essential, if we really are to understand the dynamics of speciation and radiation in families and genera in both Karoo and Fynbos, to begin to look at the role of pollination in driving diversification. Mesembs have a very generalised floral structure; colour divergence between genera, however, is relatively high. As the system was not found to be as generalised as expected, and colour was seen to be an important factor in determining insect choice, we should begin to investigate if this variable plays any role in the diversification of this plant family.

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