The Birds, the Bees and Erica:
Vulnerability of Plant-pollinator Communities in Fragmented Fynbos Landscapes

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Thesis submitted in partial fulfilment of the requirements for the degree Masters of Science in Conservation Biology by coursework and dissertation

February 2016

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ACKNOWLEDGEMENTS

Special thanks to my two supervisors, Professor Jeremy Midgley and Dr. Mark Brown. I am very grateful to them for agreeing to take on this project and for their expertise and guidance. The Brown family, and Daniël and Chrissie Cloete welcomed me into their home and I will be forever grateful for their kindness and support. My upmost gratitude also goes to Daniël Cloete for all his help in the field and unwavering support and encouragements. Daniël and his wife helped me keep my head above water at times when this thesis went out of control. Thanks also go to Alan Lee for his precious time and advice with my statistical analysis and for his corrections on my thesis drafts. Susie Cunningham answered my endless emails with tireless patience and invaluable insights. Her help throughout the Conservation Biology programme, and on how to make the most of R codes, definitely helped me get through this Masters. Ross Turner spent hours sharing his vast knowledge about *Erica* species with me. Sandy Steenhuisen and Gabriella Fleury made time out of their own busy schedule to review my drafts and give me very useful suggestions.

I would like to acknowledge and thank SANParks, the Southern Crags and Redford conservancies, and Cape Pine for allowing me to conduct my research on their lands while I was under the Natures' Valley Trust banner. Brittany Arendse and Kellyn Whitehead from the Natures' Valley Trust provided much needed technical support in the field. The Percy FitzPatrick Institute of African Ornithology allocated funding to this project.

Finally, my parents have been my rock and believed in me throughout this journey. Without their support, love and guidance this study would not have been possible. It is to them that I owe my deepest gratitude.

ABSTRACT

Habitat fragmentation has been identified as a major cause of pollination mutualism collapse that can eventually impinge on plants' reproductive success (e.g. through pollen-limitation). Agriculture, timber plantations, invasion by alien trees and the urbanisation of the southern Cape lowlands have transformed and fragmented large parts of the former distribution range of many *Erica* species. Recent evidence suggests that in the Fynbos biome, small remnants of natural and disturbed vegetation are likely to display depauperate plant communities. In the present study, it was found that *Erica* species richness declined significantly as patch size decreased. Limited nectar resources available on those small 'islands' might not be enough to attract essential bird and insect pollinators. Insect-pollinated species were more impacted by reduced patch size than the bird-pollinated ones. Further investigation using *Erica discolor* showed that for this widespread ornithophilous species, pollination mutualism still occurred in smaller fynbos patches. These findings stressed the importance of conserving small fragments for maintaining remnant plant populations, which can act as reliable food sources for avian pollinators. In addition, to cope with the effects of pollen limitation, highly resilient plant species have evolved and adopted different compensatory mechanisms. From a short-term perspective, adopting compensatory reproductive strategies (e.g. autonomous self-pollination, vegetative growth, and generalised pollination systems) could reduce dependence on specific pollinators and increase the chances of a species being able to persist through a period of low-pollinator abundance.

The prevalence of autogamy and geitonogamy as alternatives to xenogamy was assessed in six different obligate seeder *Erica* species in the eastern coastal part of the Cape Floristic Region. Despite the long history of plantation-based timber production that fragmented the study area, and the subsequent possible pollinator loss, none of the species analysed in this study have adopted autonomous self-fertilisation as a response mechanism. *Erica sessiliflora* was the only species that showed a high compatibility for self-pollen. The species under investigation in a breeding system conducted here were also incapable of vegetative propagation and were plants targeting specific animal taxa (e.g. birds or insects) for successful pollination. Having limited compensatory mechanisms, further degradation of their habitat and weakening of their ecological interactions could be extremely detrimental to these *Erica* species' reproductive success.

1 INTRODUCTION

1.1 Pollination in Peril: Habitat Fragmentation

The importance of pollinators to many terrestrial plant communities, through the provision of crucial ecosystem services, is undeniable (Aguilar et al. 2006; Wolowski et al. 2013). A large number of flowering plants rely on animals for pollination and successful reproduction (i.e. approx. 308 006 species which make 87.5% of all angiosperms; Ollerton et al. 2011; Anderson et al. 2014). The disruption of pollination mutualism can have dire evolutionary and ecological effects on the species participating in these interactions (Traveset & Richardson 2006). With the apparent global declines in pollinator richness and density, a growing body of literature has highlighted concurrent declines in flowering plants that depend on them (Kluser & Peduzzi 2007; Potts et al. 2010; Regan et al. 2015). According to Thomann et al. (2013), a lack of pollinators will generally exacerbate pollen limitation (i.e. insufficient pollen receipt causing a decline in plant fecundity). This could in turn lead to a reduction in plant reproductive success and the eventual extinction of natural plant populations (Thomann et al. 2013; Melin et al. 2014).

A direct anthropogenic change, which can potentially affect pollination patterns and cause population decline in both animal and plant species, is habitat fragmentation (Pauw 2007; Kongor 2009). In the aftermath of reduced patch size, increased isolation, and decline in plant and animal populations due to habitat fragmentation, a cascade of detrimental effects (e.g. change in disturbance regimes and microclimate, edge effect, genetic deterioration, change in biological processes, and altered species interactions) is often triggered (Lindenmayer & Fischer 2006). Eventually, local populations can become more vulnerable to environmental and demographic stochasticity and species abundance and richness is permanently altered (Hobbs & Yates 2003). In the case of a severe decline in pollinator abundance and richness, significant pollination deficiency (reduced visitation rates and pollen loads) within a plant community would be expected (Aguilar et al. 2006; Pauw 2007). Aguilar et al. (2006) observed a highly significant correlation between fragmentation and pollen limitation, which can reduce reproductive success and subsequently increase extinction risks. Amongst representatives of the Orchidaceae and Amaryllidaceae families in the Cape Floristic Region (CFR), evidence of reproductive failure due to pollen limitation has already been identified (Johnson & Bond 1997). There are numerous ways in which animal-mediated pollination mutualism can be disrupted by fragmentation (Harris & Johnson 2004). Lack of pollinators as a result of declines in the availability of food resources, nesting sites, or larval host plants may eventually decrease flower visitations (Rathcke & Jules 1993; Winfree et al. 2011). Also, pollinators may respond negatively to increased altered abiotic and biotic factors in small fragments and their surrounding matrix (e.g. change in fire frequency and increased alien invasion) and thus avoid these areas (Rathcke & Jules 1993; Montero-Castaño & Vilà 2012).

1.2 Different Pollination Systems Influencing Vulnerability

Plant species dependent on a particular pollinator taxon might be able to tolerate the impacts of landscape modification better than other species (e.g. bird- versus insect-pollinated species; Montero-Castaño & Vilà 2012). For example, obligate nectarivorous birds have been reported to depend on plant species with a specific set of floral characteristics (pollination syndromes), for instance: long-tubed corolla, diurnal anthesis, concentrated sucrose rich nectar and landing platform (Valido et al. 2004; Johnson & Nicolson 2008; Curti & Ortega-Baes 2011). These vertebrate pollinators would usually not be expected to broaden their diet to non-typical plant species (e.g. alien species or mass flowering crops) to a large extent, but rather track the plant mutualists they have adapted to and/ or evolved with (Montero-Castaño & Vilà 2012; Neuschulz et al. 2013). Nevertheless, unexpected behavioural change in nectarivorous birds like sunbirds have been observed in the Old World, allowing them to ocasonally hover feed when presented with alien plants (e.g. hummingbird-pollinated plant *Nicotiana glauca*) (Geerts & Pauw 2009). Moreover, birds generally have high energy requirements, and if need be, are able to cross matrices of transformed hostile landscape (high mobility compared to insect pollinators) to track and pollinate flowering plant species in small isolated fragments (Symes et al. 2001; Neuschulz et al. 2013).

In contrast, Goulson et al. (2002) and Gross et al. (2010) found that key generalist insect flower-visitors (e.g. honey bees, *Apis mellifera*, which was by far the most abundant insect pollinator incidentally observed feeding on the insect-pollinated study species in the present study) are capable of broadening their diets to include the most abundant and novel floral resources (e.g. alien plants or mass-flowering crops). Since all their food and energy requirements could be met nearer to their immediate fynbos foraging grounds (e.g. in agriculturally developped surrounding matrices), such important insect-pollinators could ignore small, less accessible patches (Townsend & Levey 2005; Osborne et al. 2008; Bartomeus & Winfree 2011). Moreover, Lander et al. (2011) mapped the effective movement of a family of insect pollinators (Syrphidae) and found that these pollinators were waylaid in resource-rich areas (e.g. agricultural lands) between native vegetation patches. The Circle principle proposed by Lander et al. (2011) dictates that these alternate pollinator-attractive areas could draw pollinators away from fragments with natural habitats and resources. This might explain further why insects would not deliver pollination services to plant populations in small isolated fragments (Bartomeus & Winfree 2011).

Additionally, impacts of habitat fragmentation on pollination mutualisms have been documented to be mostly detrimental to highly specialised plant species compared to more generalised species (Pauw 2007). Small populations of *Aloe pruinosa* have shown resilience to habitat fragmentation owing to their generalised pollination system (pollinated by both bird and insect species belonging to different functional types; Wilson et al. 2009). Specialised plants would be more vulnerable to drastic biotic changes in the landscape as they would have no replacement pollinators in the event that their only group of functionally similar effective pollinators (or only pollinator species for highly specialised plant species) go locally extinct (Geerts & Pauw 2012). Pauw (2007) found that an oil-collecting bee, *Rediviva peringueyi*, was absent from small urban conservation areas (<385ha). Correspondingly, seed set failed in six species of highly specialised oil-secreting plant species that rely exclusively on *R. peringueyi* for pollination. On the other hand, *Hemimeris racemosa*, a less specialised oil-secreting species yielded high seed set irrespective of the presence of *R. peringueyi* and the size of the patch in which the plant populations occurred (Pauw 2007). This was partly attributed to the fact that in essence, *H. racemosa* had replacement pollinators (other species of oil-collecting bees) to deliver the pollination service that *R. peringueyi* was no longer providing in smaller conservation areas (Pauw 2007). Additionally, in areas with low oil-collecting bees' abundance, Pauw (2005) recorded higher occurrence of homostylous (i.e. cluster of style and stamens arranged together) *H. racemose* morph individuals that can also reproduce through autogamous fertilisation. Geerts & Pauw (2012) also found that in the specialist geophyte *Brunsvigia litoralis*, habitat fragmentation and urbanisation significantly reduced seed production by impacting populations of a specific pollinator. They demonstrated that because malachite sunbirds, *Nectarinia famosa* (*B. litoralis*' sole effective pollinator), were only recorded in larger rural fragments, seeds were also only produced in these areas but not in smaller urban patches where these particular sunbirds were absent (Geerts & Pauw 2012). The implications of a cessation in sexual reproduction in *B. litoralis* might however take several decades to lead to local extinction in small fragments, as senescence of its geophytic bulbs is a lengthy process (Geerts & Pauw 2012).

In spite of the abovementioned potential risk conferred by a specialised pollination system, more than 44 species of long-tubed ornithophilous plants within the CFR are known to have a reproductive strategy dependent exclusively on malachite sunbirds (Anderson et al. 2005; Geerts & Pauw 2009, 2012). Through an analysis of 25 plant-pollinator networks, Bascompte et al. (2003) found in general that pollination webs are asymmetrically specialised. Highly specialised plant species tend to be dependent on generalist pollinators (i.e. animal species with broad floral preferences) (Bascompte et al. 2003). Specialised plant species would most certainly be sensitive to the loss of generalist pollinators (Rathcke & Jules 1993). However, these losses are expected to be uncommon as generalist pollinators can be buffered against extinction by switching or alternating their floral preferences depending on nectar availability (Bascompte et al. 2006; Dorado & Vázquez 2014). In the CFR, Rebelo (1987) reported that a single generalist nectar-feeding avian species could visit and pollinate around 35 specialised plant species.

1.3 Compensatory Mechanisms

Changes at the plant community level due to the exclusion of species that are the most reliant on pollinators could be anticipated with the loss of key flower visitors. Consequently, the loss could result in pollen limitation (Pauw & Bond 2011). Not all species respond equally to the same pressures imposed by declining pollinator populations (Knight et al. 2005). While studying the effect of a gradient of pollen limitation on a guild of 15 oil-secreting orchids (Coryciinae), Pauw & Bond's (2011) findings supported the idea that species clonal abilities could increase their persistence in pollinator-poor communities. Bond (1994) argued that obligate outcrossers, exhibiting a highly specialised pollination system, and those depending solely on seeds for propagation, would be strongly prone to extinction. To reduce this risk, such species would often end up adopting compensatory mechanisms like increased self-compatibility or vegetative persistence to buffer against the possible demographic consequences of pollinator loss (Bond 1994; Thomann et al. 2013).

Pauw (2007) also concluded that the plant species that are most resilient to the loss of pollen vectors would persist longer in a community than other species following habitat fragmentation. Substantiating previous findings, a meta-analysis by Aguilar et al. (2006) found that self-incompatible species were impacted more by habitat fragmentation than self-compatible species. In the United Kingdom, Biesmeijer et al. (2006) reported a drop in wild entomophilous plants since the 1980s, which correlated with the decline of their pollinator species. In particular, obligate out-crossers were found to be more vulnerable (mean relative change in species diversity) than self-compatible species (Biesmeijer et al. 2006). Bond (1994) argued that very few species face high risks of extinction as plant species tend to adopt at least one low risk trait (e.g. facultative clonal reproduction) as a ‘safety net’ to compensate for other traits that are strongly dependent on a particular pollination vector (Bond 1994). For instance, as they are resprouting geophytes, several orchids have low seed dependency and are able to persist long after the extinction of their specialised pollinator (Anderson et al. 2014). Ashman et al. (2004) further argued that even in the event of severe pollination limitation, the viability and abundance of plants that are long-lived (or capable of vegetative reproduction) will not necessarily be reduced shortly after their pollinator population collapses. Considering that self-compatibility has the potential to lessen plants' dependence on pollinators, species may escape chronic pollen limitation by evolving self-compatibility (Baker 1967). Knight et al. (2005) confirmed that self-incompatible plants were more pollen limited than self-compatible species. Also, breeding system shifts from obligate outcrossing (xenogamy) to autonomous self-fertilisation (autogamy) could provide a 'safety net' in case of severe pollinator collapse (Bond 1994). Using a greenhouse experiment in which pollinator abundance was manipulated, Bodbyl Roels & Kelly (2011) demonstrated that in populations of *Mimulus guttatus*, a predominantly outcrossing wildflower, there was an increase in autonomous self-fertilisation after five generations without pollinators. The latter selection experiment provided evidence of rapid adaptation towards increased selfing in a plant species undergoing pollen limitation (Bodbyl Roels & Kelly 2011). It was only in an environment where hummingbirds have been reported scarce that its dependent, specialised plant species, *Gesneria reticulate,* displayed autonomous selfing. This finding suggested that autonomous self-fertilisation could be utilised as a fallback mechanism for specialised plant species (Marten-Rodriguez & Fenster 2008).

Fynbos vegetation is particularly known for its dependence on fire (Kraaij 2012; Kraaij et al. 2013). However, because of low nectar availability, the abundance of nectarivorous birds (e.g. sunbirds and sugarbirds), in tandem with flower visitation are significantly reduced in post-fire fynbos vegetation compared to unburnt areas (Geerts 2011; Geerts et al. 2012). The abundance of nectar-feeding bird species (*Anthobaphes violacea*, *N. famosa* and *Promerops cafer*) is highest in older fynbos (Chalmandrier et al. 2013). Geerts (2011) found that the abundance and richness of nectar-feeding birds could take at least four years to reach substantial levels after a fynbos fire. Some plant species will nevertheless reach reproductive maturity before pollinator abundance is fully re-established. For example, as little as two years is needed for Ericaceae to reach maturity (Le Maitre & Midgley 1992). Adopting geitonogamy (i.e. the transfer of compatible pollen from a flower to another on the same plant and subsequent fertilization of ovules) in addition to cross-pollination as a reproductive strategy could be advantageous for some plant species (Roberts et al. 2014). However, geitonogamy still requires a vector for the transfer of pollen from anthers to a stigma (Wilcock & Neiland 2002). Unlike autonomous self-fertilisation, geitonogamy cannot therefore provide reproductive assurance in times of low pollinator abundance, for instance, post-fire (Elle & Hare 2002; Geerts et al. 2012). Nevertheless, in unstable fire-prone environments, geitonogamy might form part of a bet-hedging strategy that permits even small clusters of genetically similar plant populations to generate a substantial seedbank with minimum self- or cross-pollination (Roberts et al. 2014). Hence, augmenting the probability of seed production even with low visitation frequencies

(Vaughton 1995).

Despite the reproductive advantages that self-compatibility could confer when pollinators are few, many plant traits have nevertheless consistently evolved mechanisms to prevent self-pollination (Knight et al. 2005). The wrong timing of self-fertilisation can be costly (Elle & Hare 2002; Goodwillie & Ness 2005, 2013). The occurrence of selfing (autogamy and geitonogamy) prior to opportunities for outcrossing and competing selfing (i.e. occurs along with outcrossing) can reduce chances of cross-fertilisation (Elle & Hare 2002). Ovule pre-emption in species capable of self-fertilisation might depress seed set when some of the selfed seed subsequently abort because of inbreeding depression, and rendering the ovules unavailable for fertilization by cross pollen (Wilcock & Neiland 2002). It has been put forward that if selfing is not maintained as a secondary choice to outcrossing while a pollinator is available, disproportionate inbreeding depression (i.e. reduction of plant vigor) might result (Elle & Hare 2002). Janzen's (2001) concept of the 'living dead' supported the need for out-crossing and a reassessment of Bond's (1994) perspective (i.e. low conservation importance of mutualisms). It may take several generations, over hundreds of years, for highly persistent resprouting or long-lived plant species to disappear completely after the disruption of their mutualistic interactions (Janzen 2001). Without functional sexual reproduction, however, such species' long term persistence would eventually be jeopardised because of factors such as higher chances of inbreeding depression, deleterious mutation accumulation, and demographic stochasticity in small remnant populations (Janzen 2001).

1.4 Specifics of Current Research

In this thesis, I worked with a sub-set of species belonging to the genus *Erica*, the largest genus in the CFR that consist of more than 680 species (Oliver & Oliver 2002; Pirie et al. 2011). Only few observational and experimental breeding system studies have focussed on the vulnerability of *Erica* species to environmental perturbations thus far (Geerts & Pauw 2010; Turner 2012). Turner (2012) also stated that little was known about the ecological vulnerability of insect versus vertebrate pollination within the Cape *Erica* species. With pervasive landscape disturbances threatening at least 181 Cape *Erica* species and their respective pollinators, a better understanding of the diverse breeding systems (characteristic of the *Erica* genus) would be useful in steering conservation strategies in the right direction (Rebelo 1992; Turner 2012). Large parts of the former distribution range of many *Erica* species have been subjected to a multitude of anthropogenic and ecological perturbations, including habitat fragmentation, alien plant invasion and disturbed fire regimes (Rebelo 1992; Cowling et al. 2009). Where the present study was conducted (i.e. the eastern coastal part of the CFR), the natural habitat of wild pollinator and plant communities has been severely transformed and fragmented by agriculture, plantations of alien trees (e.g. *Pinus pinaster* and *Pinus radiata*), natural spread of alien trees (e.g. *Acacia mearnsii*), and the urbanization of the southern Cape lowlands (Rouget et al. 2003; Cowling et al. 2009; Kraaij et al. 2011; Baard & Kraaij 2014). As a result, many of the remaining typical-fynbos species populations occur in small remnants of natural and transformed vegetation (Rouget et al. 2003).

For the purpose of the current study, field surveys and field-based pollination experiments were conducted. I aimed to address the following questions using the data collected:

1) Does *Erica* species richness change with increased levels of habitat fragmentations (with respect to fragment size)?

   I predicted that with decreased patch size, *Erica* species richness would decrease (assuming plant population size decreases with decreasing patch size).

2) As patch size is reduced, are bird-pollinated *Erica* species less vulnerable than insect-pollinated species?

   I predicted that species richness of bird-pollinated *Erica* species would be less affected than insect-pollinated species as avian pollinator-plant mutualism would be more resilient to habitat fragmentation (Neuschulz et al. 2013).

3) Does a decrease in patch size affect pollinator visitation and seed set in the widespread ornithophilous species, *Erica discolor*?

   Bird-pollinators have higher mobility and energy requirements, and a less flexible association with available nectar resources compared to insect-pollinators (Johnson & Nicolson 2008; Montero-Castaño & Vilà 2012). They would thus be expected to track their food source and by the same means maintain pollination in small isolated fragments (Symes et al. 2001; Neuschulz et al. 2013). Consequently, a decrease in patch size was expected to not significantly reduce pollinator visitation nor seed set production in the widespread bird-pollinated species, *Erica discolor*.

4) Have either bird- or insect-pollinated members of the *Erica* genus in the study area adopted self-compatibility and the ability to self autonomously?

   The last prediction was that the breeding system of bird-pollinated species would be different from that of insect-pollinated species. Insect-pollinated species might be more at risk of losing their pollinators in fragmented habitats than bird-pollinated species (Montero-Castaño & Vilà 2012). Therefore, the former, but not the latter, could have adopted autonomous selfing to buffer against a lack of pollinators in the environment (Bodbyl Roels & Kelly 2011).

2 METHODS

2.1 Study Sites

For the fragmentation studies, a total of 20 patches composed of montane mesic proteoid fynbos habitat (Vlok et al. 2008) were identified and their boundaries delineated using remote sensing data in both Google Earth Pro V7.1.4.1529 and ArcGIS 10.2.2 (ESRI 2014), and on-site ground-truthing (Fig. 1). Of the 20 patches, three were large continuous stands of fynbos (mainland patches >100ha) and 17 were patches smaller than 100 hectares (Fig. 1; Appendix A). The individual patches chosen ranged in size from two hectares to 337 hectares (Fig. 1; Appendix A). Fragmentation in the study area, through afforestation and cultivation, has been recorded since the early 1900s (Kraaij et al. 2011; Sandberg 2013). Although exact dates were not available, the 17 fragments used here would have been isolated from mainland patches for over a century (Kraaij et al. 2011). The study area was located between the Keurbooms River and the Bloukrans River (Fig. 1). It encompassed a matrix of natural fynbos, stands of invasive alien vegetation, agriculture, farmsteads, *P. pinaster* and *P. radiata* plantation, and rural and urban settlements (Kraaij et al. 2011). Detailed information describing the fragmentation matrix of each patch is given in Appendix A. All of the patches were situated in areas composed of old-growth fynbos vegetation with either no alien species or very few of them (some patches were recently cleared of *P. pinaster* and *Hakea sericea*) (Appendix A; Kraaij et al. 2011). The last fire that burnt the entire area occurred in 1998 (Kraaij et al. 2013). In 2005, a small section of one of the study patches, P4, (Fig. 1; Patch no. 18; Appendix A) was impacted by a fynbos fire (D. Cloete Pers. Comm.).

All *Erica* populations used in the breeding system experiment were within a practical walking distance from an accessible road or trail. Breeding experiments were performed at three different locations within the study area. Along the Covie hiking trail in Covie Main patch (Fig. 1; Patch no. 20; Appendix A), a minimum of 20 individuals per flowering *Erica* species available were selected. Four *Erica* species *Erica discolor*, *Erica densifolia*, *Erica sessiliflora* and *Erica penicilliformis*, were treated in Covie. On the Kalanderkloof trail in Salt River East patch (Patch no. 19; Appendix A) and at the Nature's Valley rest camp (lat. 33.972296°S, long. 23.562471°E), 20 *Erica formosa* plants and 20 *Erica scabriscula* individuals were chosen respectively. Populations of the six abovementioned *Erica* species were chosen from populations in stands of mature fynbos where possible (except for the only easily accessible population of *E. scabriscula* near to Nature's Valley rest camp which was a small population bordered by the Groot River and indigenous forest vegetation).

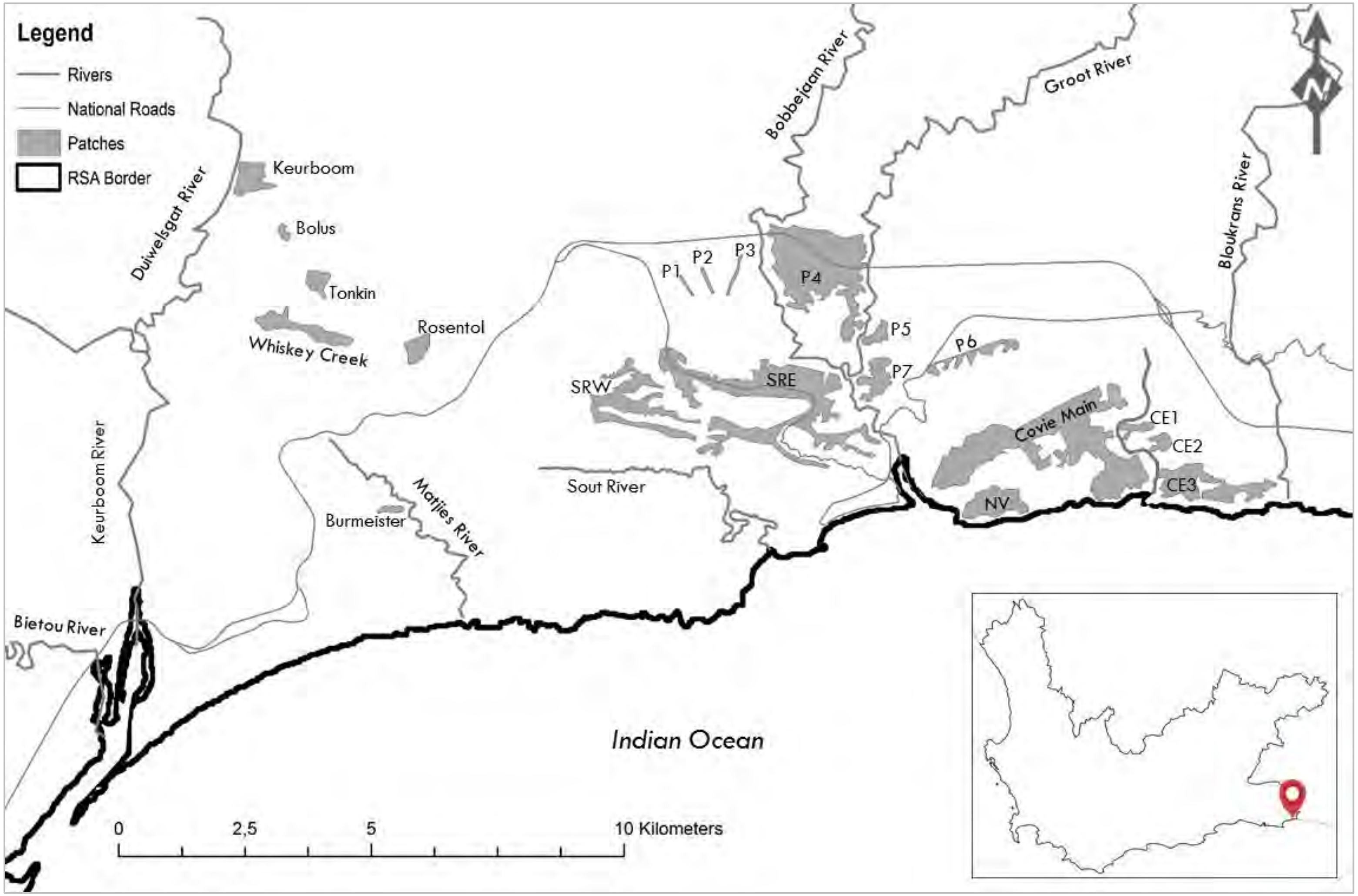

Figure 1. Map of the study area showing the location of the 20 patches chosen, major roads, and rivers. Inset map of the location of the study area within the Western Cape Province, South Africa. Scale 10km.

2.2 Study Species

From September to December 2015, 20 sites were surveyed for the presence of all identifiable flowering *Erica* species. Species were identified in the field where possible and plant samples were collected for unknown species. An *Erica* species identification key in conjunction with local botanical experts, were used to identify the species found (Smuts 2012; R. Turner Unpublished Data). A list of every *Erica* species recorded in the study and their respective breeding and pollination system, and pollinator taxa is provided in Appendix B. *E. discolor* (Fig. 2C), which is particularly common in the study area (Smuts 2012), was used to further investigate impacts of habitat fragmentation. Since *E. discolor* occurs in all 20 of the fynbos patches that were visited, it was the ideal study species for assessing the effect of habitat fragmentation on its pollination by birds.

Species selection for the breeding system experiments was based on which *Erica* species were in flower in and around Nature's Valley (lat. 33.976754°S, long. 23.562155°E) from September to December 2015. The pollination syndrome of the six species under investigation was established by use of literature and local botanical experts (Oliver and Oliver 2002; Smuts 2012; R. Turner Unpublished Data). In this study, species with long tubular flowers (15 to 30mm) - *E. densifolia, E. sessiliflora* and *E. discolor* (Fig. 2) - were considered to be pollinated by short-billed sunbirds (Smuts 2012). The plant species with small corollas (2 to 4mm) were all considered to be insect pollinated species (pollinated by a suite of insect species within different functional types) - *E. penicilliformis, E. scabriscula* and *E. formosa* (Fig. 3; Smuts 2012). All *Erica* species recorded in this study were obligate seeders (R. Turner Unpublished Data; Appendix B).

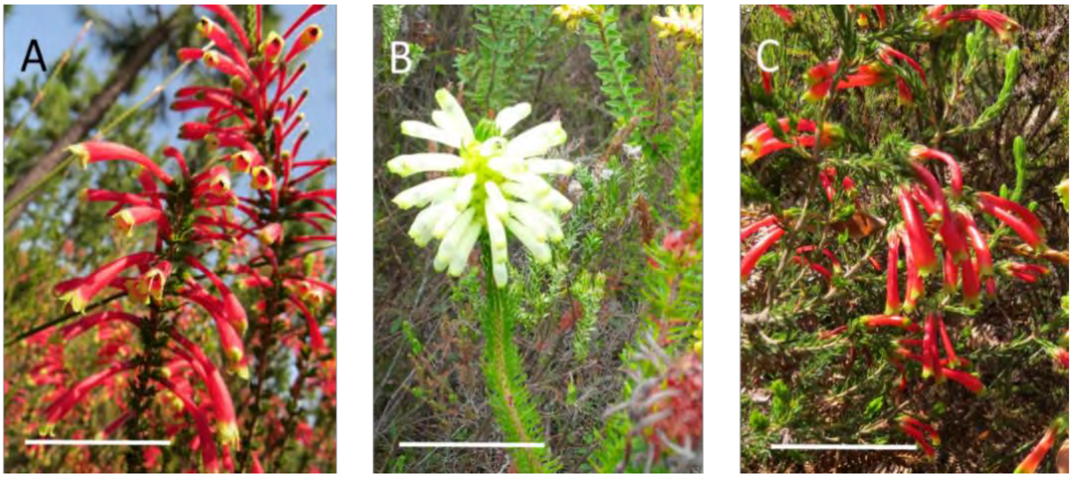

Figure 2. Bird pollinated *Erica* species. (A) *Erica densifolia*. Scale 40mm. (B) *Erica sessiliflora*. Scale 30mm. (C) *Erica discolor*. Scale 40mm.

Figure 3. Insect pollinated *Erica* species. (A) *Erica penicilliformis*. Scale 8mm. (B) *Erica scabriscula*. Scale 8mm. (C) *Erica formosa*. Scale 8mm. (D) *Apis mellifera* pollinating *Erica scabriscula*. Scale 8mm.

2.3 Fragmentation

Species Richness

A preliminary survey (wandering transect) was undertaken in the largest study patch (Covie Main; 336.62ha; Appendix A). Data collection was adapted from the methods used in Sandberg (2013). It was assumed that *Erica* species were randomly distributed across the patch. Occurrence data of *Erica* species were captured by walking a wandering transect through the Covie Main patch and recording species until the rate of finding new species plateaued at zero new species per 100 meters covered. At this point, I assumed that all the *Erica* species present in this patch were found. Although this approach could be qualitative to some extent, it enabled a greater patch coverage given the dense old-growth fynbos vegetation in the study area (Sandberg 2013). The cumulative number of species with increasing distance covered was plotted to obtain the minimum distance that needed to be covered to find the highest number of species in the largest patch (Appendix C). After covering approximately 3800 meters an asymptote was reached relative to the maximum number of species found in a patch (Appendix C). Based on this observation, it was determined that wandering transects in the fynbos patches could be terminated after covering a minimum of about 11 meter per hectare using the equation below:

(Eq. 1)

$$Distance\ to\ cover = \frac{Distance\ to\ reach\ species\ number\ asymptote\ in\ Covie\ Main(m)}{Size\ of\ Covie\ Main\ patch\ (ha)}$$

This ratio was used as a rough measure to standardise survey effort across bigger patches (>50ha) especially. To optimise the chances of finding every flowering species in the fragments less than 50 hectares, a minimum of 550 meters was covered in each of these patches (Appendix A). For all site surveys conducted, a Suunto Ambit2S GPS watch was used to track the path taken, and record the survey starting point, walking distance covered and ending point. The data recorded were imported into ArcGIS 10.2.2 (ESRI 2014) for viewing and analysis when required. The presence or absence of *Erica* species was recorded for each patch (Appendix E).

‘Snapshot’ View of Pollinator Visitation

To quantify visitation rate, a proxy for direct observation (i.e. disturbed anther ring technique) was utilised in this study (Geerts & Pauw 2010). Geerts & Pauw (2010) determined that the probing beak of a visiting sunbird disturbed the anther ring of two bird-pollinated species (*E. versicolor* and *E. perspicua*). Before being visited by a pollinator, the anther thecae of mature flowers are connected laterally at their pores into a perfect ring (un-tripped anther ring) through which the style is exerted (Fig. 4B). No pollen is released until the anther ring is ruptured. It was also confirmed that sunbirds deposit a significant pollen load on the stigma of the flower while visiting it for nectar (Geerts & Pauw 2010).

In December 2015, populations of *E. discolor* were scored for tripped anther ring in 20 different fynbos patches of varying sizes. At each site, 10 plants were selected at random and for each, an apical inflorescence (containing either 3 or 4 flowers) was scored for tripped anther rings (Fig. 4C). To standardise for flower age, all the flowers (per inflorescence) scored were mature, opened flowers, with receptive stigma (shiny and sticky) exerting out of the corolla by at least three to four millimetres (Fig. 4A). The presence or absence of nectar in the chosen flowers was also noted. This was done to confirm that flowers with an intact anther ring were mature enough to attract birds using nectar (J. Midgley and R. Turner Pers. Comm).

Seed Set Success

Seed set samples of *E. discolor* per patch were also collected. In October 2015, approximately 10 fruits (one fruit from 10 different randomly chosen plants) were collected in each of the 20 above mentioned patches. Seed set across patches was standardised by only picking ripe fruits (red coloured fruits; Fig. 4D). Available uneaten ripe fruits were collected across all patches. This was done as an attempt to standardise for maturity of fruits across patches and negate the seed predation factor from the sampled fruits. The fruits collected and subsequent seed sets obtained provided an approximate measure of the effectiveness of natural pollination frequencies in each of the 20 patches. In this study, it was implied that multiple visits would be needed to provide adequate pollen load to offset pollen limitation (Engel & Irwin 2003; Waites & Ågren 2004). With each successive pollinator visit, viable seed set was expected to increase (Engel & Irwin 2003).

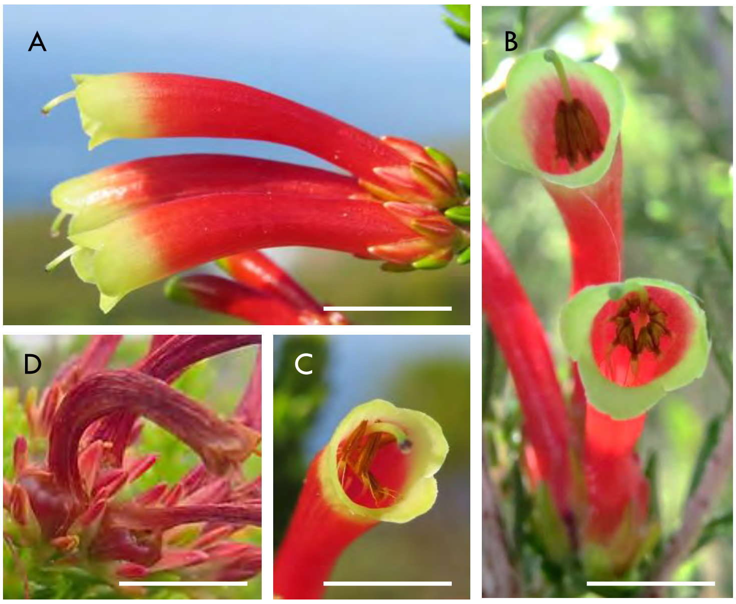

Figure 4. Photographs of *E. discolor* showing A) inflorescence with extended receptive stigma, scale 10mm, B) intact anther ring, scale 10mm, C) tripped anther ring, scale 10mm and D) red mature fruits, scale 10mm.

The fruits produced were dissected and the viable and unviable seed numbers were recorded. The viable seeds were distinguishable from unviable ones by their larger size and dark deeper colour (similar to example in Fig. 5). Viable and unviable seeds per fruit collected was scored using an image processing and analysing software package (Image-J; Rasband 2015). Photographs of every seed set were taken (similar to example in Fig. 5) and analysed in Image-J (Abramoff et al. 2004; Rasband 2015). Size and/or colour traits pertaining to the appearance of viable and unviable seeds in each seed set were measured. An output with the count of viable and unviable seeds in accordance with their respective visual characteristics was generated per fruit collected using the Image-J software (Abramoff et al. 2004). In order to test the accuracy of the Image-J results, a direct comparison (t-test) between the automated and manual seed set counts was done for 30 fruits of *Erica discolor*.

2.4 Breeding Systems

Controlled hand-pollination experiments were conducted to determine the prevalence and efficiency of autogamy (seeds set without pollen transfer to stigma), geitonogamy (seeds set after self-pollination) and xenogamy (seeds set after cross-fertilisation) within the six different

Erica species. From the 8th of September to the 29th of December 2015, budding inflorescences were bagged, hand-pollinated when opened, re-bagged and thereafter their ripe fruit were collected.

Four treatments were applied to each of the selected mature plants *in situ* to determine their ability to set seed under different conditions. The four treatments were: 1) cross-pollination, 2) self-pollination with manual pollen trasfer, 3) autonomous self-pollination, and 4) open-pollinated control. Each of these four treatments was applied on all selected plants once. There was a minimum of 20 replicates (individual plants) per species. To make sure that hand-pollination was done on unvisited flowers, inflorescences in the bud stage were bagged using total pollinator exclusion bags (made of bridal veil). Due to being enclosed in bags, no natural pollinators could reach and contaminate the stigma of flowers chosen for crossing, selfing and autonomous selfing (Arendse 2014). For every species the procedure for conducting the four different treatments was similar. Pollen used for out-crossing was obtained from neighbouring plants that were at least 5m distance from hand-pollinated flowers to ensure that the out-crossed counterparts were genetically dissimilar from each other (Arendse 2014). Anther rings of un-pollinated flowers were disturbed over a clean petri-dish to collect pollen. Using a micro dissecting spatula, pollen was applied to the receptive stigma (sticky to the feel and shiny surface) of a marked flower. Blue or green wool was used to tag the cross-pollinated flowers (n = 20 per species). For self-pollination, pollen from the plant containing the flower being ‘self’ treated (n = 20 per species, orange or purple wool) was put on the marked flower’s receptive stigma. The spatula surface was rinsed with clean water and dried after each pollen application. Flowers (n = 20 per species) were bagged but left un-manipulated to test for autogamy in the different species. After treating the flowers, they were enclosed again to prevent further pollination by either insects or birds.

Treated flowers were left in the bags to allow for fruits to mature for a minimum of six weeks. Toward the end of the ripening period, fruits were checked periodically to ensure that harvesting was done before natural dispersal of the seeds occurred (seeds expelled when fruit is completely mature). Since the fruits and seeds of *E. sessiliflora* can persist in ‘fruiting bodies’ on the old stem even after seeds are mature, they were left to ripen for a longer period (approximately 10 weeks) compared to the other species. The majority of the treated replicates (in all six *Erica* species) produced usable data. However, a few fruits expelled their seeds before collection (such replicates were discarded in the data analysis).

Control treatment seed set for each species was determined by collecting ripe fruits from un-bagged and un-manipulated flowers (n = 20 per species). Available uneaten ripe fruits were selected to negate any seed-predation factor from the experiment. The control seed set provided an approximate measure of the effectiveness of natural pollination frequencies on a particular flower. In this study, it was implied that each subsequent pollinator visit increased viable seed set. The proportion of viable seeds per plant (viable seed set) was established from the dissection of ripe fruits and their developed seeds. According to Dudash & Fenster (1997), to accurately answer pollen limitation related questions, it is most appropriate to use viable seed set as a response variable. Viable seeds are visually different from aborted ones in that they look plump and are larger in size compared to their shrivelled and smaller counterparts (Fig. 5; Turner 2012; Arendse 2014). The latter physical seed appearances were used as a proxy for evaluating seed viability. Counts of viable and unviable seeds were obtained for all seed set.

Viable and unviable seeds per flower for each treatment was scored either using the Image-J software package (Rasband 2015) or by visual manual count. For bird-pollinated species, pictures of every seed set were taken (Fig. 5) and fed into the software (Abramoff et al. 2004). Seeds of insect-pollinated species were counted manually with the help of a light microscope (X10 magnification). The colour or size of seeds from insect-pollinated plants could not be determined reliably from photographs. The Image-J software could thus not be utilised in this study for the seeds of insect pollinated species due to their very small size relative to seeds from ornithophilous plant species. Seed set from all species studied appeared to have significant size difference between their viable and unviable seeds

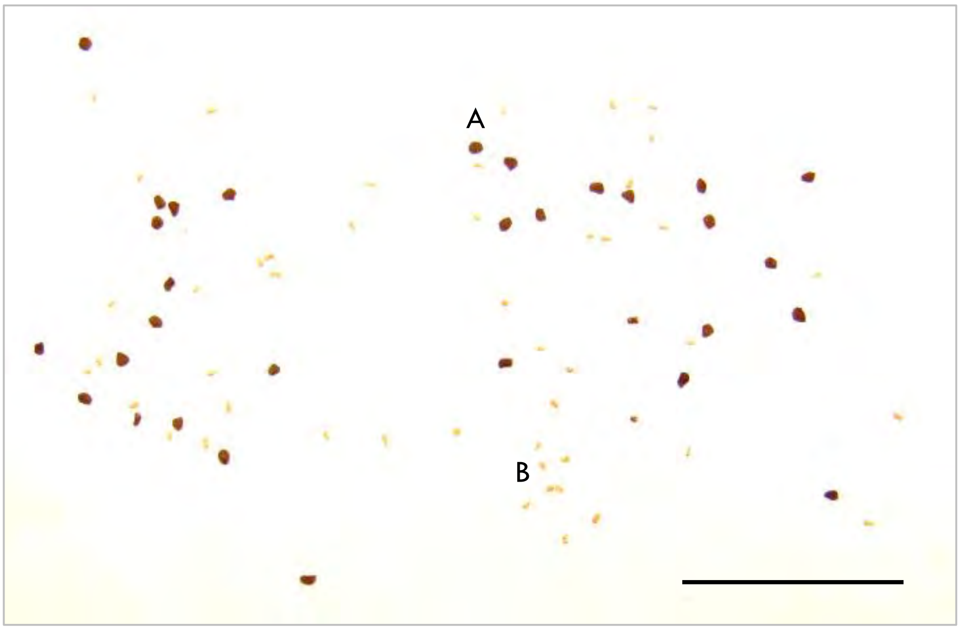

Figure 5. Photograph of seed set produced by cross-pollination treatment (*Erica sessiliflora* seeds in this example) showing the size difference between viable and unviable seeds. A) Plum seeds recorded as viable. B) Shrivelled and smaller seeds recorded as unviable. Scale 20mm.

2.5 Data Analysis

2.5.1 Statistical Analysis

The software package R 3.2.3 (R Core Team 2015) was used to perform the statistical analysis in this study. In addition, a binary matrix nestedness temperature calculator, BINMATNEST was used to detect nested patterns (Rodriguez-Girones & Santamaria 2006).

Relationship between Species Richness, Patch Size and Pollinator Taxa of *Erica* Species

A linear model (lm) was used to assess the relationship between the number of *Erica* species recorded on sites of varied patch size and two factors (patch size and species' pollinator taxa, i.e. insect vs bird pollinated). Before being added to the model, 'patch size' data were log transformed to reduce data distribution skewness and to fulfil the prerequisite of a Normal distribution (Emerson & Stoto 1983). To validate the fit and assumptions of the linear model, Global Validation of Linear Models Assumptions (gvlma) package in R was used (Pena & Slate 2006).

Quantification and Determinants of Nestedness

BINMATNEST was used to calculate species nestedness with the presence-absence data of the *Erica* species survey (Appendix E; Rodriguez-Girones & Santamaria 2006). The extent of the deviation of the data set (see matrix in Appendix E) from an ideal nested structure was indicated by a temperature value, *T*, generated by the BINMATNEST calculator. *T* can range between zero (perfectly nested matrix) and 100 for a completely disordered matrix (Rodriguez-Girones & Santamaria 2006; Li et al. 2013). To detect statistical significance with the *T* obtained in the present study, this value was generated for and compared against the distribution of three null models, which were generated by randomised sampling of the matrix (Rodriguez-Girones & Santamaria 2006; Sebastin-Gonzlez et al. 2010).

To determine which of the patch parameters (i.e. size and isolation) in conjunction with species richness, possibly contributed to a nested pattern, a Spearman's rank correlation analysis was conducted in R using patch size, patch isolation and species richness ranks (Sebastin-Gonzlez et al. 2010; Li et al. 2013).

Patch size effect on *E. discolor* pollinator visitation success

A generalised linear mixed model (GLMM) with binomial errors was fitted for the pollination visitation data (proportion of visited flowers with increasing patch size) using the glmer function (*lme4* package in R; Bolker et al. 2008; Bates et al. 2015). The cbind command was used to bind together two response variable vectors, namely: number of visited flowers per plant and number of non-visited flowers per plant, into a single object comprised of successes and failures respectively (Bates et al. 2015). The latter regression model was based on a Laplace approximation (logit scale). Patch size was added as a categorical predictor, and patch ID added as a random intercept to account for the pseudo-replication at the patch level (10 plants samples per patch).

Patch size effect on *E. discolor* seed set

A linear model (lm) was used to assess the relationship between the seed set yielded by plants on patches measuring less than 100 hectares and patch size. The same lm was repeated to also obtain a regression trendline between seed set on patches larger than 240 hectares and patch size. Based on a visual model (scatterplot; Fig. 8), illustrating a natural break in patch size smaller than 100 hectares (small patch size ≤97.68ha) and larger than 240 hectares (large patch size ≥245.41ha), the 20 patches were split into the two groups analised separately here (17 small and three large patches). Subsequently, a non-parametric Wilcoxon rank sum test with continuity correction was used to compare between the averaged proportion of viable seed sets of the samples belonging to the '<100ha' group and the '>240ha' group. No intermediate sized patches (ranging between 97.68ha and 245.41ha) were available for this study (Appendix A). Therefore, a continuous regression analysis could not be used with the entire dataset collected here. This would have overlooked any trend that intermediate sized patches might have shown if more data were available.

Hand-pollination experiment

Prior to comparing the seed-sets obtained with four hand-pollination treatments (i.e. cross-pollination, self-pollination requiring pollen transfer, autonomous self-pollination, and control), homoscedasticity of the data for each *Erica* species was verified using the Bartlett's test (Snedecor & Cochran 1989). Since the variances were not homogeneous ($P < 0.05$), the nonparametric pairwise multiple-comparison test, Dunn's test with Bonferroni adjustment, was performed for comparisons of seed-sets between paired treatments (Dinno 2015).

2.5.2 Genetic Self Incompatibility

To determine the degree of self-incompatibility for each of the *Erica* species studied here, two indices (1. index of self-incompatibility, ISI; 2. index of autonomous self-pollination, IAS) were calculated (equations used adapted from Steenhuisen & Johnson 2012 and S. Steenhuisen Pers. Comm.).

Equation for Index of Self-Incompatibility:

$$ISI = 1 - \frac{Proportion\ viable\ seeds\ for\ flowers\ pollinated\ with\ self\ pollen}{Proportion\ viable\ seeds\ for\ cross\ pollinated\ flowers} \tag{Eq. 2}$$

Proportions of self- and cross-pollinated seed set were obtained from hand pollination experiments (for ISI equation). An ISI value close to zero indicated full self-compatibility, while an index ≥0.2 indicated self-incompatibility (Zapata & Arroyo 1978; Steenhuisen & Johnson 2012; S. Steenhuisen Pers. Comm.).

Equation for Index of Autonomous Self-pollination:

$$IAS = \frac{Proportion\ viable\ seeds\ for\ unmanipulated\ bagged\ flowers}{Proportion\ viable\ seeds\ for\ flowers\ pollinated\ with\ self\ pollen} \tag{Eq. 3}$$

Proportions for viable seed set obtained from the un-manipulated bagged flowers and self-pollinated flowers were used in the IAS equation. IAS values ranged from zero (completely reliant on a vector for self-pollination) to one (fully capable of setting seeds by through autogamy). Any species scoring an IAS >0.2 was considered to be capable of autonomous selfing (Zapata & Arroyo 1978; Steenhuisen & Johnson 2012; S. Steenhuisen Pers. Comm.).

3 RESULTS

3.1 Fragmentation

Species Richness and Patch Size

With an increase in patch size, the total number of *Erica* species being recorded per patch, irrespective of which taxa pollinates them, also significantly increased ($F_{1, 18} = 16.88$, adj. $R^2 = 0.46$, $P < 0.001$) (Fig. 6). This pattern also held true for both insect-pollinated ($F_{1, 18} = 19.82$, adj. $R^2 = 0.50$, $P < 0.001$) and bird pollinated species ($F_{1, 18} = 7.54$, adj. adj. $R^2 = 0.26$, $P = 0.01$). The slope of the increase in number of species as patch size increased for insect-pollinated *Erica* species was however significantly higher than that of bird-pollinated species ($F_{3, 36} = 47.48$, adj. $R^2 = 0.78$, $P < 0.01$) (Fig. 6). See Appendix D for full results of statistical analyses.

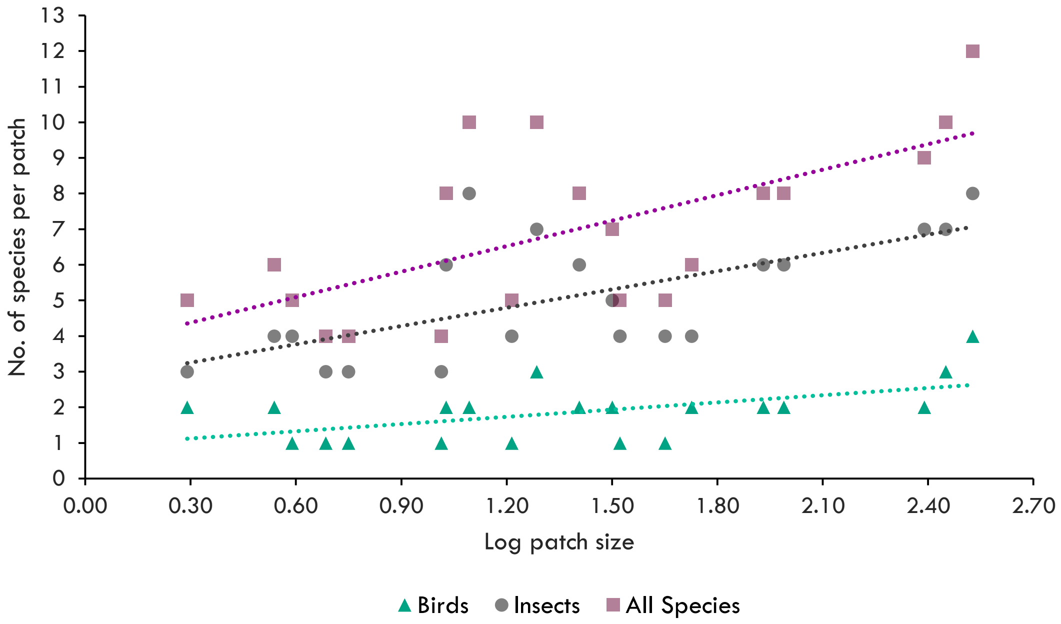

Figure 6. Scatterplot showing the relationship between the number of *Erica* species recorded and increasing log size of patch for 20 different patches. ▲ denoted the number of species that were pollinated by birds in each patch. ● showed the number of insect pollinated *Erica* species and ■ the total number of species irrespective of their pollinators' taxa. See Appendix D for full results of statistical analyses.

Nestedness & Determinants

The nestedness temperature T of *Erica* species in the study area was 17.34°C. When compared against all three randomised simulated null models' temperature value, the degree of nestedness (denoted by $T = 17.34$°C) was significantly different ($P < 0.05$). *Erica* species in the study area thus showed a nested pattern across patches (Table 1). The Spearman's rank correlation coefficients showed that both patch size (df = 19, *r* = 0.65, *P* = 0.002) and patch isolation (df = 19, *r* = – 0.60, *P* = 0.005) had a significant effect on *Erica* species nestedness.

Table 1. Nestedness analysis of the *Erica* species survey data set. See Appendix E for data set matrix. Nestedness temperature = 17.34 ˚C.

Null model	*P* value	Mean temperatures (˚C)	Variance
1	< 0.05	54.24	23.25
2	< 0.05	25.03	11.48
3	< 0.05	38.84	25.32

Pollinator Visitation vs Patch Size

The results showed that there was no significant change (GLMM, z-value = – 0.76, SE = 0.002, *P* >0.05) in the mean proportion of flower visitation success per plant as patch size increased (Fig. 7). Across all patches, the proportion of flowers visited per plant was high on average (≥0.67 ± 0.15) (Fig. 7).

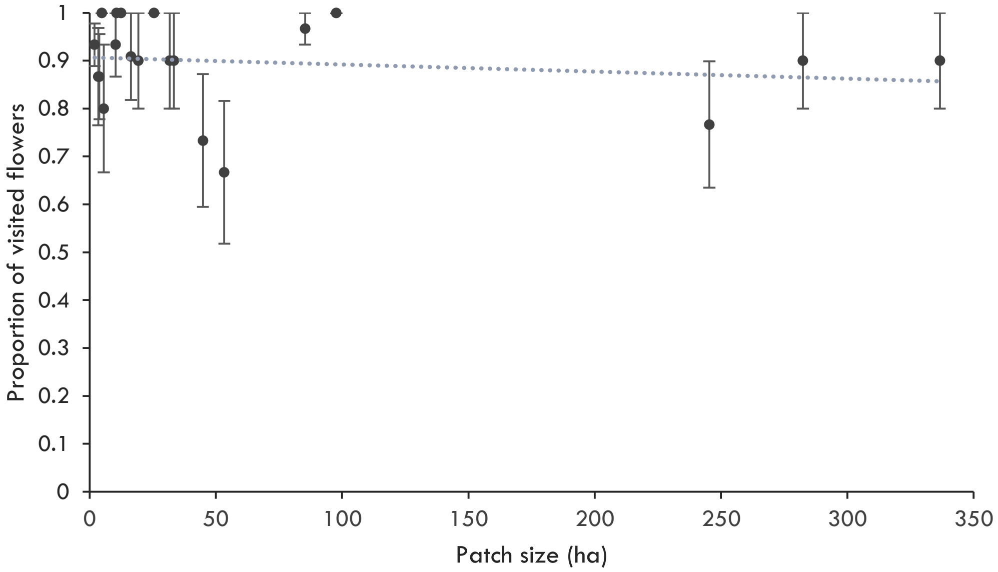

Figure 7. Scatterplot showing the relationship between mean visited flower per plant per patch for *Erica discolor* and increasing size of patch for 20 different patches. Standard Error bars shown for each patch and trendline shown.

Seed set vs patch size

There was no significant change in the proportion of viable seed set with patch size for plants on neither '<100ha' patches (df = 167, SE = 0.001, t-value = 0.41, *P* >0.05) nor those on patches '>240ha' (df = 27, SE = 0.001, t-value = – 0.31, *P* >0.05) (Fig. 8). The natural break illustrated in figure 8 between the two sets of patches justified the greater than 240 hectares versus less than 100 hectares patch size groups used for the following box plot and Wilcoxon rank sum test (Fig. 9). The mean for the averaged proportion of viable seed set of the 17 patches measuring '<100ha' was significantly bigger (W = 3118, $P < 0.01$) than the mean of the three larger patches ('>240ha' group) (Fig. 9).

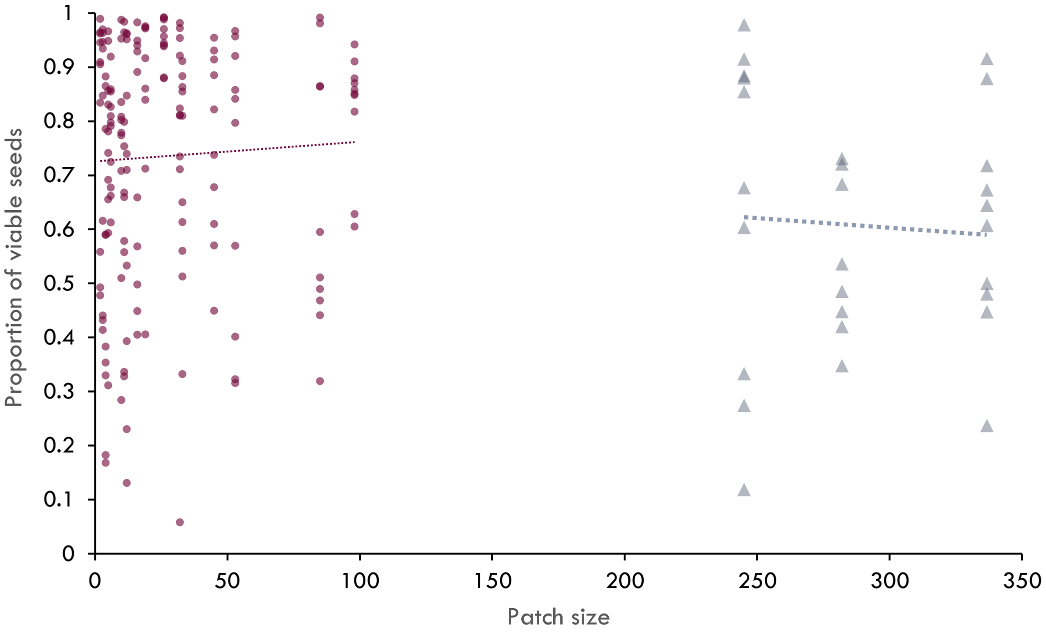

Figure 8. Scatterplot to illustrate the distribution of data across 20 patches of different sizes. Relationship between proportion of viable seed set per *Erica discolor* plant per patch shown. Trendlines given for both patches belonging to the <100ha (17 patches) and >240ha groups (3 patches).

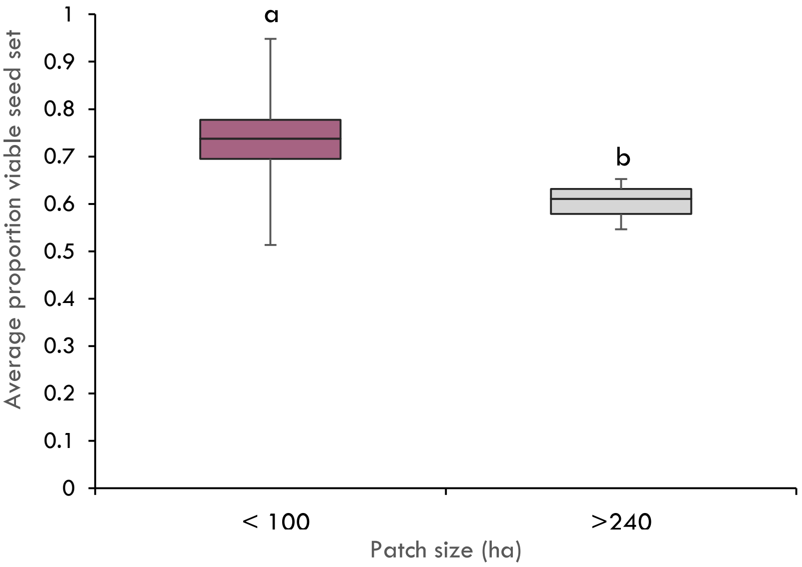

Figure 9. Boxplot showing averaged proportion of *Erica discolor* viable seed sets (approximately 10 fruits per patch) for patches < 100ha (17 patches) and > 240ha (three patches). Lower case letters indicate a significant difference between averaged proportion of viable seed set of the two patch groups at the 0.05 level. Standard Errror bars shown for each group.

Validation of Image J

There was no significant difference in viable seed count obtained using the automated (Image-J; Rasband 2015) or manual counts (df = 29, SE = 0.05, t-value = 1.66, P >0.05), validating the use of this technique.

3.2 Breeding System

Based on IAS values, none of the six species studied appeared to be capable of setting many seeds without pollen vectors (IAS <0.2; Table 2). Across all species, the viable seed set yielded by the autonomous self-pollination treatment was relatively low (≤0.08 ± 0.03 proportion of viable seeds per fruit) and significantly different (Bonferroni adj. P <0.05) from the ones produced by cross-pollination (≥0.36 ± 0.06 proportion of viable seeds per fruit) (Fig. 10; Appendix F).

As for the ISI results, five species (*E. formosa*, *E. peniciliformis*, *E. scabriscula*, *E. discolor* and *E. densifolia*) of the six species showed incompatibility to self-pollination (ISI >0.2; Table 2). These five *Erica* species yielded a significantly lower seed set (≤0.27 ± 0.08 proportion of viable seeds per fruit; Bonferroni adj. P <0.05) with the self-pollination treatment than with the cross-pollination treatment (≥0.47 ± 0.06 proportion of viable seeds per fruit; Fig. 10; Appendix F). Both self- and cross-pollination treatments yielded similar seed set (0.43 ± 0.04 and 0.36 ± 0.06 proportion of viable seeds per fruit respectively; P = 0.82) for *E. sessiliflora* (Fig. 10D; Appendix F). Given its high potential for producing viable seed sets when self-pollinated and an ISI of roughly 0 (–0.16 ± 0.17), *E. sessiliflora* would be fully capable of selfing. In all cases, the cross-pollination treatment seed set yield (≥0.36 ± 0.06 proportion of viable seeds per fruit) was similar (Bonferroni adj. P <0.05), if not significantly higher (for *E. scabriscula*, Bonferroni adj. P = 0.02; Fig. 10C) compared to that of the control treatment (≥0.29 ± 0.05 proportion of viable seeds per fruit) (Fig. 10; Appendix F).

Table 2. Index of autonomous self-pollination (IAS) and of self-incompatibility (ISI) based on mean seed set data of six *Erica* species. Standard Errors provided for both indices.

Species	IAS	SE (IAS)	ISI	SE (ISI)
E. formosa	0	0	0.79	0.10
E. peniciliformis	0.11	0.07	0.56	0.15
E. scabriscula	0	0	0.91	0.05
E. sessiliflora	0.19	0.08	-0.16	0.17
E. discolor	0.09	0.05	0.73	0.08
E. densifolia	0.14	0.01	0.86	0.02

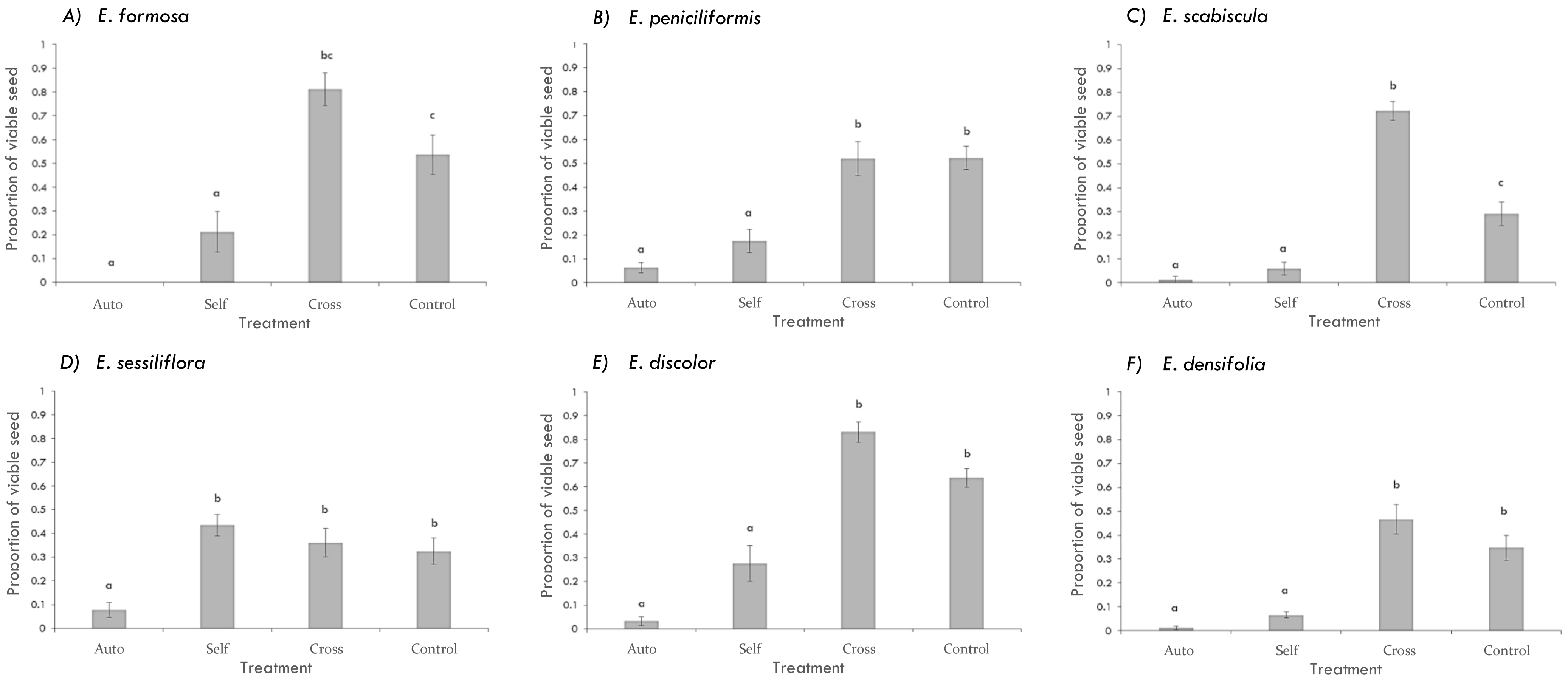

Figure 10. Seed sets per flower of six *Erica* species obtained with four different treatments 1. cross-pollination, 2. self-pollination with manual pollen transfer, 3. autonomous self-pollination, and 4. control. Lower case letters indicate significant differences between treatments at the 0.05 level. Standard Errror bars shown for each treatment. See Appendix F for full results of statistical analyses.

4 DISCUSSION

4.1 Impacts of Fragmentation

In line with other findings (Rathcke & Jules 1993; Pauw 2007; Pauw & Bond 2011), an overall decline was observed in plant species richness (both bird- and insect-pollinated species) with decreased patch size (Fig. 6). Moreover, the results obtained showed a strongly nested pattern in *Erica* species assemblage across the study area. This provides further proof that nestedness is common in fragmented habitats (Wright et al. 1998; Li et al. 2013). Patch size and isolation were statistically significant correlates of the nestedness patterns of *Erica* species across fragments and could possibly explain the distribution pattern of *Erica* species.

A closer examination of how *Erica* species richness changes with patch size revealed that the number of insect-pollinated species declined significanly more with increased fragmentation than did the bird-pollinated species number (Fig. 6). This might have been due to different responses to fragmentation across pollinator taxa (Winfree et al. 2011). Montero-Castaño & Vilà (2012) argued that whether or not vertebrate and insect pollinators provide ecosystem services across a disturbed, fragmented landscape would depend on their respective foraging distances and behaviours. Nectarivorous birds have greater energy requirements and larger foraging areas compared to most flower-visiting insects (Osborne et al. 2008; Montero-Castaño & Vilà 2012). In KwaZulu-Natal, avian pollinators were found to have high mobility and regularly cover long distances across a fragmented landscape to track available food plants (Symes et al. 2001a; Neuschulz et al. 2013). This long-distance movement of avian pollinators (but not of insect pollinators) could account for the pollination services provided to bird-pollinated *Erica* species not collapsing regardless of patch size and isolation (Wilson et al. 2009; Sandberg 2013). Because of their lower mobility than birds and in some situations the Circle principle, the movement of insect pollinators to smaller isolated fragments could be impeded (Osborne et al. 2008).

Further results obtained with *E. discolor,* one of the most common *Erica* species found in the study areas (Smuts 2012), substantiated the above argument that bird-pollinated species might be less vulnerable than insect-pollinated species to the loss of pollinators (Winfree et al. 2011; Montero-Castaño & Vilà 2012). Visitation frequencies were high and did not differ across patches of varying size for this bird-pollinated species (Fig. 7). *E. discolor* has floral traits (long-tube corolla with sucrose rich nectar) that specifically target sunbird species belonging to the same functional type (Barnes et al. 1995; Johnson & Nicolson 2008; R. Turner Pers. Comm.). Relying on common fynbos generalist sunbird species with large foraging grounds (e.g. *Cinnyris chalybeus* and *Anthobaphes violacea*; Geerts 2011), populations of specialised bird-pollinated *E. discolor* (Turner Unpublished Data) are unlikely to decline due to a lack of pollinators (Bascompte et al. 2006).

Viable seed set of *E. discolor* collected in smaller patches (<100ha) proved to be significantly higher than in patches larger than 240 hectares (Fig. 9). This finding indicated that in smaller patches, pollination mutualism in this particular ornithophilous species was still occuring despite the long history of fragmentation in the area (Kraaij et al. 2011). In bigger patches, the sheer number of available nectar resources could have saturated the available pollinator pool (Johnson et al. 2012; Turner 2012). Being presented with an abundance of choice, flower visitors could have overlooked some infloresences and visited some less (Johnson et al. 2012). This could possibly explain the lower seed sets (result of lower pollen load) obtained in the bigger patches (Engel & Irwin 2003; Waites & Ågren 2004; Johnson et al. 2012).

However, disentangling different plant reproductive attributes' (e.g breeding systems and pollination system specificity) influences on the outcome of landscape alterations remains challenging (Harris & Johnson 2004). Different plant species within the same functional group could respond to similar disturbance factors differently depending on their respective life history traits (Harris & Johnson 2004). In the present study, the pollinator visitation frequencies and the viable seed set across patches for *E. discolor* (Fig. 7 & Fig. 9) did not fully corroborate the decreasing trend observed for bird-pollinated species richness with decreasing patch size (Fig. 6). However, it could have assisted in explaining the weaker relationship species richness has with patch size in bird pollinated species, when compared to insect pollinated species (Fig. 6).

4.2 Safety Nets

Autonomous Self-fertilisation

In the present study, none of the *Erica* species showed evidence of autogamy (IAS ≤0.176; Table 2; Fig. 10). This result did not corroborate the prediction that in order to adapt to higher vulnerability to reduced pollinator abundance in the fynbos (Rebelo 1987; McCall & Primack 1992; Johnson & Bond 1997; Pauw & Hawkins 2011), insect-pollinated *Erica* species' reproductive strategies (but not bird-pollinated species') would adopt autonomous selfing so as to decrease dependence on cross-pollination (Bodbyl Roels & Kelly 2011). Due to the putatively long lifespan (longevity >50 years) of members of the *Erica* genus (van Wilgen & Forsyth 1992), the populations studied in this study might not have generated sufficient generations since fragmentation for selection to have resulted in the evolution and/or adoption of different mating system. Furthermore, there was no sign of pollen limitation for the majority of the *Erica* species (except for *E. scabriscula*) studied here. The control (open flower) seed set was similar to the cross pollination treatment seed set (Fig. 10). The breeding experiment was done in the largest patches (Salt River East and Covie Main considered as 'mainland' in this study; Appendix A) for all the species except for *E. scabriscula*. A small population of available *E. scabriscula*, which was not in a large stand of fynbos, was located near the Nature's Valley Rest camp. The difference in site characteristic might have contributed to the significantly lower control seed set compared to the seed set of the cross treatment in *E. scabriscula* but not in other species (Fig. 10). Nevertheless, irrespective of this site variability, all six species did not exhibit autonomous selfing (Table 2).

Despite the small sample size of species (n = 6) used in this study, the finding suggests that *Erica* species are still reliant on pollinators' intervention for effective fertilisation. Similar conclusions were drawn by Arendse (2014) who also obtained low autonomous selfing indices (IAS) with 15 other *Erica* species. For many *Erica* species, costs associated with the shift to an autonomous self-fertilising mating system may be higher than the benefits (Elle & Hare 2002; Arendse 2014). Favouring out-crossing instead of selfing could moreover increase seed set through higher pollen quality, and through improved seed germination and seedling survivorship (Elle & Hare 2002). Thus, independently of its association with strong pollen limitation, the benefits of self-incompatibility might outweigh the costs of reduced pollen receipt (Knight et al. 2005). However, this theory has never been tested further in the wild for *Erica* species with respect to the eventual fitness of developed plants (or germinating seeds) that originated from either selfing or outcrossing treatments (Turner 2012).

Adoption of Geitonogamy

The breeding system experiment also reported no significant difference in seed set between geitonogamy and xenogamy in one of the *Erica* species studied, *E. sessiliflora* (Fig. 10D). The ISI value further confirmed genetic self-compatibility in *E. sessiliflora* (Table 2 ; Zapata & Arroyo 1978; Steenhuisen & Johnson 2012). This particular bird-pollinated species is the only known serotinous *Erica* species (Bond & van Wilgen 2012). It is an obligate seeder which retains its' seeds in canopy stored 'fruiting bodies' until after a fire (Marais 2012). Only in the post-fire environment will seeds of non-sprouting serotinous species be dispersed in a single pulse, taking advantage of the nutrient flush in the soil, light and space for successful seedling recruitment (van Wilgen & Forsyth 1992; Pausas & Keeley 2014). Given *E. sessiliflora*'s unique life history trait (i.e. serotiny) and its strong fire dependence, it might have exploited or even favoured traits associated with pre-adaptation for self-compatibility as a bet-hedging strategy (Bond & van Wilgen 2012; Roberts et al. 2014). Therefore, increasing seed production and reducing the reproductive risks associated with low pollinator abundance and genetically dissimilar conspecific plants abundance in the post fire period (Vaughton 1995; Geerts 2011; Roberts et al. 2014).

The similar viable seed set obtained with the self- and cross-treatment in the present study (Fig. 10D) may also imply a lack of preference for 'self' or 'cross' pollen in *E. sessiliflora*. This finding may be a consequence of *E. sessiliflora* populations having a history of inbreeding that led to purging of genetic load (Vaughton 1995; Lienert 2004). Moreover, in similar fire-dependent species like the Proteaceous shrub *Grevillea macleayana*, post-germination selection in favour of outcrossed seedlings may compensate for the initial lack of parental mate choice if resources are limited when it comes to germination (Briggs & Leigh 1996; England et al. 2003; Roberts et al. 2014). Further studies looking at genotype frequencies within seedling cohort would be needed to determine if post-germination selection occurs in *E. sessiliflora*.

4.3 Study Limitations & Future Directions

A potential limitation that could have affected the results obtained in this study was the difficultly of moving through dense stands of old-growth fynbos vegetation. Due to the physical nature of the patch, it was not possible to follow a pre-set randomised transect or quadrat (e.g. line transect, sampling grid or nested plot) for the preliminary or *Erica* species patch fragmentation surveys. A ‘wandering’ transect, whereby an available relatively accessible survey route was followed and recorded was conducted. Despite efforts made to also cover densely packed vegetation, this method could have been biased towards finding *Erica* species that were closer to more opened paths, but not those present in impenetrable fynbos. Moreover, due to time and labour constraints, the sampling effort in patches bigger than 50 hectares could not be as extensive as in smaller patches (distance covered in smaller patches relative to patch size was bigger than in bigger patches relative to patch size).

In addition, a larger species sample size for the breeding system experiment could have provided a more accurate representation of actual distribution of several breeding systems within bird- and insect-pollinated *Erica* species. This was not possible in this study due to time constraints. Further similar breeding system investigation in a range of patches with various sizes, isolation and disturbance frequencies would be required to determine if habitat fragmentation itself could cause an evolutionary increase in autonomous selfing abilities. Within specific members of the *Erica* genus, exhibiting a variety of reproductive strategies and different degrees of specialised mutualisms with pollinators, the possible loss of pollinators through habitat fragmentation is expected to have discrete influences depending on different plant species (Bond 1994). Contrary to the species examined in this study, looking at putative highly specialised species may reveal more cases of facultative autonomous selfers (Goodwillie et al. 2005; Turner 2012). Also, together with plant and pollinator density data, this may yield greater insights into the capabilities of plant populations with different reproductive strategies to buffer the impacts of habitat fragmentation.

Lastly, the proportion of viable seed set per fruit in this study, could only be visually evaluated using the physical appearance of presumed viable seeds (plum and large seeds) as a viability measure. Additional germination experiments would have been useful to confirm if these characteristics were accurate proxies for viability (Arendse 2014). However, germination experiments could not be done within the scope of this thesis because of time constraints.

5 CONCLUSION

Conservation Implications of Present Study

The results of this study highlight the resilience of bird-pollinated *Erica* species to the impacts of fragmentation. Conserving continuous stands of fynbos for the preservation of higher insect-pollinated *Erica* species richness was also suggested here. The habitat of many plant and pollinator species is being lost at an alarming rate in the lowland fynbos (Cowling et al. 2009; Kraaij et al. 2011). Higher species diversity has been documented to provide a buffer against the collapse of ecological interactions (Valiente-Banuet et al. 2014). A severe loss of species interactions, due to the current elevated rates of anthropogenic disturbance and landscape modifications, could eventually lead to the deterioration of ecosystem functionality (Boyer & Jetz 2014). More than a fourth of all *Erica* species in the CFR (>181 of the approx. 680 extant species) are currently threatened (Rebelo 1992; Turner 2012). The extinction of such species could have dire cascading consequences not only in their immediate surroundings but also on a broader scale, through the loss of interactions with their respective mutualistic partners (Valiente-Banuet et al. 2014). For example, many bird-pollinated *Erica* species have developed specialised pollination systems that rely heavily on birds like the Fynbos endemic orange-breasted sunbird (Geerts 2011; Geerts et al. 2012). A decline in the population of one partner in the mutualistic relationship is bound to also cause a decrease in the counterpart's population given that the loss of species richness in an area eliminates the choice for alternative ecosystem service providers (e.g. redundant species; Memmott et al. 2004; Boyer & Jetz 2014; Ceccon & Varassin 2014). Here, small fragments of remnant native vegetation proved to be important foraging grounds for avian nectarivores. Pollinator visitation frequencies were high across all patches of *E. discolor* regardless of patch size. Answers as to whether small fragmented vegetation remnants are still important foraging grounds for pollinators in the fynbos (or are they ignored by pollinators), could help to determine more accurately if these fragments are worthy of conservation efforts when labour and financial resources are limited.

Understanding the factors behind biodiversity loss is crucial to accurately advise conservation practitioners (Valiente-Banuet et al. 2014). In order to devise the best possible strategies for the restoration and management of fynbos populations, it is essential that conservation ecologists take into consideration aspects of plant-pollinator interactions (Kearns et al. 1998; Ceccon & Varassin 2014). In the context of the Garden Route National Park, an inclusive management approach, and stewardship agreements with owners and managers of remnant fynbos patches and surrounding matrix of land use types (e.g. private land, commercial forestry and conservancies), are useful to ensure that 'healthy' and functional fynbos populations are maintained (Kraaij 2012; SANParks 2014). The results from the present study provides evidence to support this approach to integrated landscape level conservation of fynbos habitat. In particular the results indicate that even small patches of fynbos are important especially in terms of providing nectar resources to nomadic nectarivorous sunbirds (Symes et al. 2001a). Creating interconnected corridors between 'mainlands' and 'islands' of fynbos vegetation could also help maintain structural, compositional and functional biodiversity in smaller fragments (Samways et al. 2010).

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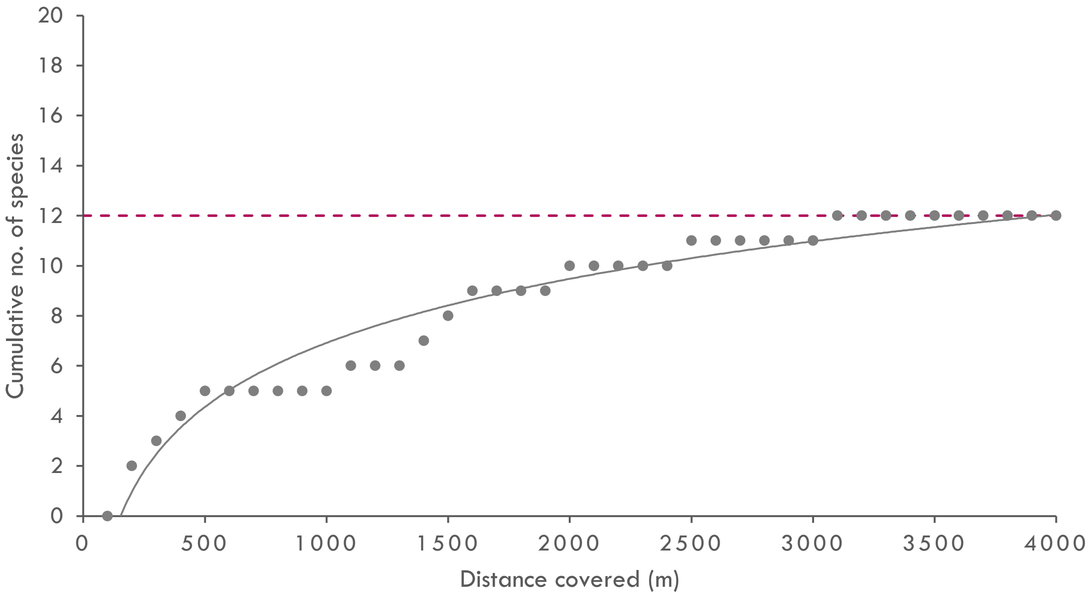
APPENDICES

Appendix A. Patch features and fragmentation matrix of 20 study sites located between the Keurbooms River and the Bloukrans River, Western Cape, South Africa. The distance to the nearest large patch for the three largest natural ‘mainland’ sites is indicated as 0.

Patch no.	Patch name	Size (ha)	Distance to nearest large patch (m)	Location coordinates		Fragmentation matrix
				Latitude	Longitude	
1	Patch 1 (P1)	1.95	1120	33.94073°S	23.51887°E	Pine plantation
2	Patch 2 (P2)	3.46	1230	33.93907°S	23.52092°E	Pine plantation
3	Patch 3 (P3)	3.89	620	33.94089°S	23.52808°E	Pine plantation
4	Bolus	4.84	7680	33.93244°S	23.43249°E	Ravine with aliens; cultivated land
5	Burmeister	5.62	5790	33.98245°S	23.45389°E	Indigenous forest; cultivated land
6	Covie East 1 (CE1)	10.33	140	33.96671°S	23.61839°E	Indigenous forest with aliens; ravine
7	Covie East 2 (CE2)	10.67	280	33.97063°S	23.62081°E	Indigenous forest; pine plantation; Klip River
8	Patch 5 (P5)	12.41	170	33.96114°S	23.55844°E	Pine plantation; forested valley; Groot River
9	Tonkin	16.41	6700	33. 94251°S	23.43782°E	Indigenous forest; fynbos with aliens; cultivated land
10	Rosental	19.33	4550	33.95681°S	23.45969°E	Ravine with aliens; farmstead
11	Patch 6 (P6)	25.55	1520	33.95849°S	23.56806°E	Indigenous forest; pine plantation; cultivated land
12	Patch 7 (P7)	31.71	310	33.95749°S	23.57081°E	Pine plantation; ravine with aliens; Bobbejaan River
13	Keurboom	33.37	8290	33.92314°S	23.42685°E	Pine plantation; farmstead; ravine
14	Whiskey Creek	44.86	6070	33. 94530°S	23.43296°E	Indigenous forest; farmstead; ravine
15	Nature's Valley (NV)	53.34	280	33.98326°S	23.57765°E	Indigenous forest; coastline
16	Covie East 3 (CE3)	85.32	210	33.97350°S	23.62820°E	Ravine; coastline and coastal forest; Bloukrants River
17	Salt River West (SRW)	97.68	180	33.96772°S	23.50423°E	Salt River; indigenous forest; farmstead
18	Patch 4 (P4)	245.41	0	33.94097°S	23.54496°E	Indigenous forest; ravine
19	Salt River East (SRE)	282.37	0	33.97018°S	23.54665E	Salt River; Groot River; indigenous forest
20	Covie Main	336.62	0	33.97021°S	23.60530°E	Klip River; indigenous forest; coastline; farmstead

Appendix B. Fourteen *Erica* species recorded in the study area and their respective breeding and pollination system, and pollinator taxa (R. Turner Unpublished Data).

Species	Pollination specialisation	Pollinator taxa	Breeding system
E. discolor	Specialist	Bird (sunbird)	Obligate seeder in study-area Resprouter on inland mountains
E. sparsa	Generalist	Insect	Obligate seeder
E. formosa	Generalist	Insect	Obligate seeder
E. scabriscula	Generalist	Insect	Obligate seeder
E. uberifolia	Generalist	Insect	Obligate seeder
E. copiosa	Generalist	Insect	Obligate seeder
E. densifolia	Specialist	Bird (sunbird)	Obligate seeder
E. seriphiifolia	Generalist	Insect	Obligate seeder
E. caniculata	Generalist	Insect	Obligate seeder
E. glandulosa	Specialist	Bird (sunbird)	Obligate seeder
E. peniciliformis	Generalist	Insect	Obligate seeder
E. tricep	Generalist	Insect	Obligate seeder
E. sessiliflora	Specialist	Bird (sunbird)	Obligate seeder
E. Nabea	Generalist	Insect	Obligate seeder



Appendix C. Species area curve for the cumulative number of species recorded with every 100m covered during the wandering transect. The dash line represent the asymptote (maximum number of species recorded in Covie Main patch. Note: The area was measured in terms of distance covered using a wandering transect.

Appendix D. Results of linear models assessing the relationship between log of patch size (fixed effect/independent variable) and number of species present per patch (dependent variable) for *Erica* species pollinated by all taxa, insect pollinated species and bird pollinated species. Parameter estimates, Standard Error, t-values, p-values and adjusted R^2 from the models are provided.

	df	Estimate	Standard Error	t	p	Adj. R^2
All taxa						
(Intercept)	18	3.449	0.912	3.780	0.001*	-
Log patch size	18	1.071	0.261	4.108	<0.001*	0.455
Insect-pollinated						
(Intercept)	18	2.528	0.612	4.133	<0.001*	-
Log patch size	18	0.778	0.175	4.452	<0.001*	0.498
Bird-pollinated						
(Intercept)	18	0.920	0.373	2.468	0.024*	-
Log patch size	18	0.293	0.107	2.747	0.013*	0.256
Bird vs. insect-pollinated						
(Intercept)	36	0.920	0.507	1.817	0.078	-
Log patch size	36	0.293	0.145	2.022	0.051	-
taxa insect	36	1.608	0.717	2.244	0.031*	-
log patch size: taxa insect	36	0.486	0.205	2.372	0.023*	0.781

* Significantly different from intercept mean (at significance level of 0.05)

Appendix E. Presence-absence matrix for 14 *Erica* species in 20 fynbos patches, arranged in order of maximum nestedness according to BINMATNEST calculator (Rodríguez-Gironés & Santamaria 2006). Shaded square = species occurrence.

Species	Patch size																			
	5.62	4.84	10.33	33.37	16.41	44.86	1.95	3.89	3.46	31.71	53.34	25.55	10.67	85.32	97.68	12.41	245.41	19.33	282.37	336.62
E. discolor	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
E. sparsa	▨	▨	▨		▨	▨	▨		▨	▨	▨	▨	▨	▨	▨	▨			▨	▨
E. formosa				▨	▨	▨		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
E. scabriscula		▨	▨	▨	▨					▨		▨	▨	▨	▨	▨	▨	▨	▨	▨
E. uberifolia	▨			▨		▨				▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
E. copiosa		▨		▨	▨	▨	▨	▨	▨		▨			▨	▨	▨	▨	▨	▨	▨
E. densifolia							▨		▨	▨		▨	▨	▨	▨	▨	▨	▨	▨	▨
E. seriphiifolia							▨	▨	▨	▨		▨			▨		▨	▨	▨	▨
E. caniculata	▨		▨					▨				▨				▨	▨	▨	▨	
E. glandulosa											▨								▨	▨
E. peniciliformis													▨	▨				▨		▨
E. tricep																▨	▨			
E. sessiliflora																		▨		▨
E. Nabea																				▨
Total no. of species	4	4	4	5	5	5	5	5	6	7	6	8	7	8	8	9	9	10	10	12

Appendix F. Results of Dunn's test to determine the differences in the mean proportion viable seed set of six *Erica* species under four different treatments. *P*-values displayed have been corrected using the Bonferroni method.

E. formosa

	Auto	Cross	Natural
Cross	<0.001*	-	-
Natural	<0.001*	0.138	-
Self	0.416	<0.001*	0.089

E. sessiliflora

	Auto	Cross	Natural
Cross	0.005*	-	-
Natural	0.007*	1.000	-
Self	<0.001*	0.820	0.526

E. peniciliformis

	Auto	Cross	Natural
Cross	<0.001*	-	-
Natural	<0.001*	1.000	-
Self	0.249	0.004*	0.001*

E. discolor

	Auto	Cross	Natural
Cross	<0.001*	-	-
Natural	<0.001*	0.165	-
Self	0.112	<0.001*	0.020*

E. scabriscula

	Auto	Cross	Natural
Cross	<0.001*	-	-
Natural	<0.001*	0.019*	-
Self	0.174	<0.001*	0.037*

E. densifolia

	Auto	Cross	Natural
Cross	<0.001*	-	-
Natural	<0.001*	1.000	-
Self	0.064	0.001*	0.010*

* Significantly different from paired treatment (at significance level of 0.05)