Certain Syndrome or Complex Conundrum?: the Pollination of *Erica lanuginosa*

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Absract:

The flower of *Erica lanuginosa* has a tightly closed corolla, held in place by hinged sepals. with a dull reddish-pink colour which makes make it hard to determine a likely pollinator. Rodent trapping and pollen analysis of faecal matter showed it unlikely to be pollinated by a rodent. Flowers excluded from external pollination showed no seed set, hence it is not considered to be self-pollinated. Nectar analysis are inconclusive as an indicator of pollination syndrome. Entomophily by a robust insect with a medium length proboscis is considered unlikely due to phenology and morphology of the flower. Ornothiphily is a possibility as stem thickness correlates with previous studies investigating the correlation between stem thickness and pollination syndromes. The pollination syndrome of *Erica lanuginosa* remains indeterminate by I hypothesize that, due to phenology, thick supportive, stem and large quantities of nectar and close-formed flower, which needs to be manoeuvred open, its pollinator is likely a short-billed generalist-feeding bird restricted by food choice during the winter months.

Introduction:

Erica is the biggest genus within the Fynbos vegetation type (Siegfried *et al* 1985, Schuman and Kirsten 1992). Of the 760 known *Erica* species, 90% occur in the Cape Floristic Region (Schuman and Kirsten 1992). This high richness in *Erica* is useful in exploring adaptive radiation of pollination syndromes and its affect on the species richness within the Cape. For example, Johnson *et al* (1998) argued that changes in pollinators correlated with changes in floral morphology in *Disa*. There have been few pollination studies in *Erica* to use as a basis to determine whether explosive speciation is due to radiation in pollination niches. Given this absence, one way to determine the role of pollination divergence as a mechanism for massive speciation is to look at the likely diversity of syndromes within the genus. For example, Rebelo and Siegfried (1985) used the colour and morphology of *Ericas* to group the flowers into syndromes correlating with a pollinator guild. There are three main pollination syndromes of *Ericas* which occur in the south-western Cape. Insect pollination is the most dominant, accounting for 80% of the species. A minority of 5% are wind pollinated and 15% of species are pollinated by birds (Siegfried *et al* 1985). Within insect pollination, many sub-types occurred such as by long-tongued flies (rhinomyiophily) and hovering moths (sphingophily) (Rebelo *et al* 1985).

A further pollination type not previously considered for *Erica* is rodent pollination. Rodent pollination is widespread in Proteaceae in the Cape and has been found in several other families, for example Wester *et al* (2009) found *Aethomys namaquensis* to pollinate *Whiteheadia bifolia* (Hyacinthaceae) and Letten and Midgley (2009) found *Acomys subspinosus* to be the chief pollinator *Liparia parva*. *Erica*s have some traits which are suggestive of rodent pollination. *Erica lanuginosa* has a downward pointing close-formed flower, 14-18mm in length. It is shaped much like an upside-down teardrop, with the corolla lobes held closed by hinged sepals (Schumann and Kirsten 1992). This arrangement means the flower needs to be forcibly opened, suggesting rodent pollination. The colour of the flower is dull and unobtrusive which is an attribute often associated with rodent pollination (Rebelo and Breytenbach 1987). Rebelo and Siegfried (1985) acknowledge its complex morphology and question whether it might be insect-pollinated.

Bird pollination in *Erica* flowers can be identified by floral colouration. Rebelo and Siegfried (1985) looked at monomorphic and polymorphic colour syndromes within *Erica*. They showed that ornithophilous flowers tend to have brightly coloured flowers, and where they have monomorphic colour systems they tend to be red, yellow and green as well as orange if the flower has a polymorphic colour system. Red, pink and white polymorphs tend to indicate entomophilous flowers, while pink and purple are common for monomorphic entomophilous flowers (Rebelo and Siegfried 1985). The mature flower of *E. lanuginosa* are mostly a dark pinkish-red when they are mature and whitish-green when immature (pers. obs.) which could conform to a bird syndrome.

Nectar sugar composition and concentration have also been found to be specific to various pollinators. Ornithophilous flowers show a tendency towards containing highly dilute nectar with low sucrose levels. Pyke and Waser (1981) state the average sugar concentrations for nectarivorous hummingbirds in North America to be 23.2% for birds and around 36% for bees. The reason for the dilution of nectar consumed by birds is unknown but it has been suggested that the nectar provides water, in addition to carbohydrates, to the birds (Nicolson 2001 cite Baker 1975). Nectar volume, sugar concentration and sucrose proportions have also been shown to differ according to the feeding habits of the pollinator bird. Johnson and Nicolson (2008) found that plant species pollinated by generalist birds, which occasionally feed on nectar tend to have high volumes of nectar with low sugar –concentrations and decreased proportions of sucrose. Species pollinated by exclusive nectarivores tend to have relatively low volumes of concentrated nectar, a higher percentage of which consists of sucrose.

Variation in growth forms can also be used as a means of determining pollinator for *Ericas*. Siegfried *et al* (1985) found that Erica plants which were pollinated by birds had significantly thicker stems in order to support the weight of the pollinating bird. When comparing assumed pollinators and flowers shape, Rebelo and Siegfried (1985) found that the shape and length of flower corollas could also be divided into pollination syndromes. Small, open-mouthed flowers are suggestive of wind pollination, while tubular flowers often indicate bird pollination. Tube shapes with narrow openings may indicate rhinomyophily (pollination by a long-probiscid fly) and ovoid/bulb-shaped flowers are suggestive of entomophilous flowers. *E. lanuginosa* is classed as having an ovoid shape which could indicate insect pollination (Rebelo and Siegfried 1985).

Due to the peculiar morphology and colour of *Erica lanuginosa* there are several possible pollinators. The corollas of the flower are pressed closed suggesting a need for a dexterous animal to open it (Schumann and Kirsten 1992), such as a rodent, bird or a robust insect. *E. lanuginosa* also has sturdy branches which would support the weight of a larger pollinator such as a bird or rodent. Geoflory is common in rodent pollinated plants, especially in Proteaceae (Letten and Midgley 2009), and *E. lanuginosa* is fairly short plant making it easy for a ground-dwelling rodent to access its branches (Rebelo and Breytenbach 1987; which Schumann and Kirsten 1992). In *Protea*, there are several rodent pollinated species, often have restricted distributions if they are rodent pollinated and extensive distribution where they are bird-pollinated (Rebelo and Breytenbach 1987). *Erica lanuginosa* has a restricted (Schumann and Kirsten 1992) suggestive of rodent pollination. Furthermore, I hypothesise in favour of rodent pollination due to the fact that *Erica lanuginosa* flowers in winter which is common in rodent pollination (Rebelo and Breytenbach 1987).

Here, I explore several possible pollination syndromes. Rodent pollination is tested for as well as self-pollination. Morphological features, such as corolla length and stem thickness, as well as nectar composition features are also analysed in order to determine a likely pollinator. I also compare data with that of past papers of proxy pollination syndrome indicators to help determine a likely pollinator.

Materials and Methods

Rodent Pollination:

The study was performed in July and August which is the flowering season of *E. Lanuginosa*. Rodent traps were laid for five nights during the flowering season; 37 traps were laid for two nights at Vogelgat Nature Reserve and 15-22 traps for three nights at Morning Star Farm. Captured rodents were identified, weighed and their faeces removed from the traps. The faeces were preserved in 90% ethanol. They were then manually pulped in the ethanol, vortexed in order to spin the pollen grains to the surface of the mixture. The process was repeated with several drops of fuchsin dye in order to stain the pollen grains. A drop was taken from the surface of the mixture and placed on a slide with a drop of glycerol to keep the slide moist. Slides were analysed at 400 x magnification, by running four transects through each slide and counting observed pollen grains.

Self pollination:

Bridal veil with a mesh gap size of > 1.3mm was used to sew bags which were tied over undisturbed/ unopened flowers, such that a potential pollinator could not reach the flower. Flowers were used as controls if they occurred on the same individual plant and were undisturbed. Controls, were marked with wire, however, many of the controls were lost and another flower was then picked as a 'pseudo-replicate.' The first set of exclosures were unsuccessful as they were removed too early to unambiguously determine seed set. As a result, the process had to be repeated and hence the flowers were excluded late in the flowering season. This meant that few flowers were left undisturbed, so a small sample size of 10 flowers was used seed set was classed from 1(none) to 4(dehisced).

Morphometrics and avian pollination

Tube length, seed set in flowers with disturbed and undisturbed anthers, maturity of anthers and distance between stigma and anther ring were measured in 30 flowers taken from individual plants. The reason for comparing disturbance of anther ring with seed set was to determine whether or not a pollinator was necessary for pollination i.e. can seed set occur without the anther ring being disturbed. This was done by comparing presence/absence of ring disturbance with presence/absence of seed set.

To help determine whether or not *E. Lanuginosa* has an avian pollinator we measured stem thickness at 5,10 and 15cm from the tip of inflorecences of specimens from the Bolus Herbarium, University of Cape Town, South Africa and compared the results with the regression curve obtained by Rebelo *et al* (1985).

Observations were made from stationary points form 1-7m meters away from flowering plants with unpollinated flowers in an attempt to observe potential bird pollinators in the morning or insect pollinators during the day. Observations were made during early morning periods 6:45 – 10:00 am as well as midday periods 12-14:00pm and late afternoon 16-17:00pm on two consecutive days in August.

Nectar analysis:

Nectar was extracted from 15 flowers taken from 10 individual plants. Nectar was extracted using a diabetic syringe and volume was measured using capillary tubes. Sugar content quantified using a refractometer. A subset of nectar from 10 flowers were used to determine the sugar composition and was processed using the HPLC (high-performance liquid chromatograph) method as outlined in Brown *et al* (2010). Nectar values were plotted in a ternary plot with the percentages of glucose, fructose and sucrose, as obtained for several other *Ericas* by Barnes *et al* (1995)

Statistical analysis

Histograms were compiled in Windows Excel 2007. Chi-squared tests and ternary plots were performed in Statistica 9.0.

Study site

Erica lanuginosa grows on the Kleinriver mountains in near Hermanus, in the Western Cape, South Africa (Schumann and Kirsten 1992). Two sites were visted, Vogelgat Nature Reserve (34°22'38.51" S 19°19'18.43"E) and Morning Star farm (34°23'47.48" S 19°25'21" E). At Vogelgat the flowers occurred along the path from Lanuginosa Rock to quite some way after Washington Head. Both sites are entirely comprised of fynbos, a shrub-like fire-prone vegetation type and *E. lanuginosa* plants tended occur around rocky outcrops, as indicated in the literature (Schumann and Kirsten 1992). The Morning Star farm site tended to have taller specimens of *E.lanuginosa*, possibly due a long respite in fires in the area.

Figure A: A Map showing the two sites visited, Vogelgat Nature Reserve and Morning Star Farm. The Map was compiled using Google Earth 2010.

Results

	mean	Standard deviation
distance between anthers and stigma (mm)	2.14	1.12
corolla length (mm)	14.66	1.03
nectar sugar concentration (%)*	28.37	11.3
nectar volume (µm)**	2.43	1.28

Table 1: averages and standard deviations for morphometrics and nectar analysis (n= 30, *n=12, **n=10)

were

There are very low trapping rates for both of the sites visited (figure 1.1). Most of the rodents captured were *Acomys subspinosus* and two were the vlei rat, *Otomys irroratus* (figure 1.2). Pollen was only found in the faeces of three *Acomys subspinosus* individuals. In all cases there was very little pollen evident in the faecal matter, the highest amounting to only 10 grains and the other two only having 1 and 2 grains respectively.

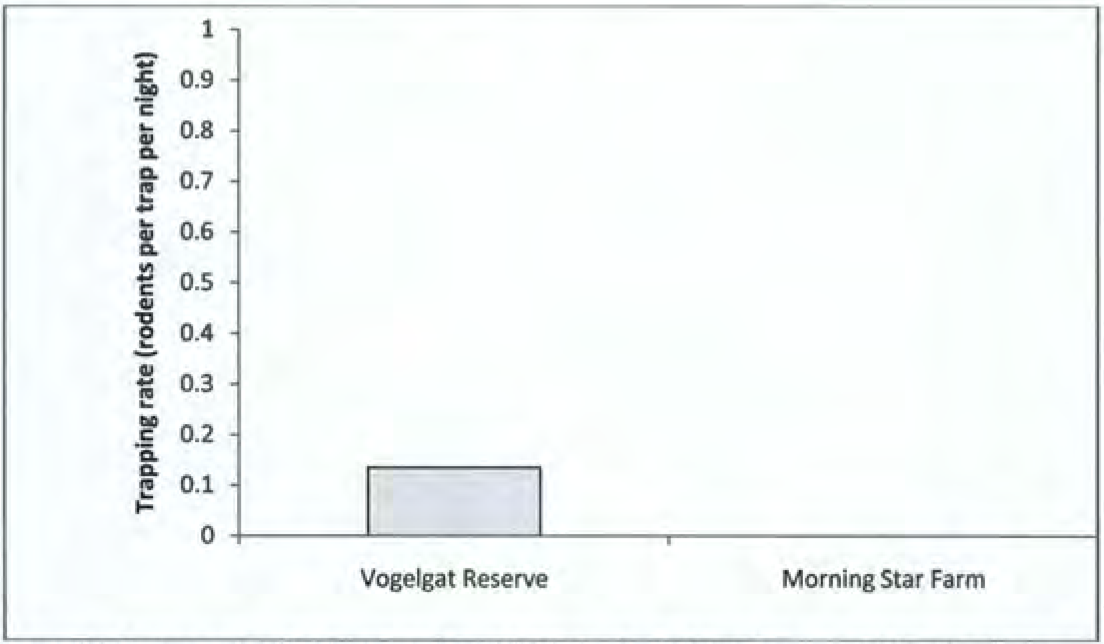

Figure. 1.1 Capture rate each of the two sites visited, Vogelgat Nature Reserve and the other farm. There are no captures in the other farm and few in the Vogelgat Nature Reserve.

The exclosures showed no seed set while 60% of the controls had set seed (figure 1.3). Many of the original controls had been lost, and so pseudo –replicates were used. Natural seed set measured from the general population was also 60%.

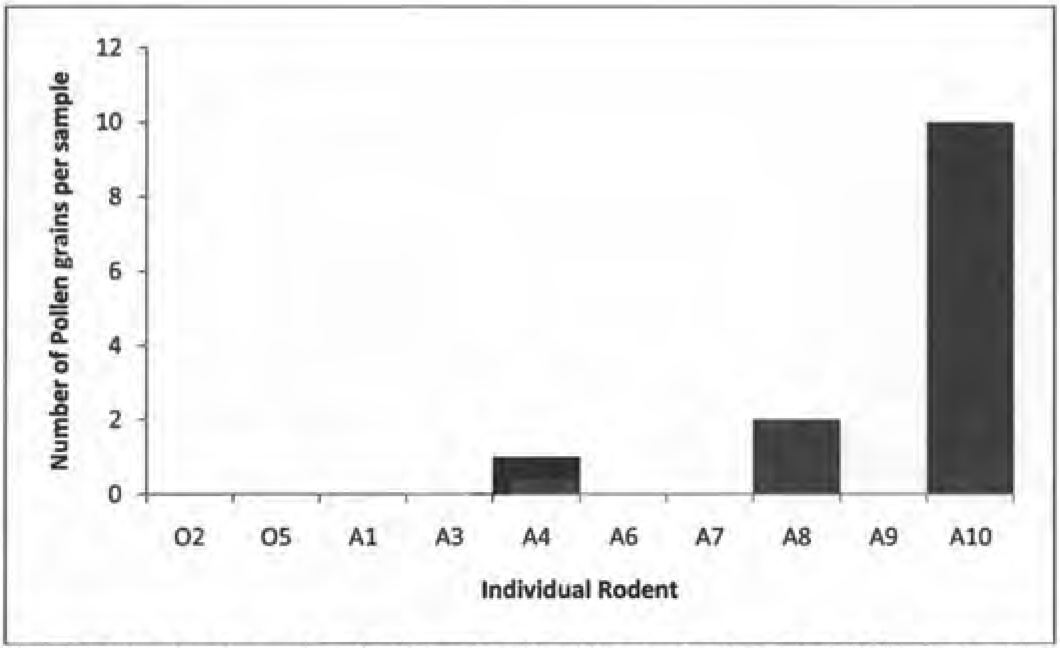

Figure 1.2 showing the number of pollen grains found in each faecal sample. 'A' denotes that the sample was taken from *Acomys subspinosus* and 'O' denotes *Otomys irroratus*.

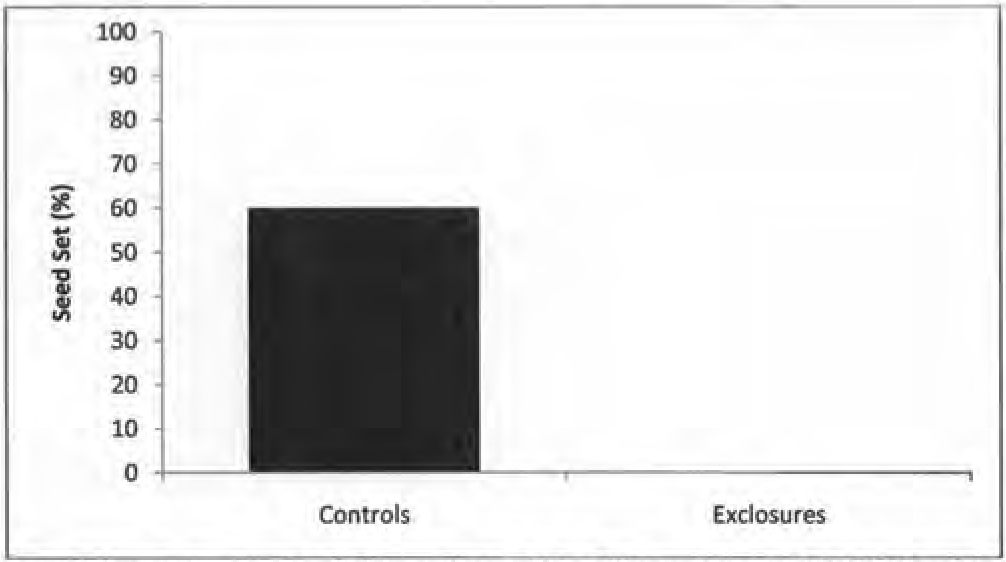

Figure 1.3 percentage seed set in flowers from which pollinators were excluded (0%) and in the natural seed set in the controls (60%).

When testing the proficiency of using anthers ring disturbance as a means of determining The χ^2 test yielded a p-value of 0.521, showing this relationship between anther ring disturbance and seed set to be insignificant.

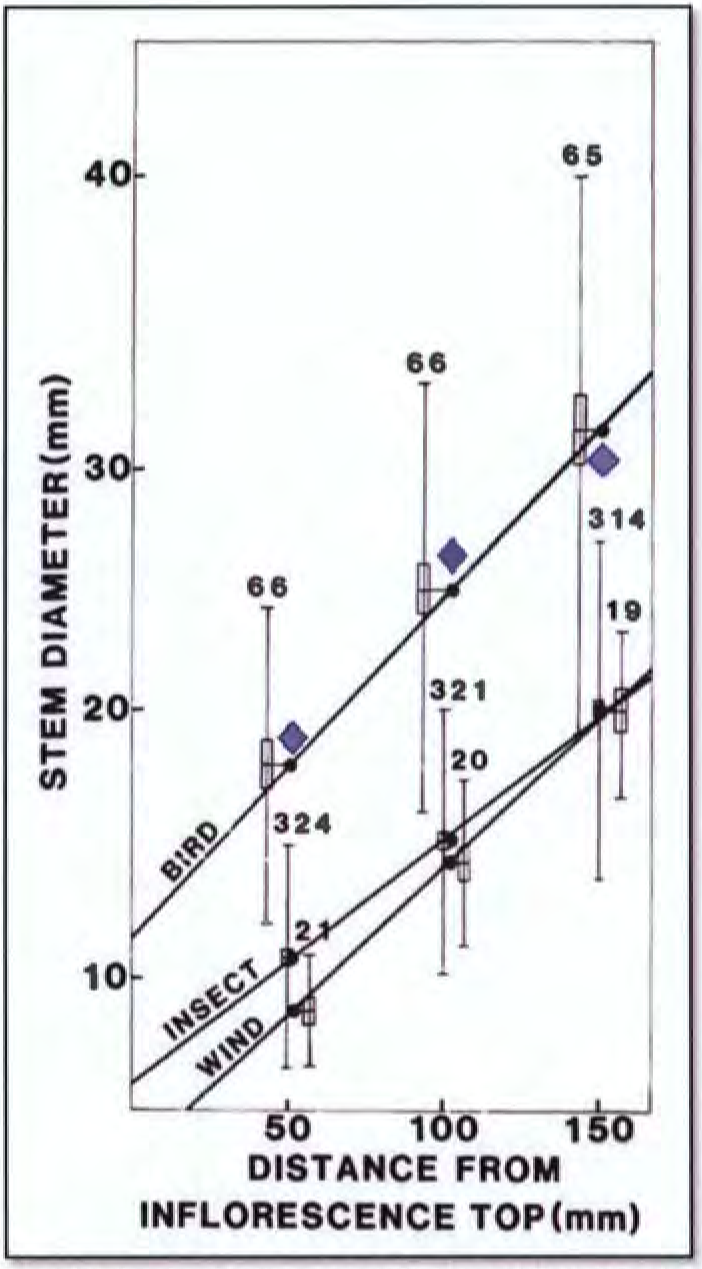

Figure 1.4 showing the average stem thickness of *Erica lanuginosa* (diamonds:◆) at 3 distances(5,10 and 15cm) from the base of the inflorescence, plotted on a graph of several Erica species taken from Siegfried *et al* (1985).

In figure 1.4 I have modified Siegfried *et al* (1985)'s graph of stem diameter of *Ericas* at several distances from the tip of the inflorescence by including the averages for the herbarium specimens of *E. lanuginosa.* The values for *E. lanuginosa* sit close to the graph line for bird pollination. The graph lines for insect and wind pollination are positioned much lower down on the graph.

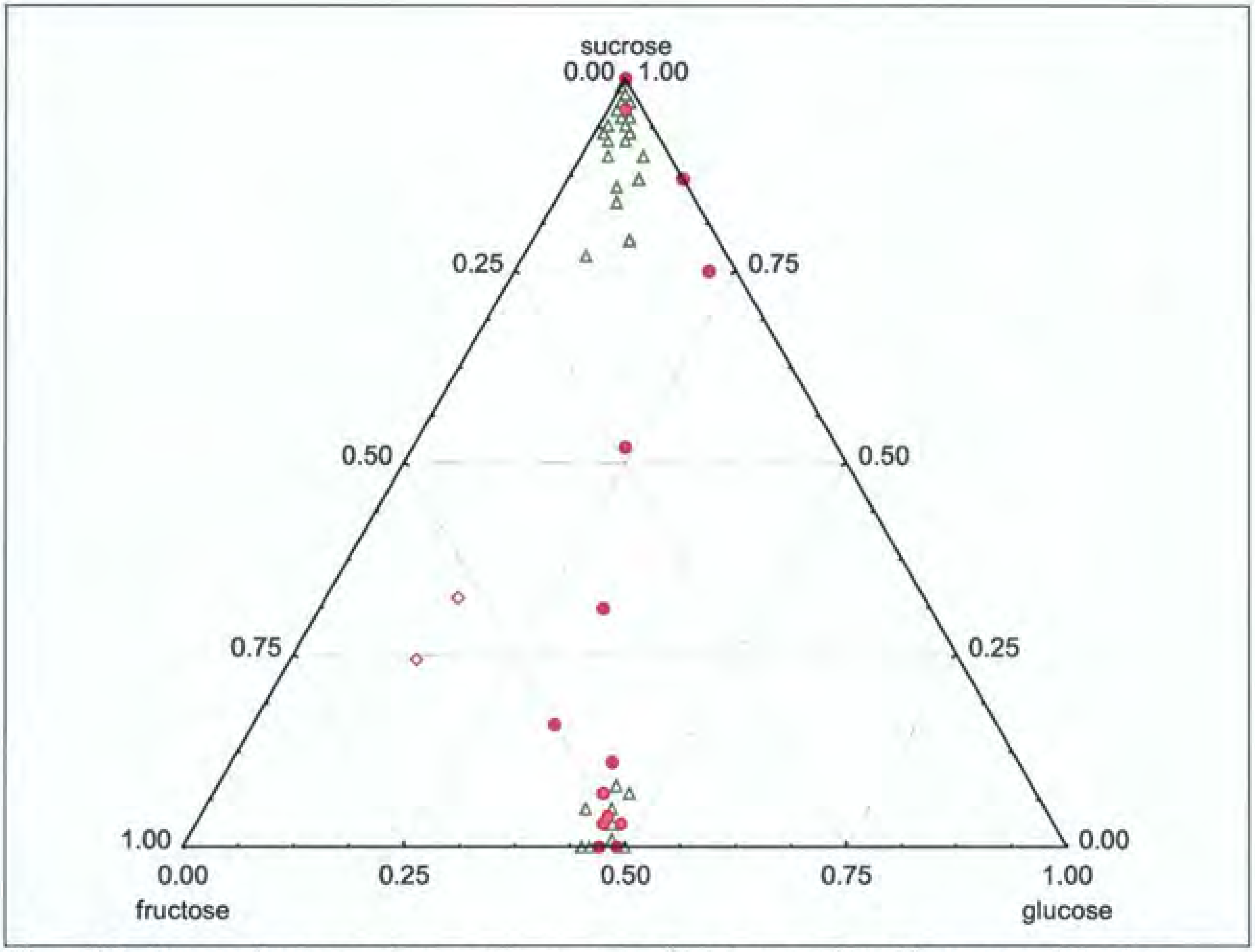

Figure 1.5 glucose, fructose and sucrose percentage of total sugar plotted in ternary plot using values obtained from Barnes *et al* (1995) analyses of the nectar of 61 species of *Erica*, to which were added two sets of values obtained from *Erica lanuginosa*(◇). Barnes et al (1995)'s data points are plotted according to perceived pollinator; insects(●) and birds (△)

The nectar sugar proportions from the bird pollinated flowers are split into two groups, one which is dominated by sucrose and one which has equal proportions of glucose and fructose (hexose dominant) as discussed by Barnes *et al* (1995) . The insect-pollinated *Ericas* show no grouping pattern, but are dispersed between the two bird guilds. *Erica lanuginosa* lies separate from both the bird and the insect pollinated flowers (figure 1.5). Instead it shows reasonably high (28%) proportion of sucrose without being sucrose dominant.

Discussion

The low trapping rates at Vogelgat Nature Reserve and the absence of trapped rodents at the Morning Star Farm does not support the hypothesis that *Erica lanuginosa* is rodent pollinated (figure 1.1). Furthermore, of the ten rodents captured (8 *Acomys subspinosus*; 2 *Otomys irroratus*) only three showed any signs of *Erica* pollen and all showed very low quantities of pollen. Biccard and Midgley(2009) found that rodents pollinating *Protea nana* had several hundred pollen grains in slides of the faeces. The maxim obtained for my slides of rodent faeces was a singular instance of 10 pollen grains, a comparatively low number. Suggesting a low likelihood of a rodent being responsible for the pollination of *Erica lanuginosa* and that the presence of pollen in the faeces was more likely due to proximity of the rodents to the flower.

The exclosures were used to test the possibility of *E. lanuginosa* being self-pollinated. Despite the fact that the loss of several controls necessitated the use of pseudo- replicates, there was no seed set in individuals from which pollinators had been excluded and high seed set (60%) in the controls, making it unlikely that *E. lanuginosa* is self-pollinated (figure 1.3).

The anther ring disturbance had no correlation with seed set (p= 0.521), suggesting that looking at anther ring disturbance is not a good way to determine if a pollinator is responsible for seed set. The anthers appeared to shrivel as they aged making it impossible to determine whether the misalignment of anthers was due to age or animal disturbance. In addition, some anther rings showing minor or ambiguous disturbance had seed set which, considering that our exclosures showed no self-pollination, must have been due to a pollinator. It is possible that anther ring analysis may yield false positives, where anthers appear disturbed due to aging when they have not been, and as well as false negatives where there is little evident disturbance despite the fact that the flower has been visited. This lack of unambiguous disturbance may be due to anther appendages which force the anthers back into place after they have been disturbed, a trait common to entomophilous flowers (Rebelo and Siegfried 1985).

The shape of *Erica lanuginosa* is not immediately indicative of a particular syndrome. Its upside-down teardrop shape is pressed closed (Schumann and Kirsten 1992), which could require an animal to open it. Its closed-formed flower and lack of everted stamen make it unlikely to be wind pollinated (Rebelo and Siegfried 1985). Having explored the idea of rodent as well as autogamy, insect pollination should also be considered as an option. During experimental and *ad hoc* observations, no insects were seen visiting the flowers. Rebelo and Siegfried (1985)'s analysis of flower shape lead them to conclude that it could be insect pollinated. Presumably such an insect would need to be fairly robust in order to open the flower and have a fairly long proboscis as the mean corolla length is 14.6mm. However, two factors make it unlikely to be pollinated by an insect such as a long-tongued fly. Firstly, the number of insects available during the cold, wet winter months in which *E. lanuginosa* flowers is likely to be quite low (Rebelo and Breytenbach 1987). Secondly, the flower hangs downwards which presumably would make it impossible for a long-tongued fly to penetrate the flower, as it would have to manoeuvre it's proboscis upward, a feat it is unlikely to be able to accomplish, particularly as long-tongued flies do not land during pollination (Rebelo *et al* 1985).

The stem thickness of *Erica lanuginosa* correlates well with Siegfried *et al* (1985)'s measurements of bird pollinated stem diameter. Siegfried *et al* (1985)'s showed that *Ericas* which were pollinated by birds had stems which were significantly thicker than necessary in order to support the weight of the bird, instead of the weight of just the flowers. This would suggest that *Erica lanuginosa* is ornithophilous.

However, when *E. lanuginosa*'s sugar proportion are plotted with Barnes *et al* (1995)'s data for 61 other species of *Erica* we find that it does not fit with either of the two bird guilds, exclusive and part-time nectarivores. The percentage total sugar was 28.37% (SD±11.3) which is between values Pyke and Wasser (1981) give for hummingbirds and bee pollinated flowers, which is 23.2% and 36.0% respectively. The majority of the total sugar is comprised of fructose (57%) but this is far greater than the amount of glucose (15%) that it contains. Hence while it is hexose dominant it does not align with the hexose-dominant bird guild due to greatly unequal proportions of fructose and glucose.The fact that it is hexose-dominant and closer to guild of generalist feeders means that if it is bird pollinated it is likely to be by a non-specialized nectarivore.

The average corolla length measured was 14.6mm which is distinctly shorter than the beaks of chief avian pollinators. Rebelo (1987) records the beak length of several dominant pollinators, Southern double-collard, Orange-breasted, Malachite and Cape sunbirds, to have average culmen lengths of over 18mm, and mostly above 20mm. This would mean that if such a bird were to pollinate *E. lanuginosa* then the pollen would sit on the beak instead of the facial feathers which is possibly a better surface to adhere to. The orange-breasted sunbird, a prolific pollinator, was seen during observations but didn't visit *Erica lanuginosa*. Cape White-eyes have beaks which are potentially the appropriate length (12-15mm; Geets and Pauw 2009). They are generalist feeders and have been known to rob nectar from flowers with tube lengths which exceed their beak length (Geets and Pauw 2009). This is consistent with the hypothesis that if it is bird-pollinated it is likely to be so by a generalist feeder.

It may seem peculiar that a generalist feeder would be the pollinator of such a unique, close-formed flower. However, considering *Erica lanuginosa* flowers in the winter, a period when there is unlikely to be many insect available and limited seed as food sources, nectar would be the best available food source for the pollinator (Rebelo and Breytenbach 1987). This would suggest that *Erica lanuginosa* has adapted to suit the pollinators needs, more than the pollinator has adapted to suit its needs.

In conclusion, our results indicate that *Erica lanuginosa* is unlikely to be pollinated by rodents due to low presence, both of rodents and of pollen within their faeces. The exclosures show that the flower is not self pollinated. This is further outlined by high nectar sugar levels which the plant is unlikely to waste energy producing if it underwent obligate autogamy. Entomophily is unlikely due to phenology and positioning of the flower. The analysis of the thickness of the plant's stem implies ornothiphily despite the nectar sugar analysis is ambiguous in this regard. Birds are the most plausible pollinator, and could either be an exclusive nectarivore such as the Orange-breasted sunbird or a generalist feeder, such as the Cape White-eye which is restricted in variety of available food during winter months (Rebelo and Breytenbach 1987, Geets and Pauw 2009).

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