Variation in breeding systems, floral morphology and nectar properties in three co-occurring *Erica* species with contrasting pollination syndromes

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ABSTRACT

The genus *Erica* is the most species rich in the Cape Floristic Kingdom, yet there are limited data on the various flower-pollinator interactions and breeding systems for the majority of these species. Until recently research has focussed on predictive floral traits, including flower shape, length of corolla and corolla opening to identify likely pollinators in this genus. Field observations provide an empirical test of such predictions.

This study investigated three species of *Erica* and compared their pollination and breeding systems and floral biology. Research, including field experiments and pollinator observations was undertaken in the Vogelgat Private Nature Reserve, Maanschynkop Nature Reserve and Boskloof farm in the vicinity of Hermanus in the Western Cape Province of South Africa. Field observations were conducted to determine what flower- visitor interactions occurred, while nectar volumes and sugar concentrations were measured to determine the value of the reward to the different visitors. Selective exclusion and breeding system experiments were carried out to determine whether these *Erica* species were capable of autonomous self-fertilization or whether they are pollinator dependent for breeding success.

The unusual morphology of *Erica lanuginosa* suggested rodent-pollination. Field observations, including photographs of visits to plants in the field, the presence of pollen in scat sample and selective exclusion and breeding system experiments identified rodents, primarily *Acomys subspinosus* to be the primary pollinator.

Long-proboscid flies of the family Nemestrinidae were found, on the basis of field observations to be responsible for pollination of the endemic *Erica aristata.* This was supported by with nectar volume and sugar concentration samples which are consistent with other long-proboscid fly-pollinated plant species. Selective exclusion and breeding system experiments undertaken confirmed that *Erica aristata* required a pollinator to set seed.

Observations and breeding trials revealed bird-pollination in *Erica sessiliflora.* Nectar volume and sugar concentrations in *Erica sessiliflora* were in line with other sunbird-pollinated plant species, providing the necessary rewards for sunbirds visiting this species.

Specialised pollination by single pollinators was found in all three species and results from breeding system experiments show that out-crossing is important. Further research into

pollinator-flower interactions in the genus *Erica* is necessary, not least to understand more fully the conservation importance of specific pollinators.

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This thesis, submitted for the degree of Master of Science in the Faculty of Science, Rhodes University, Grahamstown, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.

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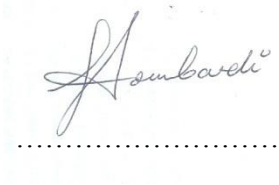

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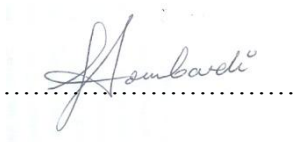
Author contributions:

Giorgio C Lombardi conceived the poster, collected and analysed data, and, Craig I. Peter, Jeremy J. Midgley and Ross C. Turner contributed valuable comments on the poster.



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CHAPTER 1: Introduction

The Cape Floristic Kingdom (CFK) is an internationally recognised biodiversity hotspot with more than 9000 plant species in a small area of approximately 90 000km² (Schnitzler et al. 2011). In comparison, the United Kingdom has only 1 500 flowering species in an area of 245 000km² and Australia which is 100 times larger in extent than the CFK has only 3 000 species.

The fynbos biome is situated at the southernmost tip of South Africa. This region has a typical Mediterranean climate with hot, dry summers and mild wet winters. In this relatively small geographical area, the fynbos biome is unique in comparison to other Mediterranean ecosystems in its large species diversity. Much of this diversity originates from a few extremely specious clades (Linder and Hardy, 2004).

Erica, the largest genus in the CFK, has the most spectacular floral diversification with approximately 760 recognised taxa. These have been arranged into 43 sections according to corolla morphology (Guthrie and Bolus, 1905). Many of the sections therefore are not composed of "natural" relationships (Oliver and Oliver, 2005), but rather are associated broadly with different floral morphologies. Only recently has a partial *Erica* phylogeny been published (Pirie et al. 2011). This phylogeny confirms that the sections in *Erica* are not natural, because there has been widespread floral convergence.

Very little is understood about *Erica* reproduction including pollination, breeding systems and seed dispersal because of the size of this genus, lack of field expertise, site inaccessibility and uncertain taxonomy (Geerts and Pauw, 2009, 2010; Turner et al. 2011a and Turner, et al. 2011b).

This study aims to address some of the deficiencies by examining three *Erica* species with contrasting pollination biology

1.1 Pollination syndromes

One of the current debates focuses on the degree of specialisation in plant-pollinator relations (Wasser et al. 1996; Ollerton, 1996). There has been interest in the plant adaptive traits in relation to pollinators and scepticism over the view of a highly specialised tendency in pollination systems as opposed to a wider generalisation in pollinations systems (Wasser et

al. 1996). Hence an adaptive link between floral traits and observed pollinators might not be as simple as previously imagined (Herrera, 1996; Wilson and Thompson, 1996; Johnson and Steiner, 2000). Pollination syndromes are clearly in need of more critical examination using field observations and experiments (Johnson and Steiner, 2000). Waser et al. (1996) questioned the general views that pollination systems tend towards specialisation. Specialisation is defined as the structural adaptation of floral parts for a particular function, in this case pollination and often associated with single species interactions. They conclude that actual pollination systems are more often generalised and dynamic than previously reported. What appears to be the rule rather than the exception is the use of several plant species by a pollinator and several pollinator species by a plant. Despite exceptions that of *Babiana*, (Goldblatt and Manning, 2006) similar findings have been reported by Schnitzler et al. (2011) for the Cape. My study of three species, will help build on the existing literature to begin to understand if interactions between *Erica* pollinators are predominantly generalised or specialised.

1.2 Plant mating systems

Who mates with whom and how often (Barret et al. 1996) is a key topic in plant reproductive biology. In selfing, pollination can occur by either vector-mediated (facilitated) or autonomous (Goodwillie et al. 2005). Lloyd (1979), Lloyd and Schoen (1992) describe three modes of autonomous selfing, namely, occurring before the flower opens, occurring during competition for outcrossing or delayed selfing after outcrossed pollen opportunity has passed. Kalisz and Vogler (2003) however make the distinction that self-pollination does not fall strictly into three classes, as autonomous self-pollination timing is continuous and can be related to floral development stage.

Self-incompatibility systems are known from diverse families and are the principal anti-selfing mechanism in flowering plants (De Nettancourt, 1997; Weller et al. 1995; Kao and McCubbin, 1996; Charlesworth and Awadalla, 1998; Charlesworth, 2000; Takayama and Isogai, 2005). Self compatibility is often correlated with dispersibility. For example Baker's Law states that colonization by self-compatible organisms is more likely because colonisation will likely produce isolated individuals. Isolated self-compatible organisms are more likely to produce progeny without pollination vectors and uniparentally (Cheptou, 2012).

Facilitated self pollination entails transfer of pollen from anther to stigma within a flower by pollinators. Facilitated selfing can provide reproductive guarantees when mates are scarce

and when pollinators are limiting (e.g. Busch and Delph 2012, Anderson et al. 2003). When pollinators are scarce and out-cross pollen is limiting, a selfing mechanism may arise. Lloyd (1979, 1992) reported that delayed selfing provided reproductive guarantee, had no gamete discounting costs and therefore in most cases is the preferred solution to pollen limitation. Lloyd (1980) reported a greater percentage of selfing amongst colonising species in comparison with species that are related and occupy more stable habitats. This model has been popular because of the link between dispersal and mating system. However Cheptou (2012) emphasises that Baker's Law and its explanation is more complex than previously argued. For example selfing may be associated with changes in floral biology, life history and ecology (Barret et al. 1996).

1.3 Distribution of selfers

Surprisingly there has been an evolutionary move in many plant groups from out-crossing to selfing (Stebbins, 1974). Schemske and Lande (1985) argued that selfing occurred more commonly. In contrast Goodwillie et al. (2005) indicated that there was a much higher reporting of selfing plants than previously stated. For example the frequency of mixed mating has increased from 31% in Schemske and Lande's report to 42% in Goodwillie et al. (2005). Mixed mating is defined as hermaphrodite plant species that reproduce by both self-pollination (selfing) and cross-pollination (outcrossing) (Goodwillie et al. 2005). Aide, (1986) and Barrett and Eckert (1990) demonstrated that animal pollinated taxa are twice as likely to display a mixed mating system than abiotically pollinated species.

Many factors are likely to be involved in determining plant mating systems. For example, plant density and spatial distribution, timing of flowering, inbreeding depression, pollen limitation and populations that contain self-compatible and self-incompatible flowers, may all play a role (Stone, 2002; Masuda et al. 2004, Schoen and Lloyd, 1984, Goodwillie et al. 2005). Further investigations are required to determine the succession of trait transitions and evolutionary pressures driving the transition to selfing or mixed mating. For example do the traits such as small flower size, reduced separation of anthers and stigma evolve simultaneously with self-fertilization or sequentially (Karron et al. 2012)? There is almost no breeding system information available for *Erica*. For example Turner et al. (2011 a and b) failed to determine whether *E. halicacaba* and *E. hanekomii* could self-pollinate or not.

1.4 Pollination in *Erica*

The "typical" *Erica* is a perennial shrub, varying in height from several centimetres up to about 2m with a few species reaching six meters. *Erica* leaves are small, narrow and folded, with edges rolled abaxially. This fine leaf morphology is referred to as 'ericoid' and is one of the defining characteristics of Fynbos vegetation. The flowers have three bracts, four sepals and a corolla that has many forms; tubular, flask-shaped, globose, bell or cup-shaped. Eight anthers are present possessing a bi-lobed anther which opens by means of two pores. Two appendages are often associated with these anthers. An ovary is usually situated on a visible disc and comprises four chambers, each bearing numerous ovules (Schumann and Kirsten, 1992).

Oliver (2000) separates the *Erica* species into 5 major groups based on the size of the corolla:

- Group A – corolla 10 mm long or longer
- Group B – corolla 5 to 9 mm long
- Group C – corolla 2.5 to 5 mm long; anthers included within the corolla
- Group D – corolla 2.5 to 5 mm; anthers and stigma exserted beyond the corolla
- Group E – corolla less than 2.5 mm

Three broad pollination systems have been typically recognised in the Cape *Erica*: insects, birds and wind (Rebelo and Siegfried, 1985; Rebelo, 1987; Oliver 2000; Oliver, 2002). It is estimated that 80% of all *Erica* species are insect-pollinated, 15% bird-pollinated and 5% wind-pollinated (Rebelo et al. 1985).

Recently a rodent-pollinated species was documented (Turner et al. 2011a). In the light of this new knowledge Turner et al. (2011a) have recalculated the different *Erica* pollination systems; Insect-pollination is now estimated at 69.7 %, bird-pollination at 14.9 %, wind-pollination at 11.6 % and rodent-pollination at 0.1 %. Among the poorly represented wind–pollinated species, *Erica hispidula* and *Erica muscosa* are the most widespread (Turner et al. 2011a).

Mating systems have a not been thoroughly investigated in the Cape *Erica* species. Therefore it is imperative that more selective breeding experiments are carried out in the field on a variety of species.

1.5 Evidence for Rodent Pollination in *Erica*

In the Cape Floral Kingdom pollination by rodents is well established (Wester et al. 2009; Wiens, et al. 1983) and, as noted above, has recently been documented in the Ericaceae (Turner et al. 2011a). The pollination of flowers by non-flying mammals (to distinguish these from bats) was first mentioned by (Kerner, 1895, v. 2, p. 230) and was discussed nearly 80 years ago by Porsch (1934, 1935, 1936a, 1936b). The subject was not given further attention, until Morcombe (1968) suggested pollinator relationships between various proteaceous flowers and non-flying mammals in the south-western Australian flora. Rourke and Wiens (1977) reviewed the problem and noted that various floral traits, convergent in Australian and South African Proteaceae suggested adaptations for pollination by non-flying mammals. The following year Wiens and Rourke (1978) offered substantial evidence for pollination by non-flying mammals (mostly rodents) in two species of South African proteas. The first evidence (pollen and anther remains in scats) of rodents visiting a South American plants, *Cajophora coronata (Loasaceae)* was documented by Cocucci and Sérsic (1998), although rodents were not the exclusive pollinators.

Rodent pollination systems have been reported in six other Cape lineages namely *Massonia* Thumb. Ex Houtt and *Whiteheadia* Harv. Asparagaceae *sensu* APG III 2009 or Hyacinthaceae s.s.) (Johnson et al. 2001; Wester, et al. 2009; *Colchicum* L. (Colchiceae) (Kleizen et al. 2008), *Liparia* L. (Fabaceae) (Letten and Midgley, 2009) and *Hyobanche* L. (Orobanche), (Wester, 2011) and in other genera of the Proteceae such as *Leucospermum arenarium* Rycroft. (Johnson and Pauw, 2014). Few detailed studies of pollination biology have been performed for the Cape *Erica* species (e.g. Collins, 1983; Geerts and Pauw, 2010; Turner, 2011a). *Erica lanuginosa* has puzzled pollination biologists for many years, especially the pollination function of the unique, closed flowers. A preliminary phylogeny (Pirie et al. 2011) suggests that *E. lanuginosa* is closest to a group of insect-pollinated species, many of them in the former "minor-genera" with, indehiscent-fruit and with tiny flowers. The sturdy morphology, dull coloured, pendulous flower heads and copious production of nectar are consistent with traits of rodent pollinated plants. I therefore tested whether *Erica lanuginosa* is pollinated by rodents.

1.6 Long-proboscid fly pollination

In the last two decades it has become obvious that long-proboscid flies are important pollinators of plants in the Cape Floral Kingdom of South Africa (Goldblatt and Bernhardt, 1990; Johnson, 1992; Johnson and Steiner, 1995; Goldblatt and Bernhardt, 1995; Johnson and Steiner, 1997). Long-proboscid fly pollination as defined by Goldblatt and Manning (2000a) is unique to two regions in the world; southern Africa and the Himalayan region. In southern Africa, long-proboscid flies have been estimated to be the primary pollinators of 25% of regional species of *Pelargonium* and approximately 10% of regional Iridaceae (Goldblatt and Manning, 2000a; Coombs and Pauw, 2009).

Pauw et al. (2009) show in experiments with *Lapeirousia anceps* (Iridaceae) that there is a correlation between both the proboscis lengths of long-proboscid flies (volume of nectar consumed) and their impact on long-tubed flowers (contact with anthers and stigmas). Proboscis and floral tube length co-vary between different populations within the same species and this trait matching has been regarded as a reciprocal adaptation between the flower and the fly (Anderson and Johnson, 2008; Pauw et al. 2009). Anderson et al. (2010) demonstrated that 31 insect species were involved with 147 plant species suggesting that a small variety of specialist animals were visiting many specialised plant species.

Flowers visited by long-proboscid flies share common traits such as long, narrow corolla tubes, an absence of discernible scent, cream or pink colour and purple "nectar guides", as well as small amounts of dilute nectar, (Johnson and Steiner, 1997).

Specialist pollinators such as long-proboscid flies may be associated with low levels of flower visitation which can lead to "pollen limitation". This can usually be tested for by determining if supplemental hand-pollination leads to increased fecundity, (Turner et al. 2012). Selective pollen addition experiments in the field are vital to clarify whether a plant species is pollen limited.

Floral cues such as colour and shape are thought to guide pollinators particularly those with long proboscides towards rewards. These guides are cues consisting of a varying assortment of converging lines or dots, or markings around the corolla opening. They usually contrast strongly with the rest of the corolla. This contrast may also include patterns in the ultraviolet (UV) spectrum in which case the patterns may be indistinct or invisible to the human eye. Generally these visual cues increase foraging efficiency, by reducing search and flight times.

They may also enhance placement of pollen. This would place a high selective premium on foraging efficiency and could explain why they form tightly specialized associations with particular plant species that flower precisely during the few weeks that the adult flies are on the wing (Hansen et al. 2011). Hansen et al. (2011) conclude that approaches to flowers without floral guides by long-proboscid flies resulted in only five out of 64 (7.8%) successful attempts to probe the perianth tube. It is surprising that there is little information on the visual physiology of Diptera and even economically important Diptera have not been well studied (Woodcock, et al. 2014).

The morphology of *Erica aristata* including its long narrow, sticky perianth, the absence of scent and presence of nectar guides leads to the expectation of long-proboscid fly pollination in this species. Field observations were made of visits to the flowers, proboscis lengths were measured and compared to perianth length and breeding experiments were carried out to test this hypothesis.

1.7 Bird pollination

Bird pollinated plant species have a broad geographical distribution, occurring from southern Africa to Israel, India, Australia, New Zealand, tropical Asia, the Pacific (and North and South America). There is only a single known example of a native bird-pollinated plant from Europe (Spain) *Anagyris foetida* (Fabaceae) (Proctor, et al. 1996; Ortega-Olivencia et al. 2005). Bird pollination extends up to 4000 meters above sea level in the mountains of South America and East Africa and bird-pollinated species are distributed across diverse ecosystems, from tropical forests to grasslands and wetland environments.

Several families of birds have evolved nectar feeding habits and serve as pollinators. These include; hummingbirds (Trochilidae in the new world), sunbirds and spider-hunters (Nectariniidae in the old world) and honey-eaters (Meliphagidae in Australia, New Guinea and various Pacific islands).

The syndrome of ornithophily describes a combination of common floral traits found in flowers pollinated by birds. Included in these traits are firm textured floral tubes, unscented, tubular, red, orange and sometimes pink coloured flowers with sturdy perching posts to accommodate the long curved beaks and weight of specialist birds (Anderson et al. 2005). The nectar of bird-pollinated flowers have specific attributes, these include large nectar volumes and dilute sugar concentrations.

There is ample data that support findings that red and pink tubular flowers are indicative of bird-pollination (Faegri and van der Pijl, 1979). Even though there are noticeable similarities, a wide variety of morphological types are displayed by bird-pollinated plant species in the Cape flora (Geerts and Pauw, 2009). This variation can be attributed to adaptations for pollen placement on different body parts of foraging birds. Johnson (1995), Pauw (1998), Johnson and Brown (2004), Wester and Claßen-Bockhoff (2006) and Botes et al. (2008) suggest that flowering plants are adapted for pollen placement on various parts of the bird's body i.e. on the tongue, bill, crown, throat or feet. In each case, different floral morphology is required to effect precise pollen placement. Rebelo (1987) further divided bird-pollinated flowers into two classes; brushes - congested inflorescences of many small flowers, typical of Proteaceae or simple tubes – single flowers typical of Iridaceae and Amaryllidaceae, amongst many others. According to Goldblatt and Manning (2006) birds usually insert their entire bill into the tubular flowers and the deposit of pollen is usually on the head feathers.

Three morphological subgroups have been suggested based on traits of specific groups of bird-pollinators in the New World. Geerts and Pauw (2009) suggest that plants pollinated by hermit hummingbirds (subfamily Phaethorninae) are adapted for birds with obvious long, decurved beaks. Secondly, non-hermit hummingbirds (subfamily Trochilinae) possess straight, short beaks. The third subgroup includes plants that are adapted for generalist perching birds. These birds have broad, short beaks. According to Snow and Snow (1972), Kress (1985) and Westerkamp (1990) floral tubes of appropriate lengths correspond to the morphological subgroups referred to above.

It has been suggested that hummingbirds and passerines such as sunbirds select for different nectar properties in the flowers that they pollinate (Baker and Baker, 1983, 1990). Two diverse sunbird pollination guilds have been proposed in the south-western Cape (Geerts and Pauw; Johnson et al. 2009). The first involves short-billed birds and the other the long-billed Malachite sunbird. These two groups are defined by their floral dimensions with short-billed birds are excluded from obtaining nectar from long tube flowers legitimately, but being able to rob these flowers by piercing their bills through the tube and extracting the nectar.

In addition, Johnson and Nicolson (2008) demonstrate that there is a distinction between generalist and specialised bird pollination systems. Flowers pollinated by specialised passerine nectarivores (sunbirds) have nectar volume ranging from 10 to 30 µl, nectar concentration ranging between 15 to 25% w/w and sucrose content is between 40 to 60% of

the total sugar, similar to those found in hummingbirds. In comparison, the flowers pollinated by generalist birds are noted to have large nectar volumes ranging from 40 to 100 µl, exceptionally dilute nectar concentrations (8 to 12% w/w) and nominal sucrose with sucrose making up 0 to 5% of total sugar. These findings illustrate that sunbirds are highly specialised nectarivores and the plants that they forage on rely on a small suite of birds that feed on nectar. In contrast the plants that have generalist bird-pollination (weavers, bulbuls, orioles) may rely on a wider suite of birds that favour omnivorous feeding behaviour. These results are mirrored in American plants that are pollinated by hummingbirds and generalist passerines (Johnson and Nicolson, 2008).

Barnes et al. (1995) revealed that 29 of the 37 ornithophilous *Erica* species had dominant-sucrose nectars with a mean sucrose proportion of 93.8 ± 6.2% (Mean ± SD). Johnson and Nicolson, (2008) later showed that bird-pollination was associated with a sucrose proportion of between 40-60%. As their results did not correspond to those of (Baker and Baker, 1983, 1990), Barnes et al. (1995) concluded that pollination syndromes could not be deduced from nectar types alone. In my study I investigated nectar attributes.

Bird-pollinated plants in the Cape Floral Kingdom are estimated to make up 4% or 318 species. It is startling that so little research has been performed on most of these plants. There have been suggestions that there are correlations between sunbird bills and perianth tube lengths (Rebelo and Siegfried, 1985; Goldblatt and Manning. 2006). Geerts and Pauw (2009) emphasise that similar correlations for plants in the Cape Floral Kingdom can be made and they have arranged the specialist nectar feeders according to three bill groups, namely:

- Long, narrow curved bill group including Cape Sugarbirds *Pomerops cafer* and Malachite Sunbirds *Nectarinia famosa* with bills 29-36 mm in length.
- Short, narrow curved bill group including Southern Double-collared Sunbirds *Cinnyris chalybea* with bills 18-23 mm in length and Orange-breasted Sunbirds *Anthobaphes violacea* with bills 20-23 mm in length.
- Broad uncurved beaks, the generalist nectar feeders including Cape Weavers *Ploceus capensis* and Cape White-eyes *Zosterops virens* with bills 12-15 mm in length.

Geerts and Pauw (2009) indicate that Malachite Sunbirds pollinate a large variety of long, tubular flowers in the Cape Floral Kingdom. The differences in habitat preferences indicate that Cape Sugarbirds frequent tall, dense stands of post-fire Proteaceae shrubs and Malachite

Sunbirds prefer more open vegetation (Rebelo, 1978). These findings also correlate to the heavier body mass of the Cape Sugarbird and their preference with sturdier, shrub-type, nectar rich Proteaceae floral component and the lighter body structure of the Malachite Sunbird prefers open vegetation provides for the long-perianth tubed flowers of the light structure of Iridaceae and Amaryllidaceae where the Malachite Sunbird is typically observed.

Malachite Sunbirds compete aggressively for resources and even though there may be higher numbers of Double-collared Sunbirds in the same habitat, Malachite Sunbirds exclude the smaller bodied Sunbirds (Geerts and Pauw, 2009). Malachite Sunbirds forage on the long-tubed flowers as they have larger volumes of nectar than the short-tubed flowers (Stiles, 1975, Goldblatt and Manning, 1999, Kaczorowski, Gardener and Holtsford 2005, Geerts and Pauw, 2009).

Rebelo et al. (1985) postulated that Orange-breasted Sunbird were the sole pollinators of bird-pollinated Cape *Erica* species. Geerts and Pauw (2009, 2011) reveal that Double-collared Sunbirds do forage amongst certain *Erica* species, including *Erica cruenta, Erica discolor* and *Erica perspicua.* In their study Geerts and Pauw (2009) showed that the smaller Sunbirds could not replace the Malachite Sunbird as a pollinator if Malachite Sunbirds were absent and this would have ecological consequences for those plant species that were reliant on Malachite Sunbird pollinator services. The current study examines the possibility of bird-pollination in *Erica sessiliflora*.

1.8 Hypotheses

This study tested the following primary hypotheses:

That the unusual morphology and dull colouration of the corolla in *Erica lanuginosa* represents an adaptation for rodent-pollination. In addition breeding experiments were carried out to test if this *Erica* species relies on rodents for breeding success.

In *Erica aristata*, the sticky outer corolla tube, unscented, long narrow perianth with floral guides suggested that this species was likely to be pollinated by long-proboscid flies. This hypothesis was tested by visual observation and measurement of nectar volumes, sugar concentrations and breeding experiments.

Birds were hypothesised to be likely pollinator in *Erica sessiliflora* on the basis of the sturdy tubular floral morphology. Breeding experiments and visual observations were used to test this hypothesis.

The *Erica* species under investigation are narrow endemics, localised to the Klein River Mountains, and were therefore thought to have specialised pollinators. Breeding systems and pollinator exclusion experiments were carried out to test this hypothesis.

CHAPTER 2: Materials and Methods

All three *Erica* species have contrasting floral morphologies and thus are likely to have different pollinators. They all largely co-occur in Vogelgat Private Nature Reserve. Field studies for the determination of pollinators in *Erica lanuginosa* were carried out in the Vogelgat Private Nature Reserve, Hermanus, Western Cape Province, South Africa. *Erica aristata* study sites were in the Vogelgat Nature Reserve and Maanschynkop Nature Reserve, adjacent to Vogelgat Nature Reserve; these reserves are 125km east of Cape Town.

As a consequence of a devastating fire burning the vegetation of Vogelgat and surrounding areas in December 2012, a population of *Erica sessiliflora* was found on Boskloof Farm, Akkedisberg Pass, east of Stanford and 15km east of Hermanus and this served as a third study site. The Vogelgat and Maanschynkop Nature Reserves and Boskloof Farm fall within the Fynbos Biome (Mucina and Rutherford, 2006). The dominant lithological class within the biome is sandstones of the Peninsula Formation of the Table Mountain Group and the broad soil pattern class within the biome are quartzite (Whittle-Herbert 1990).

2.1 Study sites

2.1.1 Erica lanuginosa

Field studies for *Erica lanuginosa* were conducted between early April and the end of July 2012, in the Vogelgat Nature Reserve. The primary study site was on Washington Ridge at Selago Rocks; (34° 23’ 31.3”S, 19° 18’ 42.5”E, 265 m above sea level). The area was rocky and had a steep southern aspect. The population of *E. lanuginosa* at this site was made up of between 200 and 300 mature, flowering plants.

2.1.2 Erica aristata

Field studies were conducted between early August and the middle of September of 2012, in the Maanschynkop Nature Reserve. The selected site was on Lex’s Gully, a series of zig zag pathways on the southern slopes, with a steep aspect; (34° 23’ 36.25”S, 19° 19’ 31.90”E). The site had a healthy mature plant population of well over 200 plants.

2.1.3 Erica sessiliflora

The study site co-ordinates are 34° 24 12.2”S and 19° 40 54.2E on the farm Boskloof. The site has flat, north-west facing aspect. The population numbered approximately one hundred individuals.

2.2 Study Species

2.2.1 Erica lanuginosa

Erica lanuginosa was first described by Andrews (1806); *lanuginosus* Latin translation, “with woolly down”, referring to the hairy covering on the sepals and corolla. This species is endemic to the Kleinriver Mountains of the Southern Overberg region, Western Cape Province, South Africa, occurring on rocky outcrops from Hermanus towards Stanford, as well in the Akkedisberg Pass. Generally it grows near rocks or on rocky out crops, where it forms a spreading mat-like growth, but it may also be found in the open where individuals usually grow erect to form low shrubs about 350 mm in height. It flowers from April until August.

The unusually shaped flowers of this species do not look like that of an *Erica*. It has downy sepals that are green, tinged reddish brown. The lobes of the corolla, which are also covered with, soft hairs, are tightly pressed together at the tips to form a sharp “beak” and are split almost right down to the base of the flower, where the angle between them bears a short triangular tooth. (Figure 3-1A). The flowers hang down in clusters with a cone-shaped corolla that is 14 to 18mm in length and dull red brown in colour (Schumann and Kirsten, 1992).

Intriguingly, Inge Oliver, in her unpublished systematic diagrams of the genus *Erica* (South African National Biodiversity Institute, Compton Herbarium, Kirstenbosch) noted an unusual “fold” at the base of each corolla lobe. This is a unique character within the genus (Figure 3-1B). This can also be seen in the cross section of Baker and Oliver (1967, plate 154)**.**

Anthers are coupled laterally together, forming a ring-like structure through which the stigma is exserted. When a pollinator probes the perianth in search of a nectar reward, this anther-ring is broken (usually termed “triggered”) and the pollen is released via anther pores. Visibly ‘triggered’ anthers are a sign that the flowers have been visited by a pollinator (Turner et al. 2011).

2.2.2 Erica aristata

Erica aristata, Andrews (1807); *aristatus* = with an awn; referring to the awns at the ends of the leaves. This species forms a well branched shrub reaching a height of 600 to 700mm and bears umbels of usually four flowers at the tips of its branches. The flower is purplish-pink, with eight darker veins running longitudinally to a slightly constricted dark purple throat. The corolla is tubular, 25 to 30 mm long, and the lobes turn back at the mouth, giving it the appearance of an old-fashioned, frilly white bib. The outer surface of the corolla tube is also extremely sticky, giving the flowers a glossy white sheen (Figure 3-3A). The anthers have a distinctive 'double chin'.

Distribution is limited to areas above 300 - 600m above sea level in the mountains between Hawston and Stanford in the Southern Overberg region. It flowers from early August to October (Schumann and Kirsten, 1992).

2.2.3 Erica sessiliflora

Erica sessiliflora Linnaeus f. 1781; *sessiliflora* = sessile flower referring to the lack of a pedicel. This species is distinct because it is the only known serotinous *Erica*; when the flowers die and shrivel up the sepals remain green and increase in size, protecting the developing ovaries. The hard sepals gradually turn red and this "fruiting head", which resembles a fungal growth, may remain on the plant for years until fire stimulates the fruit to dehisce. Shrubs may reach 2 m in height and they bear greenish white flowers packed tightly into short spikes at the end of the branches. The corolla is tubular and between 16-30 mm long and the anthers are positioned in its mouth and have long awns and a short 'chin' (Figure 3-5A)

Erica sessiliflora is widespread in the southern Cape, as far east as Humansdorp. Plants occur in moist, seepage areas and flowers are borne from April to September (Schumann and Kirsten, 1992).

2.3 Pollinator observations

2.3.1 Erica lanuginosa

In 2011, 16hrs of observations were carried out during different periods of the day. Plant pollinator interactions were observed from 7:30 until 13:00 for 6 days in early April 2012. A

total of 26 hrs were devoted to observation of any bird-plant interactions, as well as any other interaction with possible pollinators.

To test the hypothesis of rodent-pollination in *E.lanuginosa*, Bird Cam 2.0, 8mp digital motion sensor cameras were set up to remotely monitor individual plants to expand the suite of observations of flowers and to include nocturnal observations. Between 18 April 2012 and 30th June 2012 a total of 74 days of monitoring with the aid of motion cameras was achieved.

To confirm the identity of rodents imaged by cameras as well as to determine pollen loads of pollinators, PVC gutter type traps were used to capture rodents in the vicinity of *E.lanuginosa* plants. These traps are made of sections of square PVC down pipe, 300mm in length x 60mm width x 75mm in height. They have a rear, detachable, clear Perspex window for easy access. Inside this PVC tube there is a sensitive aluminium trap plate that is held in place at the front aluminium door, by way of a small latch. The animal is lured into the trap with a mixture of peanut butter and oats. Once the animal steps onto the sensitive plate, the front door closes, capturing the animal. Thick (5mm) thermal plastic was wrapped around each trap to insulate the tube and keep the animal warm. Traps were in place for four days, but were left unset during the heat of the day to avoid stressing animals. Traps were sprung in 6 out of the 16 traps and had their rolled oats taken, but showed no signs of rodents in the traps, giving indications of some malfunctioning on the trap mechanism or the ability for the rodent to escape.

All rodent interactions were conducted under the guidance of the Rhodes University mammal ethics committee certificate number: ZOOL-05-2013

Captured rodents were removed from traps at 8:00 the following morning, identified and released at the position of their capture. Scats were retrieved from the traps and placed in zip-lock plastic sleeves and trap number, locality, date and rodent species recorded. Scats were collected to determine whether the rodents had ingested any pollen whilst preening themselves

Scats were transferred to separate labelled eppendorf tubes with 70% alcohol and stored at 4 ^{o}C until analysis. For pollen analysis, the droppings were crushed and the tubes placed in a vortex shaker for 30 seconds for five minutes to separate heavier faecal matter from the lighter pollen grains. Pollen samples from the supernatant were treated with three drops of

fuchsin red dye, mounted in a small amount of stain on a slide and covered with a cover slip. Pollen grains were counted under a compound microscope.

On the morning of the 31st May 2012 one of the *Acomys subspinosus* that had been caught was brought down to Base Camp to conduct observations in a terrarium. The glass terrarium (460 x 300 x 230 mm) was filled with local gravel, indigenous plants, water and an egg box filled with soft tissue paper for the rodent to retreat to. The egg box had a large enough opening to enable the rodent to freely move in and out. Observations started at 20:00 and continued for 4 hrs after fresh branches of *Erica lanuginosa* bearing open flowers were placed in the terrarium. In the morning this individual was returned to the original site of capture, and released. An *Otomys irroratus* was captured on the 2nd of June 2012 and taken for terrarium observations under the same set of criteria as *Acomys subspinosus*.

2.3.2 Erica aristata

Initial observations of plant pollinator interactions were made in 2010, from the 4th of August to the 22nd of September. A total of 17 hrs of observations were carried out during different periods of the day. In 2011, between the 3rd August and the 17th September a total of 23 hrs of observations were carried out. In early April 2012, a total of 26.5 hrs was spent observing any pollinator-plant interactions. Once flies were seen probing the corolla tube, a butterfly net was used to capture them. The net was quickly placed over the flowers and the top of the net was raised to enable the fly to fly into this space. Captured flies were killed using an ethyl acetate killing jar and mounted. One of the flies was sent to Dr. Barraclough at the University of KwaZulu-Natal for identification.

2.3.3 Erica sessiliflora

Field observations started on 16th April 2013 and were concluded on the 29th of April 2013. Observations began at 7:00 and lasted until midday. A total of 14 hours of observations were carried out over 5 days. The number of visitors probing flowers for this period was counted, recording visitor type, species and sex. Photographs of flower visits by pollinators were recorded where possible (See Figure 3-3A and 3-3B).

2.4 Plant morphology

To investigate the match between flower and pollinator morphology, different floral traits including corolla length, corolla width, anther length, stigma length, and stigma-anther distance were measured using an electronic calliper.

Means were calculated for corolla length, corolla opening and anther/stigma distance of the three different *Erica* species (Table 3-1) and compared to the corresponding measurements of pollinators. Mean rostrum lengths for pollinating rodents were sourced from Wiens et al. (1983). Mean Orange-Breasted Sunbird culmen lengths were calculated from data in Geerts and Pauw (2009). Mean long-proboscid fly proboscis lengths were calculated from specimens caught during the current study.

2.4.1 Erica lanuginosa

Images were taken with a Nikon D90 digital camera, 50 mm, F1.8 lens with close-up filters of twenty individual flowers from twenty individual plants. A metal ruler included in each digital image indicated the scale and the following measurements were recorded from the photographs: corolla length, corolla width, anther length, sigma length, stigma- anther distance.

2.4.2 Erica aristata

In 2011 and 2012 the following measurements were taken from ten fresh flowers one from each of ten separate, randomly selected plants (2011) and twenty flowers from twenty separate plants (2012). Measurements include corolla length, corolla diameter at top, corolla diameter at base, flower opening, diameter of floral skirt, stigma exsertion beyond floral skirt, style length, anther length, anther filament, stamen length.

Average proboscis length, body length, wingspan and thorax diameter were determined for the three long-proboscid flies captured.

2.4.2.1 Reflectance spectra of *Erica aristata* flowers

Many pollinators perceive different colours to humans and flowers may provide ultra-violet signals for their pollinators (Hansen et al. 2011). To quantify any ultra-violet signals of the flowers *E. aristata* flowers the reflectance spectra of the various floral parts were measured using an Ocean Optics S2000 spectrophotometer (Ocean Optics Mini, Dunedin, Florida

USA), coupled to an Ocean Optics Mini-Deuterium tungsten halogen light source (200-1100nm) as described in (Peter and Johnson 2008).

The reflectance spectra of the following floral parts were measured for three flowers, each from a different individual plant:

1. Petal tip (white)
2. Petal base (pink base)
3. Corolla Tube (distil) (pink)
4. Corolla Tube (white)

Reflectance was measured as a percentage between 300 and 700 nm and the standard deviation plotted.

To determine whether flowers possess patterning in the UV region of the spectrum, flowers were photographed with a B+W 403 black filter (Jos. Schneider Opstische Werke, Bad Kreuznach, Germany). This removes all wavelengths of light above 400 nm. Konica 400 ISO black and white film (Konica-Minolta, Tokyo, Japan) was used as it is sensitive to near-UV (350-400 nm). The grey scale of Kevan et al. (1973) was used to judge the exposures.

2.4.3 Erica sessiliflora

Morphological measurements of *Erica sessiliflora* flowers included: corolla length and width, calyx length and width, style length inclusive of ovary, style length, anther positioning, stigma-anther distance and corolla aperture.

2.5 Nectar properties

Nectar volume, concentration and sugar ratio may represent an adaptation for specific pollinators (Baker and Baker 1983, 1990, Barnes et al. 1995, Johnson and Nicolson 2008). Flowers were not bagged prior to nectar sampling. Nectar samples were therefore collected from specimens of each species and the percentage sugar concentration and nectar volume analysed.

Nectar sugar concentrations were sampled using an Eclipse hand-held 0 to 50% sucrose equivalent refractometer (Bellingham and Stanley LTD).

2.5.1 Erica lanuginosa

Nectar from 60 separate individual flowers, each from a different plant were sampled throughout the population using a gas tight syringe, accurate to 2 µl. In 2012 a further test was conducted on nine flowers using disposable, calibrated 1 ml, micro-syringes accurate to 0.005 ml.

In addition nectar samples for *Erica lanuginosa* were spotted onto four 7.0 cm Whatman filter papers and dried for later determination of constituent sugars using HPLC methods, which were carried out by the University of Cape Town, as described by Brown et al. (2009).

2.5.2 Erica aristata

Nectar from thirty flowers from separate plants was sampled throughout the population using disposable, calibrated 1 ml, micro-syringes accurate to 0.005 ml.

2.5.3 Erica sessiliflora

Nectar from 16 flowers from separate plants was sampled throughout the population using disposable, calibrated 1 ml micro-syringes.

2.6 Exclusion and breeding system experiments

Breeding experiments were carried out to selectively exclude possible pollinators by installing fine mesh bags over the flowers in bud stage. Subsequent treatments tested whether these species of *Erica* required pollinators for seed set or if autonomous self-pollination is possible in the absence of a pollinator.

All flowers with exception of open control treatments were bagged in bud stage with fine mesh bags and then subjected to one of the following treatments:

- Bagged in bud stage to test for autonomous self-pollination - flowers were bagged in bud stage and left unmanipulated to test for the possibility of non-facilitative autonomous self-pollination.
- Facilitated self-pollination – after flowers opened, stigmas were pollinated by hand with pollen from the same flower using a clean dissection needle. The flowers were then rebagged to exclude further pollinator interaction.

- Geitonogamy – after flowers opened, stigmas were pollinated by hand with pollen from another flower on the same plant using a clean dissection needle. The flowers were then rebagged.

- Cross pollination (xenogamy) – after flowers opened, stigmas were pollinated by hand with pollen from a different plant using a clean dissecting needle and the flowers rebagged.

- Open control – individual unbagged flowers were tagged and left untreated in order to allow for natural pollination to occur.

After two months, fruit were removed from the plants to determine seed set. This was determined by opening the ovary carefully under a stereomicroscope and counting viable seeds. Bags that were lost were noted and bags of different colours were used to differentiate treatments.

2.6.1 Erica lanuginosa

In 2011, breeding system experiments were carried out in mid June. Vouchers in the Vogelgat Herbarium show that flowering occurs as early as 14th of April so two further breeding system experiments were carried out in early April 2012. The first breeding system experiment in 2012 involved the sampling of eighty separate flowers from twenty different plants. Sixty separate flowers from twenty different plants were sampled in the second breeding system experiment.

To determine the role of rodents in pollination and also to see whether *Erica lanuginosa* was capable of autonomous seed production, twenty, separate, mature plants were selectively bagged with fine mesh bags. A single flower was selected in the bud stage and all the other flowers that may interfere with autonomous self-pollination treatment were removed. Twenty separate, single uncovered flowers were marked as controls.

In order to determine whether the species was capable of self-pollination, anther tripping was observed before self-pollination treatments were conducted as described by (Geerts and Pauw, 2010; Turner et al. 2011). Around the selected flower which had not been anther tripped, neighbouring flowers were cut away to exclude any possible pollination interference, as neighbouring anthers may spray pollen. By carefully triggering the anthers with a dissection needle, pollen was transferred to the stigma and thereafter the individual flower

was enclosed in a fine net bag. Each needle was cleaned after pollen transfer in order to avoid any pollen contamination between treatments.

A further experiment was done to determine cross pollination (xenogamy). Here again flowers were selected with their anther rings intact and neighbouring flowers cut away from around the selected flower. With the aid of a dissection needle pollen from a different plant was captured on the needle tip and transferred to the selected stigma and the flower was then bagged. Each needle was cleaned after pollen transfer in order to avoid any pollen contamination.

2.6.2 Erica aristata

Exclusion and breeding experiments were conducted as for *Erica lanuginosa* (2.6.1). Initial investigations in 2010 included the following treatments:

1. Bagged in bud stage (testing for autonomous self-pollination) – up to 4 flowers were sampled from each of 46 different plants, totalling 176 treatments.
2. Facilitated self-pollination - 4 flowers were treated from 2 different plants each, totalling 8 flowers.
3. Cross-pollination – 4 flowers were treated from 2 different plants each, totalling 8 flowers.
4. Open control - up to 6 flowers were treated from each of 46 different plants, totalling 163 flowers sampled.

The treatments undertaken are described above. In the 2011 flowering season 20 separate plants were selected, with four flowers treatments per plant, and flowers being examined and treated as described above (2.6). In 2012 twenty plants were selected and four different treatments per plant were undertaken. These included:

1. Bagged in bud stage, testing for autonomous self-pollination
2. Facilitated self-pollination
3. Cross pollination
4. Open control

A total of 80 flowers were investigated across the four treatments.

An attempt was made to determine if cross pollination would occur after emasculation. Here the corolla of unopened flowers was cut away to expose the anthers and the stigma. Carefully, the anthers were cut away and the anther ring dismantled, without causing any pollen to dislodge onto the stigma. Thereafter these flowers were inspected for fruit set.

2.6.3 Erica sessiliflora

Breeding system experiments for *E. sessiliflora* were similar to those of rodent exclusion and breeding system experiments described for *E. lanuginosa* (2.6.1). These included:

1. Bagged in bud stage, testing for autonomous self-pollination
2. Facilitated self-pollination
3. Geitonogamy
4. Cross-pollination
5. Open control
6. Seed set from previous season (as *E. sessiliflora* is unique and retains old fruit from the previous season covered by hard succulent sepals)

In these experiments 10 different plants were chosen and 5 flowers of each treatment per plant were performed i.e. 5 different treatments on a plant x 10 plants, plus 18 separate plants were assessed for seed set in the previous year.

2.7 Role of insects in *Erica lanuginosa*

Holes were often seen in the corollas of *Erica lanuginosa* and these might represent nectar robbery caused by either flying insects such as carpenter bees as suggested by Rebelo et al. (1985) or by crawling insects (Turner et al. 2011b).

To determine if robbing caused a reduction in seed set, all flowers were counted and those that were robbed (holes bitten into corolla wall) were noted and marked. Seed set of these flowers were recorded and compared to the seed set of the unrobed flowers on the same plants.

In 2011 a total number of 1391 flowers were examined for robbing from 20 separate plants. In 2012 a total of 2567 flowers were examined for robbing from 20 separate plants.

It was noted that many of the flowers in the bagged exclusion experiments were robbed and on 3 of the plants a corrugated card board flashing was fastened by way of bin wire around the base of the stems. This was to encourage any weevils to hide in these structures. Weevils feed nocturnally and then go down to base of the plants during the day. This method is used on deciduous and vine farms to determine the numbers of these pest insects prior to a chemical treatment and the subsequent efficacy of these treatments. Corrugated card flashings were opened and checked for any signs of Curculionidae at each observation.

To elucidate whether bees or ants acts as robbers, Plantex® was placed onto 10cm of the base of the stems of 6 free standing plants. All vegetation around these plants was removed to prevent any crawling insects reaching the flowers. Two flowers with anthers intact per plant were tagged. These flowers were observed for any robbing every time the site was visited. One month later holes in their corollas were counted. Twenty eight flowers were examined for seed set. A further six separate plants representing a total of 685 individual flowers were examined.

2.8 Statistical analysis

Standard analyses were performed using Microsoft Excel and Past 3 (Hammer, 2001).

An index of self-incompatibility (ISI) was calculated using the following equation:

$$ISI = 1 - \frac{\text{relative selfed success}}{\text{relative outcrossed success}}$$

Relative pollination success is defined as the proportion of fruits set to flowers pollinated (Raduski et al. 2012).

Kruskal-Wallis one-way analysis of variance was used to determine significance between treatments in the breeding system experiments and between the nectar volumes and sugar concentrations of the three *Erica* species. This analysis is comparable to ANOVA, comparing the medians of several univariate groups. It can also be regarded as a multiple-group extension of the Mann-Whitney test (Zar, 1996). It does not assume normal distribution, but does assume equal-shaped distribution for all groups. Data were analysed in Past 3 using

Kruskal-Wallis to determine significant differences between treatments followed by Mann-Whitney posthoc pairwise comparison. (Hammer et al. 2001).

CHAPTER 3: Results

3.1 Pollinator observations

3.1.1 Erica lanuginosa

After a total of 42 hours in 2011 and 2012 of day-light field observations, no pollinators had been seen interacting with flowers of *Erica aristata* suggesting that there may be a nocturnal pollinator present. Camera traps confirmed *Aethomys namaquensis* probing *Erica lanuginosa*. (Figure 3-1C).

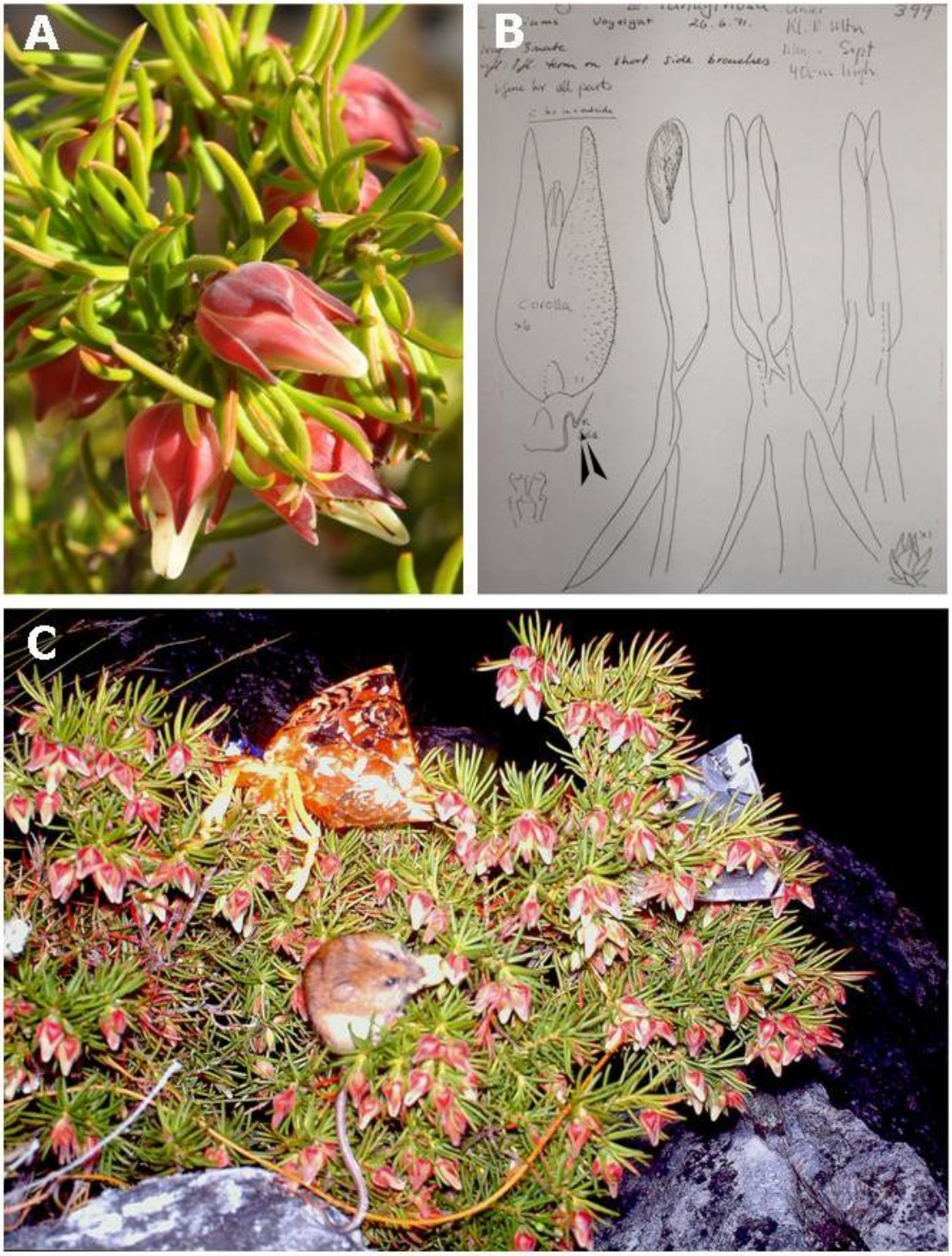


Figure 3-1: A. Individual flowers of *Erica lanuginosa* showing their tightly apressed corolla lobes. B. Schematic drawing by I. Oliver depicting the 'hinge'(arrow) at the base of the corolla which allows movement of corolla lobes. C. *Aethomys namaquensis* probing *Erica lanuginosa* on Washington Ridge, Vogelgat Nature Reserve on 25th April 2012 at 19:57.

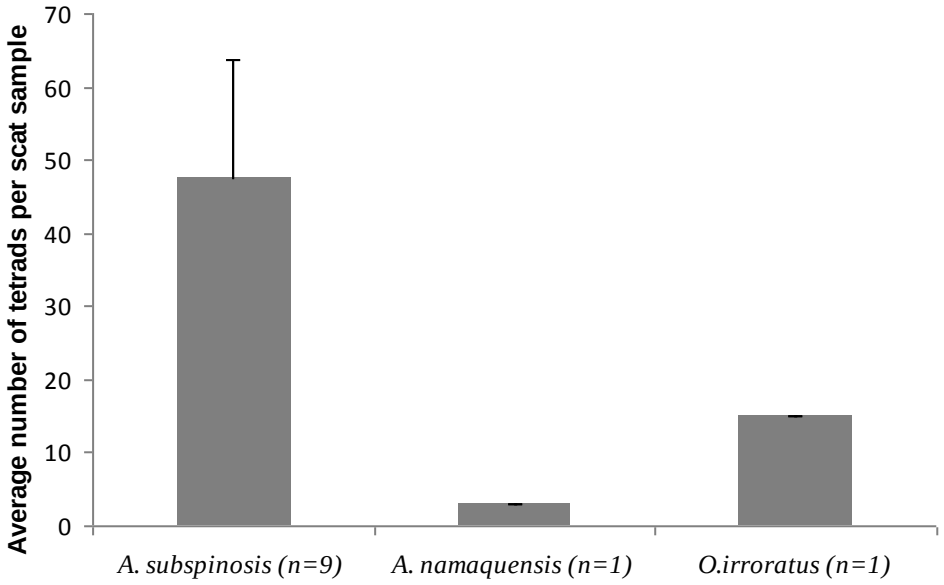


Figure 3-2. Average number of *Erica lanuginosa* tetrads per scat sample for three rodent species. Error bars indicate standard error.

A day after placing out the camera-traps a rodent was photographed, with what appears to be a sepal in its mouth. Digital camera traps recorded fourteen images of rodents visiting two different plants on seven separate days at two different sites in the study area. *Aethomys namaquensis* was the first rodent photographed (Figure 3-1C) prising the corolla open. This represents the first ever field photograph of a rodent-pollinating an *Erica.*

Subsequently, three different species of rodents, *Aethomys namaquensis*, *Acomys subspinosus*, and *Otomys irroratus* were captured in PVC gutter traps. *Acomys subspinosus* accounted for the majority of all the rodents trapped representing 9 out the total of 11 rodents caught. Scat samples taken from traps were examined for the presence of pollen and all species showed evidence of pollen tetrads in scats (Figure 3-2). The virtual absence of co-flowering species of *Erica* at the trapping site, suggest that these tetrads are likely those of *Erica lanuginosa.*

None of the rodents that were viewed in a terrarium showed any inclination to feed on *Erica lanuginosa* nectar. *Aethomys namaquensis* (n=1) and *Acomys subspinosus* (n=1) had a flight response and only wanted to escape the terrarium. *Otomys irroratus* (n=1) on the other hand attempted to chew everything including flowers and branches.

Ants (minor of *Camponotus maculates)* were seen crawling all over the flowers, none penetrating flowers. It was therefore decided to conduct an exclusion and breeding experiment for any possible pollination by ants. (See section 3.5).

3.1.2 Erica aristata

Observations were conducted for a total of 68.5 hours on 20 days between 2010, and 2012 during different times of the day, during different climatic conditions and at two different sites. Only long-proboscid flies of the family Nemestinidae (Figure 3-3B) were observed pollinating this species and a total of 35 individuals were seen although only 8 were visiting flowers, each flower on a different plant (Figure 3-4). Visitation rates were therefore very low and visitations fleeting with each fly spending one to two seconds per flower and then visiting the next flower before flying off rapidly to another plant elsewhere. Long-proboscid flies visiting a flower were observed to put their whole head into the flower.

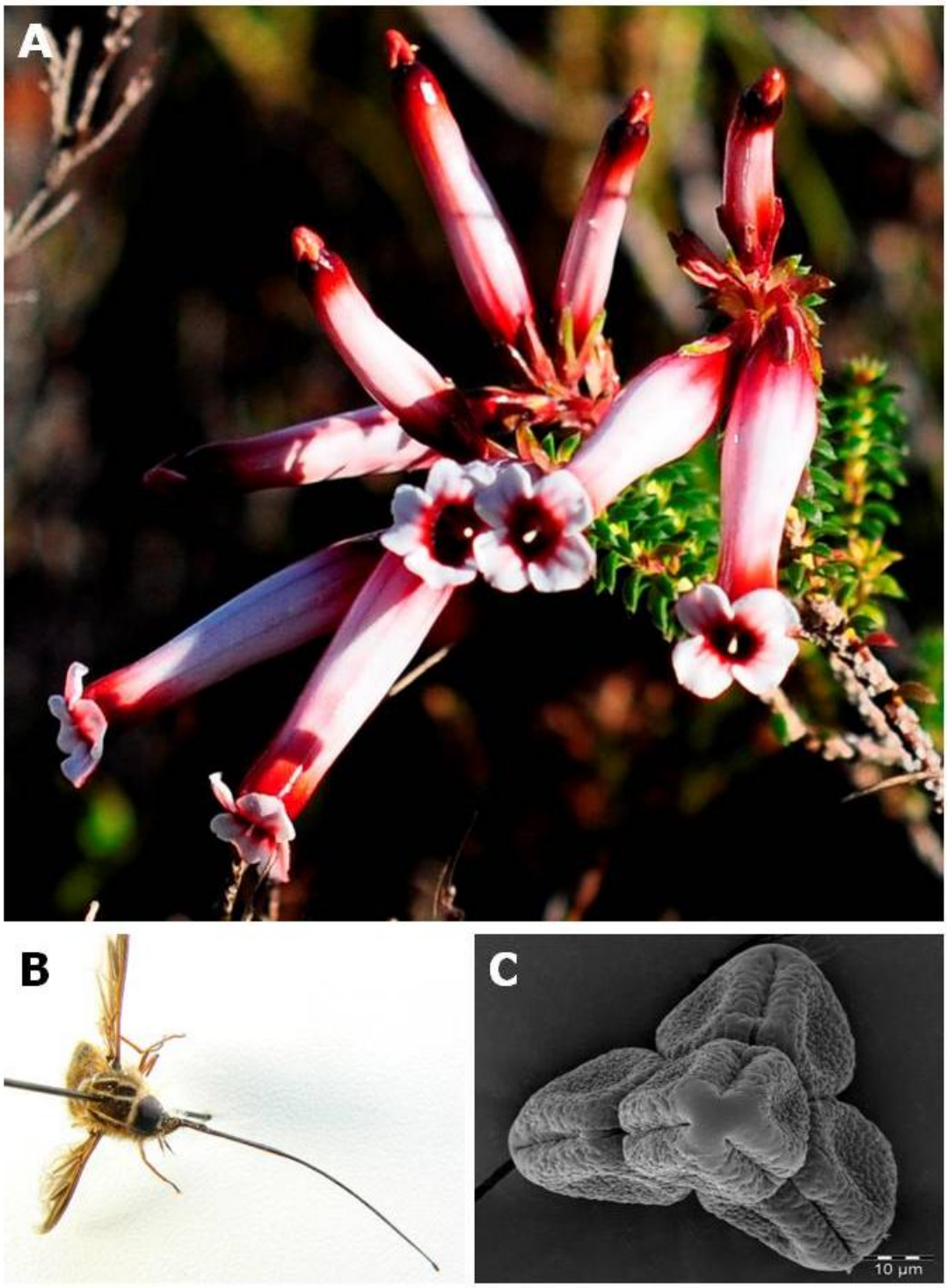


Figure 3-3: A. Flowers of *Erica aristata* showing their long tubular corolla tube with distinct white and pink to red colouration. White petals form a 'skirt' surrounding the pink corolla opening forming a "bulls eye" pattern. B. Unidentified long-proboscid fly (Nemestinidae) that was caught while visiting *Erica aristata* flowers. C. Typical pollen tetrad of *Erica aristata* imaged by scanning electron microscope. These pollen grains are present on the body, head area and along proboscis of the long-proboscid fly.

Only three flies were caught whilst visiting *Erica aristata* flowers, one was mounted and sent to D. A. Barraclough (School of Biological and Conservation Sciences, Howard College, University of KwaZulu-Natal, Durban, South Africa) and awaits description (Figure 3-3B). Long-proboscid flies were seen hovering over plants for periods longer than 20 minutes, after *Erica aristata* had ceased flowering. On some occasions flies perched on *Restio* species in the vicinity of the flowering *E. aristata* but were not observed visiting *Restio* flowers.

No images of any pollinators were obtained from the camera traps as the camera traps are likely not sensitive enough to be triggered by flies.

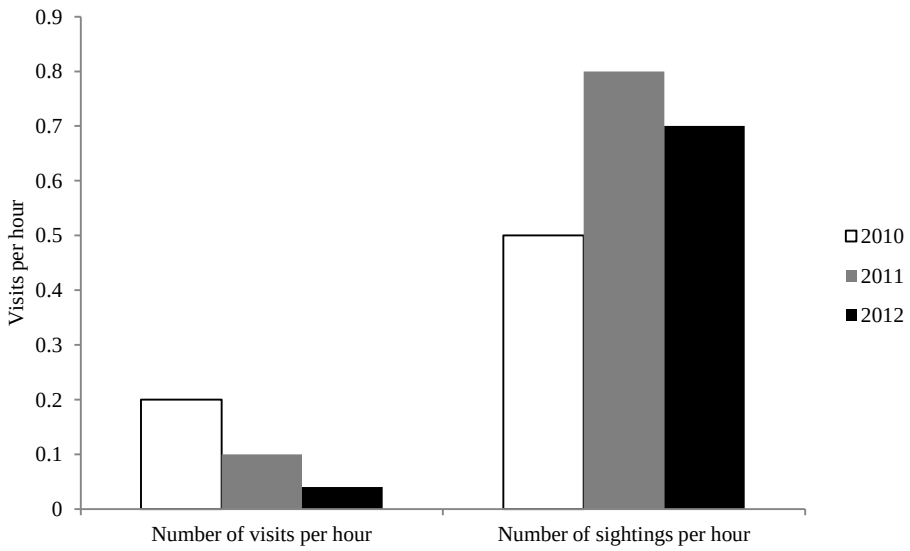


Figure 3-4: Comparative field observations of long-proboscid fly (LPF) visits to *Erica aristata* between 2010, 2011 and 2012.

Results from the two different study sites, one in Vogelgat Nature Reserve and the other in Maanschynkop Nature Reserve revealed that visitation rates by the long-proboscid flies were low at both sites in both 2011 and 2012 (Figure 3-4).

3.1.3 Erica sessiliflora

Figure 3-5: A. Male Orange-Breasted Sunbird, *Nectarinia violacea* probing flowers of *Erica sessiliflora*. B. Female Orange-Breasted Sunbird, *Nectarinia violacea* visiting flowers of

Erica sessiliflora amongst different coloured mesh exclusion bags used in breeding system experiments.

After viewing stands of plants for five days for a total of fourteen hours, fourteen different foraging bouts by birds were seen on *Erica sessiliflora* (Figures 3-5A and 3-5B). The majority of birds seen were sunbirds and in particular the Orange-Breasted Sunbird, *Nectarinia violacea.* Other birds seen but not visiting *Erica sessiliflora* were the lesser Doubled Collared Sunbirds, *Nectarinia chalybea* and Malachite Sunbirds, *Nectarinia famosa*. There was a higher visitation rate (50 visits in 14 hours of observation hours) of a number of sunbird species to co-occurring *Erica discolor.* This included the Orange-Breasted Sunbird, *Nectarinia violacea*, the lesser Doubled Collared Sunbird, *Nectarinia chalybea* and Malachite Sunbird, *Nectarinia famosa*. Females of the Orange-Breasted Sunbirds were the most frequent visitors to the flowers of *Erica sessiliflora*.

3.2 Plant Morphology

Table 3-1: Mean floral traits for three *Erica* species compared for corolla length, opening and anther/stigma distance.

	Pollinator	**Pollinator measurements (mm)**	**Corolla length (mm) n = 20**	**Corolla opening (mm) n = 20**	**Anther/stigma distance (mm) n = 20**
Erica lanuginosa	Rodent	10 (rostrum)#	13.5	0.1	2.6
Erica aristata	Long-proboscid fly	23.91 (proboscis length)$	33.6	3.9	0.9
Erica sessiliflora	Sun birds	20-23 (culmen)*	26.5	3.2	3.4

Wiens et al. (1983) * Average Orange-Breasted Sunbird culmen length (Geerts and Pauw 2009). $ Current study

Floral traits of the three *Erica* species were compared to establish whether there is a correlation between the length of the corolla and corolla opening and the morphology of pollinators probing the respective flowers. Table 3-1 indicates the long corolla tube in *Erica aristata* and *Erica sessiliflora* require a pollinator with a long proboscis or beak to enter the corolla tube to reach the nectar. The corolla opening in *Erica lanuginosa* is effectively absent suggesting a specialist pollinator that can prise open the corolla is required (Table 3-1). In all cases the corolla tube is longer than the relevant structure of the pollinator.

The length of the proboscis of the long-proboscid fly in Table 3-1 suggests that there is a correlation between the corolla length and the proboscis, even though the proboscis length (mean 23.9 mm) does not match the corolla length (mean 33.6 mm), observations note that the long-proboscid fly enters the large (mean 3.9 mm) corolla opening, inserting its whole head whilst probing for nectar. This is supported by Figure 3-3B indicating pollen grains on the proboscis and body of the long-proboscid fly. Whilst this opening does not allow the larger thorax (mean 6.2 mm) to enter the floral tube. Typical *Erica* tetrad pollen grains were collected on the Long-proboscid flies captured visiting the flowers (Figure 3-3C) providing evidence that these are the likely pollinators of *Erica aristata.*

Table 3-2: Morphological traits for two of the three long-proboscid flies captured in this study.

	Individual 1	**Individual 2**	**Mean (mm)**
Proboscis length (mm)	22.5	25.3	23.9
Body length (mm)	35.8	39.0	37.4
Wing span (mm)	33.7	38.4	36.0
Thorax width (mm)	5.2	7.1	6.2

3.2.1 Reflectance spectra in *Erica aristata*

There is a high UV reflectance exhibited by the white petal tips. UV reflectance is low in the in the pink petal (bases), gradually increasing to 20% in the 650-700 nm wavelength range. (Figure 3-6).

The white colour of the corolla tube in *Erica aristata* had a high reflectance UV (40-60%) in comparison (almost zero UV reflectance) in the pink colouration in the corolla (Figure 3-7).

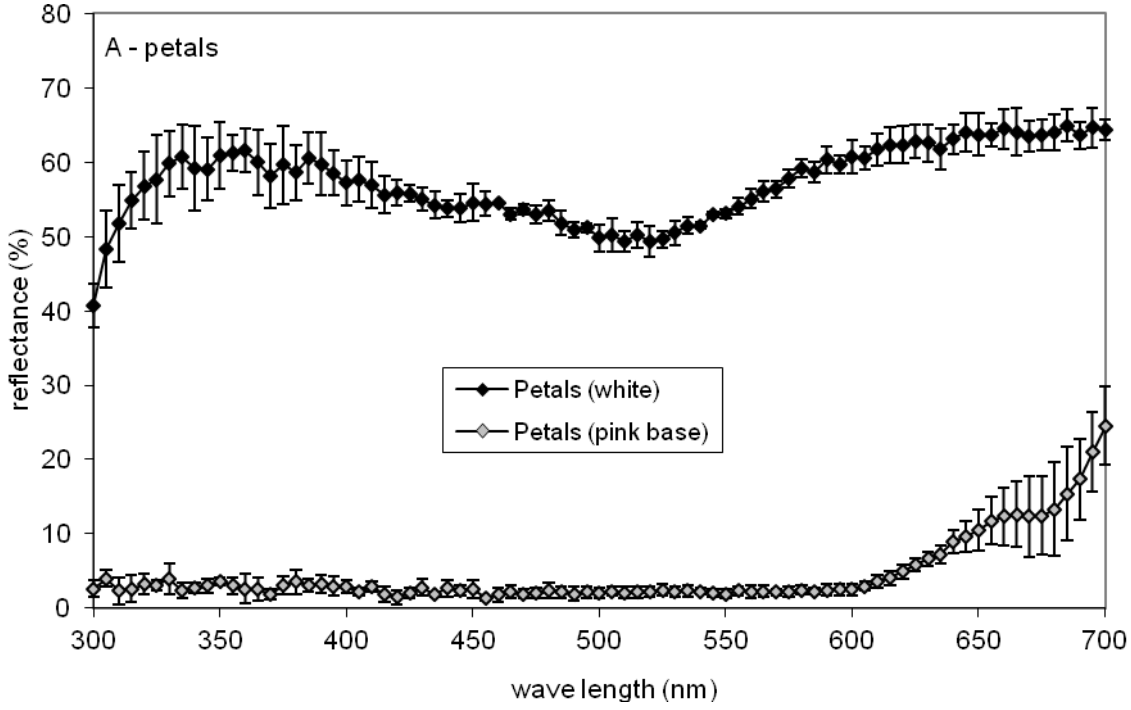


Figure 3-6: Spectral reflectance percentage comparisons in *Erica aristata* between white petal tips and pink petal bases (n=3) across the 300-700 nm wavelength range. Bars indicate standard deviation.

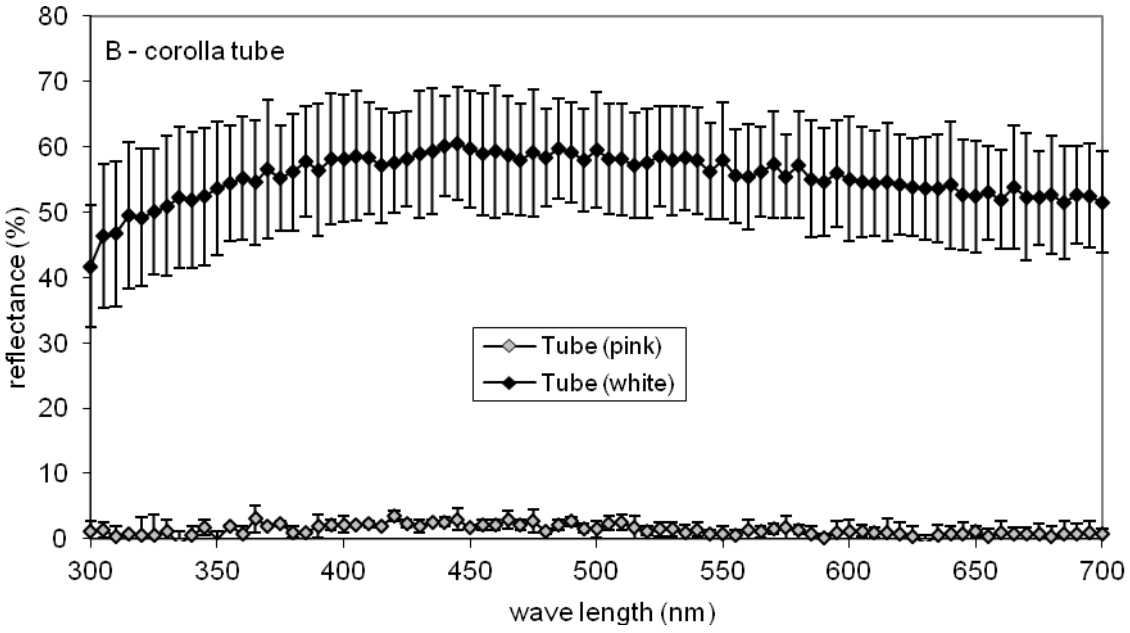


Figure 3-7: Spectral reflectance percentage comparisons in *Erica aristata* between white and pink sections of the corolla tubes (n=3) across the 300-700 nm wavelength range. Bars indicate standard deviation.

UV photography supports the quantitative measurements (Figures 3-6 and 3-7), and only the anthers and stigma appear different in the UV (Figure 3-8).

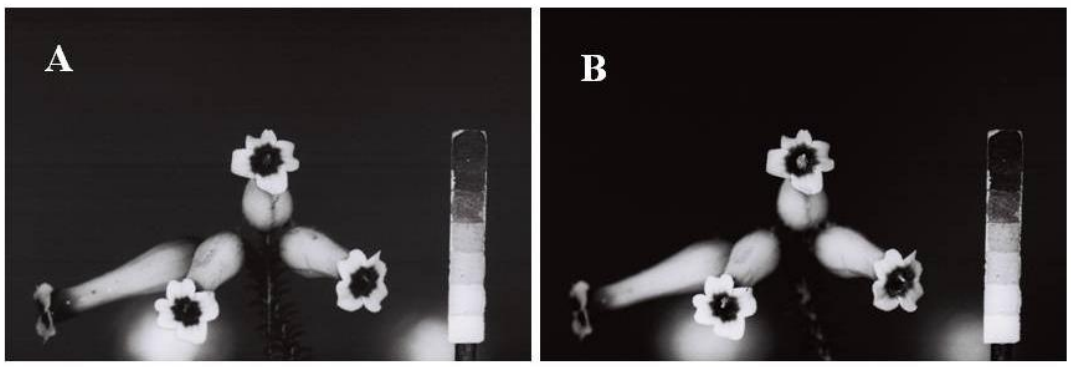


Figure 3-8: A) Image of *Erica aristata* flowers photographed in the near UV (~350 to 400 nm). B) Image of *Erica aristata* flowers photographed in the Human Visual Spectrum (~400 to 780 nm).

UV photography supports the quantitative measurements (Figures 3-6 and 3-7), and only the anthers and stigma appear different in the UV (Figure 3-8).

3.3 Nectar Properties

Similar nectar volumes were obtained from flowers of *E. lanuginosa* (mean: 2.22 µl ±1.04 SD) and *E. aristata* (mean: 2.31 µl ± 1.35 SD) (Figure 3-9). Flowers of *E. sessiliflora* had higher nectar volumes than the other two species (mean: 3.36 µl ± 1.45 SD).

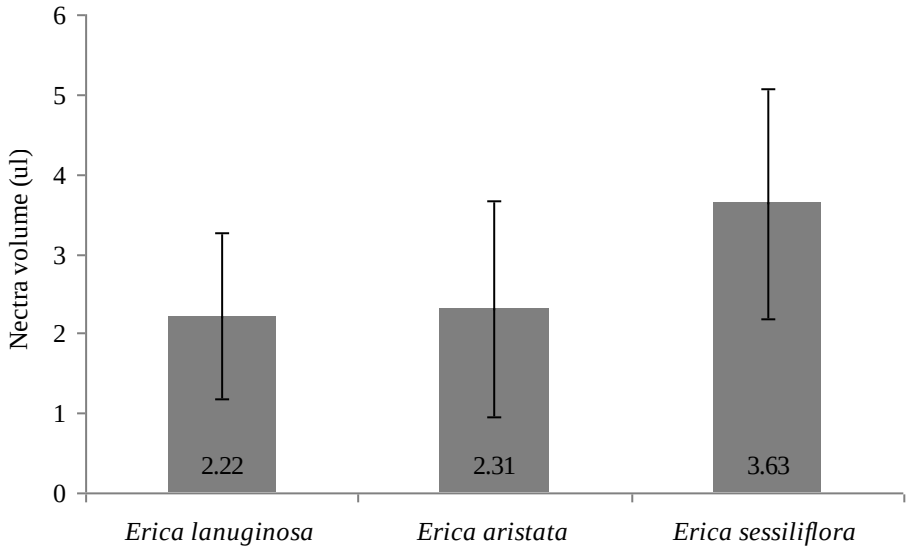


Figure 3-9: Mean nectar volume comparisons between *E. lanuginosa* (n=12), *E. aristata* (n=30) and *E. sessiliflora* (n=16). Numbers give the mean for each species, bars indicate standard deviation.

There are significant differences in nectar volume between the three species ($H = 8.9$, $p < 0.001$). Posthoc Mann-Whitney pairwise comparisons show that *Erica lanuginosa* is significantly different to *Erica sessiliflora* ($p = 0.02$) and *Erica aristata* significantly different to *Erica sessiliflora* ($p = 0.006$). No significant difference between nectar volume of *Erica lanuginosa* and *Erica aristata* were noted ($p = 0.9$).

Figure 3-10 reveals that in *Erica sessiliflora* sugar concentrations are low at 15.2 % ± (2.64 SD) in comparison to *Erica lanuginosa* (29.7 % ± 10.46 SD) and *Erica aristata* (33.8 % ± 9.98 SD) and these later two species have similar sugar concentration values ($H = 29.4$, $p < 0.001$).

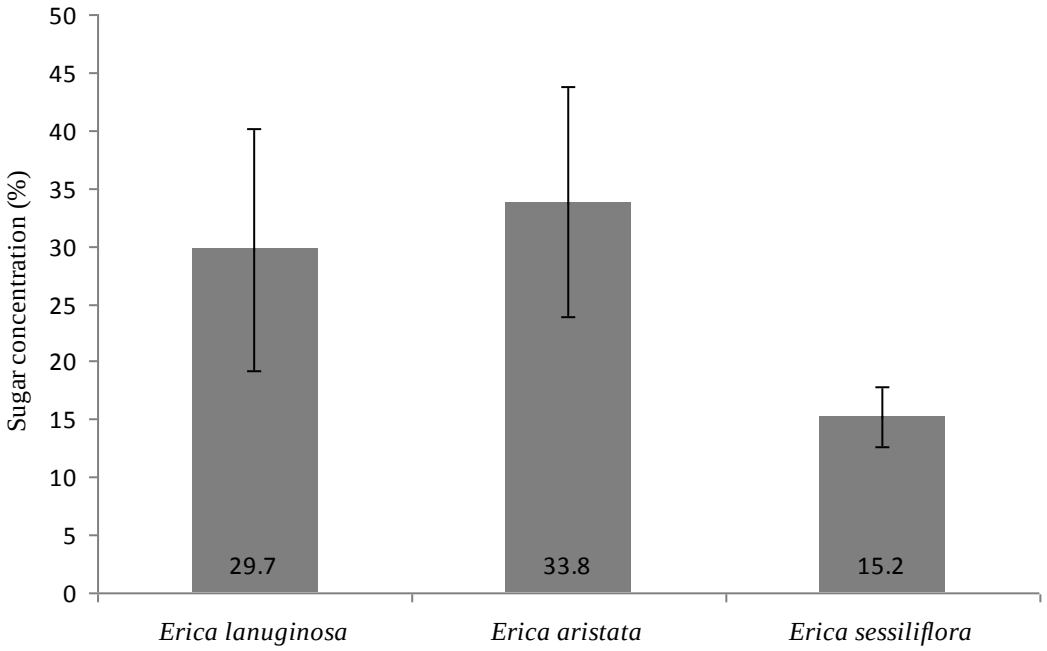


Figure 3-10: Percentage mean sugar concentration comparisons between *E. lanuginosa* (n=12), *E. aristata* (n=30) and *E. sessiliflora* (n=16). Numbers indicate mean sugar concentration and bars indicate standard deviation.

There are significant differences in nectar concentration between the three species ($H = 8.9$, $p < 0.001$). Posthoc Mann-Whitney pairwise comparisons show that *Erica lanuginosa* is significantly different to *Erica sessiliflora* ($p < 0.0005$) and *Erica aristata* significantly different to *Erica sessiliflora* ($p < 0.0002$). No significant difference between nectar volume of *Erica lanuginosa* and *Erica aristata* were noted ($p < 0.2$).

Table 3-3: Comparisons of mean nectar volume (µl) and sugar concentrations (%) in *E. lanuginosa*, *E. aristata* and *E. sessiliflora.*

	Erica lanuginosa (n = 12)	***Erica aristata*** (n = 30)	***Erica sessiliflora*** (n = 16)
Mean nectar volume (µl)	2.2	1.2	3.6
Mean sugar	29.6	23.1	15.2

concentration (%)			
Range nectar volume (µl)	0.33 – 3.5	0.4 – 6.5	1 – 6
Range sugar concentration (%)	11.8 - 49	15 – 47.5	11 - 22

The average nectar sugar composition from 10 different flowers, from different plants, separated into two different samples in *Erica lanuginosa* comprised means of glucose 15%, sucrose 28% and fructose 57%. In *Erica sessiliflora* nectar sugar composition sampling conducted by (Barnes et al. 1995) resulted in glucose 46%, sucrose 5% and fructose 49%.

No nectar sugar composition data were available for *E. aristata*.

3.4 Exclusion and breeding system experiments

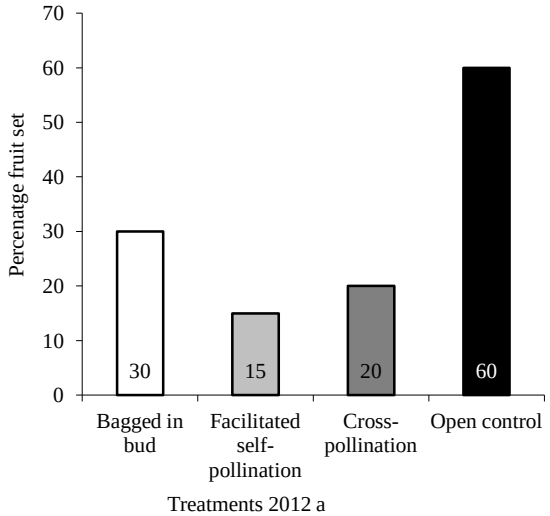
3.4.1 Erica lanuginosa

In 2011 fruit set in both bagged in bud (autonomous selfing) and open control treatments was high. Bagged in bud treatments had a 40% fruit set, while the open controls had a fruit set of 42.5%. The calculated value of self-incompatibility (ISI) was low, 0.25, suggesting that *Erica lanuginosa* is self-compatible. The 2012a bagged in bud treatment showed relatively high percentage of fruit set (30%). This was also shown in Figure 3-12 where bagged in bud treatment showed a high mean seed set (26.8 seeds per fruit) in comparison to lower mean seed set for facilitated self-pollination treatment (13.90 seeds per fruit) and cross-pollination treatment (14.3 seeds per fruit)(2012a experiment). However, the open controls has a high mean seed set in both of the 2012 experiments (76.6 and 124.8 seeds per fruit respectively) which correspond to the high percentage fruit set shown in Figure 3-11 (60% and 90%). Figure 3-11 shows that there is high percentage fruit set and high mean seed set for bagged treatments (2012a experiment). However the second 2012 experiment (2012b) showed no fruit or seed set indicating that there was no autonomous selfing occurring. Mean seed set values were low in all treatments with the exception of open controls, showing that *Erica lanuginosa* has a single mating system and a high degree of inbreeding depression. No values

of index of self-incompatibility were calculated for the 2012b experiment. Unfortunately no cross-pollination treatments were preformed in the 2012b experiment due to the lack of flowers late in the season, however cross-pollination treatments in the first 2012a experiment showed fruit set (20%) and (relatively low) mean seed set (14.3 seeds per fruit).

The 30% fruit set in bagged in bud treatments maybe due to the possibility of inadvertent hand manipulation whilst placing and replacing fine mesh bags. Therefore two further experiments were conducted to investigate the possibility of autonomous self-pollination in *Erica lanuginosa*. In the first trial only five flowers were bagged in bud (autonomous selfing) and resulted in no fruit set. The second experiment had treatments with 20 flowers in each group, including flower bagged in bud (20), flowers with a facilitated selfing treatment (20) and open controls (20). No fruit set was found in bagged in bud treatments, selfing was limited and fruit set low (10%), whereas the open controls had high fruit set, and 19 out of 20 flowers produced fruit set or 95% fruit set (Figure 3-11). Although no cross-pollination treatments were possible in the 2012b (Figure 3-11), cross-pollination treatment in 2012a indicated a 20% fruit set.

In the previous experiments in 2011 and 2012a which did not incorporate exclusive bagging in bud, the procedure may have inadvertently triggered anther release while bagging flowers. What is evident is that this species has a closed corolla and it is not very easy to determine whether the anthers are intact. The only way to be certain that there is no self-pollination is to bag flowers in bud stage.



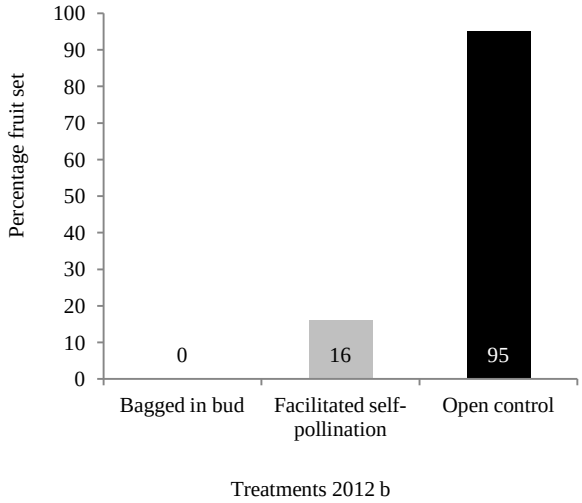


Figure 3-11: Results of two *Erica lanuginosa* - breeding experiments in 2012, fruit set as a percentage, (n=20) for each treatment. Numbers in the bars indicate percentage fruit set per treatment.

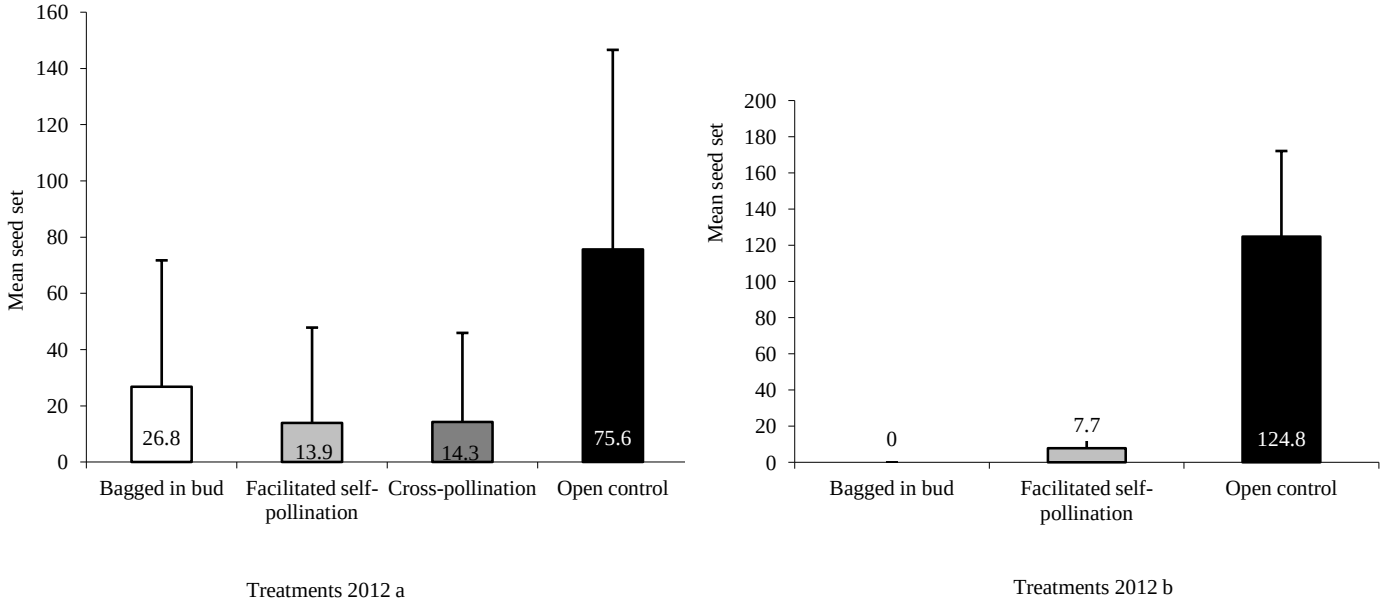


Figure 3-12: Mean seed set values for two experiments in 2012 for *Erica lanuginosa.* Numbers in bars indicate mean seed set per treatment. Error bars indicate standard deviation.

Figure 3-12 indicates mean seed set in 2012a treatments *Erica lanuginosa*, bagged in bud (26.8 ± 44.9 SD), facilitated self-pollination (13.9 ± 33.9 SD), cross-pollination (14.3 ± 31.7 SD) and open control (75.6 ± 70.9 SD). In the second experiment 2012b bagged in bud (0), facilitated self-pollination (7.7 ± 21.6 SD), cross-pollination (no treatment available) and open control (124.8 ± 47.3 SD).

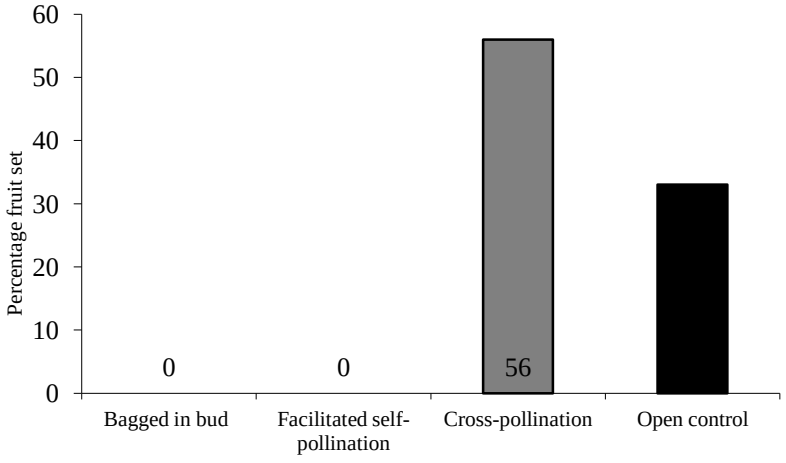
There was a significant difference in seed set in the *Erica lanuginosa* 2012a breeding system experiment ($H = 10.38$, $p = < 0.001$). Mann-Whitney post-hoc pairwise tests show there are significant differences between bagged in bud stage and open control, ($p = 0.016$); facilitated self-pollination and the open control ($p = 0.002$) and cross-pollination and open control ($p = 0.003$). These significant differences substantiates that a pollinator is required to pollinate *Erica lanuginosa.*

Similarly there were significant differences in *Erica* lanuginosa 2012b exclusion and breeding system experiments ($H = 36.54$, $p < 0.001$). Mann-Whitney post-hoc pairwise tests

show there is no significant difference in seed set between bagged in bud stage (autonomous selfing) and facilitated self-pollination (p = 0.072). Significant differences were found between bagged in bud stage and open control (p = 0.001), facilitated self-pollination and open control (p = 0.001), and bagged in bud stage and open control (p = 0.001) treaments indicating that there is little autonomous selfing occuring and some capacity of self-pollination, supporting the measurements of ISI calculated above. Significant difference between the open control and bagged in bud stage and facilitated self-pollination treatments indicated that a pollinator was necessary to pollinate flowers in their un-bagged, natural condition.

Cross-pollination values were lower than open control. It could have been that hand cross-pollination technique was not ideal and I may have hand cross-pollinated too early and stigma was not receptive.

3.4.2 Erica aristata



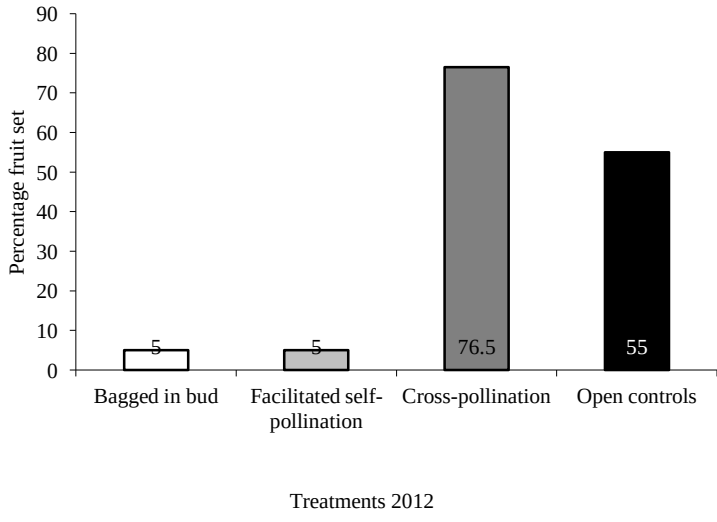


Figure 3-13: *Erica aristata* Comparative percentage fruit set 2011 (n=120) and 2012 (n=80) for exclusion and breeding system experiments. . Numbers above the treatments indicate percentage seed set per treatment.

Cross-pollination in both 2011 and 2012 resulted in a high percentage fruit set (56%), (76.5%) (Figure 3-13). Bagged in bud (autonomous selfing) and facilitated self-pollination is not significantly different in seed set, whilst cross-pollination has significantly higher seed set.

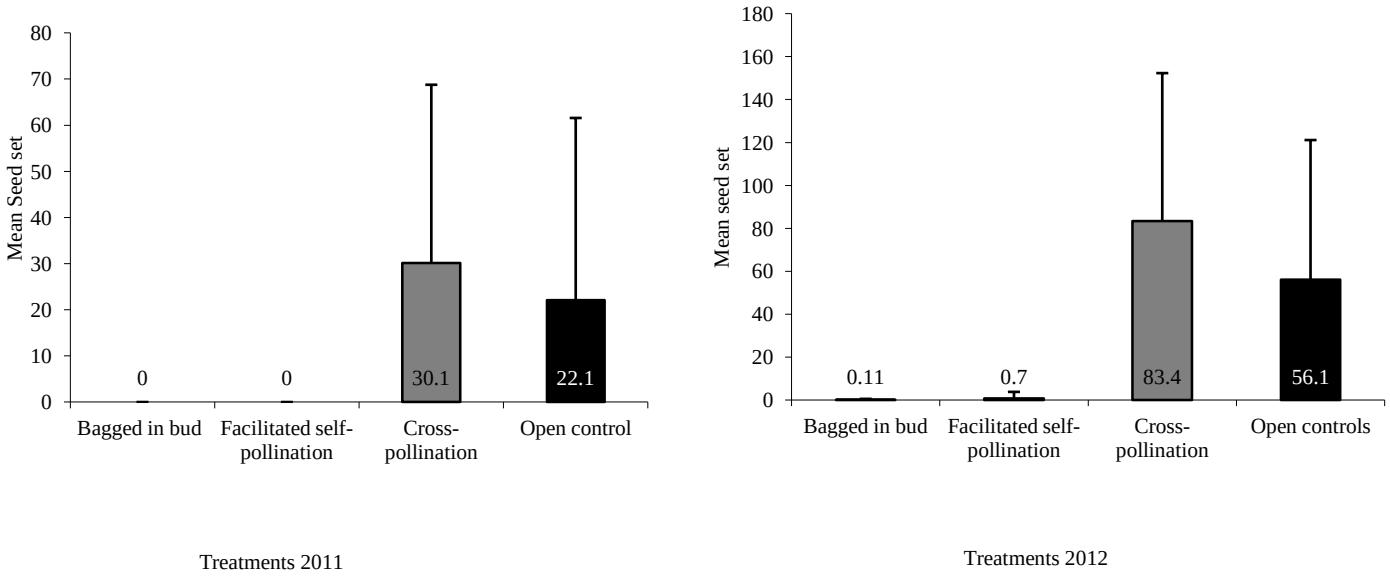


Figure 3-14: Results of mean seed set in *Erica aristata* in 2011 and 2012. Values in bars indciate percenatge seed set. Error bars indicate standard deviation.

The calculated ISI value in 2011 was 1 and in 2012 0.99 indicating that *Erica aristata* is self incompatible. Cross-pollination in both 2011 and 2012 resulted in high numbers of seed set (30.1 ± 38.7 SD), (83.4 ± 69 SD) (Figure 3-14). Bagged in bud (autonomous selfing) and facilitated self-pollination is not significantly different in seed set, whilst cross-pollination has significantly higher seed set.

There was a significant difference in seed set across the treatments in the *Erica aristata* breeding systems ($H = 23.53$, $p < 0.001$). Post-hoc Mann-Whitney pairwise comparisions show there is no significant difference between between bagged and facilitated selfing treatments ($p = 1$) ruling out autonomous self-pollination and supporting the ISI calculations above that show *E. aristata* is self-incompatible. There are significant differences between bagged in bud stage and the cross-pollination treatment ($p < 0.0001$) and bagged in bud stage and open controls ($p < 0.001$) indicating that *Erica aristata* require the serves of a pollinator.

Emasuclation experimentation was not possible as once the anther ring was removed the stigma had no support structure and swayed vigouruosly in the wind and shriveled up and died and no seed set occurred.

3.4.3 Erica sessiliflora

Bagged in bud (autonomous self-pollination treatment) and facilitated selfing are not significant in producing seed set ($H = 64.57$, $p < 0.001$) (Figure 3-13). Significant differences in seed set indicated that cross-pollination was necessary, indicating that a pollinator being necessary to produce seed set. Significant differences in seed set occurred between bagged in bud in stage, facilitated self-pollination and the previous year flower stalks.

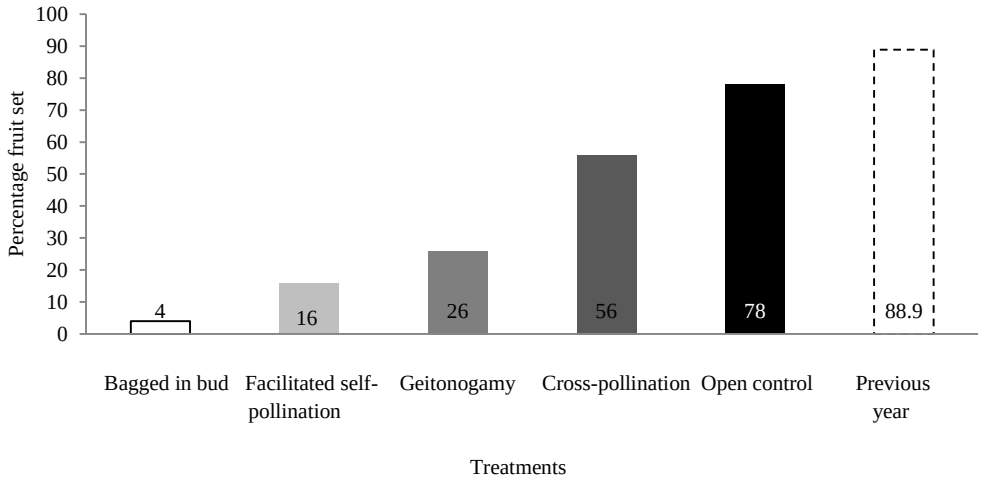


Figure 3-15: Percentage fruit set in *Erica sessiliflora.* Numbers above the treatments indicate percentage fruit set.

Large differences were found between fruit set values in bagged in bud stage (autonomous self-pollination) and all the other treatments (Figure 3-15). Bagged in bud stage resulted in

4% fruit set, facilitated self-pollination 16% , geitonogamy 26%, cross-pollination 56%, open control 78% and previous year 88.9%. There was no significant difference between facilitative self-pollination and geitonogamy; cross-pollination and open control.

The index of self-incompatbility value (ISI) of 0.71 indicates there is a measure of selfing in *Erica sessiliflora.*

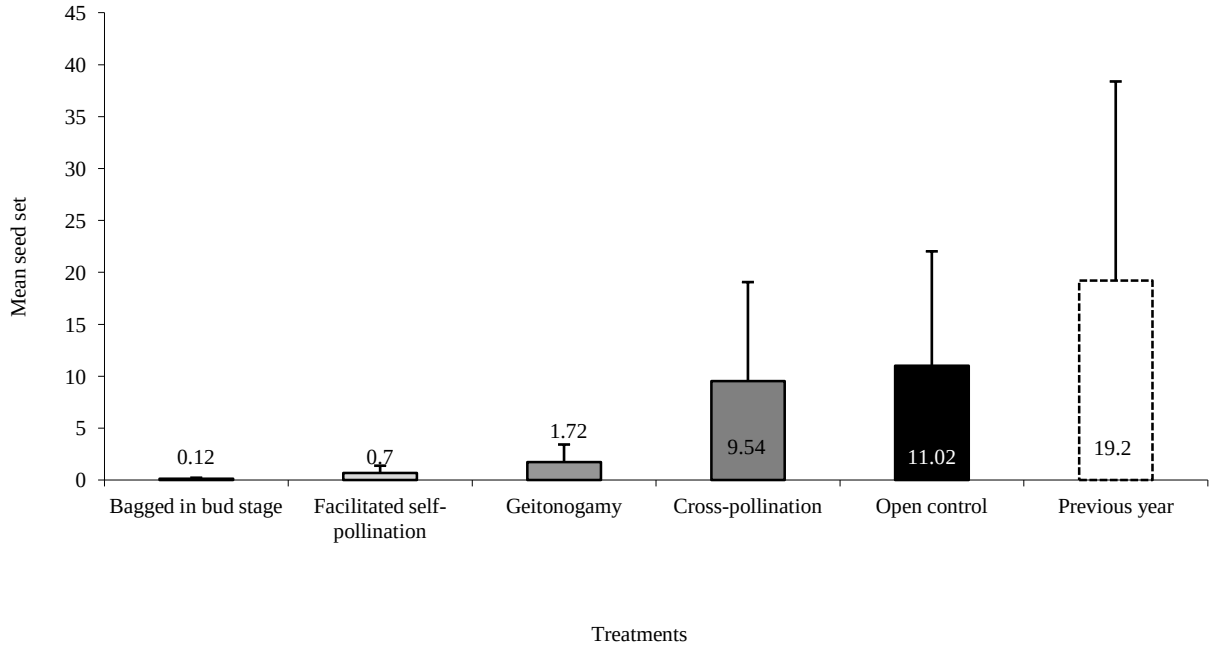


Figure 3-16: Mean seed set values in *Erica sessiliflora.* Numbers in bars indicate mean seed set. Bars indicate standard deviation.

There were significant differences in seed set resulting from the different treatments in the *Erica sessiliflora* breeding systems experiments ($H = 23.53$, $p < 0.001$). Bagged in bud stage resulted in a mean seed set of (0.12 ± 0.72 SD), facilitated self-pollination (0.7 ± 2.44 SD), geitonogamy (1.72 ± 3.65 SD), cross-pollination (9.54 ± 14.9 SD), open control (11.02 ± 12.18 SD) and previous year (19.2 ± 11.42 SD).

Post-hoc Mann-Whitney pairwise comparisons show significant differences between bagged in bud and facilitated self-pollination treatment ($p = 0.046$) indicating that autonomous selfing is limited. There was no difference between cross-pollination and open control values ($p = 0.085$). The absence of autonomous self-pollination and the siginificant difference

between this treatment and the manual cross-pollination treatment indicate the importance of pollinators for seed set.

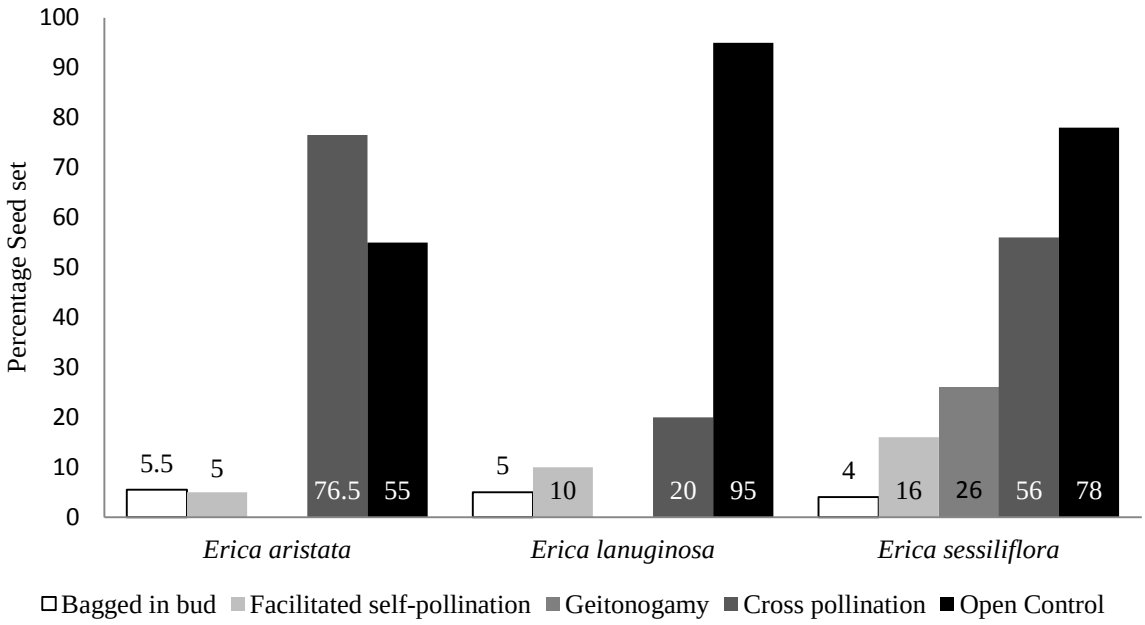


Figure 3-17: Percentage fruit set comparisons between Erica lanuginosa, Erica aristata and Erica sessiliflora.

Low levels of autonomous self-pollination is possible in both *Erica lanuginosa* (10%) and *Erica sessiliflora* (16%). Relatively high fruit set resulting from cross-pollination in both *Erica lanuginosa* (20%) and *Erica sessiliflora* (56%) is therefore principally a result of the services of pollinators but a degree of mixed mating is possible.

In *Erica lanuginosa* an ISI of 0.03, indicates that selfing is possible. In *Erica aristata* ISI = 1 which suggests that this species is self-incompatible. In the case of *Erica sessiliflora* the ISI value = 0.7, which indicates that this species not completely self-incompatible but shows the possibility of mixed mating.

3.5 Role of insects in *Erica lanuginosa*

In the 2011 and 2012a experiments (Table 3-4) of a total of 3958 flowers that were examined for robbing, 648 had holes in the corolla (robbed) indicating that 16.4% had been robbed.

A second experiment in 2012b was carried out in 28 flowers to investigate whether plants that had ants excluded by placing Plantex® around the base of each stem could self pollinate. Seventeen of the 28 flowers produced seeds. As the ants were excluded from entering flowers it may indicate that ants did not play a role in pollination but another pollinator was likely involved. In the same experiment, 685 flowers were examined for robbing, but no sign of

robbing was observed. As there was 60% seed set, it is likely that rodents were the pollinators.

Table 3-4: Examination of flowers for robbing in *Erica lanuginosa* 2011, 2012a and 2012b

	2011	**2012a**	**2012b**
Total numbers of flowers examined	1391	2567	685
Total flowers robbed	295	353	0
Percentage robbed	21.2	13.8	0

There were no observations of any weevils taking refuge in the card board flashing during the experiments. However copulating weevils belonging to the primitive weevil group Brentidae: Nanophyinae were observed on other free standing plants whilst conducting rodent trap experiments and identified. Plantex® exclusion experiments (Table 3-4) indicated that crawling insects did not play any role in seed set. Seed set is therefore most likely a result of rodent visits.

CHAPTER 4: Discussion and Conclusion

Data collected shows that all three species are probably specialised for one or a few pollinators. *Erica lanuginosa* was shown to be pollinated by rodents, making it only the second species in the genus known to use rodents as a pollen vector.

Erica aristata is equally unusual, being pollinated by long-proboscid flies. This is the first documented case of long-proboscid fly pollination in *Erica*. In contrast bird-pollination is well known in the genus and is demonstrated here for *Erica sessiliflora*.

With the exception of nectar volume in *Erica lanuginosa*, nectar volumes and nectar concentrations in all three species were in line with expectations in the literature.

All three species were shown to be primarily outcrossing, but with some evidence of limited self-compatibility, which may be due to inadvertent anther tripping during experimentation.

4.1 Pollinator observations

4.1.1 Erica lanuginosa

The sturdy shrub-like morphology, relatively large dull coloured, pendulous flowers and abundant production of nectar are all consistent with rodent pollination. However *Erica lanuginosa* does not possess all the characteristics typically expected in rodent-pollinated species as it does not have a musky odour, (Wiens et al. 1983). These findings together with those by Turner et al. (2011a) show that rodents may be more prevalent as pollinators in the genus *Erica* than previously expected.

The absence of day time observations of visits to *Erica lanuginosa* caused the search for possible nocturnal pollinators to be extended into the night. Visits by *Aethomys namaquensis* to the flowers were captured on the first camera trap installed, apparently probing for nectar (Figure 3-1C). Fourteen different images of rodents were subsequently recorded on camera. These photographs represent the first images taken of rodents visiting *Ericas* acquired under natural field conditions and are some of only a few observed natural visits by rodents to any flower (e.g. Hobbhahn & Johnson (2013), Johnson et al. 2011). This study's findings where rodent exclusion experiments resulted in significant reduction in seed set are consistent with those of Turner et al. (2011a), working on *Erica hanekomii* as well for a number of other

rodent-pollinated plants (Biccard and Midgley 2009, Letten and Midgley 2009, Johnson et al. 2001, Wester et al. 2009 and Johnson and Pauw 2014).

The rocky habitat preferences of both *A. subspinosus* and *A. namaquensis* corresponded to field study sites of *Erica lanuginosa* in Vogelgat Nature Reserve on Washington Ridge at Selago Rocks. The rocky habitat requirement of *A. subspinosus* and *A. namaquensis* has been reported by Wiens et al. (1983), Fleming and Nicholson (2002) and Letten and Midgley (2009).

The behavioural pattern of the photographed *Aethomys namaquensis* on *Erica lanuginosa* was similar to that of *Acomys subspinosus* visiting the flowers of *Erica hanekomii*, as reported by Turner et al. (2011a). Their data show the rodent clasping the flowers whilst lapping the nectar, but not destroying the flowers. Captive rodents often destroy flowers in terraria and hence images from wild interactions as filmed by remote camera traps are important to fully understand behaviour of rodents when interacting with flowers. As with the study by Turner et al. (2011a), abundant *Erica* pollen tetrads were found in the scat samples of multiple rodent individuals from three species captured in the vicinity of *Erica lanuginosa*.

Pirie et al. (2011) included *Erica lanuginosa* in a clade with many Erica species that are ostensibly insect-pollinated although Pirie et al. (2011) used floral syndromes to classify these species. Therefore the present examination in the field of *Erica lanuginosa* contributes important pollinator information and it will be interesting to see if the position of *Erica lanuginosa* in the phylogeny remains the same with the addition of molecular information.

Ants and thrips were repeatedly observed in the flowers but selective bagging experiments where flowers were bagged with mesh big enough to admit these visitors failed to set seed, ruling these insects out as meaningful pollinators.

There were no weevils present in the cardboard flashings and the exclusion experiment utilising Plantex® resulted in no seed set. This indicates that crawling insects do not play a role in the pollination of *Erica lanuginosa.*

4.1.2 Erica aristata

In their review of insect pollination in the Cape Flora of South Africa, Whitehead et al. (1987) were the first to recognise long-proboscid fly pollination as a unique pollination system (Goldblatt and Manning, 2000).

Recent investigations indicate that long-proboscid flies are important pollinators of plants in the Cape Floral Kingdom of South Africa (Goldblatt and Bernhardt, 1990; Johnson, 1992; Johnson and Steiner, 1995; Goldblatt et al. 1995; Goldblatt and Manning, 2000; Newman et al. 2014). A wide variety of floral species across numerous families were found to be pollinated by long-proboscid flies. To illustrate this variety the following plants species are known to be pollinated by the long-proboscid fly species: *Cyrthanthus leptosiphon, Nerine humilis, Brunsvigia grandiflora* (Amaryllidaceae), *Wahlenbergia guthrie* (Campanulaceae), *Pelargonium carneum* (Geraniaceae), *Geissorhiza fourcadei, Gladiolus engysiphon, Tritoniopsis antholyza, Babiana dregei, Hesperanth latifloia, Lapeirousia dolomitica subsp. dolomitica, Romulea hantamensis, Sparaxis metelerkampiae, Aristea spiralis, Watsonia borbonica, Ixia paniculata, Nivenia stenosiphon* (Iridaceae), *Orhtosiphon tubiformus, Plectranthus ambiguous* (Lamiaceae), *Disa draconis* (Orchidaceae), *Zaluzianskya microsiphon* (Scrophulariaceae), (Goldblatt and Manning, 2000; Newman et al. 2014). Recent preliminary evidence has even raised the possibility of long-proboscid fly pollination in *Protea punctata* (Johnson et al. 2012). This shows that there is abundant convergence in many lineages and hence long-proboscid flies are important drivers of floral diversity in South Africa.

Previous studies indicate that long-proboscid flies pollinate flowers with long narrow corolla tubes, an absence of any discernible scent, cream or pink colour, and purple "nectar guides" (Hansen et al. 2012). Another common feature in species with such flowers is the small amount of relatively dilute nectar in the flowers (Johnson and Steiner, 1997; Goldblatt and Manning, 2000). Findings in the present study indicate that all of the above traits are present in the flowers of *Erica aristata* (Tables 3-1, 3-2, 3-3, Figures 3-3A, 3-9).

Field observations of pollinators of *Erica aristata* suggest visits were infrequent. Unfortunately, the captured specimens remain unidentified by the expert on the group, although it is clear that these are likely a single species and belong to the Nemestrinidae. Goldblatt and Manning (2000) proposed recognising three separate long-proboscid fly pollination guilds namely *Prosoeca peringueyi* guild, *Moegistorhynchus-Philoliche* guild and

Prosoeca ganglbaueri guild. Newman et al (2014) describe a fourth, the *Prosoeca longipennis* guild. The plants that belong to each of these guilds show very little overlap with those of other guilds in terms of the pollinating flies or flowering season which coincides with the period flies are on the wing. They further explain that within each system there are well defined guilds of plants species that have one or occasionally two fly species as their sole pollinator. When taking these factors into account it is likely that *E. aristata* can be grouped into the *Prosoeca peringueyi* guild. It may reveal that this long-proboscid fly may belong to a new long-proboscid fly pollination guild.

The stickiness of the corolla makes it impossible for crawling insects to enter the flowers either legitimately or as a robber. Similarly, winged flower visitors notably carpenter bees, with tongues shorter than corolla tubes or sunbirds with bills wider than the corolla tube openings would similarly be discouraged from resorting to nectar robbery by piercing the base of the corolla because of this stickiness.

4.1.3 Erica sessiliflora

Observations indicated that there were low visitation rates by sun birds in the case of *Erica sessiliflora* in comparison to the co-occurring *Erica discolor*. This may be due to the fact *Erica discolor* has higher nectar sucrose content than *Erica sessiliflora* (Barnes et al. 1995). Turner et al. 2011b suggested that observational data does not support exclusive floral niches occupied by specific sunbird species in Fynbos. It was noted that in the study region *Erica sessiliflora* has two flowering times. In the summer period, flowers are not produced in profusion in comparison to plants flowering in winter.

Heystek et al. (2014), working on *Erica perspicua* concluded that *Anthobaphes violacea* under aviary conditions preferred pink flowers over white coloured flowers; 95% of their first choices were to pink inflorescences and they visited and probed more pink inflorescences and flowers. This preference was not confirmed in the field by the present study and Carlson and Holsinger (2013) suggested sun birds and sugar birds do not show any preference to either white or pink inflorescences.

4.2 Plant morphology

Comparisons of floral traits for the three *Erica* species indicate that there is a relationship between plant morphology, notably length of the corolla and diameter of corolla opening and the morphology of the pollinator probing the flower. Long corolla lengths in both *Erica*

aristata and *Erica sessiliflora* require a pollinator with a long proboscis or beak respectively to enter the corolla and reach nectar. Flowers are typically adapted to have tubes a little longer than the tongues/beaks of the pollinators to ensure deep probing and hence optimal anther or stigma contact (Anderson et al. 2010).

4.2.1 Erica lanuginosa

The corolla of *Erica lanuginosa* has a small opening, indicating that a specialised mechanism to open the corolla is necessary to reach the nectar and this is consistent with the interpretation that this species appears is adapted for pollination by rodents. The unusual fold at the base of each corolla lobe acts as a hinge to facilitate the opening of the corolla lobes. The corolla is likely opened by manipulation of a rodent's forelimbs, thereby allowing for nectar foraging by rodents. Similar manipulations by rodents of *Erica hanekomii* flowers by rodents have been recorded by Turner et al. (2011a).

The reduced nectar-stigma distances as observed by Johnson and Pauw (2014) ensure a good fit between the rodent rostrum and flower, enabling easy access to nectar reward. This has also been found to be the case in many non-flying mammal pollinated Proteaceae (Wiens et al. 1983).

4.2.2 Erica aristata

The length of the proboscis of the long-proboscid fly examined cannot reach the nectar unless the fly pushes its whole body into the corolla to reach its food supply (pers obs). This trait can be readily understood in terms of a mechanism that was suggested by Darwin (1862) and later developed and tested by Nilsson (1978; 1988). Selection will favour longer corolla tubes when they cause the pollinator to insert its entire proboscis into the flower and thus make maximal contact between reproductive structure and the pollinator (Johnson and Steiner, 1997).

To avoid the external sticky outer (abaxial) surface of the corolla of *Erica aristata*, the long-proboscid fly is able to move its long proboscis from a trailing position in flight into a forward orientated position for probing flowers. With the aid of the floral guides the long-proboscid fly is able to precisely insert its proboscis into the long corolla tube to reach the nectar.

In a survey conducted by Penny (1983) the majority of insect-pollinated flowers possessed nectar guides and that there were contrasts between nectar guides and the petals, largely conspicuous in the insect-visible spectrum. Nectar guides are thought to be important for long-proboscid flies to accurately orientate their tongues for probing flowers. For example, Hansen et al. (2012) indicate the importance of white arrow markings on the corolla tube for the proboscis insertion by the long-proboscid fly pollinator in *Lapeirousia oreogena*. These white arrows could be considered functional 'nectar guides'. The UV reflectance of the white floral frill at the mouth of the corolla in *Erica aristata* as well as the UV absorbent centre of the corolla entrance acts as a "bull's eye", directing the proboscis of the pollinator to the opening of the corolla and the main white corolla acts as a guide to the pollinator to a reward. This is supported by Waser et al. (1985) who suggested that a strongly contrasting colour pattern at the centre of the flower would help guide pollinators to concealed nectar rewards. The pink markings on the corolla tube in *Erica aristata* are UV absorbent and contrasts with the high UV reflectance of the corolla tube (white) to enable the fly to orientate itself visually from a distance. This increases foraging efficiency, by reducing search and flight times as concluded in experiments conducted by Hansen et al. (2012). Similar findings by Leonard and Papaj (2011) showed the presence of nectar guides reducing the bumble bee (*Bombus impatiens*) pollinators' handling time, leading to an increased visitation rate and promoting pollen transfer. Medel et al. (2003) showed that insect pollinators preferred flowers with large corollas and obvious nectar guides in contrast to hummingbirds which preferred flowers with small corollas and small guides. In the same study it was found that flowers with nectar guides projecting toward the corolla tube had a higher chance of insect visitation. According to Hansen et al. (2012) markings did not affect long-distance flower choice foraging but markings were important at close range to orientate the long-proboscid fly to probe effectively. The visual cues are important for these specialised pollinators to enabling them to guide their long, non-retractable proboscis towards a nectar reward and thereby increasing levels of seed set.

4.3 Nectar properties

4.3.1 Erica lanuginosa

The mean nectar volume (2.2 µl) found in *Erica lanuginosa* was lower than found by Turner et al. (2011a) and Johnson and Pauw (2014) in other rodent-pollinated species. The mean nectar concentration of (29%) did correspond to findings reported by Turner et al. (2011a).

Wiens et al. (1983) reported high nectar sugar concentrations of 36% in *Protea* pollinated by non-flying mammals, which is similar to the present results for *Erica lanuginosa.* A recent study by Johnson and Pauw (2014) in *Leucospermum arenarium* indicated similar nectar volumes and concentrations.

Baker and Baker, (1979) reported a sucrose rich nectar composition in rodent-pollinated proteas in comparison to sucrose poor bird-pollinated proteas. However, Cowling (1978) found nectar with high sucrose content in *Protea longifolia* which is bird pollinated. Findings in the present study indicate high fructose (57%), medium sucrose (28%) and low glucose (15%) in *Erica lanuginosa*. These findings were in contrast to results for rodent-pollination reported by Turner et al. (2011a) in *Erica hanekomii* of high sucrose content (61%), medium fructose (29%) and low glucose (7.9%) concentrations. This indicates there may not be a consistent pattern of nectar volumes, nectar sugar concentrations and sugar compositions in rodent pollinated species. This may be expected in such systems where flower nectar is a minor consistent of the total diet of these generalist mammals.

Comparisons of nectar in rodent pollinated plant species in the Cape flora (Table 4-1) indicate that nectar volume in *Erica lanuginosa* is lower than the mean. Nectar sugar concentration in *Erica lanuginosa* (29.6%) close to the mean of six of the rodent-pollinated Cape plants for which data is available (29.1%, Table 4-1). Mean sucrose content were high in the six of the plant species in table 4-1, which confirmed results for rodents by Baker and Baker (1979). This is also consistent with research conducted in South America on *Cajophora coronata (Loasaceae)* by Cocucci and Sérsic (1998).

4.3.2 Erica aristata

The mean nectar sugar concentration found in *Erica aristata* of 33.8% is in line with findings reported by Goldblatt and Manning (2000), who reported an average nectar concentration of 32% for plants (*Pelargonium* species) with flowers adapted for long-proboscid fly pollination with similar low nectar concentrations.

Mean nectar volume for *Erica aristata* was found to be 2.3 µl indicating low nectar volumes are present in the flowers of this species. These values are consistent with values ranging from 1.1-5 µl found in flowers of 120 species pollinated by long-proboscid flies (Goldblatt and Manning, 2000). Nectar sugar composition in *Erica aristata* has not been documented and needs further investigation.

Long-proboscid flies have been described as high-speed, continuous foragers (Pivnick and McNeil, 1985) which should prefer nectar with sugar concentrations between 30 and 50%. *Lapeirousia* species in Namaqualand (Goldblatt et al. 1995) and various other species typically visited by *Prosoeca* species only produce nectar with sugar concentrations of 20–30% (Goldblatt et al. 1995). Kim et al. (2011*)* show that optimal nectar concentration is strongly related to the drinking style of foraging insects, being higher for viscous dippers, e.g. bees, than suction feeders, such as long-proboscid flies, thus possibly offering an explanation for the relatively dilute concentrations of nectar in *Prosoeca* species nectar plants (Woodcock et al. 2014). Higher concentrations increase viscosity and may set an upper limit for maximum nectar concentrations. Results from *Erica aristata* show low nectar volumes and medium nectar sugar concentrations suggesting similarities to findings of Pivnick and McNeil (1985). This implies that the low nectar volume in *Erica aristata* may have a significant influence on promoting out-crossing as flies need to move amongst numerous individual flowers to find sufficient nectar to support their metabolism.

Table 4-1: Nectar properties of rodent-pollinated plants in the Cape Flora

Taxon	Pollinator	Nectar volume (µl) x±SD	Nectar concentration % x±SD	Fructose (%)	Glucose (%)	Sucrose (%)	Xylose (%)	Reference
Erica lanuginosa	*Acomys subspinosus; Aethomys namaquensis*	2.2 ± 1.04*	29.6 ± 2.7	57	15	28	0	This study
Erica hanekomii	*Acomys subspinosus*	9.0 ± 7.5‡	24 ± 6.59	29.8 ± 3.5	7.9 ± 0.5	61.4 ± 3	0	Turner et al. (2011a)
Leucospermum arenarium	*Gerbillurus paeba* *Rhabdomis pumilio*	9.9 ± 1.88*	26.1 ± 12.02	1.63 ± 0.79	0.54 ± 1.06	97.8 ± 1.06	0	Johnson and Pauw (2014)
Protea humiflora	*Acomys subspinosus; Aethomys namaquenisis*	8.8*	37.8 ± 6.9	30.5	14.5	48.3	6.75	Wiens et al. (1983): Nicolson and Van Wyk (1998)
Protea nana	*Myomyscus verrauxii; Aethomys*	> 1000‡	29.4 ± 6.2	30	5	64	1	Nicolson and Van Wyk

	namaquensis							(1998): Biccard and Midgley (2009)
Protea cryophila	*Acomys subspinosus*							
Liparia parva subsp.parva	*Acomys subspinosus*	Not reported	33.3 ± 7.2	33.3	34	27.6	0	Wiens et al. (1983)
Massonia depressa	*Gerbillurus paeba; Aethomys namaquensis*	10.7 ± 6.2*	19.7 ± 5.1	31.7	0	41.7	0	Van Wyk (1993): Letten and Midgley (2009)
Whiteheadia bifolia	*Aethomys namaquensis*	17.7 ± 26*	± 20	32	23	42	3	Johnson et al. (2001)
Mean		7.9 ± 5.9*	27 ± 13.9	66	27	7	0	Wester et al. (2009)
*Amount per flower		8.4~	29.1	34.5	14.1	47.8	1.2	
‡Amount per inflorescence								

~ >1000 values not included.

4.3.3 Erica sessiliflora

The mean nectar volume found in *Erica sessiliflora* was 3.6 µl, which according to Turner (2012) is typical for sunbird pollinated *Erica* species and nectar sugar concentration range of 11-22% was consistent with the typical preference of sunbirds (20-25%) as reported by Lotz and Nicolson (1996) and Brown et al. (2010). Barnes et al. (1995) documented nectar sugar composition in *Erica sessiliflora* and found ratios of glucose 46%, sucrose 5% and fructose 49% which is in contrast to findings of Baker and Baker (1983, 1990) who showed 29 out of the 37 bird-pollinated *Erica* species had sucrose dominated nectars. Barnes et al. (1995) indicated that bird-pollinated *Erica* species had sucrose dominant nectars (93%) and sunbird-pollinated *Ericas* having much higher nectar sucrose levels than many hummingbird pollinated flowers.

Erica sessiliflora was found to have low sucrose content (5%) (Barnes et al. (1995). According to Barnes et al. (1995), *Erica discolor* had sugar content ratios of fructose (1%), glucose (2%) and sucrose (97%) and this may be the reason why visitations of sunbirds to *Erica discolor* were more frequent in the present study than in the case of *Erica sessiliflora.*

4.4 Exclusion and breeding system experiments

4.4.1 Erica lanuginosa

In 2012 autonomous self-pollination and facilitated self-pollination experiments with *Erica lanuginosa* resulted in little seed set, whereas cross-pollination and open controls had high seed set, indicating the presence of a pollinator for this species to seed set, with considerable inbreeding depression (Figure 3-11). Johnson and Pauw (2014) found that flowers of *Leucospermum arenarium* that were bagged in bud, i.e. a treatment testing for autonomous self-pollination treatments, yielded no seeds and therefore concluded that this species does not undergo autonomous self-pollination. These results were confirmed in the second 2012 *Erica lanuginosa* breeding experiments. It became clear that during experimentation it is critical to avoid inadvertently triggering anthers, which is thought to have resulted in seed sets in non-facilitative self-pollination, facilitative self-pollination and geitonogamy treatments in the previous experiment in 2010. This inadvertent triggering gives the incorrect impression that the flowers were selfing and ignores the importance of pollinators for seed set.

Differences in population structures have been suggested as the single most important factor regulating the evolution of pollination by non-flying mammals in Proteaceae (Wiens et al. 1983; Turner et al. 2011a). This 'restrictive population hypothesis' involves the shift from bird-pollination to rodent-pollination in rodent-pollinated Proteaceae. This may apply to *Erica lanuginosa* with rodents providing a more reliable pollination service for this very localised *Erica* species.

Rodent pollinators provide an efficient service for *Erica lanuginosa*, accounting for 95% seed set rates in open controls. This is similar to results reported by Turner et al. (2011a) for *Erica hanekomii*, where *Acomys subspinosus* was shown to be an efficient pollinator resulting in 73.5% seed set recorded in the field. This suggests that in *Erica lanuginosa* fecundity rates are not pollen-limited. This is in contrast to most other Cape plants (Johnson and Bond, 1997).

Thrips were seen inside corollas when fruits were opened to conduct seed set counts. However, autonomous self-pollination experiments resulted in low numbers of seed set and hence any role of ants and thrips in the pollination of *Erica lanuginosa* is likely to be minimal.

4.4.2 Erica aristata

It is evident from the results (Figure 3-12) that *Erica aristata* is likely specialised for pollination by long-proboscid flies. This is supported by the fact that little or no self-pollination was observed, and high seed set being found in manipulated cross-pollination treatments.

In 2010 high fruit and seed set in bagged in bud treatments was a result of experimental failure, whereby I did not take note of anther tripping prior to bagging and therefore cross-pollination may have occurred prior to my exclusion. This failure in technique also resulted in high fruit and seed set in facilitated self-pollination treatments. In 2011 bagging technique was refined. Exclusion with fine mesh bags was also carried out very early in the season when flowers had not opened. Artificial out-cross treatments matched or had higher seed set than the open controls, evidence for at least some pollen-limitation in this species. (Chittka and Thompson, 2001). This can be supported by the low long-proboscid fly visitation observed.

The stickiness of the corolla, the depth of the nectaries, the reflectance characteristics of the flowers and the results of the pollinator exclusion treatments all indicate that *Erica aristata* flowers are likely to be exclusively pollinated by long-proboscid flies.

Many *Erica* flowers are as sticky as *Erica aristata*, a trait that has been interpreted by Vlok and Schutte-Vlok, (2003) (in a popular account of an unpublished study) as a mechanism to reduce transpiration in *Erica* and by Herrera et al. (1984) and Turner et al. (2011b) as an adaptation to deter nectar robbing by ants in Mediterranean plant species. The anti-transpiration hypothesis is unlikely to apply for *Erica aristata* which occurs on steep moist south facing slopes, supporting the possibility that stickiness is an anti-robbery adaptation.

Pollination is a critical ecosystem function, especially in the Western Cape and the Cape Floristic Region and therefore more research in to the role played by long-proboscid flies is required. Specialised plant species pollinated by a single species of insect are clearly at risk in comparison to those plants that are pollinated by a suite of different pollinators.

The conservation status of these flies is of utmost importance and therefore more field research is needed to better understand their biology.

4.4.3 Erica sessiliflora

Low seed set in open flowers compared to artificially crossed plants (Figure 3-13) revealed that this species is pollen limited. Insufficient pollen deposition (pollen limitation) on stigmas and resource limitation may cause plants to produce fewer seeds than the ovules that they produce. (Bierzychudek, 1981; Sutherland, 1986; Johnston, 1991; Campbell and Halama, 1993; Burd, 1994; Asman et al. 2004; De Waal, 2010). Pollen limitation may arise either because the quantity or quality of pollen delivered to stigmas is insufficient to fertilise all ovules (Knight et al. 2005; Aizen and Harder, 2007). As many as 63% of flowering plants species are pollen limited, largely due to variable and/or unpredictable pollinator service (Burd, 1994; Johnson and Bond, 1997; Larson and Barrett, 2000; Knight et al. 2005; Weber and Goodwillie, 2009).

In the present study on *Erica sessiliflora*, autonomous selfing is possible; however high percentages of seed set in cross-pollination (xenogamy), in comparison to low seed set in facilitative self-pollination is an indication that a pollinator is required for significant fruit set. Birds were observed probing the flowers for nectar legitimately and thus considered the most likely to provide pollination services to *Erica sessiliflora*.

As had occurred in experimental trials in *Erica halicaba* (Turner et al. 2011b) seed set in autonomous self-pollination treatments in *Erica sessiliflora* resulted in significantly lower seed set in comparison to those flowers that were exposed to natural pollinators. This supports the hypothesis that flowers of *Erica sessiliflora* require a pollinator to produce seeds.

It is typical that flowers having bright colours i.e. red, orange and sometimes pink are bird-pollinated (e.g. Anderson et al. 2005; Johnson, 1995; Manning and Goldblatt, 2005; Pauw, 1998). However this may not be as straightforward as often stated. More recently, Rodriguez-Gironés and Santamaria (2004) suggest that bees can "see" the red end of the spectrum. Numerous tubular *Erica* species have brightly coloured flowers that are red, pink, white, orange, yellow and green, with almost 50% being selectively bird-pollinated species that have bi-coloured or tri-coloured forms (Oliver and Oliver, 2002; Rebelo et al. 1985). Rebelo et al. (1985) concluded that the Orange-breasted Sunbird was the sole pollinator of ornithophilous Cape *Erica* species, this however has been disputed by Geerts and Pauw (2009, 2010). They have shown that the Southern Double- collared Sunbirds *(Cinnyris chalybea)* visit flowers of *Erica cruenta, Erica discolor and Erica perspicua.,* and Heystek et al. and Van der Niet et al. (2014) show that the Malachite Sunbird (*Nectarinia famosa)* visits flowers of *Erica perspicua* and *Erica plukenetii* respectively, thus suggesting that it is not only one specialist passerine nectarivore that has imposed selective pressure upon bird-pollinated *Erica* species.

Geerts and Pauw (2009) concluded that there was a correlation between culmen length and flower length in 'short' and 'long billed' sunbird pollination syndromes. Mean corolla lengths of *Erica sessiliflora* are 26.5 mm, (23-29 mm; n=16) which corresponds with *Anthobaphes violacea* culmen lengths of 20-23mm (Rebelo, 1987, in Tuner et al. 2011b). The shorter length of the culmen results in the bird inserting its head deeply into the opening of the corolla and hence the pollen is loaded onto its upper, feathered head parts rather than its beak. Based on this *Erica sessiliflora* is consistent with the hypothesis of specialised bird-pollination primarily by *Anthobaphes violacea*.

Data supporting the hypothesis that flowers of *Erica sessiliflora* are adapted for bird-pollination include repeated observation of legitimate pollination behaviour and seed set in open controls compared with non-facultative autonomous selfing treatment (bagged in bud) and small volumes of nectar are consistent with trends in other flowers pollinated by sunbirds (Johnson and Nicolson, 2008) and correlation between flower tube and culmen lengths

(Geerts and Pauw, 2009). Given the green-white coloured flowers with relatively long corolla tubes, one other possible mode of pollination is moth-pollination. However the flowers remain unscented at night to the human nose and the corolla tubes are relatively wide, suggesting this is unlikely.

4.5 The role of insects in the pollination of *Erica lanuginosa*

The presence of ants on photographs from the camera traps indicated high activity but breeding experiments confirmed no seed set by ants (Table 3.3) Ants are known nectar thieves (Fritz and Morse, 1981; Haber et al. 1981; Herrera et al. 1984) and as such have neutral or negative effects on plant fitness (Turner et al. 2011b).

4.6 Conclusion

Data was obtained that supported the hypothesis that flowers of *Erica lanuginosa, Erica aristata and Erica sessiliflora* rely on the services of pollinators to set seed. For *Erica lanuginosa* these include flowers of that are likely adapted for rodent pollination, only the second example of a rodent pollinated *Erica* species. Evidence includes closed corolla with hinged corolla lobes, no day time pollinator observations, repeated camera imagery of rodents visiting flowers, high nectar volumes and pollen grains in scat samples. Breeding experiments confirmed that pollinators are necessary for fruit set in *Erica lanuginosa.*

In *Erica aristata,* data supports the possibility of flowers adapted for pollination by long-proboscid fly species, the first time long-proboscid fly pollination has been shown in the genus *Erica*. These include the correlation of insect morphology with flower morphology; repeated observations of long-proboscid fly probing flowers for nectar, captured specimen bearing pollen grains of *Erica aristata* adhered to their long proboscis and head areas; low nectar volumes; and nectar concentrations matching those of other long-proboscid fly pollinated taxa.

Data supporting the hypothesis that *Erica sessiliflora* is bird-pollinated includes the match between bird and flower morphology and repeated field observations of birds probing the flowers for nectar.

The selective exclusion and breeding system experiments resulted in low numbers of seed set in all autonomous selfing and facilitated selfing treatments in all three species. High numbers

of seed set in cross-pollination and open controls confirm that these *Erica* species rely on out-crossing and therefore require pollinators to produce viable seed set.

Pollination is a critical ecosystem function, especially in the Western Cape and the Cape Floristic Region and therefore more research on the role played by long-proboscid flies is essential. Plants pollinated by a single insect are clearly at risk in comparison to those plants with more generalist pollination systems that are pollinated by a suite of different pollinators (Turner, 2012).

Both *Erica lanuginosa* and *Erica aristata* are narrow endemics, restricted to the Klein River Mountains and Akkedisberg Mountains and more detailed research should be conducted in order to fully understand their conservation status. The identity, biology and conservation status of the long-proboscid fly requires further detailed research to understand its life-cycle and therefore the importance to *Erica aristata* as a mutualist.

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