Population dynamics of translocated *Frithia humilis*, an endangered sandstone endemic

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PREFACE

Translocation of plants in South Africa is still poorly studied and seldom used as a conservation tool. Thus, the translocation of *Frithia humilis* for conservation purposes can be seen as a first for South Africa. Consequently, a monitoring programme was initiated to assess the feasibility of translocation as a conservation tool for this species. This study is considered to be the second phase of the monitoring program and aims to determine the long term feasibility of translocation, since the previous study determined whether *F. humilis* could survive the translocation process and successfully reproduce at the receptor sites.

The objectives were to study the population to quantify and compare the:

(i) pollination system over time and between receptor and control sites;

(ii) fecundity over time and between receptor and control sites; and

(iii) population structure over time and between receptor and control sites.

The dissertation is divided into seven chapters. **Chapter** 1. Discusses project history, species account, aims and objectives, hypotheses and dissertation layout. **Chapter 2.** Discusses translocation challenges, factors influencing success and failure and guidelines. **Chapter 3.** Describes the study area, study sites and methodology. **Chapter 4**. The findings of observations and identification of potential pollinators are given and primary and reserve pollinators are suggested. **Chapter 5.** Fecundity of translocated populations is investigated and discussed along with habitat characteristics influencing the health of translocated populations. **Chapter 6.** Investigates and discusses population structure and health. **Chapter 7.** Concludes project findings and presents suggestions for further study.

Findings from this study contributed to the protection of a natural and translocated population from destruction due to renewed mining interest.

I would like to thank the following persons for their contributions to this study:

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ABSTRACT

Frithia humilis Burgoyne is an endangered succulent and edaphic specialist that is endemic to the Rand Highveld Grassland between the towns of Middelburg, eMalahleni (Witbank) and Bronkhorstspruit. The species is restricted to sedimentary Dwyka and Ecca sandstone rock plates (Karoo Supergroup). Underlying these rock plates are valuable coal deposits and as such coal mining has become the greatest threat to this species.

In 2008 a population of *F. humilis* was discovered at the Inyanda Coal mine (Exxaro mining group), north of eMalahleni, before mining activities commenced. *In situ* conservation was impossible due to open cast mining practices. Thus an alternative solution was required to save the population. Consequently Exxaro, in cooperation with the South African Biodiversity Institute and the Mpumalanga Tourism and Parks Agency, translocated the population to three receptor sites within the species' natural distribution range. The first receptor site's substrate consisted of the typical Ecca sandstone habitat and received the majority of plants. Two smaller groups of plants were also translocated to two different receptor sites for experimentation. The substrates of these atypical habitat types consisted of outcrops of the sedimentary Wilge River Formation (Waterberg Group) and the igneous felsite outcrops of the Rooiberg Group (Transvaal Supergroup).

Translocation is still a controversial method of conservation due to its numerous challenges and varied results. However it is useful for species preservation, population augmentation and research purposes. Furthermore, as pressure on natural habitats and endangered species increases, translocation may inevitably become a vital part of conservation methodology. Since no similar conservation effort has been made for a succulent plant species in South Africa, a monitoring program was initiated in 2010 to establish whether translocation is a feasible method of conservation for *F. humilis*. Subsequently, a repeatable monitoring programme was established to gather baseline data and to monitor post-translocation progress. In addition, a population at Ezemvelo Nature Reserve also occurring on Dwyka and Ecca sandstone, was chosen as control for comparison with translocated populations. In this study, population monitoring was continued with the purpose of supplementing existing knowledge of pollinators, investigating fecundity at the various receptor sites and determining the health of the population structure for the translocated populations compared to a control population.

Observations for pollinators were made at two of the receptors sites and captured using hand nets. Insect identification was done with the help of international experts while examination for pollen was done using a stereomicroscope and scanning electron microscope.

The fecundity of translocated populations was established based on count data which was analysed using a linear mixed model. The number of flowers, fruits, seedling, sub-adults and adults were compared to a control population and between populations. Habitat variables influencing the health of translocated plants were also investigated using non-metric multidimensional scaling and principal component analysis.

Population structure at various receptor sites was analysed in terms of size-class distribution using linear regression analysis, Permutation Index, Simpson's index of dominance and quotient analysis. Results were compared to a control population and between translocation populations.

Initial qualitative results revealed that pollinators were generalists consisting of bees and flies (Apidae, Megachilidae (Hymenoptera) and Bombyliidae (Diptera)). Observations for pollinators reinforced previous findings that pollination is not a limiting factor to population reproduction. Carriers of *F. humilis* pollen included *Notolomatia* sp. (Bombyliidae), *Paragus* sp. (Syrphidae), *Ammophila* sp. (Sphecidae), *Lipotriches* sp. and *Seladonea* sp. (Halictidae) and *Quartinia* sp. (Masarina). These species extended the list of visitors to *F. humilis* flowers and support the standing Mellitophilous pollination syndrome, while also presenting the possibility of an alternative syndrome. Several primary pollinators were suggested, mostly bees, based on the distribution and number of observations, while reserve pollinators were identified from several different genera. Fruit production as a percentage of adult plants indicated that pollination was more successful at receptor sites than the control population, though this may be density related. Furthermore, the high percentage of fruit production suggested that one or more of the observed insect species is likely an effective pollinator and may be confirmed from among those observed in this study.

Population analysis was based on new and previously collected data. In addition to investigating the fecundity of translocated populations, specific habitat conditions were identified which influenced the health and recruitment of plants, particularly in the typical receptor site. Reproduction was found to be stable and functional for all populations. Results in terms of numbers of seedlings, sub-adults and adults varied between translocated populations. Generally, populations showed declines for all life stages, though these were not as severe for some as for others. Habitat quality was found to have the greatest influence on population performance. Although flowering and fruiting was occurring at high percentages for the translocated populations seedling and sub-adult numbers have been declining over time. This is ascribed to lack of suitable micro-habitats for seedling establishment, and patch deterioration and competition limiting the establishment of sub-adults. These problems occurred at both a-typical and typical geologies, though less so at the latter, in patches which maintained habitat conditions similar to that of the control population.

The health of translocated populations was determined by examining size class distribution, population slope, stability and evenness, and comparing it to a control population. It was determined that some instability and variation within even the natural populations may be normal. Two of the translocated populations showed relatively healthy size class distributions, population slope, stability and evenness, while for the other two these variables did not fall within the limits set by the control population, indicating unhealthy population structures. The healthiest populations occurred on the typical *F. humilis* geologies while the unhealthiest populations occurred on a-typical geologies.

Despite past indicators supporting translocation as a feasible conservation tool, results from this study suggest that translocation is not a long term conservation solution for *F. humilis s*ince current trends suggest continued habitat and consequential population deterioration. Further studies have to be conducted on the species' habitat requirements and the selection of suitable receptor sites. Until our understanding of the habitat preferences of the species is sufficiently increased the translocation of this species is strongly discouraged in favour of *in situ* conservation.

Keywords: Plant translocation, conservation, *Frithia humilis*, endangered, succulent, population dynamics, pollination ecology, South Africa.

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CHAPTER 1

INTRODUCTION

1.1 Project history

South Africa is highly dependent on its coal resources which account for 73% of the country's primary energy generation (Jeffrey, 2005; Subramoney *et al.*, 2009). This makes it an important contributor to South Africa's electrical supply (95%) and economy. The Witbank Coalfield in Mpumalanga is over a century old and still one of the most important coalfields in South Africa, providing 50% of South Africa's coal (Hancox & Götz, 2014). Owing to the nearing depletion of these coal resources (Jeffreys, 2005), and the steady demand for coal in South Africa, the remaining coal deposits are increasingly targeted for exploitation. Many of these valuable coal deposits of the Witbank Coalfields (Cairncross, 2001) underlie the Dwyka and Ecca Groups of the Karoo Supergroup to which a threatened edaphic specialist plant species, *Frithia humilis*, is restricted (Burgoyne *et al.*, 2000b). Opencast mining is the preferred mining method since the coal deposits are relatively shallow (Cairncross, 2001). Therefore, *F. humilis* populations are increasingly coming under threat as its habitat is not simply altered but completely destroyed.

In 2009 one of the 11 remaining *F. humilis* populations was rescued from open cast mining activities and translocated from Inyanda Coal Mine (Exxaro) north of eMalahleni (Witbank), Mpumalanga, to three receptor sites. The population was found by chance on proclaimed mining land and as such was translocated as an emergency measure (Burgoyne & Hoffmann, 2011). *In situ* conservation was impossible since the mining licence had already been finalised and the area prepared for open cast mining and subsequent destruction. The mining company in question, Exxaro Mining Group, along with Mpumalanga Parks and Tourism, approached the South African National Biodiversity Institute (SANBI) to plan the translocation, select receptor sites and assist in the translocation process (Burgoyne & Hoffmann, 2011). Translocation was believed to be a feasible option since other genera of the Aizoaceae (*Aridaria*, *Drosanthemum* and *Psilocaulon*) had been successfully translocated in the Succulent Karoo (Blignaut & Milton, 2005).

SANBI and Exxaro selected three habitats deemed suitable as receptor sites for the translocation of the *F. humilis* population. The largest number of plants (3991) was translocated to a site with identical geology to that of the donor site, while 86 and 788 were respectively translocated to a-typical geologies for experimental purposes (Burgoyne & Hoffman, 2011). In 2010 a post-translocation monitoring project was initiated with funding from Exxaro and under the auspices of the North West University (Harris, 2014).

To comply with South Africa's membership of the World Conservation Union (IUCN) and the IUCN guidelines for species translocations, the following was considered during the planning and execution of the translocation process (Burgoyne & Hoffman, 2011; IUCN, 2013):

(i) Availability of basic ecological information for *F. humilis* (Burgoyne *et al.,* 2000a; Burgoyne *et al.,* 2000b; Burgoyne, 2001; Harris, 2014).

(ii) Buy-in from stakeholders that supported the translocation project and assisted in long-term population protection such as local farmers and investors, and avoidance of social or economic impacts potentially caused by such a project avoided.

(iii) Permission from relevant authorities (Mpumalanga Tourism and Parks Agency).

(iv) An action plan for the preparation of translocation sites, the translocation process and the post-translocation monitoring project - the latter of which is continued in this study.

1.2 Species account

Frithia humilis Burgoyne, known in the vernacular as Fairy Elephant's Feet, is a succulent 'window' plant belonging to the family Mesembryanthemaceae (Aizoaceae), commonly referred to as "mesembs" or "vygies" (Burgoyne *et al.,* 2000a, Burgoyne *et al.,* 2000b). It is one of only two species belonging to the genus *Frithia* N.E. Br., the other being *F. pulchra* N.E.Br. In 1968 the name *Frithia pulchra* var. *minor* was published in the Dutch journal *Succulenta* (De Boer, 1968). However, this name was considered invalid since type material was not mentioned (Burgoyne, 2001). In 2000 it was described as a distinct species by Burgoyne *et al.* (2000b). The Latin derived name, *humilis*, meaning 'smaller than others of its kind', refers to its significantly smaller size compared to *F. pulchra*.

Frithia is one of only a few mesemb genera to be found in South Africa's summer rainfall region. The vast majority of the mesemb family occurs in the winter rainfall region of the Succulent Karoo Biome of southern Africa (Chesselet *et al.*, 2002; Ihlenfeldt, 1994). *Frithia* meta-populations can be considered allopatric since they can be found in two distinct regions separated by about 150 km. *F. pulchra* can be found in the Magaliesberg mountain range between Rustenberg and Hartbeespoort Dam in the North-West Province and Gauteng. *F. humilis*, on the other hand, occurs in the Rand Highveld Grassland between Middelburg and Emalahleni (Witbank) in Mpumalanga and Bronkhorstspruit in Gauteng (Burgoyne *et al.,* 2000b, Burgoyne, 2001).

The extent of occurrence also differs greatly between the two species. *F. pulchra* occurs in an area of 285 km^2 and *F. humilis* in an area of 2987 km^2. Despite this larger extent of occurrence, *F. humilis* only has an area of occupancy of 2 ha, while *F. pulchra* occupies an area of 13.25 ha (Burgoyne, 2001). Due to the small area of occupancy of *F. humilis* relative to that of *F. pulchra*, the meta-population of the former is smaller, despite localised high population numbers in preferred habitat.

Both species prefer coarse, shallow and well drained sedimentary soils as their growth medium (Burgoyne *et al.*, 2000b). *F. pulchra* grows in quartzites of the Magaliesberg Formation of the Transvaal Sequence. Plants anchor themselves in the cracks of rocky outcrops, but are not limited to this micro-habitat as they can also be found in course gravel away from outcrops (Burgoyne, 2001). *F. humilis* prefers shallow soils derived from the Dwyka and Ecca Groups of the Karoo Supergroup. It only grows on the edges of flat rock plates where such soil collects after rainfall and provides the crucial micro-habitat for seed germination and establishment. The medium has a high content of organic matter, occasionally lending it peat-like properties. This most likely keeps the medium somewhat cooler than that of *F. pulchra*, meaning plants are protected from extreme heat while soil moisture is retained for longer (Burgoyne *et al.,* 2000b, Burgoyne, 2001, Burgoyne & Hoffman, 2011).

Both species occur at altitudes between 1360 and 1620 m above sea level and receive rainfall varying between 700 and 800 mm per year (Burgoyne *et al.*, 2000b; Ihlenfeldt, 1994). Summers are warm while winters are dry and cold with occasional frost.

In response to the challenges of its xeric habitat, *F. humilis* is seasonal and responsive to moisture. The roots are more fibrous than those of *F. pulchra* which may help to insulate the plant against the summer heat. The leaves of the plant are succulent and contractile (Burgoyne *et al.*, 2000b, Burgoyne, 2001). The stem is significantly reduced.

Leaves of *F. humilis* are arranged spirally, are cylindrical in shape and have flattened windowed tips. The leaves are about 15 mm long and only protrude above the ground when turgid. Leaf colour is green when turgid turning brown or purple when dehydrated. The leaf epidermis consists of distinctly arranged rows of idioblasts which shrink lengthwise and allow the leaf to contract up to one third of its length during times of drought (Burgoyne, 2001). The windowed tips therefore allow light to penetrate the leaf surface and travel through the window cells to the chlorenchyma tissue. This occurs even when the leaf has contracted entirely beneath the soil surface to reduce heat exposure and water loss (Bennet *et al.*, 1988; Egbert *et al.*, 2008; Simpson & Moore, 1984).

Flowers appear from spring to summer on a short stalk, or more usually, no stalk at all. Flowers open at mid-morning and remain open until mid-afternoon (Burgoyne *et al.*, 2000b; Smith *et al.*, 1998). Very little research has been done on the pollination biology of *F. humilis*. The previous study by Harris *et al.* (2016) established that *F. humilis* is pollinated by generalist pollinators belonging to the orders Lepidoptera, Hymenoptera and Diptera. Fruits are barrel shaped and hydrochastic, opening and closing repeatedly on wetting and drying. Capsules tend to be somewhat fragile, depending on environmental condition, and disintegrate soon after ripening.

F. humilis is assessed as endangered (EN B1, 2b, c, d) (Burgoyne *et al.*, 2000a; Raimondo *et al.*, 2009). *F. humilis* has an extent of occurrence of <3000 km^2 and an area of occupancy <20 000 m^2. Populations are under threat from overgrazing and livestock trampling, invasion by alien plants and collection for the horticultural trade (Raimondo *et al.*, 2009). Mining has recently become the greatest threat as three of the 11 known sub-populations have been destroyed by mining activities (McCleland, 2014).

Figure 1.1 ***Frithia humilis*** **during the growing season with exposed, finger-like leaves (a).** ***F. humilis*** **plant retracted into the soil during prolonged dry periods in winter (b). Flowers of** ***F. humilis*** **are either white or tinged with pink (c), compared to that of** ***F. pulchra*** **which is larger and magenta coloured (d). (*****F. pulchra*** **photo: Angus, 2006).**

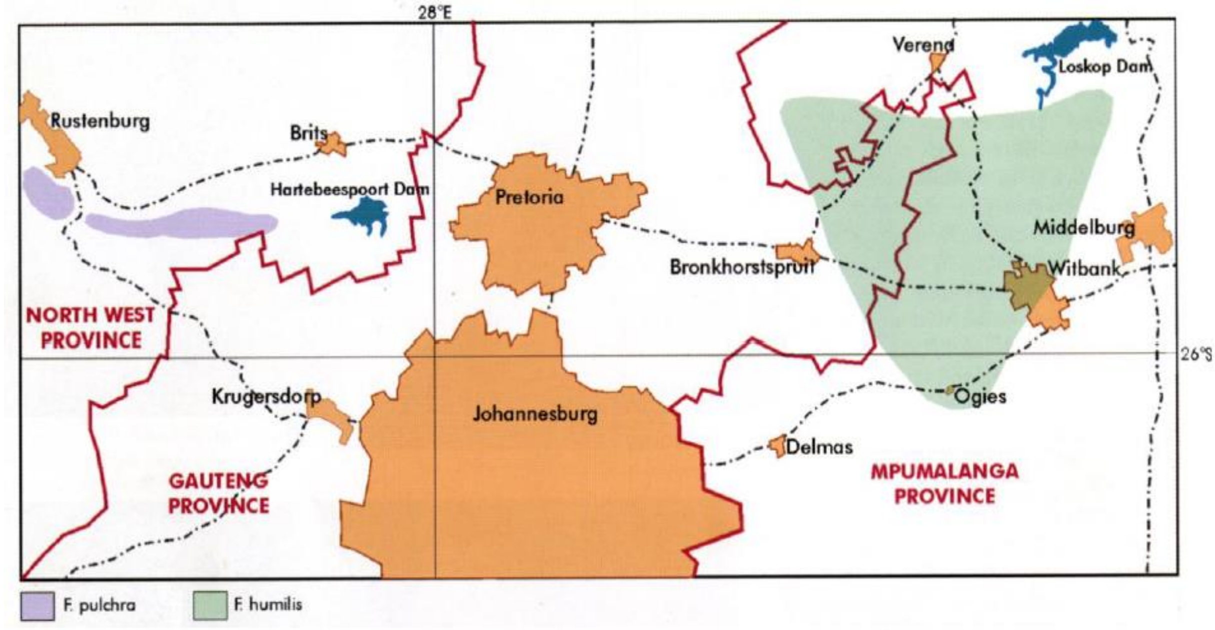

Figure 1.2 Allopatric distribution ranges of *Frithia pulchra* and *F. humilis*, both within the summer rainfall region of South Africa (Burgoyne *et al.*, 2000a).

1.3 Rationale

The remaining habitats of *F. humilis* are under continual threat due to the coal deposits underlying the species' preferred habitat. This causes a problem for *in situ* conservation or translocation efforts. The continued monitoring of this translocation project will verify whether translocation is a viable conservation method for this species in the future, as well as contribute knowledge regarding the translocation of mesembs in South Africa and translocation projects in general. The identification of alternative habitats could provide a lifeline for the species if threatened populations can be moved to habitats which are safe from mining interests, such as atypical geological habitats within protected areas. This line of inquiry is especially worthwhile since a healthy natural population of *F. humilis* is known to occur on conglomerate substrates rather than the typical Dwyka and Ecca sandstones.

About 90% of flowering plants are believed to be dependent on pollinators for reproduction (Kearns & Inouye, 1997; Menz *et al.*, 2011). Understanding the breeding system of a plant species is therefore of vital importance to any conservation program (Wilcock & Neiland, 2002). Despite this, the reproduction of very few reintroduced species is understood (Godefroid *et al.*, 2011). For Aizoaceae, even outside the conservation context, very little is known about the family's pollination biology (Peter *et al.*, 2004). For these reasons it is crucially important to understand the pollination biology of *F. humilis* to maximise the potential for success in future translocations. A previous study by Harris *et al.* (2016) concluded that flowers of *F. humilis* are pollinated by generalist pollinators belonging to the orders Lepidoptera, Hymenoptera and Diptera. However, further information is required to accurately describe the pollination system of *F. humilis* and how it may impact the translocated populations.

1.4 Aim and Objectives

Long term monitoring is a requirement to assess the success of a translocation project (Godefroid *et al.*, 2011; Maschinski *et al.*, 2004). The primary aim of this study is to continue the assessment of the translocated *F. humilis* populations at three receptor sites. The objectives were to study the population to quantify and compare the:

(i) pollination system over time and between receptor and control sites;

(ii) fecundity over time and between receptor and control sites; and

(iii) population structure over time and between receptor and control sites.

1.5 Hypotheses

F. humilis, like many other mesembs, is believed to be self-incompatible (Ihlenfeldt, 1994), thus requiring cross-pollination. Harris *et al.* (2016) reported that *F. humilis* is pollinated by generalist insects. Considering that the receptor sites are located within the natural distribution range of *F. humilis* (Burgoyne & Hoffman, 2011), the first hypothesis therefore suggests that primary pollinators will be present at the receptor sites and will be carrying *F. humilis* pollen. If this first hypothesis is supported, and considering that previous population studies have revealed an increase in the number of translocated individuals that flower (Harris *et al.*, 2014), then the second hypothesis proposes that fruit set will increase over time and so will the seedling numbers. Since recruitment success is positively correlated with fruit set, then it is expected that the reproductive success (hypothesis 1 and 2) will stabilise the population numbers at the receptor sites (Brys *et al.*, 2003; Eriksson & Ehrlén, 1992). From this the third hypothesis states that, over time, the population structures of the receptor sites will become comparable to that of the control population. If supported, then it can be accepted that the receptor site populations have become sustainable over the short term and suggesting that translocation is a viable option for the species.

1.6 Format of dissertation

This dissertation is comprised of six chapters and complies with the North-West University guidelines for a standard dissertation. All references cited in this dissertation are recorded in the reference list at the end of the dissertation. Chapters 4, 5 and 6 (results and discussion) were adapted from manuscripts that will be submitted to scientific journals. Thus, duplication of literature, methodology and results for this purpose was unavoidable in some instances within these chapters.

Chapter 2: Literature review

The literature review will discuss challenges and considerations for the application of translocation as well as current guidelines and suggested criteria to maximise the chances of translocation success. Translocation in terms of implementation and as a conservation tool along with factors associated with the success and failure of translocation projects will be discussed.

Chapter 3: Materials and methods

The overarching methods and experimental design will be reviewed in this chapter and the study areas will be described. Methodologies specific to the results and discussion chapters will only be mentioned briefly in this chapter and will be discussed in detail in the relevant chapters.

Chapter 4: Pollination biology of *Frithia humilis*

Existing knowledge of the pollination biology of *F. humilis* will be supplemented. The pollination syndrome of *F. humilis* will be inferred based on floral traits, nearest relatives and newly observed vector species. Potential primary and reserve pollinators will be suggested based on the *F. humilis* flower visitors observed in the previous study by Harris *et al.* (2016) as well as this study.

Chapter 5: Fecundity of translocated *Frithia humilis* populations

New demographic data for the translocated population was collected, added to existing data and quantified to further expand the monitoring conducted by Harris *et al.* (2014). Aspects of fecundity will be considered to investigate whether translocated *F. humilis* populations were experiencing increases in flowers, fruits, seedlings, sub-adults and adults which would indicate whether translocation is a viable conservation measure for the species. Potential reasons for the observed results will be discussed.

Chapter 6: Population dynamics of *Frithia humilis*

The size class distribution of a control population will be compared to that of the translocated populations at the receptor sites to establish the population structure of a natural *F. humilis* population. The size class distributions of translocated populations were then analysed and compared to the control population to determine the demographic health of the translocated populations.

Chapter 7: General conclusion

The current status of the *F. humilis* translocation project will be conveyed. Adapted recommendations and mitigation measures will be proposed based on the results of the latest monitoring period. Recommendations for future studies will also be presented.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In recent times human induced threats such as habitat loss, fragmentation, land use change and over exploitation have resulted in approximately 20% of the world's plant species being classified as endangered (Bontrager *et al.*, 2014; Maschinski *et al.*, 2004) while up to 91% of rare plant species, many of which are endemic and edaphic specialists, are under threat of extinction (IUCN, 2013). In the face of such dire predictions, rising demands for natural resources and continued habitat alteration, there is an urgent need to increase our knowledge of rare species and different mitigation measures required for their conservation (Heywood & Iriondo, 2003; Müller & Eriksson, 2013).

2.2 Translocation

As a consequence of the continued human impact on the environment conservationists are challenged with the problems of habitat and species loss as well as the conservation and management of the remaining natural environment and its organisms (Heywood & Iriondo, 2003). This situation is further aggravated by inadequate resources and a lack of input from the relevant authorities and the greater community (Moritz, 1999). The extent of damage and loss is such that natural recovery is rarely sufficient for re-establishing viable ecosystems or species populations. Human intervention is usually necessary in the majority of cases. While the general consensus is that the best conservation strategies will maximise the chances of reaching conservation goals there is still an on-going discussion as to how various strategies should be applied to conservation challenges (Halbur *et al.*, 2014; Maschinski *et al.*, 2004; Seddon, 2010).

While habitat restoration is an effective conservation approach, it is often insufficient for rare plant species which face different challenges such as being dispersal-limited, having short-lived seed banks or being threatened by climate change (Godefroid *et al.*, 2011). A strategy which is being increasingly used for rare species is translocation. This is the intentional movement of species from one area to another for conservation purposes (Milton *et al.*, 1999; Müller & Eriksson, 2013; Seddon, 2010). Translocation is a difficult and complicated process and as a branch of conservation biology, often termed a crisis discipline, since it is implemented in response to an extreme degree of biodiversity loss. Furthermore, translocation is often the last option available for preventing the loss of rare and endangered species and/or ensuring their recovery and conservation (Bontrager *et al.*, 2014; Heywood & Iriondo, 2003; Krauss *et al.*, 2002).

Although the goals of translocations are most often for conservation purposes, it may also be for economic or aesthetic reasons, related to anthropogenically caused habitat loss or alteration (Milton *et al.*, 1999). Examples of such translocations are reintroduction of animals to reserves in areas where they have since been extirpated, for example elephants and large carnivores, charismatic animal reintroductions such as the Arabian oryx, golden lion tamarins, peregrine falcons and vultures (Seddon *et al.*, 2007) or farming indigenous plants such as Proteas (Manders, 1989). In terms of conservation, translocations are undertaken to mitigate habitat fragmentation and interrupted dispersal mechanisms, to maintain community composition, boost meta-populations and save populations from destruction (Griffith *et al.*, 1989; Menges, 2008).

More extreme conservation suggestions include translocating climate-threatened species far beyond their natural ranges, such as re-populating North America with large African mammals for example (Donlan *et al.*, 2005) or supplementing North Atlantic fish stocks with alien species (Briggs, 2008). Such ideas are in conflict with traditional conservation goals and, as Ricciardi and Simberloff (2009a) pointed out, disregard the significance of evolutionary processes in conservation efforts. Furthermore, there are abundant data which show how introduced organisms can negatively influence an unfamiliar habitat (Ricciardii & Simberloff, 2009b). Concerns have also been raised about translocated species becoming invasive in new habitats as well as long term genetic implications in terms of inbreeding, introgression or genetic drift (Minteer & Collins, 2010).

Despite translocations remaining the last resort for many threatened organisms, the use of this approached remains heavily debated (Hewitt *et al.*, 2011; Riccaiardi & Simberloff, 2009a). The reasons for this debate are based on the goals and effectiveness of translocation (Fahselt, 2007; Riccaiardi & Simberloff, 2009b). This literature review aims to identify the challenges experienced during plant species translocation efforts in a South African context.

2.3 Definitions

Over the years terminology has changed as certain terms have gained or lost popularity. The situation is further complicated when new terms are changed, used inconsistently or to replace an obsolete or unpopular term. In response to this many authors have taken to clarifying terminologies or promoting and defending new or popular terms (Armstrong & Seddon, 2010). It has now become necessary to confirm terminologies generally accepted by the academic community and official organisations such as the International Union for Conservation of Nature (IUCN).

Since the IUCN’s 1987 Position Statement on Translocation of Living Organisms, guidelines and terminologies in later versions of this and similar documents have been updated several times (IUCN, 2013; IUCN, 1987). Initial definitions were basic and only covered concepts in the broadest sense. Translocation “…is the movement of living organisms from one area with free release in another” (IUCN, 1987) and “…requires completely removing naturally occurring mature plants from one spot and re-establishing them elsewhere” (Allen, 1994).

The latest IUCN Guidelines for Reintroductions and Other Conservation Translocations has updated the term “translocation” to “…the human-mediated movement of living organisms from one area, with release in another.” (IUCN, 2013). Due to this definition, translocation can be seen as a primary term covering various methods concerning the movement of organisms (Armstrong & Seddon, 2010; IUCN, 2013). This includes all types of movements of organisms from various sources either accidental or intentional. The motives for these translocations may be political, recreational, commercial or for conservation purposes. For this reason a more specific definition has been presented: “Conservation translocation is the intentional movement and release of a living organism where the primary objective is a conservation benefit…” (IUCN, 2013). This has been generally accepted since various authors refer to this definition or come to the same point (Armstrong & Seddon, 2008; Jusaitis, 2005; Kraus *et al.*, 2002; Menges, 2008; Pérez *et al.*, 2012).

Confusion usually arises from the inconsistent use of the terminologies considered under the term “translocation”, for this reason clarification of the subcategories is required:

2.3.1 Species introduction

Numerous authors have referred to this definition of introduction found in various IUCN publications (Moritz, 1999; Seddon, 2010). The latest definition for introduction, according to the IUCN (2013), states “(Conservation) introduction is the intentional movement and release of an organism outside its indigenous range.

Two further definitions may be categorised introduction, namely “assisted colonisation” and “ecological replacement”. Assisted colonisation has synonyms such as assisted migration or managed relocation (Minteer & Collins, 2010). However, as Seddon (2010) pointed out, the term migration implies a back and forth movement of organisms which an introduction is definitely not. Managed relocation also seems incorrect since relocation and translocation are synonymous and any form thereof is naturally managed.

Assisted colonisation is a conservation strategy mostly suggested for organisms which face habitat alteration through climate change. This takes the form of translocations outside an organism's natural range or contiguous range extensions. The only justification for assisted colonisation provided by the IUCN guidelines is "…where protection from current or likely future threats in current range is deemed less feasible than at alternative sites." (IUCN, 2013; Moritz, 1999). This justification has been provided to discourage poorly conceived translocation attempts and suggestions which having the past been made such as assisted colonisation for intercontinental and interoceanic movements. Such translocations are strongly discouraged for their generally undeterminable consequences for the introduced species and ecosystems of the receptor sites (Ricciardi & Simberloff, 2009a; Ricciardi & Simberloff 2009b).

When reintroduction is no longer possible due to the extinction of a species which performed an important ecological function then ecological replacements are made. "Ecological replacement is the intentional movement and release of an organism outside its indigenous range to perform a specific ecological function." (IUCN, 2013). In such cases, a substitute species is introduced to replace the lost function (Atkinson, 2001; IUCN, 2013). While using a functionally equivalent species is possible, a species or subspecies closely related to the species lost is most preferable (Seddon, 2010) to minimise the risk of unintended consequences.

2.3.2 Reintroduction

"…is the intentional movement and release of an organism inside its indigenous range from which it has disappeared." (IUCN, 2013). The primary goal of reintroductions is to restore self-sufficient populations of a species within its historical range. (Guerrant & Kaye, 2007; Godefroid *et al.*, 2011; IUCN, 2013). Information from pre-historic ranges is increasingly providing valuable information for restoration targets. At present the implied assumption is that if extirpation occurred within a certain range in a relatively recent timeframe then reintroductions are performed within those ranges, since they may be recent acquisitions and thus to some extent act as a precaution against drastic habitat change (Seddon, 2010).

2.3.3 Reinforcement

"…is the intentional movement and release of an organism into an existing population of conspecifics." (IUCN, 2013). The aim of this type of translocation in terms of plants is to enhance population growth and genetic diversity or to overcome dispersal barriers experienced by existing populations (Moritz, 1999; Seddon, 2010). When it comes to reinforcement using foreign stock there are significant genetic consequences such as inbreeding and outbreeding to consider.

Translocation will be used as a general term in this project for the purpose of continuity since it was used to the same effect in the previous study on *F. humilis* (Harris, 2014), since the same definition is also supported by Godefroid *et al.* (2011) and because this conservation effort does not fall within one of the abovementioned categories. The purpose of the *Frithia* population's relocation was to avoid the loss of a population of *F. humilis*, conserve the species and to test the feasibility of future conservation on typical and a-typical habitats while still within its area of occurrence.

2.4 Success and failures of translocations

Since so many translocations end in failure or only partial success (Godefroid *et al.*, 2011), it is useful to study the factors of success and failure in order to learn from them. Translocation shares many of its goals and methodologies with reintroduction, the only significant difference being source material. Reintroductions use material sourced from seed banks and botanical gardens while translocations use existing material sourced from within the species range.

Before translocation failures can be addressed an understanding of success has to be established. It is of course important to realise that the lowest expectations would provide the highest success rate. Therefore, if translocated organisms are supposed to replicate their natural counterparts, high standards have to be maintained, no matter how the success criteria differ between translocation projects (Bullock, 1998; Fahselt, 2007). Only when a translocated population is able to function like a natural population in every respect can the translocation process be deemed truly successful.

Translocation success can be evaluated according to their biological purposes and project purposes. Biological purposes are concerned with conservation, augmentation or establishment of a new population. Project purposes are concerned with the ways in which the biological purposes are pursued and achieved. The best way to achieve both these purposes is to perform translocations as scientific experiments with specific hypotheses that need to be tested (Gordon, 1996; Guerrant & Kaye, 2007; Jusaitis, 2005; Menges, 2008). In most cases translocations have to meet conservation and experimentation goals, thereby automatically including both purposes (Godefroid *et al.*, 2011). Certainly, translocations would achieve success sooner if research focused on answering the necessary questions in terms of species recovery and ecosystem restoration, rather than providing descriptive accounts (Armstrong & Seddon, 2008).

The most basic criteria for success, and certainly the first questions that come to mind, are whether a population survives and whether it reproduces (Godefroid *et al.*, 2011). While this is true, the monitoring of more specific criteria is necessary for an accurate estimate of success

(Menges, 2008). Many scientists refer to the methods suggested by Pavlik (1996) for measuring the success of a translocation project.

Success can be measured in terms of abundance, extent, resilience and persistence. The first two goals may occur over a shorter time span of one to ten years, while the last two can only be established over a period of several decades (Pavlik, 1996). Most assessments in terms of the first two goals focus on survival, followed by growth and fecundity (Menges, 2008). Resilience refers to the ability to survive serious environmental disturbances. A species may have higher resilience based on its genetic diversity, seed or vegetative dormancy and ability to resprout after fires. Persistence is a combination of the three previous measures, indicated by a population's ability to sustain itself and function within the ecological community (Guerrant & Kaye, 2007). Recruitment is also a very important point of focus and the ultimate measure of success, but is rarely included in translocation studies (Godefroid *et al.*, 2011).

Armstrong and Seddon (2008) suggested that establishment also be considered an important factor in translocations. Persistence implies that a population increases only in density in a geographical area rather than increasing its range. This differentiation is suggested since even though populations may persist at a certain location, the conditions that enable persistence do not necessarily enable establishment.

Very few studies concerning the success of translocations in general have been made. Most reports of the success or failure of a project are included in the study of only a single species or of several species within a study (Drayton & Primack, 2000; Guerrant & Kaye, 2007; Jusaitis, 2005). Furthermore, there is a lack of documentation for translocation attempts that end in failure. Successful reports are mostly published due to a bias towards positive results and a possible disinterest in negative results (Fahselt, 2007; Godefroid & Vanderborght, 2011). However, failed translocation attempts represent valuable learning opportunities for future translocation projects (Menges, 2008). Plenty of useful information is also held back in internal reports and difficult-to-access grey literature (Hodder & Bullock, 1997). For this reason Godefroid and Vanderborght (2011) amongst others have suggested an internet database enabling access to all aspects of various translocation projects. Several smaller databases have already been compiled for this purpose, however, these are small, local and may be out of date.

In an attempt to characterise reintroduction success rates Godefroid *et al.* (2011) collected information on 249 reintroduction attempts from questionnaire surveys as well as from published literature. Their analysis indicated that the rates of survival, flowering and fruiting were mostly quite low. However, a lack of data for seed production and recruitment prohibited a more comprehensive assessment of population viability. Despite this, success rates reported in the literature were significantly higher than reported by respondents. A similar disparity between literature and unpublished records was noted by Pérez *et al.* (2012), who reported that three years was an insufficient period over which to gauge long term success. Similarly Guerrant and Kaye (2007) felt that five years was insufficient to make long term predictions on several of their own reintroduction projects. This was partially based on the fact that while one species survived for ten years another went extinct in the wild for a second time after 15 years of survival.

Godefroid *et al.* (2011) established that the reasons for failure were mostly unknown, due to unsuitable habitat, predation or degraded habitat, in other words monitoring and environmental factors. Such problems have their origin in the poor understanding of the biology and ecology of the species in question (Scade *et al.*, 2006). These trends indicate that translocation of species is in general not a successful conservation strategy based on current procedures.

Guerrant and Kaye (2007) point out that biological failure is easier to recognise than success. While this is true, success is possible, or at the very least the chances thereof can be maximised. Godefroid *et al.* (2011) reported that the source of materials, site preparation and site protection were important factors in various reintroductions. While these are important factors to consider, many other factors which may determine the success of similar projects have to be considered as well.

2.5 Factors influencing successful translocation

Success and failure are two sides of the same coin and depend on how the factors affecting translocations are handled. The factors that may have an influence on the success of a translocation can be divided into biological and methodological components (Guerrand & Kaye, 2007). Biological factors include the type of source material, number and location of material source populations and the characteristics of the translocation site. Methodological factors include the procedures for preparation, handling and planting of propagules, translocation site preparation and modification and post-translocation care (Fahselt, 2007; Guerrand & Kaye, 2007). Not all factors are necessarily of consequence for every translocation project, but most have to be considered since they may become important later on or because they overlap with other factors.

2.5.1 Source material: Seeds versus plants and single versus multiple sources

Source material for translocation can be in the form of seeds or plants from existing populations, from plants cultivated *ex situ* for reintroductions or from seed banks (Guerrant & Kaye, 2007). Various studies have shown that either seeds or plants may be suitable depending on the biology of the species in question and the techniques used for the translocation.

In most cases seedlings or adult plants have been reported to establish with greater success than seeds. This is ascribed to seed germination, establishment and survival often being the weakest links in a plants' lifecycle (Cogoni *et al.*, 2013; Drayton & Primack, 2000; Fahselt, 2007; Jusaitis, 2005; Jusaitis *et al.*, 2004; Menges, 2008). For this reason, many practitioners prefer to avoid using seeds, favouring plants for initial translocations. However, the study by Godefroid *et al.* (2011) showed that the survival of seedlings, when compared to seed, was only significantly higher in the first year after translocation. The reasons why seedlings fail are often ascribed to controllable threats, for example in South Australia it was reported that for 14 endangered plant species the greatest threats were herbivory at 86% and competition with weeds at 71% (Jusaitis, 2005).

When possible, the use of seed may in fact provide numerous advantages. To begin with, large numbers of seeds may be collected. This is especially useful for species that produce many seeds, even if establishment is low (Guerrant & Kaye, 2007). Seeds are also useful when the biology of the species in question is not understood well enough to allow translocation of adults or when translocating any remaining adults is too great a risk (Drayton & Primack, 2000). The use of seeds is also generally cheaper and less time consuming than *ex situ* cultivation of cuttings (Menges, 2008). At suitable *in situ* sites it is possible that seeds which do germinate and survive have a better chance of long-term success, since they are selected by the micro-environment. Seeds can also be distributed soon after collection, meaning new plants are better able to grow and assimilate the timing of a natural population unlike a population which has been cultivated under different *ex situ* conditions and which may be on a different time schedule. Furthermore, using seeds may reduce the risk of introducing pathogens to translocation sites (Milton *et al.*, 1999).

When there are not sufficient seeds, direct sowing may be wasteful. Consequently, propagation programs may be more effective since the most can be made of available material and new plants can be cared for more effectively than *in situ* seedlings (Guerrant & Kaye, 2007). Seedlings are generally more effective than seeds, and mature plants are generally more successful than younger plants (Menges, 2008). In addition to this, Godefroid *et al.* (2011) showed that bare root plants were more successful than plants planted with potting soil. This may be due to the differences between the characteristics of potting soil and soil from the translocation sites. Furthermore, bare root plantings also reduce the risk of introducing foreign soil fungi or other pathogens which may negatively affect the translocated plants (Milton *et al.*, 1999). Despite these advantages, transplanting may be a stressful event for the plants, exposing them to disease and herbivory (Allen, 1994). Moreover, unforeseen environmental factors or adverse weather conditions may severely affect the survival rate of transplants (Drayton & Primack, 2000). Poor horticultural practices may also have adverse effects if plants aren't hardened-off before translocation, or if weak individuals are transplanted that only managed to survive in cultivation (Fahselt, 1988).

From this it is clear that seeds have the advantage of numbers and the ability for waiting out adverse conditions. Even though survival rates are lower, newly emerged plants may be stronger and better adapted to the new environment. Mature plants on the other hand, have the advantage of establishing populations and reproducing quicker than seeds but are initially more susceptible to environmental pressures if they have not established in time.

Linked to the question of seeds or plants is the question of how many propagules are necessary to establish a viable population. While no consistent guidelines have been established for a minimum viable population, it is widely acknowledged that the smaller the population the smaller the chances of success (Armstrong & Seddon, 2008; Shaffer, 1981).

A minimum viable population is the number of individuals it would take to replicate the processes of a natural population and persist despite various pressures (Shaffer, 1981). These pressures may be demographic stochasticity related to survival and reproduction of a limited number of individuals, environmental stochasticity related to competition, herbivory, parasitism and disease, natural catastrophes such as fire, drought and flooding and gene stochasticity resulting from inbreeding, genetic drift and founder effects (Shaffer, 1981). Based on these factors it is difficult to establish what a viable minimum population is (Montalvo *et al.*, 1997), however it is possible to establish a general idea using experiments, biogeographic patterns, theoretical models, simulation models or genetic considerations. Whatever the minimum viable population may be for a given species it is clear that it should be large enough to survive the different disturbances within its context.

Whichever method is used, demographic and genetic theories both predict that the persistence time of a population increases with its initial size (Robert *et al.*, 2007), though this is not always the case, as genetic diversity cannot be guaranteed in population numbers (Fahselt, 2007). If too few plants are translocated then insufficient diversity will be retained from the donor site and genetic complications may occur (Kraus *et al.*, 2002). In populations of more than a 1000 individuals, genetic change may theoretically be the result of natural selection and gene flow (Montalvo *et al.*, 1997). For example, in the case of *Abronia umbellata* subsp. *brevifolia,* variations in DNA sequence repeats suggested that more than a 1000 plants would be necessary to conserve 90% of the diversity in a natural population (McGlaughlin *et al.*, 2002).

Despite recommendations for high numbers of transplants (between 500 and 5000) Godefroid *et al.* (2011) found that around 43% of the reintroductions in their study used fewer than 100 individuals. The use of fewer numbers are understandable since reintroductions can be expensive and labour intensive, or include species which produce insufficient propagules to allow for larger reintroductions. It could also be that conservationists do not wish to impoverish a donor population by taking too many propagules (Guerrand & Kaye, 2007). In Oregon such a case occurred when a population of *Abronia* was heavily harvested for large-scale seed reintroduction and eventually caused the decline of the donor population. Consequently, one of the reintroduced populations became the new donor population, having captured the entire genetic diversity of the source.

This raises the next consideration of whether to use single or multiple sources. In terms of the *Abronia* only one population was fit enough to act as a donor. In cases such as this where sources are limited the reasons for a single source are practical. The reasons may also be biological, since the use of multiple sources may cause harm to the translocated population by causing outbreeding (Guerrant & Kaye, 2007). However, the use of multiple sources for translocation material is considered favourable and has shown to increase reintroduction success (Cogoni *et al.*, 2013; Godefroid *et al.*, 2011; Guerrant & Kaye, 2007), and may even be necessary if a single population is too small or does not contain enough genetic diversity to sustain a new population. Multiple sources also hold the benefit of containing individuals from one source that may be more adapted to the new environment than individuals from a single source, thereby improving the chances of survival (Montavalo *et al.*, 1997).

2.5.2 Stochasticity: environmental, demographic and genetic

Stochasticity refers to the random occurrence of events. Population dynamics, as well as the persistence of a population, are generally believed to be influenced by three main forms of stochasticity, namely environmental, demographic and genetic (Shaffer, 1981). Disturbance is another sort of stochastic event which is the destruction of biomass, either due to natural or human causes. This form of stochasticity is usually brief and in some cases can be linked to environmental stochasticity. Deterministic events also fall under environmental stochasticity and may also have influences which bring about unavoidable change, such as climate shift, deforestation, pollution or natural or human caused catastrophes. These various factors are important areas of study for ecological and conservation scientists since sessile organisms are limited in their defences against these events (Silvertown & Charleswood, 2001). Thus, when attempting to conserve a species using a controversial method such as translocation, it is important to consider all the factors which may influence the success of the project and the survival of the population. These include factors that may take effect suddenly, in the short term, or factors which may have a gradual effect and only manifest in the long term, years after the translocation.

Whether a population is naturally small or small due translocation, the stochastic effects are often similar (Robert *et al.*, 2007). However, Given (1994) warned against generalisations, even though stochastic effects often seem similar, and emphasised the importance of applying a case specific approach to every conservation project. To adequately identify the stochastic effects and their influences on a population, sufficient empirical data has to be collected and combined with simulation models (Schemske *et al.*, 1994). Similarly, Fréville *et al.* (2004) stated that only long term study will reveal the influence of intrinsic (genetics, mating, dispersal etc.) and extrinsic (climate, competition etc.) factors on population dynamics.

2.5.2.1 Environmental stochasticity

Environmental stochasticity refers to changes in environmental conditions which may affect a population. These are generally continuous small to moderate disturbances, fluctuating from year to year, that affect the various life stages of plants within a population (Lande, 1993; Reed & Hobbs, 2004; Silvertown & Charleswood, 2001). Closely related to these are natural catastrophes such as droughts, fires or floods which occur as greater disturbances and at unusual times compared to a natural drought period, fire regime or flood period. In addition to this, environmental stochasticity may also refer to the presence and interactions with other organisms such as predators, competitors, diseases and parasites (Shaffer, 1981). Such events can affect the populations of an entire region, no matter their size, and especially those in ephemeral habitats, no matter their demographic characteristics (Husband & Barret, 1996).

Even though catastrophic events influence populations of all sizes, small populations are most at risk of extinction for a variety of reasons (Fischer & Matthies, 1998; Husband & Barret, 1996). Fragmentation is one such reason which makes subpopulations more vulnerable to stochasticity. Another reason is catastrophic events or deterministic processes which in turn can bring about fragmentation. A species of plant which effectively shows the impacts of environmental and catastrophic stochasticity is Furbish's Lousewart, *Pedicularis furbishiae*, which is distribution limited and subject to significant spatial and temporal disturbances of its riverside environment due to ice scour and erosion events, such as bank slumps. However, research has determined that these disturbances may act to the advantage, as well as disadvantage, of the population dynamics of the species since smaller riverbanks which do not experience disturbances have thicker vegetation cover and no individuals of *P. furbischiae* or other species found in ice disturbance zones (Gawler *et al.*, 1987; Menges, 1990). The importance of environmental stochasticity can be further proven by models produced by Menges (1992), which showed that environmental stochasticity is generally more important to the persistence of small and medium populations than is demographic stochasticity.

It is commonly believed that environmental stochasticity eventually leads to extinction. However, it might be the case with more species than originally thought that environmental stochasticity is a necessary occurrence for species survival and the coexistence of strong competitors. *P. furbshiae* is one of many species which serve as examples of plants the require clearing of space for recruitment (Grubb, 1988). Apart from making use of environmental disturbances, the theoretical mechanism for surviving environmental stochasticity is the capacity for storing reproductive potential between generations, the storage effect, thereby surviving unfavourable years or stochastic events and suddenly increasing in favourable years (Higgins *et al.*, 2000). These are familiar mechanisms such as seed banks, delayed germination, clonal growth, storage organs and long lived adults. While the case of *P. furbischiae* is only one example of the influence of environmental stochasticity, it supports the importance of understanding the ecology of a species.

2.5.2.2 Demographic stochasticity

Demographic stochasticity refers to the chance variation in the age structure of a population due to reproduction and mortality (Given, 1994). After environmental stochasticity, demographic stochasticity is the greatest threats to small and/or fragmented populations (Matthies *et al.*, 2004; Robert *et al.*, 2007). Age structure is often also closely related to environmental stochasticity and disturbances such as weather conditions and patch formation. For this reason a stable population value may never be achieved and generally, when a population falls between or bellow 25-50 individuals, population size begins to fluctuate erratically due to individual recruitments and deaths (Lande, 1993; Menges, 1992; Primack, 2006). This connection between population size and temporal variation is theoretically expected, and was confirmed by Reed and Hobbs (2004). Population size is an important factor in establishing extinction risk and conservation action, but seldom considered in the level of endangerment (Reed & Hobbs, 2004). This is often the result of generational length and the amount time devoted to the study of an organism.

Species that experience a significant amount of deaths or a low amount of recruitment are in danger of even greater demographic fluctuations in subsequent years, unless they possess a survival mechanism to buffer such effects. Populations which experience decreases due to habitat destruction, alteration or fragmentation are in danger of extinction just through the chance occurrence of a single low recruitment or high mortality event. The chance of this increases in populations that are already small, which consist of old individuals with low reproductive capacity, species that are necessary outbreeders or species that have low recruitment rates (Given, 1994; Silvertown & Charlesswood, 2001).

When a population drops below a certain threshold it may no longer be able to complete the necessary functions for its own persistence. This is known as the Allee effect and results in individuals within a population or sub-populations being further separated which in turn reduces the chances of adequate pollination and subsequent seed set (Primack, 2006). Further effects of a reduced population are the aforementioned population fluctuations, unbalanced sex ratios, reduced individual fitness, disrupted social behaviour and potential local extinction. The culmination of effects leading to an ever decreasing population is known as an extinction vortex (Fischer & Matthies, 1998; Kearns *et al.*, 1998).

However, even though there is some empirical evidence of extinction vortices for small local animal populations, there is very little for small plant populations. Consequently Matthies *et al.* (2004) conducted a 10 year study of 359 plant populations in northern Germany and found that the necessary population size for survival varied amongst the different species, and extinction of plant populations was at least partly due to stochastic processes.

2.5.2.3 Genetic stochasticity

Whether a species poses a conservative genetic architecture or not, the demographics and genetics of a population are influenced by a species spatial distribution, the local environment and the continuous disturbances and processes therein (Husband & Barret, 1996). Furthermore, the smaller a population the greater these effects become, for example Fischer and Matthies (1998) found that smaller populations of *Gentianella germanica* had significantly reduced seed set, offspring fitness, genetic variation and growth rates than larger populations as a result of inbreeding depression. However plant performance and population size have to be considered in terms of habitat quality and density dependence, since populations of different size will probably differ in habitat quality as well. This could account for what would seem to be a genetic effect, meaning any inbred offspring could be compensated for by better survival or reproductive capacity of remaining plants. None the less, the suspected inbreeding was supported by the fact that pollination between plants less than one meter apart had two thirds less survival than offspring from mates that were 10 meters apart. This in turn indicates the effect that size and density may have on successful pollination (Abrol, 2012; Kearns *et al.*, 1998; Mustajärvi *et al.*, 2001).

In a similar case with the rare edaphic species, *Centaurea corymbosa*, models were used to investigate the demographic processes and genetic characteristics in relation to the particular habitat of the species (Kirchner *et al.*, 2006). Various introduction strategies were also explored by comparing population growth and extinction risk. Due to the species self-incompatibly, the expected impacts on dynamics and viability were present; however the model revealed that this varied between the different introduction methods of single versus multiple sites. Multiple sites increased the likelihood of site suitability, as well as population growth and viability since self-incompatibility alleles were preserved. Furthermore, the link between density and pollination rate was also positively linked through self-incompatibility. Consequently, the study suggested that more than 800 seeds would be required to establish a sustainable population of this species.

The genetic consequences of small populations are either short-term, such as a genetic bottleneck, due to the founder effect (Newman & Pilson, 1997; Robert *et al.*, 2007), or because of a catastrophic event which reduced population numbers on its own, or caused an imbalance in the demographics of the population. Genetic problems can also occur over time due an insufficient growth rate or a limited carrying capacity of the habitat. In the case of a genetic bottleneck the frequency of individuals with deleterious alleles increases resulting in reduced fitness known as an inbreeding depression. On the other hand, slower genetic decline occurs as deleterious alleles build up over time within a population reducing the population's potential for adaption to environmental changes. This is called genetic drift and occurs when a population's specific adaptions are weakened through, for example, the introduction of individuals adapted to a different habitat (Silvertown & Charlesworth, 2004). Numerous examples exist of genetic issues that arise in small, reintroduced or translocated populations (Godefroid *et al.*, 2004; Hodder & Bullock, 1997; Montalvo & Ellstrand, 2000; Newman & Pilson, 1997; young *et al.*, 1996).

2.5.3 Inbreeding and outbreeding

The genetic health of a translocated population is also an important point to consider as it may have consequences for the success of a project. Linked to the use of single or multiple sources is the on-going debate on the importance of demographics of small populations or genetic diversity for the management of threatened species. This has created the belief that genetic diversity is not a primary concern (Montalvo *et al.*, 1997).

While genetics may not be a primary concern it nonetheless has to be considered for the long term health of the population. Many views have been presented as to the genetic dangers and detrimental effects of imprudent planning and selection of source material (Hodder &Bullock, 1997). Inbreeding depressions is one such danger and can be caused by a variety of factors. When a single source is used to establish a new population, or if the sources are already inbred or declining, genetic diversity can be lost, harmful mutations can occur or the population can lose its reproductive or adaptive abilities (Armstrong & Seddon, 2008; Montalvo *et al.*, 1997; Moritz, 1999). A similar population depression can occur in the form of demographic disequilibrium if the species is a natural out-breeder and the population consists of an insufficient number of plants, insufficient ratios of males to females, or if the majority of individuals in a population are self-incompatible (Fahselt, 2007; Kraus *et al.*, 2002; Robert *et al.*, 2007).

Another threat is outbreeding depression, which occurs when material is used from various separated sources or an existing population is augmented with foreign genetic material, thereby potentially introducing locally maladaptive genes (Fahselt, 2007; Montalvo *et al.*, 1997; Moritz, 1999). This may lead to the loss of specific necessary genetic traits for local adaption, resulting in the production of incompatible genes or increased genetic load. The use of multiple sources is generally discouraged in order to minimise such risks. However, while outbreeding certainly does occur, the theoretical and experimental evidence is less than for inbreeding depressions. In fact, the use of multiple sources provides the opportunity of using the available genetic diversity to increase the chances of translocation success (Hodder & Bullock, 1997; Moritz, 1999). For example in the case of the scarlet gilia, *Ipomopsis aggregate*, Heschel and Paige (1995) showed that reinforcement through pollen transfer between small populations countered inbreeding depressions.

For translocations, this means a balance has to be achieved between ensuring genetic diversity, whilst preventing genetic erosion (Menges, 2008). Research has shown that populations can be locally adaptive, which could be the result of a plastic response to the environment, since plants of the same genotype may vary phenotypically depending on conditions (Montalvo *et al.*, 1997). This local adaption has been shown to promote increased population health at sites with particular ecological conditions.

One suggestion to achieve genetic diversity and minimise adverse genetic effects is to use locally sourced material while collecting as much genetic diversity as possible (Lesica & Allendorf, 1999). Where multiple populations are unavailable, one way of ensuring some genetic diversity is to collect seeds from a single source over several seasons (Cogoni *et al.*, 2013). A similar suggestion to maximise adaptive potential of a population, is to collect material from the edges of a species range. Individuals from the species periphery may have reduced genetic diversity because of founder effects and smaller populations; however they may be more adaptive to new translocation sites (Rice & Emery, 2003). However, this depends on the stability of the source population, since Godefroid *et al.* (2011) found that sourcing material from already healthy, stable populations had a positive effect on reintroduction survival rates. Whatever the situation, the fact remains that the genetic and environmental similarities of source and receptor sites have to be considered (Montalvo & Ellstrand, 2000).

Evidence shows that effective decisions can only be made and success achieved with adequate empirical ecological data based on theory (Milton *et al.*, 1999). In most cases the requirements of the species in question is not adequately understood (Fahselt, 2007; Godefroid *et al.*, 2011; Mueck, 2000). This causes problems for rare species and species at risk since their requirements are narrower than other more common species. Since the genetic and demographic composition depends on the protocol used (Robert *et al.*, 2007), adequate species knowledge can influence the translocation process from propagules and site selection to post translation management and ultimately the success of the entire project.

2.5.4 Interspecific relationships

Interspecific relationships can be crucial for the survival of many species making the knowledge thereof important for translocation efforts (Guerrant & Kaye, 2007). These interactions range between symbiotic and mutualistic relationships involving plants, soil microbes, pollinators and seed dispersers and herbivores, pathogens and competitors. The majority of translocated species are involved in some type of mutualistic relationship (Montalvo *et al.*, 1997).

Concerning just microbial partners, several species of bacteria are already known as nitrogen fixators while others are important symbionts between fungi and plant roots. Other groups of bacteria provide benefits such as improving root permeability, increasing resistance to soil pathogens, and facilitating mycorrhyzal and root development (Fahselt, 2007). Mycorrhyzal fungi may also be important to plants by functioning as hyphal linkages which can transport carbon and nutrients between plants. On the other hand not all bacteria and fungi act as mutualists and may compete with plants for resources or even act as pathogens (Hodder & Bullock, 1997). Despite their potential importance to a translocation project, soil fauna are seldom considered in translocations.

Plant and animal interactions in the form of pollination and dispersal are crucial mutualistic relationships which are important not only to plants but their faunal partners as well (Kearns *et al.*, 1998; Montalvo *et al.*, 1997). A problem with regard to translocations such as reintroductions and assisted colonisation is that a pollinator may have disappeared from the plant's area of occurrence or never existed at the new location to begin with. Usually little is known about pollinator abundance or distribution which often fluctuates over several years. This would be a serious situation if a plant species had only a single pollinator. Fortunately there are few single species relationships and most plants have several pollinators (Kearns & Inouye, 1997). This may be the reason why plant-pollinator interactions have received so little attention (Menz *et al.*, 2011). Nonetheless, pollinators and distributors are important factors to consider in translocations as they may significantly impact on a population's reproduction and distribution (Dixon, 2009).

On the other hand, animals may also represent significant threats to translocated plant populations. Trampling can occur when large animals are present at translocation sites comprising smaller plants. A more significant problem though, is herbivory which can affect seeds, seedlings and plants of a variety of species (Jusaitis, 2005; Manders & Botha, 1989; Maschinksi *et al.*, 2004; Menges, 2008). Indeed Godefroid *et al.* (2011) concluded that herbivory was one of the main causes for deaths in translocation projects. On the other hand, herbivory which removes competition, has also been found to partially improve seedling germination in grassland restoration (Martin & Wisley, 2006).

Competition from weeds or more robust native species may also influence translocated populations. Several authors reported that weeds and pioneers may significantly influence the future of a translocated population (Griffith *et al.*, 1998; Jusaitis, 2005; Jusaitis & Polomka, 2008; Maschinski *et al.*, 2004; Menges, 2008). Negative competitive effects have also been found to occur in multi-species clumps in mine soil restoration (Blignaut & Milton, 2005). While competition is natural and often unavoidable, it can and should be controlled when attempting to establish a translocated population. Various methods have been used to reduce competition such as removing competitive plants before and after translocation, controlled burning and clipping (Godefroid *et al.*, 2011; Gordon, 1996; Jusaitis, 2005; Menges, 2008).

2.5.5 Site selection

The biological requirements that have to be met before a species can be translocated fall under the consideration of methodological factors. Site selection and preparation are the first and often most crucial factors in translocations (Abeli *et al.*, 2012). Sites may already be pre-determined as in the case of rehabilitation and some reintroductions (Blignaut & Milton, 2005) or have to be selected for translocation.

In addition to this, Seddon (2010) stated that the biotic and abiotic conditions of the donor and receptor sites should be as similar as possible, since inconsistencies between sites may reduce the chances of translocation success (Fahselt, 2007). Several studies have shown that the home-site advantage of local translocations provide an increased survival advantage over translocations from distant sites (Fahselt, 2007; Lawrence & Kaye, 2011). This is especially the case with species which naturally inbreed or have poor dispersal capabilities, making them more locally adaptable (Menges, 2008). Such requirements correspond with the physical and biological criteria set out by Fiedler and Laven (1996).

Various authors (Drayton & Primack, 2000; Guerrant & Kaye, 2007; Maschinski *et al.*, 2004) have referred to the site selection criteria proposed by Fiedler and Laven (1996). These criteria suggest that the site selection approach should be based on physical, biological, logistical and historical factors.

Physical criteria refer to aspects such as the geomorphic setting, landscape matrix (suitability of surrounding habitat), soil type (including soil chemistry), pH and texture, slope angle, aspect and albedo effect (Guerrant & Kaye, 2007). Much of the biological criteria have already been discussed as they refer to issues of genetics, competitors, herbivores and mutualistic relationships with pollinators, distributors and soil microbes. Other biological criteria which have to be considered include similar composition and structure of the surrounding flora and similar habitat function with regard to successional stages and trajectory.

The logistical aspects of translocation projects are concerned with ease of access in terms of site protection, monitoring and management. These points have much to do with land ownership since this can influence the degree of protection and levels of monitoring and management.

Historical criteria are more difficult to determine and may cause controversy regarding the future conservation of the species. There are two issues in terms of historical criteria that have to be considered (Seddon, 2010). The first is the selection of known sites as opposed to potential sites. The second is maintaining the potential site for future evolutionary change. Known sites are those from which the species has been extirpated. Potential sites are those which seem suitable but are unoccupied because the species has not yet colonised these sites or is dispersal-limited. The historical criteria also includes considering the long-term future of translocations in terms of protection and evolutionary potential (Geurrant & Kaye, 2007).

Site selection is clearly an intricate process comprising many related factors. In a nutshell a translocation site has to accommodate plants sufficiently to ensure establishment and persistence. It also has to provide enough flexibility for future species evolution, land use or management. Furthermore, these sites should fall under some form of protection from inconsiderate human interferences such as rezoning for agriculture, mining or development (Fahselt, 2007; Guerrant & Kaye, 2007). Godefroid *et al.* (2011) reported that site protection accounted for 12% of reintroduction successes.

The majority of reintroductions occur on degraded or sparsely populated sites, while translocations mostly occur on the above-mentioned potentially suitable sites. Most translocation sites including the seemingly most suitable site will differ from the donor site to some extent (Fahselt, 2007). Since the ecological requirements of many species are still poorly understood, research into species biology is crucial (Montalvo *et al.*, 1997). This is especially the case for rare edaphic specialist species which have more specific requirements than most other plants (Armstrong & Seddon, 2008).

For this reason initial experimentation has been advocated as a means of gaining crucial species information and to verify translocation as a plausible conservation strategy (Guerrant & Kaye, 2007; Gordon, 1996; Hodder & Bullock, 1997; IUCN, 1987; Jusaitis, 2005; Maschinski *et al.*, 2004). Unfortunately, due to the lack of species-specific knowledge, most translocations are simultaneously set up as experiments. Indeed Allen (1994) has stated that based on current knowledge, few translocation projects are little more than experiments. Nonetheless experiments provide valuable information concerning population establishment, genetics, growth, reproduction, dispersal and persistence (Hodder & Bullock, 1997; Montalvo *et al.*, 1997; Newman & Pilson, 1997).

2.5.6 Site preparation and translocation techniques

After site selection it may be necessary to prepare the site(s) to various degrees before translocation can take place. The main reason for site preparation is to facilitate the establishment of the translocated species. Godefroid *et al.* (2011) found that even a single type of site preparation activity increased the transplant survival rate during the first year.

Various methods have been used for site preparation depending on the species in question. In cases where desiccation is a threat, shade can be provided with nurse plants, branches, screens or leaf litter. In other cases though, the removal of shading may be necessary to improve growth (McChesney *et al.*, 1995). Soil preparation by means of cultivation can also be useful to reduce below-ground competition or to improve conditions for root development (Bazzaz, 1996). This may be particularly important for translocations using seed propagules. Above-ground competition may also be removed through weeding or controlled burning (Gordon, 1996; Pavlik *et al.,* 1993). Irrigation and fertilization may also be useful to provide an initial boost for plant establishment (Doerr & Redente, 1983). Where seed and plants may come under threat from herbivores the use of cages and fences to protect plants may be beneficial (Buisson *et al.*, 2008).

Translocation techniques may be closely linked to site preparation. Bainbridge *et al.* (1995), have established various techniques for pre-translocation cultivation of desert plants in the USA. These techniques include container characteristic such as material, size, depth and drainage, soil mixes as well as handling and planting techniques which improve the chances of survival once translocation has occurred. As with most other factors affecting translocation, the techniques used depend on the species in question. Techniques may vary between seeds, seedlings (Jusaitis, 2005; Mustard *et al.*, 1995; Navarro & Guitián, 2003), cuttings (Bontrager *et al.*, 2014), potted plants, bare-root plants or plants translocated between sites while still in their habitat soil (Bullock, 1998; Burgoyne & Hoffman, 2011). Any combination and repetition of these techniques can be used or may be necessary where it is suspected that repeated introduction is required to establish a persistent seed bank (Guerrant & Kaye, 2007).

2.5.7 Cost and timing

Other factors which require less discussion, but are no less important, are costs and timing of the translocation activities. The financial implications of any translocation are a restricting factor, since translocations are high risk and high-cost endeavours (Maunder, 1992). In any translocation effort the most cost effective approach has to be weighed against the most effective propagule types and translocation techniques (Drayton & Primack, 2000; Guerrant & Kaye, 2007). Financial considerations for post-translocation care and management are also important since funds are often limited or uncertain (Haight *et al.*, 2000).

Timing may also affect the cost of the project as well as its success. Timing is usually concerned with performing a translocation so that the species can take maximum advantage of optimal environmental conditions. Weather variables can however negatively affect a translocation, even if the right season is selected. In cases where seasonal preferences are not fully understood seeds make for a potentially useful propagule choice, since they can wait for the best conditions to germinate and minimise the risk of losing plants. In this regard experimenting with timing may also provide valuable information for future translocations (Guerrant & Kaye, 2007).

The schedule of the species in question also has to be considered since many species may separate flowering events between populations and/or their sexual phases in time (Lundberg & Ingvarsson, 1998). The result is that if a translocated population is intended to outcross with a natural population, the translocated plants may need more time to adapt to the natural environment and synchronise with the existing population. Where sexual phases are separated in time, asynchronic flowering in a population occurs to ensure cross pollination. This in turn places a minimum limit for a viable population in some species. This is this case in *Ficus* species that have species-specific relationships with pollinating wasps. Bronstein *et al.* (1990) determined that a minimum population of 95 trees is necessary to produce enough flowers over time to sustain its wasp pollinator. Hammer (1995) observed that mesemb flowers generally become receptive after four days, thus having a distinct male phase followed by a female phase (Hartmann & Dehn, 1987, cited in Zietsman, 2013). A minimum viable population, in terms of pollination at least, would therefore be one that has enough individuals to allow a sufficient number to be receptive for pollination i.e. the more flowers the better the chances that all are not un-receptive at the same time.

2.5.8 Post-translocation care and adaptive management

Activates at a translocation site often continue after the site preparation and translocation has occurred. This is frequently referred to as a post translocation care or “soft release”, since initial site preparations or management is continued to prolong the most favourable conditions for the translocation to be successful (Drayton & Primack, 2000).

If site conditions change or if the conditions were not as suitable as initially thought, then adaptive management may be necessary to improve the situation. Adaptive management can in fact be the entire purpose of an experimental translocation or form the backbone for a translocation project. Adaptive management acknowledges the incomplete understanding of natural systems and puts the lack of knowledge to good use by allowing conservationists to learn through acceptable experimentation (Rout *et al.*, 2009). However, some uncertainties are unavoidable and cannot be controlled, such as natural stochasticity. Still, unforeseen problematic issues can still present valuable learning opportunities for the improvement of future projects (McDonald-Madden *et al.*, 2010).

There are two forms of adaptive management namely active and passive. Active adaptive management explicitly focuses on learning from different management strategies and implementing lessons learnt.

Active adaptive management aims to balance management with learning about the system being managed for future implementation. It may make use of traditional experimental designs, or decision theory which requires management objectives and options, describing the state of the system, monitoring of response to different management options, identification of uncertainties and methods of finding solutions (McCarthy & Possingham, 2007; Rout *et al.*, 2009). Furthermore as part of the explicitly active approach, various methods are designed to test different management options and their outcomes.

In passive adaptive management learning is not purposely pursued and occurs by chance and may or may not be implemented (McCarthy & Possingham, 2007). Passive adaptive management includes no specific design for the purpose of learning. The primary goal of the translocation is conservation by using a single method. Any learning which takes place during the course of the translocation may be applied to improve the single translocation but not necessarily for the purpose of learning (McDonald-Madden *et al.*, 2010).

Since most cases of translocation are often limited by funding and lack of propagules, experimentation takes second place to conservation. In terms of active adaptive management it is difficult to establish how resources should be divided between experimentation and conservation (McCarthy & Possingham, 2007). It also presents significant theoretical and conceptual challenges. For example, it is difficult to design and implement studies to effectively distinguish between different management options. Furthermore it requires controversial management practices for the purpose of improving system knowledge, as well as designed monitoring programs which may be difficult to implement (Westgate *et al.*, 2013).

For these reasons the use of active adaptive management is limited despite being widely advocated (Brook *et al.* 2002; Hirzel *et al.* 2004; Sarrazin & Barbault 1996; Seddon *et al.* 2007; Stockwell & Leberg 2002 as cited by Rout *et al.*, 2009). The result is that many translocation efforts only employ passive adaptive management (if adaptive management is being used at all). This is not always problematic, since active adaptive management may not necessarily be advantageous to all aspects of the translocation project. Active adaptive management may also be unnecessary where only minor improvements can easily be achieved. Lastly, passive adaptive management can be implemented more easily and cost effectively while still gaining useful applicable information (Westgate *et al.*, 2013). Once again the choice between active and passive management depends on the specific translocation taking place.

2.5.9 Documentation and monitoring

In any sort of translocation, be it animal or plant, proper documentation and monitoring during and after the translocation is essential (Menges, 2008). During the 1990's literature focusing on the application of experimental approaches, research and monitoring was sparse, and becoming increasingly necessary (Seddon *et al.*, 2007). Published reports have generally focused little on documentation and monitoring (Drayton & Primack, 2000), with the result that the negligence of these key factors has been identified as important points of weakness in reintroduction projects (Godefroid *et al.*, 2011). Fortunately, monitoring of translocation success has increased in the last few decades along with the number of reintroductions being performed (Armstrong & Seddon, 2008).

Despite the increase in reintroduction literature it is still mostly descriptive and retrospective. For this reason, instead of monitoring being led by questions, the questions have been driven by the available data. Such unfocussed monitoring as well as failure to establish goals and questions for investigation before a translocation takes place may lead to crucial data being missed, resources allocated inadequately and an increase in the likelihood of project failure (Armstrong & Seddon, 2008).

Proper documentation and subsequent monitoring is necessary to identify correct translocation practices or to avoid mistakes (Griffith *et al.*, 1989). In this regard failed translocations are significantly under-documented (Goedefroid *et al.*, 2011). In most cases the two main criteria focussed on are establishment and persistence. Monitoring usually ends within a few years or after establishment has been confirmed. Adequate long-term monitoring is however necessary to identify the other previously mentioned success criteria (Pavlik, 1996), as well as ambiguous short-term results such as initially high survival rates followed by significant decreases over time (Fahselt, 2007; Menges, 2008). Suggested monitoring periods range from 10 years to several decades (Godefroid *et al.*, 2011) while the precise duration of a long-term monitoring program depends on the species as some may have shorter or longer life cycles than others.

Monitoring is further necessary to identify demographic parameters and population health as well as any restrictions or new threats to the population, along with any interventions or management changes that may be necessary (Seddon, 1999). It also has the possible advantage of providing unexpected supplementary information which may be beneficial to the translocation (Guerrant & Kaye, 2000).

Menges (2008) suggested that population monitoring be done according to vital signs of survival, growth and fecundity in comparison to a control population. Similarly, Pavlik *et al.* (1993) suggested that populations should be demographically monitored. In this way germination, seedling recruitment, plant growth and reproduction can be monitored for the initial success criteria. Such approaches allow for the most accurate identification of success criteria, changes in population demographics, and whether a translocated population is capable of replicating the functions of a natural one.

2.5.10 Translocation guidelines

Due to the numerous and diverse factors that need to be considered as well as the general trend of failures in translocations, various guidelines have been published in an attempt to consolidate these considerations and maximize the chances of success. The most notable and often cited guidelines are those published by the IUCN (2013; 1995; 1987; Hodder & Bullock, 1997).

These have been revised and published several times, with the latest edition published in 2013. During 1987, the IUCN started off by publishing its Position Statement on Translocation of Living Organisms, which dealt with the various forms of translocation as well as administrative concerns (IUCN, 1987). In 1995 the IUCN deemed it necessary to revise the guidelines so as to include more comprehensive attention to the various forms of translocation (IUCN, 1995). The most recent guidelines have been further extended to accommodate the increasingly interventionist and active management approaches to biodiversity (IUCN, 2013). These now include guidelines and cautions for assisted colonisation and ecological replacement, which have been added to the translocation spectrum in response to climate change and continuous habitat fragmentation and destruction.

Various other translocation guidelines have been published in the past, some relating to specific taxa and geographic regions (Hodder & Bullock, 1997). While these are often focused on animals, the principles can also be applied to plant translocations. These guidelines are, however, occasionally criticised since they have been mistakenly considered as rules which limit novel interventions (Seddon, 2010). Other guidelines again cannot determine if a translocation should take place because of a lack of clear data to suggest whether a project is feasible and likely to succeed. In addition, these guidelines do not take into account the difference between importance, need and usefulness of translocations (Pérez *et al.*, 2012).

For this reason Pérez *et al.* (2012) made a study of the necessity and usefulness of translocations in Spain using various guidelines from which they developed 10 main criteria (Table 2.1). The criteria have been further divided into levels pertaining to the necessity of the translocation, risk evaluation, and technical and logistic suitability. These guidelines were developed using studies conducted over 20 years and include numerous authors. These criteria are therefore useful and comprehensive tools for focussing any guidelines being used and evaluating the necessity and usefulness of any translocation project.

Only compliance with all ten criteria (Table 2.1) will fully justify a project and indicate that all known challenges to the project have been considered. Since some criteria may be outranked by others and since subjectivity may occur in a project assessment, Pérez *et al.* (2012) further proposed a ‘Hierarchical Decision-making System for Translocations’ which can be used to avoid these problems. Each level builds on the next and increases the likelihood of success. If the first level is not fulfilled then translocation may be of little value for the species or population in question and resources would be wasted. If the second level is unfulfilled the recipient ecosystem could experience unintended negative effects. Finally, if the third level is unfulfilled the translocation may fail without negative consequences for conservation but would still be a waste of resources (Pérez *et al.*, 2012).

Table 2.1 Levels and criteria for translocations (adapted from Pérez *et al.*, 2012).

Level	Criteria	Description/Notes
1st Level: Necessity of Translocation	1. Threat status?	The threat level and conservation status of the species/population has to be established.
	2. Threat factors removed, controlled or absent?	What are the threats to the species/population and have they been managed at the release site?
	3. Best tool?	Other management options have to be considered before translocation. If it is possible to address threats such as human impacts then *in situ* management maybe a better option.
2nd Level: Risk Evaluation	4. Risks for target species?	Is translocation an acceptable strategy for the species in question in terms of spreading disease, genetics and population stability? All threats have to be accessed and dealt with appropriately.
	5. Risks for other species or ecosystem?	Are the potential risks held by a translocated species acceptable to the recipient ecosystem and its organisms? This is particularly important to top predators that have been absent from their natural environment or species that may become invasive.
	6. Acceptable to locals?	Will a translocation cause trouble for local people or negatively affect the local socioeconomic system? Attitudes and social impacts of a translocation have to be investigated and addressed where necessary.
3rd Level: Technical and Logistical Stability	7. Maximise translocation success?	Do all the factors that might affect the survival of a population maximise the chances of translocation success? These factors are included in the previously discussed considerations.
	8. Clear goals and monitoring?	Any translocation should include clear goals, a monitoring plan and an adaptive management strategy to continually improve the chances of success.
	9. Economic and human resource support?	Since translocation is a risky and expensive method of conservation sufficient resources have to be available to meet any project demands.
	10. Scientific, governmental and stakeholder support?	Participation and cooperation between various stakeholders such as scientist, government (in the form of legislative support) and corporate entities is required for appropriate and sufficient project support.

2.6 Governing translocation in South Africa

South Africa has a strong and well-developed legislative framework for the conservation of nature and the sustainable use of biodiversity (SANBI, 2015). Various legislative documents exist pertaining to the different aspects of biodiversity conservation, on national and provincial levels. Furthermore, various provisions are made for *in situ* and *ex situ* conservation as well as for supporting biodiversity monitoring on a variety of scales (South Africa, 1997; South Africa, 2004). In addition to this, South Africa's legislation encourages its cooperation with various international conservation organisations and programmes. National and provincial legislative documents concerning biodiversity include the following:

- White Paper on the Conservation and Sustainable Use of South Africa's Biological Diversity (1997)
- National Environmental Management: Biodiversity Act (Act 10 of 2004)
- National Environmental Management: Protected Areas Act (Act 57 of 2003)
- National Biodiversity Strategy and Action Plan (NBSAP) (2005)
- National Spatial Biodiversity Assessment (NSBA) (2004)
- National Biodiversity Framework (NBF) (2008)
- National Protected Area Expansion Strategy (NPAES) (2008)
- Mpumalanga Nature Conservation Act (1998)
- Gauteng Nature Conservation Bill (2014)

While these publications make provision for biodiversity and species conservation as well as protected areas, very few guidelines or protocols have been suggested for translocation and reintroduction (Holmes & Richardson, 1999; Milton *et al.*, 1999;). Furthermore, these predominantly focus on Fynbos. However, since South Africa has expressed a desire to cooperate with international conservation organisations and programmes, translocations for other plant species would best be executed according to the guidelines proposed by the IUCN within the framework of South African Legislature.

Previous projects in South Africa in the line of plant translocation have been focussed around various mine or Fynbos rehabilitations. Forty seven translocation projects have been documented in South Africa, 24 of which include Cape Flats Fynbos species (Milton *et al.*, 1999). However, information on these projects is scarce while reasons for failure have seldom been reported. In Namaqualand a study on strip mine rehabilitation was investigated by adapting methods from natural ecological processes. This included testing the fertility of mined and unmined soil, planting and sowing two different plant species to test for the facilitation or inhibition of indigenous plant establishment, testing the effects of microsites and the translocation of three succulent species to test whether planting in clumps increased survival (Schimdt, 2002). In the Western Cape various methods have been developed to reduce stress and disease risk during translocation (Milton, 1999). Examples include *ex situ* seedling cultivation and sheltering seedlings *in situ* in the case of the Clanwilliam cedar (*Widringtonia cederbergensis*) (Higgins *et al.*, 1989; Manders & Botha, 1989). Another example is the clumping of Proteaceae which improved plant survival (Milton, 1999), contrary to the aforementioned example where clumping was not effective. Furthermore, Holmes and

Richardson (1999) have provided a conceptual framework on which protocols can be based for restoration of Western Cape Fynbos.

Currently the South African National Biodiversity Institute's (SANBI) Plant Conservation Strategy is focussed on protecting at least 75% of threatened flora *in situ* by expanding protected areas and incorporating site which contain threatened species (von Staden & Raimondo, 2015). This target is the primary approach to plant conservation since *ex situ* conservation is costly and requires plenty of man-power. Furthermore, available space at conservation facilities is limited and could probably not accommodate a sufficient genetic representation of a species. Despite this, SANBI's goal is to preserve at least 60% of threatened species *ex situ*, and to participate in the Millennium Seed bank Partnership. By 2020 SANBI also aims to have 1% of the species in *ex situ* collections in active restoration programs (Nkuna *et al.*, 2015). SANBI is also in the process of establishing a protocol for documenting restoration work and a knowledge database involving threatened species which is due in the near future. Since *F. humilis* is currently protected at the Walter Sisulu National Botanical Garden as well as the Lowveld National Botanical Garden, and is currently in an *in situ* conservation project, it can be considered as part of this 1% target.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

Various authors have stressed the importance of proper planning, management, documentation and monitoring before, during and after any translocation attempt (Armstong & Seddon, 2008; Fahselt, 2007; Godefroid *et al.*, 2011; Menges, 2008; Pavlik, 1996). This chapter will discuss the study area and methodology used in this project, as well as the project history thus far. Methodologies for specific objectives of the study can be found in the relevant chapters.

3.2 Study area

3.2.1 Grassland Biome

The Grassland Biome covers about 40% of the Earth and occurs on every continent except Antarctica (Egoh *et al.*, 2011). It shares its surface area with almost one billion people and provides various ecosystem services to people around the world (Sala and Paruelo, 1997). Furthermore, it's moderate climate and fertile soils make it the most productive and biodiverse biome in the world and the most favourable for human settlements and agriculture (Fourie *et al.*, 2015).

Grasslands are predominantly characterised by grass species which provide biomass, while the perennial forb component enhances species richness. Woody plants are rare or absent, being low-growing to medium sized shrubs at most (Carbutt *et al.*, 2011). In temperate grasslands this can be attributed to sub-zero temperatures, dry winters and regular fires (Bredenkamp *et al.*, 2002). These conditions are also the reason that many grassland species have specialised underground storage organs which allow them to survive adverse conditions by going dormant (Siebert, 2011). Rainfall in the Rand Highveld Grassland may vary between 570 - 730 mm per year with an average precipitation of 654mm (Mucina & Rutherford, 2006).

The Grassland Biome is the second largest (covers 28% of the land surface) of the nine biomes in Southern Africa (Carbutt *et al.*, 2011). Sixteen per cent of this extent falls within South African borders, covering an area of 346 174 km^2 (Rodríquez *et al.,* 2007). The majority of South African people live within this biome, particularly in the Johannesburg and Tshwane metropolitan areas (Neke & Du Plessis, 2004). Globally, grasslands are habitat to various endemic species, while South African grasslands are home to numerous endangered species of fish, birds, mammals and plants, including *F. humilis* (Neke & Du Plessis, 2004). The grasslands are however threatened by urban development, plantations, invasive species, agriculture, overgrazing, mining and climate change (Neke & Du Plessis, 2004). In South Africa these threats have transformed about 35% of the biome making it the most threatened biome in the country (Egoh *et al.*, 2011), with the immediateness and extent of transformations depending on the kind of threat (Siebert, 2011), such as mining versus subsistence agriculture. Despite the importance of this biome, only 2% of South African temperate grasslands are protected, which falls well short of international and even national targets (Carbutt *et al.*, 2011).

3.2.2 Receptor sites

Even though the impacts of mining are localised (Neke & Du Plessis, 2004) within the Grassland Biome, they are highly destructive and pose an imminent threat to species when habitats are irreversibly destroyed. The population of *Frithia humilis* studied as part of this project was threatened by such destruction before being translocated to three receptor sites as a conservation measure.

Translocation receptor sites were chosen (Burgoyne & Hoffmann, 2011) within the Rand Highveld Grassland (Figure 3.2a) (Mucina & Rutherford, 2006). Translocations were performed near eMalahleni (Witbank) and Balmoral in the Mpumalanga province (Figure 3.1). Potential receptor sites in the Gauteng province were not considered due to the significant legal implications of a trans-border translocation (Burgoyne & Hoffman, 2011). Receptor sites were chosen according to the criteria in Table 3.1 and initially searched for by helicopter and verified on site thereafter (Burgoyne & Hoffman, 2011). Each receptor site consisted of various receptor patches which were further evaluated according to individual patch characteristics before the translocations took place in July 2009.

Table 3.1 Habitat specifications considered in the translocation of the habitat specialist, *Frithia humilis*, to suitable receptor sites (Burgoyne & Hoffman, 2011).

Habitat characteristics	Significance
Geological stratum:	
i) Porosity	i) Prevention of accumulation and inundation
ii) Sedimentary rocks (Dwyka and Ecca sandstones, Karoo Supergroup)	ii) Weathering products, i.e. quartz-rich pebbles, protect vulnerable seedlings from environmental factors
iii) Plate-like outcrops	iii) Shallow 'grit pans' on flat outcrops offer and ideal micro-habitat: shallow, sandy soil, with a top layer of gravel
Slope inclination	A smaller incline prevents plants from being washed down-stream, while still facilitating seed dispersal via water flow
Species composition of the greater habitat	Similar ecological processes would presumably minimise post translocation stress
Intact habitat	Few ecological disruptions prevent further disturbance of populations
Distance from the donor site	A shorter distance leads to less disruption of genetic variation
Absence of existing *F. humilis* population	Avoidance of gene pool contamination
Protected/conserved area	Guaranteed conservation of the translocated population(s)

3.2.2.1 Goedvertrouwdt Farm

The Goedvertrouwdt Farm receptor site is located on a hill located on portions 9 and 24 of Goedvertrouwdt farm 499 JR and was at the time of the translocation set aside by the land owner as a reserve for the translocated plants (Burgoyne & Hoffman, 2011). Goedvertrouwdt is located east of Bronkhorstspruit near the Gauteng-Mpumalanga border. As a typical *F. humilis* habitat in terms of geology, this is the largest of the three receptor sites containing 2705 plants at the onset of monitoring in 2010 (Harris, 2014). Plants were translocated into receptor patches according to the availability of suitable sandstone plates (Figure 3.2b). Patches were originally named GW, GL, GM and so forth, but have since been renumbered G1.1, G1.2, G1.3 etc. for practicality (Table 3.2).

The Goedvertrouwdt site was geologically the most suitable site since the sandstone plates were of the Ecca group. Accumulated topsoil and quartzite gravel was also present at the receptor site, which are necessary for *F. humilis* seed germination and growth (Burgoyne *et al.*, 2000b). The gradient is less than 3˚ which allows plants to establish and not wash away while still allowing seed distribution by water flow. The surrounding grassland is reasonably pristine allowing for normal ecological functions to occur (Burgoyne & Hoffman, 2011).

The vegetation of the area belongs to the Rand Highveld Grassland vegetation unit (Mucina & Rutherford, 2006). A highly variable surrounding topography is characterised by plains and ridges consisting of sour grassland and a high diversity of herbs. Ridges and slopes are home to sour shrub-land, while particularly rocky hills and ridges may include sparse woodlands.

However, since the Goedvertrouwd site overlies an abandoned subterranean coal mine, major structural cracks in the underlying geology may inhibit dispersal due to water run-off into deep fissures. Fortunately the acid mine water drainage at the foot of the slope does not affect the *F. humilis* plants on the upper slope. Several alien invasive plant species are also present at the site, though currently these have no direct effect on the translocated plants.

Table 3.2 Old (Harris, 2014) and new coding system for the receptor patches at the Goedvertrouwdt receptor site, including patch size and altitude.

Old code	New code	Size (m)	Altitude (m a.s.l).
GW1	G1.1	0.82 x 3.12	1547
GW2	G1.2	1.57 x 1.65	1550
GM1	G2.1	0.56 x1.74	1556
GM2	G2.2	0.48 x 0.50	1557
GM3	G2.3	0.67 x 1.38	1556
GM4	G2.4	1.70 x 2.45	1556
GL1	G3.1	0.60 x 1.49	1556
GL2	G3.2	1.11 x 1.78	1557
GL3	G3.3	1.50 x 3.10	1555
GL4	G3.4	0.36 x 1.89	1556
GL5	G3.5	1.86 x 2.71	1554
GL6	G3.6	0.70 x 0.95	1556
GR1	G4.1	0.75 x 1.39	1552
GR2	G4.2	0.34 x 1.31	1553
GR3	G4.3	0.98 x 1.22	1552
GR4	G4.4	0.95 x 1.69	1554
GR5	G5.1	0.82 x 1.56	1561

Despite the site being donated for the project, the site is not legally protected and was sold some time after the translocation took place. In mid-2015 what seemed to be prospecting activity was taking place, suggesting that the new landowners were exploring the possibility of extracting remaining coal deposits (Hilda Kroonhof, pers. comm.).

3.2.2.2 Eagle's Rock Private Estate

Two a-typical translocations sites were also chosen for experimental purposes, one of which is at Eagle's Rock. Eagle's Rock is a privately owned wildlife reserve situated about 10 km north-east of eMalahleni. The geology of the area is of the Wilge River Formation of the Waterberg Group (Transvaal Supergroup) (Figure 3.2c). The site's sedimentary rocks are denser and less porous than the Dwyka and Ecca sandstones, meaning drainage would be potentially less than in natural *F. humilis* habitat. Since this site did not include any locally weathered quartz pebbles, it was necessary to import pebbles from the original *F. humilis* habitat (Harris, 2014).

The surrounding vegetation also belongs to the Rand Highveld Grassland vegetation unit (Mucina & Rutherford, 2006) and is similar to that of the Goedvertrouwdt receptor site.

Five translocation patches were established at Eagle's Rock (Table 3.3), contain 673 *F. humilis* plants at the onset of monitoring in 2010 (Harris, 2014). The rocky outcrops were surrounded by tall grasses with downslope areas which were inclined to water-logging, hampering population expansion. Although grazing animals, such as zebra, were present in the area, trampling seemed to be a minor threat for the population.

Table 3.3 Size and altitude of receptor patches at Eagle's Rock and Witbank Nature Reserve receptor sites.

Patch	Size (m)	Altitude (m a.s.l.)
E1.1	1.70 x 1.87	1540
E1.2	1.80 x 1.08	1540
E1.3	4.30 x 8.60	1540
E1.4	0.70 x 0.21	1540
E1.5	1.70 x 1.19	1540
W1.1	0.50 x 0.93	1551
W1.2	0.62 x 0.64	1551
W1.3	0.30 x 0.78	1551

3.2.2.3 Witbank Nature Reserve

The Witbank Nature Reserve borders on the south-eastern part of eMalahleni and lies to the north of the Witbank Dam in the Olifants River, south of the N4 motorway. The reserve covers 847 hectares and is a transitional zone between mostly Rand Highveld Grassland and Eastern Highveld Grassland vegetation units (Mucina & Rutherford, 2006). The reserve protects 11 different plant communities making it an area of high botanical diversity and conservation importance (Smit *et al.*, 1997).

The *F. humilis* plants translocated to this area were placed in a *Myrothamnus flabellifolius - Ursinia nana* herbland (Smit *et al.*, 1997). This area has 99% cover of felsic rock sheets which occur on crests and moderate slopes. Most plant species are xerophytic and grow in the cracks of the rock sheets or the limited soils which accumulate to a depth of up to 30 mm on the rock sheets. Several xerophyte and geophyte species are present (Smit *et al.*, 1997). The surrounding vegetation is largely grass-dominated.

The geology of the site is also a-typical to the preferred *F. humilis* substrate (Figure 3.2d). The felsic rock sheets are igneous in composition and belong to the Selons River Formation (Rooiberg Group) of the Bushveld Igneous Complex (Smit *et al.*, 1997). Being volcanic in origin this rock is denser and less porous than sandstone, meaning an increase in run-off, while the shallow slope of the site holds potential for seasonal flooding (Harris, 2014). Pebbles were present at the proposed site but larger, more angular and less abundant than pebbles of sedimentary origin (Harris, 2014). This site only accommodates three patches (Table 3.3). Fifty five *F. humilis* plants were counted at the onset of monitoring in 2010 (Harris, 2014).

3.2.2.4 Ezemvelo Nature Reserve

Ezemvelo Nature Reserve is an intermediary area between the inland plateau grasslands and the lowland plateau savannah. The landscape consists of rocky ridges and hills while the soil is shallow and rocky (Acocks, 1988; Louw, 1951; O'Connor & Bredenkamp, 2003, as cited by Swanepoel, 2006). The 11 000 hectare reserve contains 22 plant communities, one of which is the *Frithia humilis–Microchloa caffra* community and the only officially protected population of *F. humilis* (Burgoyne & Hoffman, 2011; Swanepoel, 2006). This population was chosen as the control due to its protection, stability/persistence and proximity to the Goedvertrouwdt receptor site. The area of occupancy for the population exceeds 1 km^2 which is a far greater area than that of the translocated populations. Six random 1 m^2 patches where chosen to be monitored.

The geology of the habitat is exposed Dwyka and Ecca Group sedimentary sandstones on a low lying hill surrounded by grassland. The sandstone plates are surrounded by grasses which facilitate the accumulation of the typical thin layer of soil and weathered quartz pebbles by acting as a barrier for rain run-off allowing sedimentation of suspended materials. This, along with the sandstone plates being of adequate size and flatness, creates extensive areas of ideal habitat for *F. humilis*. The grass species, including *Microchloa caffra*, do not encroach on the *F. humilis* habitat and provide some protection against extreme environmental conditions, resulting in plants that are bigger and more turgid than unprotected plants. These ideal conditions have allowed *F. humilis* to colonise large areas of suitable habitat at Ezemvelo Nature Reserve, as is evident in the mean plant density (517 plants /m^2) and high population numbers. Other taxa of Aizoaceae in the Succulent Karoo Biome are generally found in much smaller numbers because of ecological limitations (Ihlenfeldt, 1994).

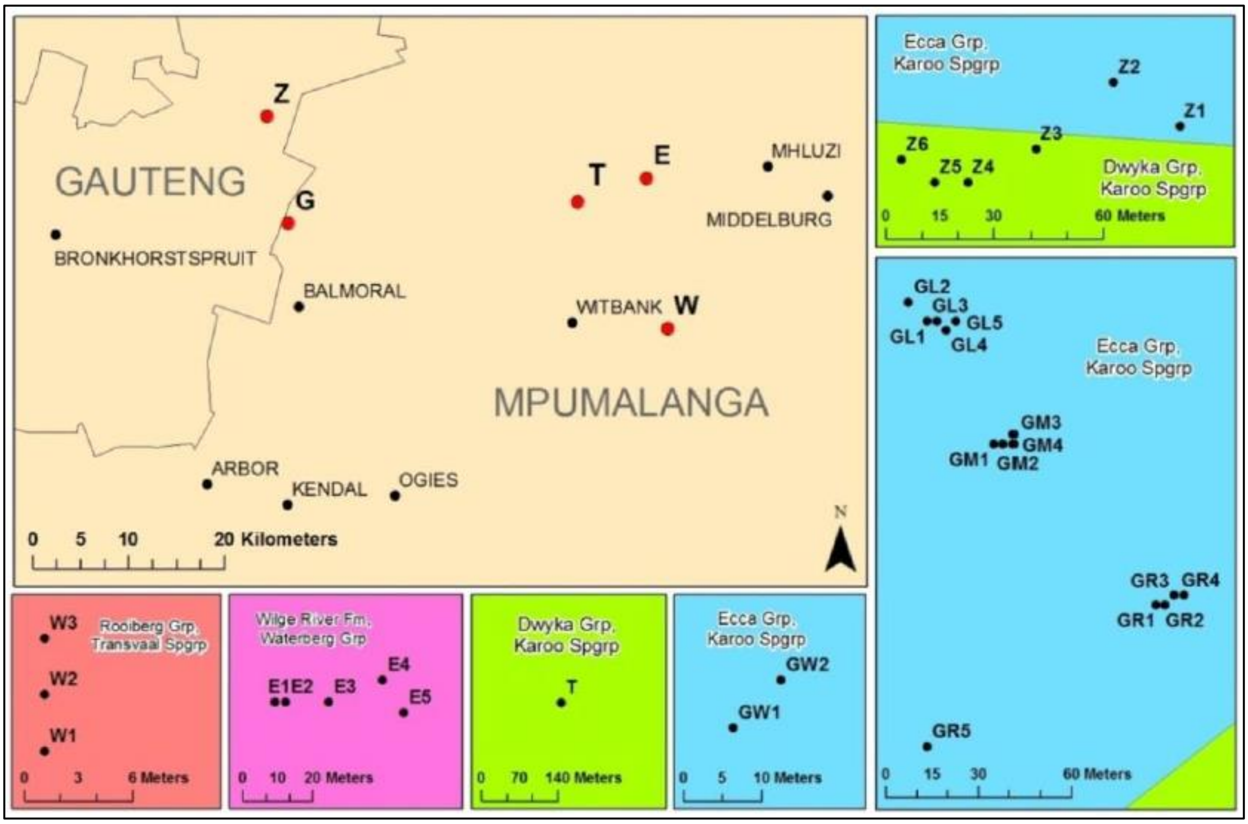

Figure 3.1 The donor population of *Frithia humilis*, indicated by T, was translocated to three suitable receptor sites (G, Goedvertrouwdt; E, Eagle's Rock and W, Witbank Nature Reserve). A natural, control population of *F. humilis* is located in Ezemvelo Nature Reserve (Z). All study sites occur on sedimentary rock, except for W which is felsic. Grp, group; Spgrp, supergroup. (Courtesy of Harris, 2014).

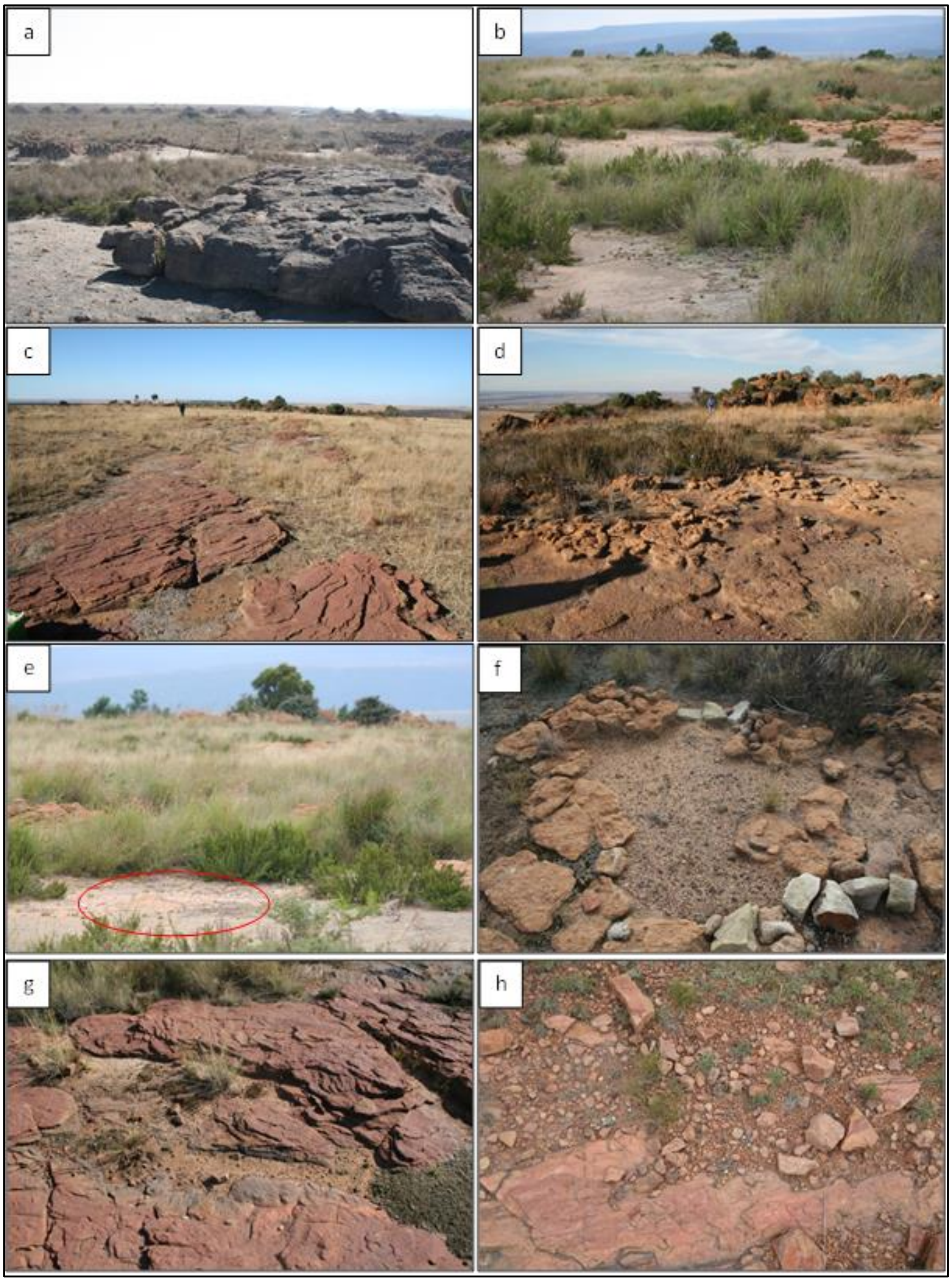

Figure 3.2 **Inyanda Coal donor site (a). Ezemvelo Nature Reserve (Control population) (b). Eagle's Rock receptor site (c). Goedvertrouwdt receptor site (d). The characteristic Rand Highveld Grassland, including rocky Dwyka outcrops. The red circle indicates typical, xeric *F. humilis* habitat consisting of shallow, well drained, eroded soil (e). A translocation patch at the Goedvertrouwdt receptor site placed on typical Ecca sandstone (f). A translocation patch at the Eagle's Rock receptor site placed on rock of the Wilge River Formation (g). A translocation patch at the Witbank Nature Reserve receptor site on rock of the Selons River Formation (h).**

3.3 Monitoring

In the past, key factors and weaknesses in translocations have gone unidentified due to poor monitoring practices (Godefroid *et al.*, 2011). To avoid this, adequate monitoring at all stages of a translocation project is crucial in establishing success or failure (Griffith *et al.*, 1989; Menges, 2008). Proper monitoring may also provide unexpected information which may be beneficial to the translocation (Guerrant & Kaye, 2000). For this reason a monitoring program has been applied to the *F. humilis* translocation project since 2010.

Menges (2008) suggested that monitoring be done according to the vital signs of survival, growth and fecundity, while Pavlik *et al.* (1993) suggested a demographic monitoring approach which aims to establish abundance, extent, resilience and persistence. The previous study by Harris (2014) determined trends in demography, flowering, fruiting and seedling recruitment to establish initial population trends and translocation success (Godefroid *et al.,* 2011; Mueck, 2000). The study concluded that sub-populations successfully survived the translocation process and that flowering and fruiting occurred, as proven by the high seedling numbers (Harris, 2014).

These conclusions are equivalent to the first two measures of success, abundance and extent, suggested by Pavlik (1996). However, these milestones, despite being the earliest to be detected, can only be established with confidence after ten years because ambiguous, short term results of high survival rates followed by decreases over time are not uncommon (Fahselt, 2007; Menges, 2008). Since this study, like many others, cannot be continued for the recommended amount of time, it has to be tentatively concluded that the translocated populations reached the first two measures of success. The next two measures of success, resilience and persistence, including establishment as suggested by Armstrong & Seddon (2008) refer to the population's ability to survive environmental disturbances and function within the ecological community and increase in range. The same monitoring techniques can be applied to the translocated populations, however data will be analysed from a population dynamics perspective with which the milestones have much in common.

3.3.1 Monitoring season

Since *F. humilis* is dormant and cryptic during the winter months (Burgoyne *et al.*, 2000b), monitoring was restricted to the rainy season from November to February, occasionally extending to March and early April. Monitoring during the growth period also meant that plants and seedlings are more easily noticed and habitat disturbance can be kept to a minimum. Monitoring for flowering is, by necessity, conducted in the late season since *F. humilis*, like many other mesemb species, flowers towards the end of the growth period (Hammer, 1995).

Depending on prevailing conditions, monitoring for flowers even took place as late as early autumn.

3.3.2 Size classification system

The monitoring in this study continues with the methods used in the previous study by Harris (2014). Since the primary aim of the study is to determine whether translocation is a suitable conservation technique by tracking the changes in translocated populations of *F. humilis*, counting individual plants in patches is used to determine population density. Density is especially useful when monitoring for recruitment or loss of plants in a population and is most sensitive to these changes (Elzinga *et al.*, 1998). Density is not, however, sensitive to plant or population condition and is thus not suited to long-lived species such as trees. It is also not suitable for short lived plants such as annuals where population fluctuations may be significant. Since *F. humilis* is neither of these, density monitoring remains a suitable method for this species.

In addition to density, size classes determined by the number of leaves are used as a source of information concerning changes in population dynamics. Counting units often used for density include stem diameter, rooted density, number of stems or leaves or cover (Elzinga *et al.*, 1998). For *F. humilis* measurement of stem diameter is impossible since most of the plant is underground. Cover or area is also impractical since attempting to measure the area of seedlings or young plants would almost be impossible without a magnifying glass. Thus, the number of leaves is the most practical counting unit. Similar methods using the number of leaves as a size indicator have been used on other forbs (Witkovski & Liston, 1997).

For *F. humilis*, the definition of size classes was guided by preliminary *ex situ* studies by Glatz *et al.* (2012), which determined that plants with less than three leaves could be considered seedlings or juvenile and non-reproductive. Relative size classes are displayed in Table 3.4. Size class 3-5 is also considered reproductive since it can be seen as a transitional stage between juveniles and plants of a reproductive size. The remaining size classes are divided, as indicated, for the sake of practicality. The final size class was defined as >30 since counting more than 30 leaves increased the chances of miscounts and became impractical time wise.

Apart from counting density and population dynamics, additional data was recorded in the form of the number of flowers, seed capsules and damage or general health for individual plants. In doing so the qualitative data for the translocated populations can be provided in a way that density counts cannot. Judging the health of the plants based on the condition of the leaves is also more significant for *F. humilis* since leaves make up a far greater portion of the plant total biomass compared to that of a tree for example (Menges, 2008; Pavlik *et al.*, 1993).

Table 3.4 Relative size classes of *Frithia humilis* plants based on the number of leaves, and the reproductive capacity of each group (Harris, 2014).

Relative size class (number of leaves)	Life stage
<3	Seedling
3-5	Sub-adult (reproductive)
6-10	Adult
11-15	Adult
16-20	Adult
21-30	Adult
>30	Adult

3.3.3 Total counts

Initial total counts were conducted six months after the translocation in December 2009 and acted as a baseline against which the translocation disturbance could be measured. Unfortunately no numbers were recorded during the translocation, but as the plants were moved during their dormant season the first count provides a very accurate benchmark. It also acted as a record of abundance per size class and as a baseline for monitoring fluctuations in the age structure and fecundity of the translocated populations. Total counts were conducted according to the size class system for all *F. humilis* populations to record the initial abundance per size class and to monitor fluctuation in the age structure and reproductive capacity of the translocated populations (Harris, 2014).

For this study, total counts were again conducted for all translocated populations the 2015 monitoring period. The total counts were conducted at fixed points within the translocation patches and the control population. A metal grid of 1x1m was placed over each of the translocation patches at each receptor site to count the number of plants per 1m^2 quadrat. Control data were generated by counting the number of plants present in six randomly placed 1m^2 quadrats inside a naturally occurring population at Ezemvelo Nature Reserve. Permanent sampling points, for the purpose of consistency, were provided for the translocation patches in the form of screws drilled into the sandstone surrounding the patches. Plants were counted according to their size class in which they fell. Flowering and fruiting data was also recorded according to the size class of the plant. In total 17 quadrats were sampled at the translocation patches at Goedvertrouwdt, five at Eagle's Rock and three at Witbank Nature Reserve.

3.4 Entomological studies

The previous study on the pollination biology of *F. humilis* by Harris *et al.* (2016), determined that *F. humilis* is pollinated by generalist insect species. The purpose of this part of the study will be to supplement the existing knowledge concerning these pollinators. Observations were made at the Goedvertrouwdt as well as Eagle's Rock receptor sites. Methods will be described in detail in Chapter 4.

3.5 Habitat assessment

Simple on-site observations along with yearly photos were taken at fixed points and used to assess changes in habitat conditions for the extent of the monitoring period (Elzinga *et al.*, 1998). These assessments allow for the detection of significant changes in the translocation environment which may be negatively affecting *F. humilis* population health.

Translocation patches were scored on a scale from one to ten based on various habitat characteristics and changes over the monitoring period. A score of 1 was considered favourable and 10 unfavourable. These data were then analysed using non-metric multidimensional scaling (NMDS) to group patches according to their characteristics using PRIMER software version 6 (PRIMER-E, 2012). The score data were further analysed using principal component analysis (PCA) in Canoco version 5 (Ter Braak & Šmilauer, 2012) to establish which characteristics corresponded with specific translocation patches. These results were used to group translocation patches at the Goedvertrouwdt receptor site since significant micro-habitat differences were noticed.

3.6 Fecundity of translocated *F. humilis* populations

To determine the next two measures of translocation success, resilience and persistence, the fecundity of the translocated populations had to be assessed in terms of flowers, fruits, seedlings, sub-adults and adults for the entire study period and compared to the control population. Analysis of flowering and fruiting provide evidence of the populations' ability to sustain the later reproductive groups and whether this is occurring at a level similar to that of the control population.

Population data were assessed using a Linear Mixed Model (LMM) model in SPSS version 21 (IBM, 2012), which could compensate for the zero values recorded in the dataset. This method of analysis was chosen since the data did not have a normal distribution due to variation and a small population size (Harris, 2014). Furthermore, data collected in one year was dependant on data from the previous year (covariance), for which LMM analysis is more suitable. Methods will be described in detail in Chapter 5.

3.7 Demographic analysis

A healthy plant population should show a healthy monotonic decline with more seedlings and juveniles than adults (Botha *et al.*, 2004; Wiegand *et al.*, 2000). To assess the demographic health of the translocated populations the control population at the Ezemvelo Nature Reserve was analysed in terms of linear regression slope, permutation index, Simpson's dominance index and quotients. Excel version 14 (Microsoft, 2010) and XLSTAT Evaluation 18.06 (Addinsoft, 2016) were used for analysis. These results were used as a baseline against which to compare the translocated populations. Methods will be described in detail in Chapter 6.

CHAPTER 4

POLLINATION BIOLOGY OF ***FRITHIA HUMILIS***

4.1 Introduction

Interactions with pollinators and seed vectors are of vital importance to flowering plants. At least 67% of flowering plant species require insects as pollinators while the remaining portion is pollinated by birds or mammals (Kearns & Inouye, 1997). In addition, at least three quarters of the world's major crops are dependent on animal pollination (Abrol, 2012). Thus it is clear that threats to crucial pollination services, such as those provided by bees, has the potential to disrupt entire food chains (Mayer, 2004). Despite this, pollination systems experience increasing pressure from human activities both directly, through poisoning and habitat destruction, and indirectly through inadvertent introduction of invasive and displacing species (Kearns *et al.*, 1998). Consequently the significance of protecting these systems is only now being realised, while the pollination requirements of many wild plant species still remain unknown.

Such knowledge of pollination systems is crucial when conserving plants by means of restoration and translocation. A clear understanding of the species' pollination biology can be a determining factor as to whether the population is only just surviving or actually persisting (Montalvo *et al.*, 1997). Apart from merely understanding the pollination biology of a species, plant-pollinator interactions have to be conserved in the translocation process to ensure reproduction and to aid in the long-term success of such projects (García-Robledo, 2010). A poorly functioning pollination system can have several genetic consequences and may sooner or later affect population viability, eventually even leading to population extinction (Kearns & Inouye, 1997). These genetic problems include inbreeding, outbreeding, genetic drift and founder effects (Armstrong & Seddon, 2008; Montalvo *et al.*, 1997; Moritz, 1999).

To minimise such problems, translocation projects have to adequately address the challenges of a potentially inadequate habitat, as well as nutrient deficiencies which may affect plant and flower health and subsequently the occurrence and availability of pollen (Mayer, 2004). Reduced flowering can affect the number of pollinators that may visit a population. Reduced flowering might limit outcrossing in small populations, resulting in pollen transfer between relatives or not being pollinated at all (Kearns & Inouye, 1997). It is generally believed that larger populations are more attractive to pollinators resulting in higher visitation and pollination (Mustajärvi *et al.*, 2001). However, studies on population density and size provided varying results. A study by Bosch and Waser (1999) on *Delphinium nuttallianum* and *Aconitum columbianum,* both partially self-compatible, found that pollination was only weakly related to plant density. Conversely, in the fully self-compatible species, *Lychnis viscaria,* pollination and reproduction were more successful in sparse populations (Mustajärvi *et al.*, 2001), though these results may have been due to greater resource availability. They concluded that both population size and density had an effect on pollination and seed production, although the effect of density was greater. Similarly, for a self-incompatible species, *Brassica kaber*, density was found to have a strong influence on pollinator visits and reproductive success (Kunin, 1997), while the size of the population had little effect.

These varying results, whether for self-compatible and self-incompatible species, or different flowering methods, support the point made by Bosch and Waser (1999) that the effects of density on flower visitation, pollination and subsequent seed production are distinctive for individual species and their pollination systems. An example of this is the case of *Cheiridopsis imitans* and *Leipoldtia schultzei*, members of Aizoaceae (Pufal *et al.*, 2008). It has been hypothesised that in Namaqualand, there are too many flowers and too few pollinators. In other words the density is too high for the available pollinators resulting in many unpollinated flowers. Pufal *et al.* (2008), suggested that light grazing or adverse climate, while directly influencing flower numbers, increased pollination success and fruit set due to decreased density. The habits of pollinating species may also play an important role in successful pollination in the sense of movement patterns, consistency and degree of outcrossing (Harris & Johnson, 2004).

The type of relationship that a species has with its pollinator is the key to the species pollination biology. Plants which are pollinated by animals act either as generalists or specialists. Generalists attract a wide variety of different pollinators while specialists often have a relationship with a single species with which it may have co-evolved (Abrol, 2012). According to the "most effective pollinator principal" (Stebbin, 1970), if a pollinator is reliably available, specialisation will occur with the most effective and abundant pollinator. Conversely, if even the most effective pollinator is unreliable then a plant prefers generalisation. Then again, a-symmetric specialisation may also occur where several generalist pollinators pollinate a specialist species or various generalist and specialist pollinators pollinate a generalist species (Vázquez & Aizen, 2004). Various factors have been considered as influences of specialisation or generalisation such as the species breeding system, successional status, abundance and life history (Johnson & Steiner, 2000).

In addition to the type of relationship, the particular arthropod ('pollinator') species also needs to be identified. Pollination syndromes have been used for many years to predict the kind of pollinators that flowers attract. These syndromes are based on the phenotypical characteristics of a flower (Abrol, 2012; Fenster *et al.*, 2004). However, pollination syndromes are a controversial subject due to the evidence for and against their accuracy (Fenster *et al.*, 2004). Ollerton *et al.* (2009) for example concluded in a global study that the pollination syndrome hypothesis could not satisfactorily describe floral diversity or predict pollinators. Conversely, Momose *et al.* (1998) provided evidence for pollination syndromes of 270 flowering plant species. While floral syndromes may have developed to target specific pollinators, Struck (1995) pointed out that this is not necessarily exclusive and pollination may occur thanks to any one of the other pollinator classes. This suggests that some pollination syndromes may develop to maximise the chance of successful pollination by a specific species while still reserving the option for chance pollination by a less suitable species.

The actual purpose of pollination syndromes is to track patterns of convergent evolution between unrelated plants rather than to replace actual observations. However, the theoretical nature of pollination syndromes often leads to exactly this, with the result that potential pollinators may be overlooked because they do not conform to the specific syndrome (Johnson & Steiner, 2000). Symes *et al.* (2009) presented various South African examples, particularly concerning Aloes, which cast doubt over the effectivity of pollination syndromes as methods of identifying pollinators. For these reasons, Ollerton *et al.* (2009) suggested using pollination syndromes with caution.

Pollen-ovule ratios are also used as an indicator of potential pollinators among Aizoaceae. These ratios indicate the pollination efficiency and therefore, potentially, the pollinator as well (Mayer & Pufal, 2007). Generally if pollen-ovule ratios are high, a plant is more likely to be xenogamous, subsequently indicating the need for an animal breeding system (Jürgens & Witt, 2014). While Mayer and Pufal (2007) successfully used pollen-ovule ratios to identify the breeding system of four Aizoaceae species, Cruden (2000) suggested using additional floral traits when pollen-ovule ratios of xenogamous species are so low as to cause uncertainty.

For the family Aizoaceae, Hartmann (1991) defined four pollination syndromes namely anemophilous (various pollinators including masarid wasps), melittophilous (bees and butterflies), phalaenophilous (moths) and psychophilous (butterflies). Each syndrome corresponds to specific flower characteristics of openness, flower length, colour, pollen, nectarines, fragrance and time when open (Table 4.1). Insect morphology often corresponds with flower characteristics which can influence pollination efficiency. This is based on insect size, hairiness and feeding and foraging apparatus. Based on the correspondence between the flower and insect morphology various potential pollinators may be suggested. Numerous orchid-pollinator relationships between flowers and sweat bees or moths are good examples, a local case being *Disa uniflora* and its butterfly pollinator, *Meneris tulbaghia* (Bond & Johnson, 1992).

Table 4.1 **Pollination syndromes of Mesembryanthemaceae (Aizoaceae) (Harris *et al.*, 2016; Hartmann, 1991).**

Pollination syndrome	Pollinator	Pollination method	Flower traits	Opening time
Anemophilous	Wide array of pollinators, including masarid wasps		• Open • Pollen abundant dry and powdery	
Melittophilous	• Bees • Butterflies	• Crawling/walking over flowers • Insertion of proboscis/crawling into cone-like flowers	• Open (cone-like in some subtypes) • Pollen abundant dry and powdery • Hidden nectaries • Bright, shiny petals (yellow, purple, or white)	Biurnal (11:00-17:00)
Phalaenophilous	• Moths		• Fragrant • Tapering petal tips • Petals shades of white, even greenish (often yellow towards centre)	Nocturnal
Psychophilous	• Butterflies	Insertion of proboscis	• Long, narrow tube • Nectar at base of tube	

In comparison to the size of the family, reasonably little research has been done on the pollination biology of Aizoaceae (Zietsman, 2013) and information is mostly general rather than specific. Most Aizoaceae flowers attract a variety of pollinators, though specialisation is not unheard of (Hartmann, 1991; Liede & Hammer, 1990; Peter *et al.*, 2004). In Aizoaceae species that produce many small flowers with reduced petals and profuse amounts of pollen, it may imply that wind pollination occurs. However, this is doubtful and requires further investigation (Ihlenfeldt, 1994). Most Aizoaceae flowers are protandrous and self-sterile, while flowering is often *en masse* and synchronised within populations thereby promoting xenogamous pollination (Peter *et al.*, 2004; Ihlenfeldt, 1994). Plant populations of mixed Aizoaceae genera have even been observed to synchronise their flowering which could indicate a combined effort to attract pollinators (Groen & Van Der Maesen, 1999).

Most data concerning the pollination of Aizoaceae is restricted to winter rainfall regions of the western part of southern Africa with few examples from the summer rainfall region (Gess &

Gess, 2004a; Harris *et al.*, 2016; Peter *et al.*, 2004 Zietsman, 2013). Aizoaceae flowers usually last for about a week and are most receptive for pollen on the fourth or fifth day (Hammer, 1995). Furthermore, flower opening and closing usually occurs diurnally and during a specific time window. This is believed to be a mechanism to exclude generalist pollinators in favour of more specific insect species. Additionally, opening and closing of Aizoaceae flowers protects the pollen from moisture which has been shown to negatively affect pollen fertility (Hammer, 1995; Peter *et al.*, 2004). Documented pollinators of this family are not known to travel far though, thereby limiting pollen exchange between populations. However, it has been observed that even a single visit may be enough for pollination to occur (Mayer & Pufal, 2007).

Understanding the pollination biology of a plant species is an essential part of successful translocation since pollination is the onset of population growth and genetic exchange (Montalvo *et al.*, 1997). In cases where a species is translocated outside its pollinators' area of occurrence, these crucial processes are disrupted (Dixon, 2009). This has implications not only for the translocated plant species, but the pollinator as well. Specialist relationships are the first and most obvious to be affected. However, even generalists have to be considered when effects such as climate change and colony collapse (e.g. bees) can have implications for translocated plant species (Memmott *et al.*, 2007). Despite the importance of pollinators and the services they provide, pollination systems have not received the necessary attention in restoration and translocation projects (Forup *et al.*, 2008), the importance of which was highlighted by Harris *et al.* (2016) on the pollination biology of *F. humilis*.

4.1.1 Pollination of *Frithia humilis*

Frithia humilis is an endangered cryptic dwarf succulent species belonging to the family Aizoaceae (Raimondo *et al.*, 2009; Burgoyne *et al.*, 2000b). It is endemic to the Rand Highveld Grassland of Gauteng and Mpumalanga, specifically in an area between Bronkhortspruit, Ogies and Middelburg. This makes it one of only a few Aizoaceae species to be found in summer rainfall regions. *F. humilis* is restricted to a micro-habitat of flat sandstone plates of the Dwyka and Ecca formations where weathered and organic materials collect in sufficient amounts to sustain the plants (Burgoyne, 2001). The leaves of the species generally grow from beneath the soil surface, seldom protruding more than 30mm above ground level. Furthermore the leaves are contractile, allowing the plant to retract into the soil where it is protected from further desiccation and frost in the winter months (Burgoyne *et al.*, 2000a). Consequently the species is only visible during the summer months of growth and flowering.

The closest relatives of the genus *Frithia* are *Delosperma* and *Conophytum*, the most likely species being *Delospema deilanthoides* and *Conophytum limpidum* (Burgoyne *et al.*, 2000a). *D.*

deilanthoides is most similar to *Frithia* in its leaf structure and the lack of covering membranes in the capsules of both. However, differences are significant, making the two genera easily distinguishable. In the case of the other relative, which looks morphologically very similar due to both having windowed leaves and bladder cells, the floral structures of *C. limpidum* and *Frithia* are also similar, apart from the sepals which differ between the species.

Conophytums are generally self-sterile with only a few weakly self-fertile species. Two species are known to be cleistogamic while only one species, *C. rugosum,* is known to be diurnal and is frequently self-fertile (Liede & Hammer, 1990). In a study of 30 *Conophytum* species (not including *C. limpidum*), Jürgens and Witt (2014) found diurnal species to be visited mostly by pollen wasps belonging to the genus *Quartinia* (Vespidae: Masarinae).

However, taxonomical work by Chesselet *et al.* (2002), based on floral nectaries, placed the genus *Frithia* under Delospermeae, making it most closely related to *Bergeranthus*, *Delosperma* and *Stomatium* rather than *Conophytum* which is placed under Ruschieae. This makes the studies by Zietsman (2013) on the reproductive biology of *Stomatium bolusiae* and Peter *et al.*, (2004) on the pollination biology of *Bergeranthus multiceps* of importance to better understand *Frithia humilis* pollination systems.

S. bolusiae is self-incompatible and exhibits crepuscular and nocturnal anthesis. This nocturnal flowering is accompanied by fragrance and rewards, which indicated the species to firstly be phalaenophilous and secondly melittophilous, therefore making use of two pollination syndromes. Crepuscular pollinators were bees while nocturnal pollinators were found to be noctuid moths (*Spodoptera* sp., Lepidoptera: Noctuidae) (Zietsman, 2013).

B. multiceps is also self-incompatible and flowers between 15h30 and 18h30. Bees were determined to be the most important pollinator to this species, placing it in the melittophilous syndrome. Other potential pollinators included butterflies, bees and hover flies belonging to the families Bombyliidae, Tachinidae and Syrphidae (Peter *et al.*, 2004).

4.1.2 Flowers of *Frithia humilis*

The self-incompatible flowers of *F. humilis* are about 15 to 20 mm in diameter and white with a yellow centre (Figure 4.1). The petals, especially toward the tip, are occasionally tinged pink.Flowers are carried on very short stalks or are entirely stalkless. The pollen (
Figure 4.3) is yellow and tricolpate (a pollen grain with three colpi or pollen apertures) (Furness & Rudall, 2004). The sepals are similar in appearance to the leaves and form a short tube (Burgoyne, 2001; Burgoyne *et al.*, 2000b). After pollination the flowers turn yellow or pink before

expiring, which may serve as an indication to pollinators that the flower is pollinated and without reward (Burgoyne *et al.*, 2000a).

Based on descriptions provided by Hartman (1991), *F. humilis* flowers could belong to the Melittophilous syndrome since they are open, petals are bright, shiny, white-ish and open diurnally (mid-morning to mid-afternoon) for several days. Pollen is also less abundant than in other melittophilous species, but is still easily accessible.

Other support for this syndrome include the sexual phase of flowers belonging the Melittophilous syndrome – these flowers are reported to have distinct male phases followed by female phases approximately four days later (Hammer, 1995; Hartmann, 1991). Stigmata are initially shorter than stamens, only elongating and emerging at the beginning of the female phase when the male phase comes to an end. This observation was supported by microscopic inspection of *F. humilis* flowers where no stigma was found at all (A. Jordaan, pers. com.). This suggests that the flowers submitted for inspection were so fresh that the stigma had not yet developed.

Furthermore, Hammer (1995) described hand-pollinating *Frithia*s as “dipping for water in a deep well”. This, along with the description of a tube formed by the sepals, further supports the proposed melittophilous pollination syndrome since flowers belonging to this syndrome have hidden nectaries.

A recent study conducted by Harris *et al.* (2016) provided initial evidence that *F. humilis* has a generalist plant-pollinator relationship with the insect families Bombyliidae (Diptera), Megachilidae and Apidae (Hymenoptera), with the mostly likely efficient pollinator being *Megachile niveofasciata*.

The aim of this chapter is to supplement the existing knowledge concerning the pollinators of *F. humilis.* Such information is valuable to understand whether seed formation can be initiated within the translocated populations. This study will further the observations of pollinators made by Harris *et al.* (2016) at Goedvertrouwdt and Eagle’s Rock sites.

Figure 4.1 ***Frithia humilis*** **flowers displaying the difference between a fresh (left) and an expired flower (right).**

4.2 Materials and methods

4.2.1 Insect observations

Qualitative observations for pollinators were made at the receptor sites of Goedvertrouwdt Farm and Eagle's Rock, applying the hand net method for capturing insects (Figure 4.2). This method was chosen since Harris *et al.* (2016) concluded that general quantitative surveys did not yield satisfactory results and that surveys should have a targeted approach. One observation session was performed per day for each population. The two patches with the most flowers were chosen at each site for observation. At the Goedvertrouwdt site one person per patch observed for pollinators for the entire period of anthesis from 11:30 am to 15:00 pm, thus seven hours of observation were conducted for this site. At the Eagle's Rock site observations were again made by one person per patch. However, inclement weather in the morning reduced the observation time by two hours. Observations were consequently made from 12:30 to 15:00, totalling five hours of observation. Table 4.2 summarises the sites at which observations were made as well as the mean number of flowers and number of insects captured since 2011.

Insects alighting on a flower or foraging on flowers were captured using a hand net and euthanized in a killing jar with ethyl acetate. These specimens were then stored in individual marked containers for later inspection and identification.

Table 4.2 Sites, mean number of flowers, hours of observation and flower visitors (and species) collected in this and the previous study by Harris *et al.*, (2016).

Source	Study site	Year	Mean number of plants (per 1 m²)	Observation time (per site)	Hours of observation	Number of flower visitors (ssp.) (per site)
Harris *et al.*, (2016)	Eagle's Rock	2011	51	Two persons making observations from 11:00-14:00 for 2011 and 2012	12	2(1)
		2012	11			
	Goedvertrouwdt	2011	40		12	12(4)
		2012	9			
	Ezemvelo (Control)	2011	119		12	28(5)
		2012	183			
This Study	Eagle's Rock	2016	14	12:30-15:00	5	4(3)
	Goedvertrouwdt	2016	11	11:30-15:00	7	4(3)

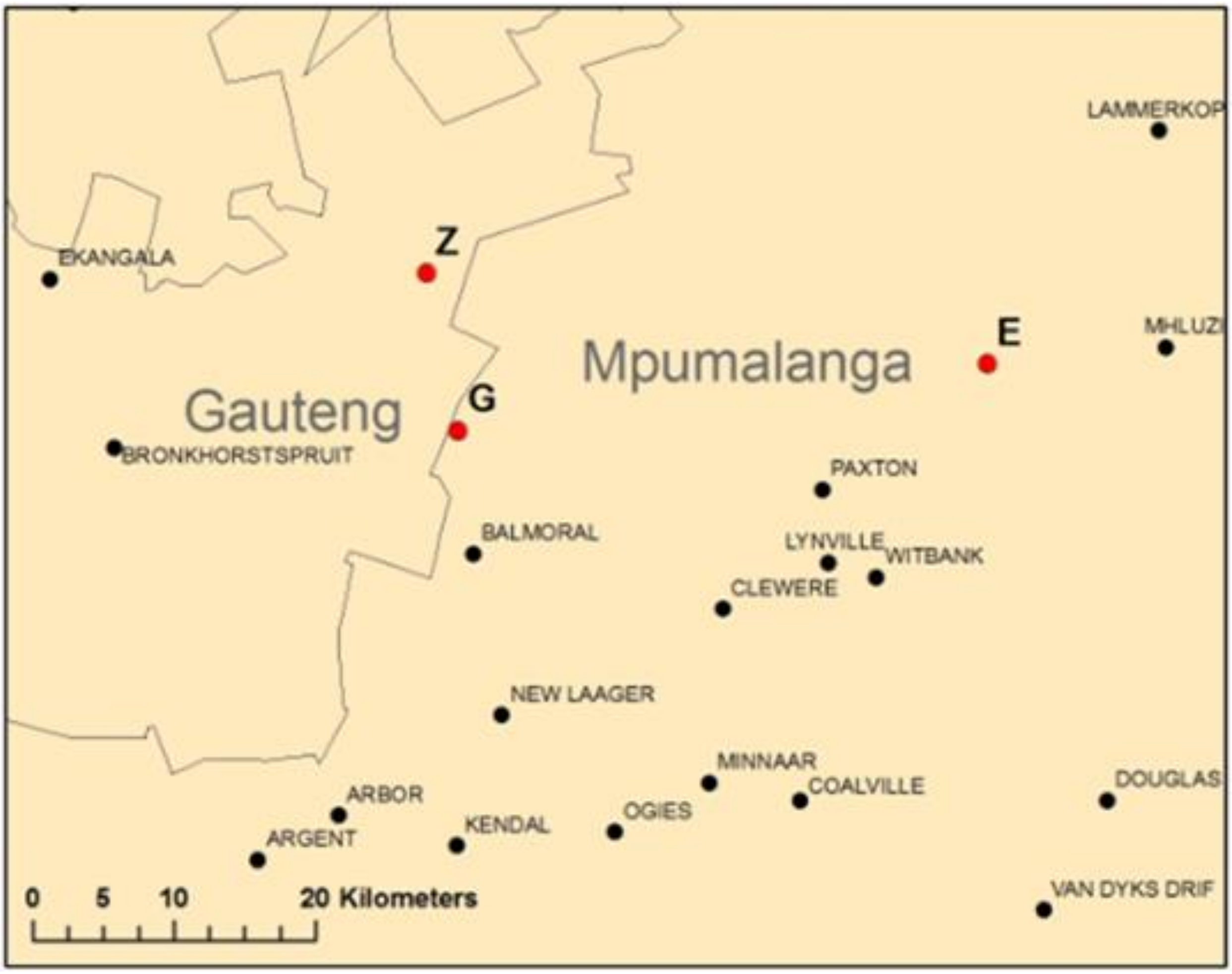

Figure 4.2 **Locations of receptors sites and the control population (G: Goedvertrouwdt; E: Eagle's Rock; Z: Ezemvelo control population) (Adapted from Harris *et al.*, 2016).**

4.2.2 Verification of pollen presence and insect identification

All captured insects were scanned for pollen. Photos were taken of the insects using a Nikon AZ1000 stereomicroscope to assist in identification and to verify whether any pollen was present on the specimens. Particular attention was paid to the head and legs where pollen was most likely to be found. Those potentially carrying *F. humilis* pollen were then inspected under a FEI Quanta FEG 250 scanning electron microscope (SEM) and micrographs taken of relevant pollen loads. All insects were coated with a gold-palladium alloy before micrographs were taken. Insects were then pinned and labelled as prescribed by Uys and Urban (2006) and submitted to the Biosystematics Division of the Plant Protection Research Institute of the Agricultural Research Council (ARC) for identification.

Sample micrographs of *F. humilis* pollen were taken using SEM (Figure 4.3) for verification against the pollen depicted in Burgoyne *et al.* (2000b). Upon verification, the pollen was compared with pollen found on the insect specimens. Investigation for pollen under the SEM was performed before species identification to prevent any possible loss of pollen from the specimens due to handling and transport. For this reason the specimens were not in their best condition for identification.

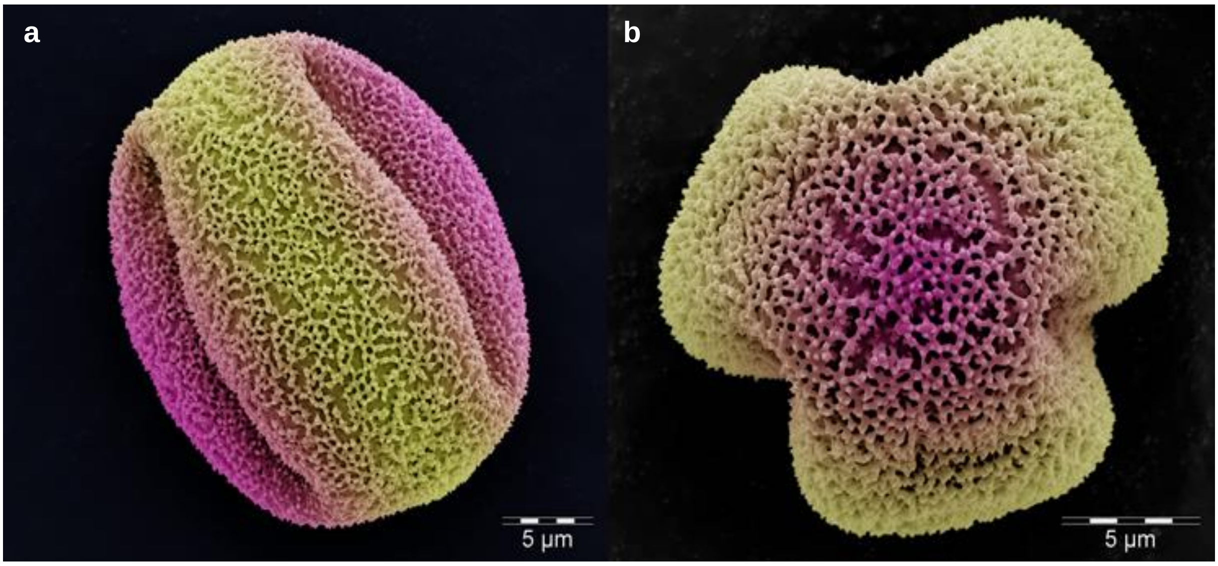

Figure 4.3 Scanning electron microscope micrographs of *Frithia humilis* pollen grains taken from different angles (a & b).

4.3 Results

Observations were possible at the Goedvertrouwd site for the entire period of anthesis, though a moderate wind was blowing. Flower anthesis at Goedvertrouwd was observed to be between 11:30 and 15:00, rather than between 11:00 and 14:00 (Harris *et al.*, 2016). Flowers opened somewhat later than suggested by Harris *et al.* (2016), possibly due to poor weather conditions. However, the time that flowers remained open, was longer than suggested, continuing for another hour before closing. Four insect specimens were observed on *F. humilis* flowers at Goedvertrouwdt and captured. Two species of butterfly were also observed to make quick, darting visits to flowers; however, it was not possible to capture these.

Poor weather at the Eagle's Rock site reduced the duration of observation time. Despite this, four insect specimens were observed to land and forage in *F. humilis* flowers and were subsequently captured.

A total of eight insects were captured comprising six different species. Four species of Hymenoptera and two species of Diptera were collected. This study adds six new species to the list of *F. humilis* visitors first observed by Harris *et al.* (2016) (Table 4.3).

4.3.1 Insect identification

Insect identification was done with the assistance of specialists from various international institutions (Table 4.4). Identification to species level was hindered by the taxonomic impediment whereby large numbers of species still require classification or have gone undiscovered (Eardley, 2016; Gess, 2016, pers. com.).

For the Goedvertrouwd site two female sweat bees (*Seladonea* sp., Halictidae), along with a thread-waisted wasp (*Ammophila* sp., Sphecidae) and a bee fly (*Notolomatia* sp., Bombyliidae) were identified as flower visitors. For the Eagle's Rock, site a female sweat bee (*Lipotriches* sp., Halictidae) was captured, along with two pollen wasps (*Quartinia* sp., Vespidae) and a hover fly (*Paragus* sp., Syrphidae) (Eardley, 2016; Gess, 2016; Jordaens, 2016; Evenhuis, 2016, pers. comm.).

Table 4.3 Flower visitors observed by Harris *et al.* (2016) and newly reported by this study.

Order	Family	Species	Common name
Flower visitors observed by Harris *et al.* (2016)			
Diptera	Bombyliidae	*Exoprosopa eluta* (Loew)	Bee fly
Hymenoptera	Apidae	*Amegilla fallax* (Smith)	Banden bee
	Halictidae	*Lipotriches* sp.	Sweat bee
	Megachilidae	*Megachile niveofasciata* (Friese)	Dauber bee
Lepidoptera	Hesperiidae	*Platylesches ayresii* (Trimen) *P. moritili* (Wallengren)	Pepper hopper Honey hopper
Newly observed flower visitors			
Diptera	Bombyliidae	*Notolomatia* sp.	Bee fly
	Syrphidae	*Paragus* sp.	Hover Fly
Hymenoptera	Sphecidae	*Ammophila* sp	Thread-waisted wasp
	Halictidae	*Lipotriches* sp. *Seladonea* sp.	Sweat bee Sweat bee
	Vespidae	*Quartinia* sp.	Pollen wasp

Table 4.4 List of captured species and identification by specialists.

Family	Genus	ID contributor	Institute	Date
Bombylyiidae	*Notolomatia* sp.	Evenhuis, N.L.	Bishop Museum, Honolulu, Hawaii, USA	8 June 2016
Halictidae	*Lipotriches* sp.	Eardly, C.	Agricultural Research Council, Pretoria, South Africa	31 May 2016
Halictidae	*Seladonea* sp.	Eardly, C.	Agricultural Research Council, Pretoria, South Africa	31 May 2016
Syrphidae	*Paragus* sp.	Jordaens, K.	Royal Museum for Central Africa, Tervuren, Belgium	8 June 2016
Sphecidae	*Ammophila* sp.	Eardly, C.	Agricultural Research Council, Pretoria, South Africa	31 May 2016
Vespidae	*Quartinia* sp.	Gess, S.K.	Albany Museum, Grahamstown, South Africa	31 May 2016

4.3.2 Presence of pollen

During SEM inspection, pollen was found in varying amounts on the insect specimens, indicating these to be pollen carriers and potential pollinators (Table 4.5). Some pollen grains were visible on the *Notolomatia* sp. (Figure 4.4), on and immediately adjacent to the mouth parts. On the *Paragus* sp. (Figure 4.5), only three grains of pollen were found on the abdomen. However, a significant clump of pollen was found directly on the anus of the specimen. Pollen found on the *Quartinia* sp. (Figure 4.6) was very sparsely distributed over its body. Significant amounts of pollen were found on the underside of the mouth parts of the *Ammophila* sp. (Figure 4.7). All three Halictidae specimens (*Seladonea* sp. and *Lipotriches* sp.) (Figure 4.8; Figure 4.9; and Figure 4.10) were carrying copious amounts of pollen, particularly in their pollen baskets.

Table 4.5 List of species with the location and load of pollen on each.

Family	Species	Pollen placement	Pollen load
Bombyliidae	*Exoprosopa eluta*	Eye and head cavity	Low
	Notolomatia sp.	Proboscis	Low
Halictidae	*Lipotriches* sp.	Pollen baskets and legs	High
	Seladonea sp.	Pollen basket and legs	High
Apidae	*Amegilla fallax*	Head, legs and eye	Medium
Megachilidae	*Megachile niveofasciata*	Abdomen	High
Sphecidae	*Ammophila* sp.	Mouth parts	Medium
Syrphidae	*Paragus* sp.	Body and anus	Low
Vespidae	*Quartinia* sp.	Head, body and legs	Low

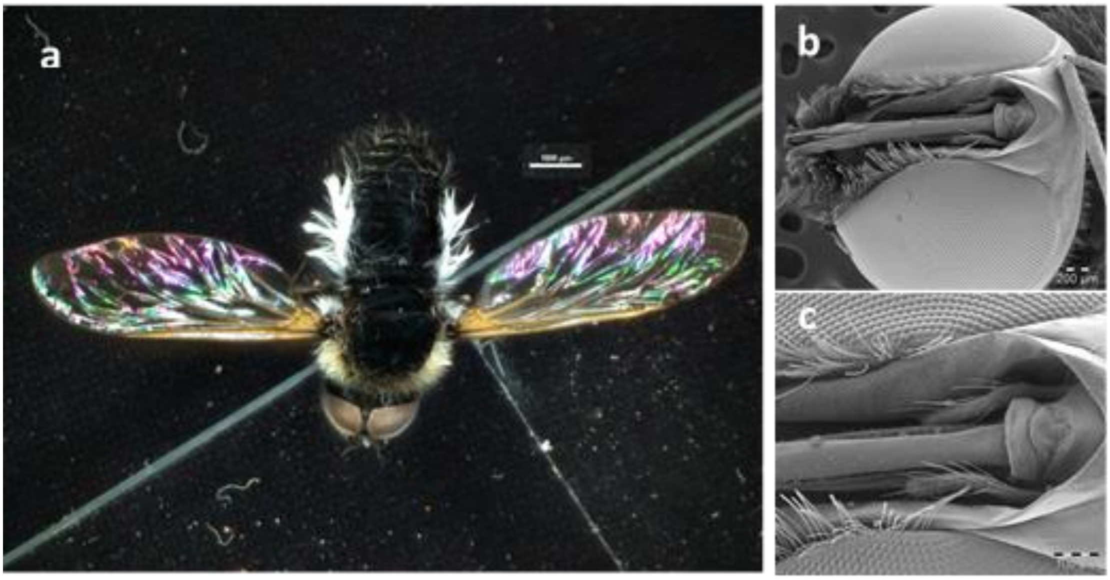

Figure 4.4 Stereomicroscope photograph of ***Notolomatia*** **sp. (Bobyliidae) (a). Scanning electron microscope micrographs of several *Frithia humilis* pollen grains on the head (b) and the mouthparts or proboscis (c).**

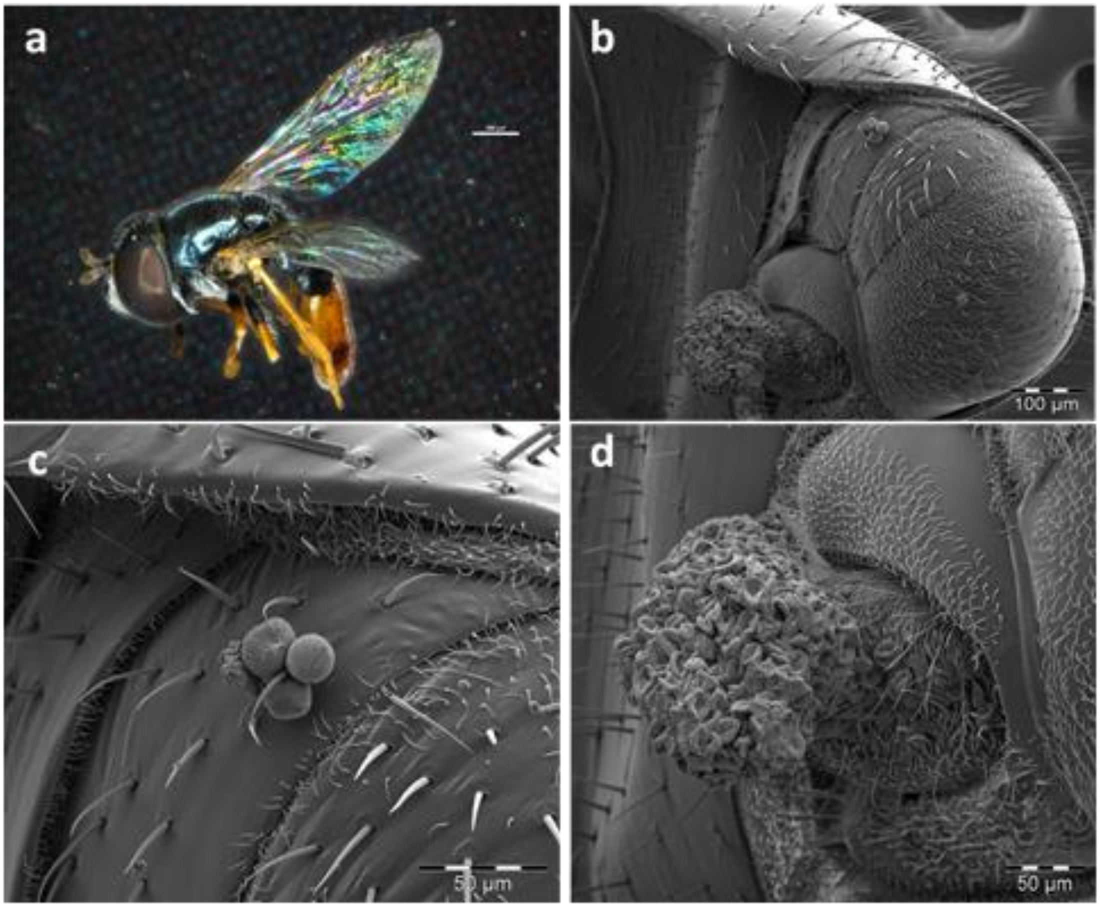

Figure 4.5 Stereomicroscope photograph of ***Paragus*** **sp. (Syrphidae) (a). Scanning electron microscope micrographs of *Frithia humilis* pollen grains on the body and anus (b), on the body (c) on the anus(d).**

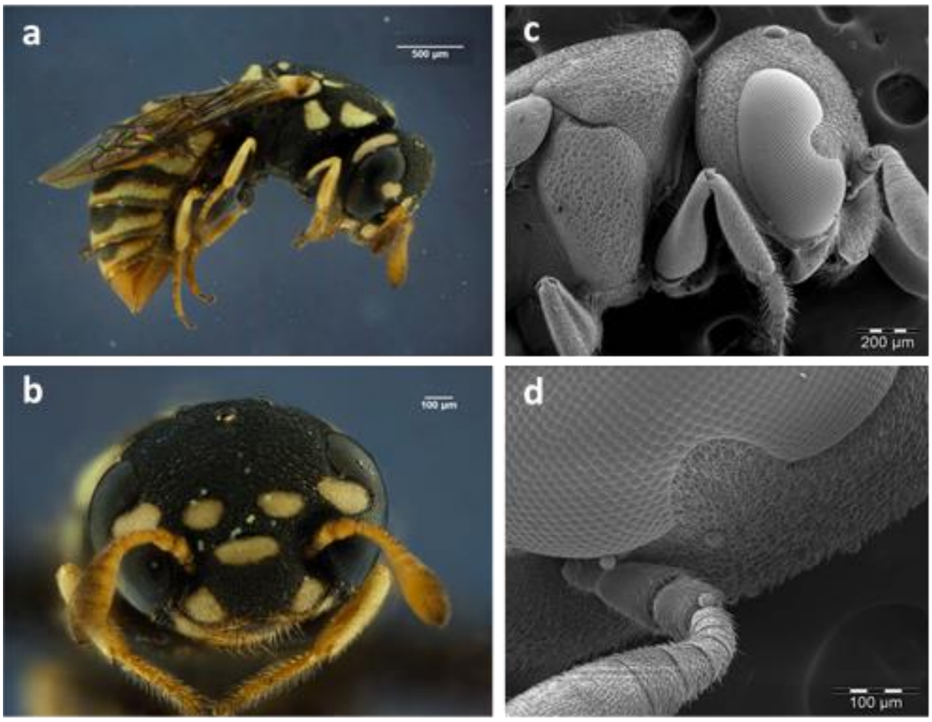

Figure 4.6 Stereomicroscope photograph of ***Quartinia*** **sp. (Vespidae) (a, b). Scanning electron microscope micrographs of several *Frithia humilis* pollen grains on the body (c) and the head and antenna (d).**

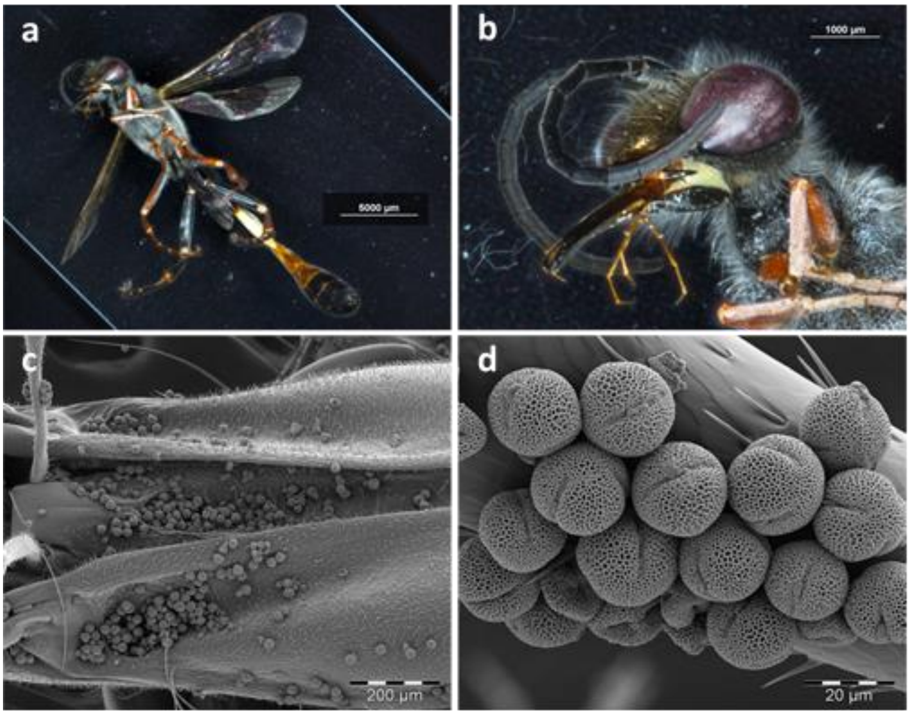

Figure 4.7 Stereomicroscope photograph of ***Ammophila*** **sp. (Sphecidae) (a, b). Scanning electron microscope micrographs of *Frithia humilis* pollen grains on the underside of the mouthparts (c) and on a maxillary palp (d).**

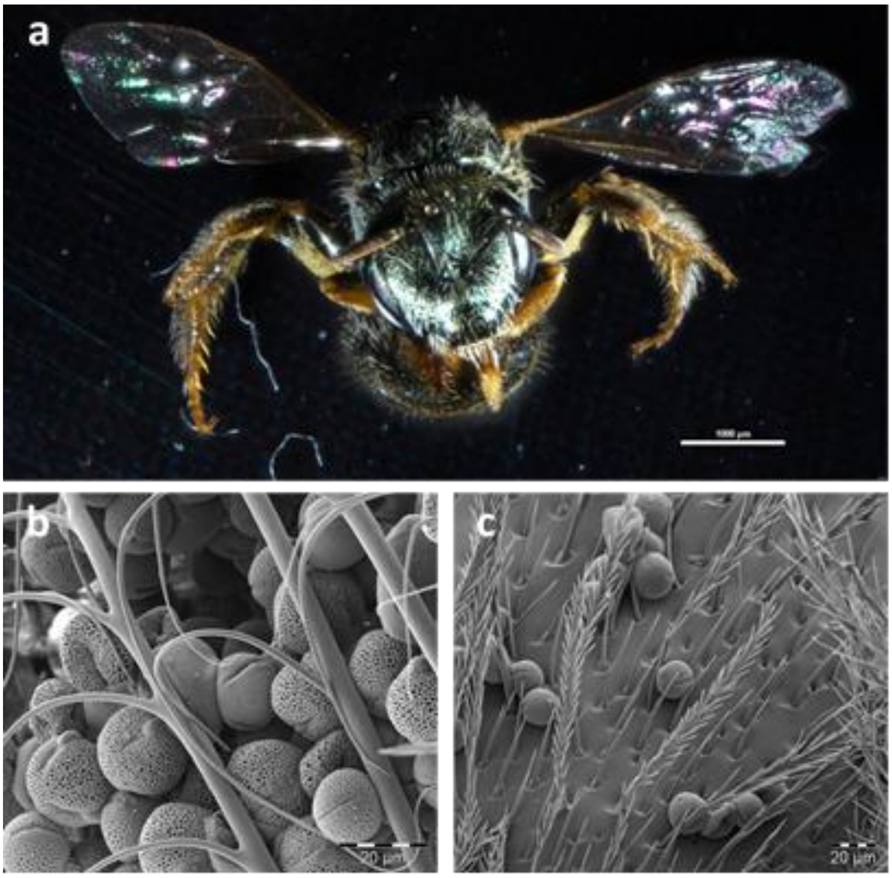

Figure 4.8 Stereomicroscope photograph of ***Seladonea*** **sp. (Halictidae) (a). Scanning electron microscope micrographs of *Frithia humilis* pollen grains in a pollen basket (b) and on the body (c).**

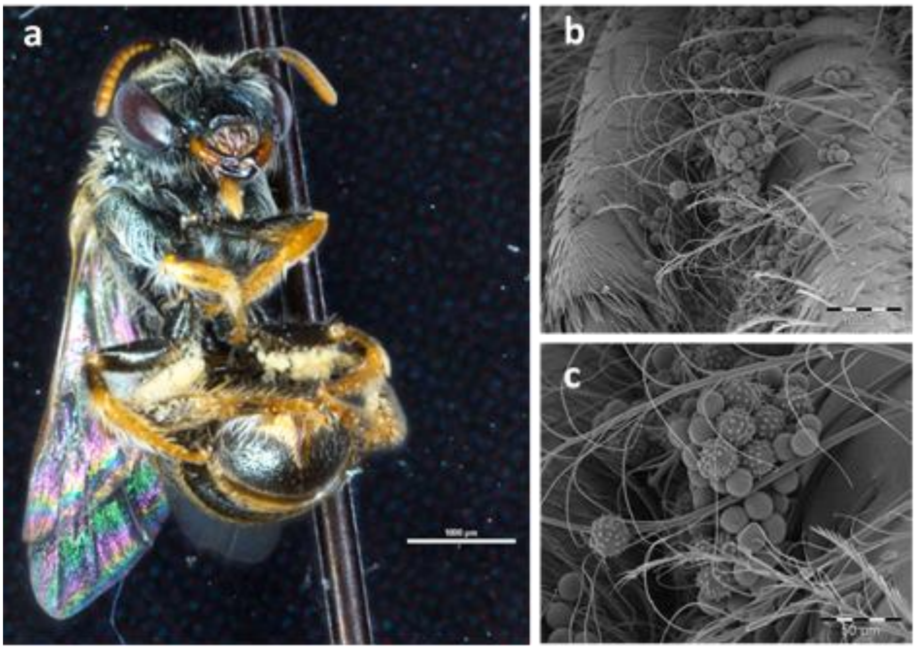

Figure 4.9 Stereomicroscope photograph of ***Seladonea*** **sp. (Halictidae) (a). Scanning electron microscope micrographs of *Frithia humilis* pollen grains in the pollen baskets (b, c).**

Figure 4.10 Stereomicroscope photograph of *Lipotriches* sp. (Halictidae) (a, b). Scanning electron microscope micrographs of *Frithia humilis* pollen grains in the pollen baskets (c, d).

4.3.3 Pollination system

The two translocation sites and the control population are situated in an area of approximately 200 km^2 in size. Within this area, three bee species (*Amegilla fallax*, *Lipotriches* sp. and *Megachile niveofasciata*) were recorded from at least two of the sites and a bee fly species, *Exoprosopa eluta*, was recorded in 33 flower visits (83% of all observations) from all three sites (Fig. 4.11). This indicates that the pollination system of *F. humilis* is dominated by generalist bee pollinators that carry medium to high loads of pollen within sites. Although the bee fly is a more regular flower visitor, its pollen load is low. At Goedvertrouwdt and Eagle Rock Estate reserve pollinators were observed. These pollinators are mostly flies and wasps, and have low pollen loads. These five species account for 17% of flower visitations.

4.3.4 Pollination success

Table 4.6 indicates the number of flowers, fruits and seedlings as a percentage of adults for 2012. This is three years after translocation when it was likely that the majority of seedling no longer originated from the seed bank but rather from recent seed production. For Z this data shows that a high percentage of plants bear flowers, yet a significantly smaller percentage of fruits are produced. At the receptor sites there are lower numbers of flowers per 1 m^2, as well as fewer flowers as a percentage of each population. Despite this, the percentage of fruit production at the receptor sites is considerably higher than that of the control population. However, it is interesting that a high percentage of seedlings are only produced at G and Z for the number of seeds produced. All of the above indicate that successful pollination is taking place.

Table 4.6 Mean number of plants, flowers, fruits, seeds and seedlings per 1m^2 for all receptor sites and the control, as well as the flower, fruit and seedling percentage of plants for 2012.

Study site		Feb-2012				Dec-2012
		Plants	Flowers	Fruits	Seeds	Seedlings
G	Mean	66.9	8.9	3.3	462	67.6
	%		13.3	37.1		14.6
E	Mean	55.8	11.4	3.4	476	10
	%		20.4	29.8		2.1
W	Mean	12.7	5.7	2	280	5
	%		44.9	35.1		1.8
Z	Mean	433.7	214.8	9	1260	191.3
	%		49.5	4.2		15.2

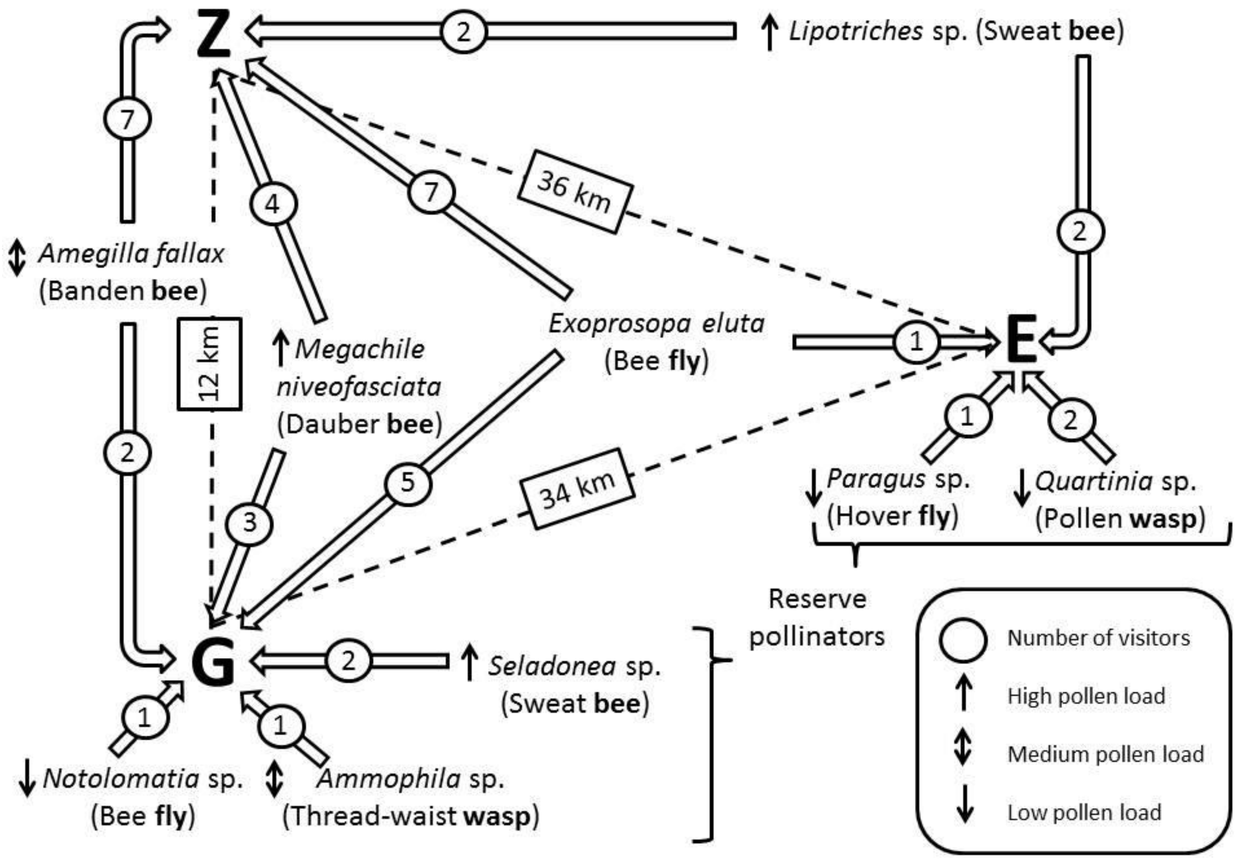

Figure 4.11 Pollination system and reserve pollinators of translocated *Frithia humilis* populations (G; E) in relation to a control population (Z). G, Goedvertrouwdt farm; E, Eagle Rock; Z, Ezemvelo Nature Reserve.

4.4 Discussion

4.4.1 Flower visitor and pollen carriers

The family Bombyliidae, including the genera Exoprosopa and *Notolomatia*, are primarily Afrotropical in occurrence (Greathead & Evenhuis, 2001). Larvae are either predators or parasitoids, while adults in most genera are adapted for nectar feeding. Females also feed on pollen for reproductive purposes and many have special adaptations for the collection of pollen (Greathead *et al.*, 2006). Bombyliids are most active during periods of sunshine which corresponds with the flowering time of *F. humilis*. Little research has been done on their role as pollinators; however it is believed that most are generalists (Szucsich and Krenn, 2002). Despite this, specialised relationships are known (Johnson & Steiner, 1997). The small amount of pollen on this *Notolomatia* specimen could be a consequence of the species (more or less interested in pollen than others), the gender of the specimen (males may not be as interested in pollen, if at all) and by the fact that the specimen was captured as soon as it landed on the flower, thereby shortening its potential visitation time.

Halictidae is the second largest family of bees with over 3500 species, many of which are pollen specialists possessing special adaptations for collecting nectar and or pollen from a few closely related plants (Danforth *et al.*, 2008). Non-apis bees in southern Africa visit numerous plant families, however Aizoaceae is amongst the top four plant families most visited, along with Asteraceae, Fabaceae and Zygophyllaceae (Gess & Gess, 2004a). While as many as 13 different plant families were visited by any one species, preferences, apparent in visitation records, were reported by Gess and Gess (2004a). *Seladonea* sp. for example, seemed to prefer Asteraceae compared to other families. However when considering the species richness and diversity of the Halictidae along with their polylectic manner of feeding it can be concluded with a reasonable degree of certainty that they are important pollinators for many seed bearing plants (Dikmen, 2007). All three of the collected specimens were observed to carry out typical pollen collection behaviour, thereby accounting for the copious amounts of pollen observed on their bodies and in their pollen baskets.

Ammophila is a genus of thread-waisted wasps in the Sphecidae family, and well known to be parasitic and predatory in nature. Spechids in general, feed on a variety of food types including nectar, honeydew and the bodily fluids of their prey. Genera with short tongues seek nectar from flowers with short corollas. Such flowers come from the families Apiaceae, Asteraceae, Euphorbiaceae and Polygonaceae. *Ammophila*, along with *Podalonia*, are two genera which have longer mouthparts thereby enabling feeding from a greater variety of flowers. Flowers recorded to be visited by *Ammophila* include *Lavandula latifolia* (Lamiaceae), *Deverra aphylla* (Apiaceae) and *Monechma* sp. (Acanthaceae) (Gess & Gess, 1991; Herrera, 1989; Weaving, 1989). Preferences for specific flowers are not unknown amongst genera of the Sphecidae, however such relationships are not as strong as in bees or masarids (Bohart & Menke, 1976). The captured *Ammophila* specimen was observed to make precise efforts to probe nectaries within the *F. humilis* flower by circumnavigating the flower in such a way that ensured contact between the anthers and the underside of its mouth parts where pollen grains were found.

All species of pollen wasp, including those belonging to the genus *Quartinia*, are nectar feeders and pollen collectors (Gess & Gess, 2010). Of the 40 species recorded visiting flowers, 75% of these were visits to Aizoaceae. Amongst the 86 described species, 13 show a marked preference for Aizoaceae. This plant family, along with Asteraceae, are the most important to *Quartinia* in terms of resources and numerous specialised relationships have been noted. *Quartinia* are generally considered to be an effective potential pollinator for many Aizoaceae species (Gess & Gess, 2010; Gess & Gess, 2004b). During the survey, one of the *Quartinia* specimens continued to forage in the *F. humilis* flower even after the hand net had been placed over it and the flower. As soon as the hand net was placed over the other specimen it left the flower, suggesting that this interruption may be the reason that no pollen was found on it. Even though little pollen was found on one of specimens, the fact that all species of *Quartinia* are pollen feeders and collectors along with the existence of close relationships, indicate that this genus may be an efficient reserve pollinator of *F. humilis* (Gess & Gess, 2010).

The larvae of the genus *Paragus* are recognised aphid predators (Hayat & Claussen, 1997), while adults are nectar feeders. Females also consume pollen since it is a rich protein source necessary for reproduction (Haslett, 1989). Pollen that is consumed and digested by all syrphids still retains its shape after passing through the gut making identification possible (Holloway, 1976). This explains the large cluster of pollen, including that of *F. humilis*, found directly on the anus of the specimen. The specimen was also not allowed much foraging time, to ensure its capture, but the pollen cluster on the anus suggests that it had been in the vicinity of *F. humilis* flowers and recently fed on pollen, perhaps the previous meal.

Though few specimens were captured during this study, these new visitor species support the hypothesis presented by Hartmann (1991) that *F. humilis* belongs to the Melittophilous syndrome, thus being pollinated mostly by bees and bee like pollinators. The Pollen Wasp Syndrome, while not as broad, falls within the Mellitophilous syndrome (Gess & Gess, 2004b), and accounts for the visits from the *Quartinia* species. Since the captured specimens are spread across numerous genera and the only similar genus captured in this and the previous study is *Lipotriches* sp., and the *Lipotriches* sp. specimen from the previous study was not found to be carrying any pollen (Harris *et al.*, 2016), the possibility of a primary pollinator is unlikely. While some of the specimens captured partially confirm the presence of similar pollinators at the different sites, the additional observations of flies and the *Ammophila* wasp make the theories of the Anemophilous syndrome and of “reserve” pollinators possible (Johnson *et al.*, 2009) plausible alternatives to the standing hypothesis of the melittophilous syndrome suggested by Hartmann (1991). In addition, the variety of insects support the suggestion made by Harris *et al.* (2016) that *F. humilis* has a broad spectrum of pollinators due to the significant distances between populations.

The distribution of the various insect genera and species amongst the different study sites in the past and present study is summarised in Table 4.7.

Even though specimens might have been shown to carry *F. humilis* pollen, it does not conclusively prove that any of these species are pollinators. Gess and Gess (2010) stress the difference between a potential pollinator and an actual pollinator, highlighting how pollen carrying insects are often automatically and incorrectly considered to be pollinators.

Table 4.7 Presence of potential pollinators at three *Frithia humilis* localities.

Location	Genus & species	Specimens observed
Goedvertrouwd	*Amegilla fallax (*Smith*)*	2
	Ammophila sp.	1
	*Exoprosopa eluta (*Loew*)*	5
	Megachile niveofasciata (Friese)	3
	Notolomatia sp.	1
	Platylesches ayresii (Trimen)	2
	Seladonea sp.	2
Eagle's Rock	*Exoprosopa eluta* (Loew)	1
	Lipotriches sp.	2
	Paragus sp.	1
	Quartinia sp.	2
Ezemvelo (Control)	*Amegilla fallax* (Smith)	7
	Exoprosopa eluta (Loew)	7
	Megachile niveofasciata (Friese)	4
	Lipotriches sp.	2
	Platylesches moritili (Wallengren)	8

4.4.2 Pollination system

Identifying a primary pollinator depends on the pollination efficiency of the species, which is defined as the amount of con-specific pollen transferred to a stigma in any one visit (Inouye *et al.*, 1994). However, determining pollination efficiency is problematic since pollination is a highly variable ecological interaction and can be influenced by factors such as pollinator species composition, species abundance across years, flower visit duration and frequency, contact with flower parts as well as pollen removal and deposition amongst others (Ivey *et al.*, 2003). Furthermore, an increasing number of studies have shown that visiting species vary in their effectiveness in transferring pollen (Adler & Irwin, 2006). For example, bees were revealed to be more effective pollinators than butterflies for the butterfly-weed, *Asclepias tuberosa* (Fishbein & Venable, 1996). However, even amongst bees, pollination affectivity varied and was not predicted by the size of the bee species as expected (Adler & Irwin, 2006). In addition to this, the amount of pollen on bees' bodies was also found to be an unreliable determinant of efficiency. Thus it was suggested that other species-specific morphological factors may be more important than previously thought. Despite this, Herrera (1987) found that, out of 29 insect species from three orders, Hymenoptera had high pollinator efficiency for *Lavandula latifoli.* Similarly five taxa of Hymenoptera were responsible for 99 percent of fruit set in a neotropical herb, compared to four taxa of Lepidoptera which were more common visitors but less effective pollinators (Schemske & Horvitz, 1984). Hymenopteran insects were found to be the primary pollinator for the generalist plant, *Asclepias incarnata* (Ivey *et al.*, 2003). However, *A. incarnate* was found to make use of an extensive variety of potential pollinators, none of which were crucial for reproduction. The variety of pollinators showed geographical differentiation as well, further reducing the chances for specialisation to occur.

With this in mind, the following species are proposed as primary pollinators of *F. humilis*: *Amegilla fallax, Megachile niveofasciata*, *Lipotriches* sp. and *Exoprosopa eluta,* thus mostly bees. While pollen load may influence the efficiency of pollination amongst bees, the difference in pollen loads between bees and the other *F. humilis* visitors was significant and evident on the micrographs, supporting the perception that bees are primary pollinators. The number of observations (which could be seen as visitation frequency) is also in favour of bees compared to the various other species (Table 4.5). Only one generalist pollinator, *Exoprosopa eluta*, was observed at the new sites (G and E) and is likely not an efficient pollinator due to the limited amount of pollen found on the eye (Harris *et al.*, 2016). This increases the possibility that this species is only a reserve pollinator despite the number of observations at the two sites.

The remaining species are suggested reserve pollinators (Table 4.5), based on the number of observations and their occurrence at only the new sites, G or E. These are primarily wasp and fly species. *Seladonea* sp. may also, however, be a primary pollinator due to high observed pollen loads, despite only two observations. While bees may be primary pollinators, the small size and isolation of *F. humilis* populations supports the idea that *F. humilis* is a generalist rather than specialist, relying on reserve pollinators as well, as is the case with *A. incarnata*.

4.4.3 Pollination efficiency

Results have indicated that pollination is more successful at the receptor sites than the control population, since a higher percentage of flowers are producing fruits. This situation may be similar to the results found by Mayer and Pufal (unpublished data) that flowers at a lower density were more likely to be pollinated than flowers at a higher density. This observation and the pollination results in this chapter could be explained by the hypothesis for pollination in Namaqualand, which states that flower density is too high for the number of available pollinators (Pufal *et al.*, 2008).

Despite high fruit and seed production, only G has shown a percentage of seedlings similar to Z. For the percentage of fruits and number of seeds produced, W and E have shown very low percentages of seedlings for their populations. This may indicate seed predation or habitat conditions which do not support seed germination and seedling development. This is further investigated in subsequent chapters of this study.

4.5 Conclusion

These latest findings build on the previous research concerning the pollinators of *F. humilis* presented by Harris *et al.* (2016). Six new species of insect have been added to the existing list of species observed to visit *F. humilis* flowers, extending this list to 11. All six of the new species have been identified as potential pollinators. Including those identified in the previous study, the list is now extended to nine potential pollinators.

The addition of newly observed pollen wasps as well as bee species supports the standing Melittophilous pollination syndrome. However the observation of two fly species and a species of thread-waisted wasp supports the Anemophilous pollination syndrome and the possibility of 'reserve' pollinators. The increased variety of visiting insects and potential pollinators is encouraging for the pollination biology of *F. humilis* at translocated populations.

Several primary pollinators have been suggested, most of which are bee species. However, several factors make it unlikely that *F. humilis* has a specialised pollinator relationship, therefore increasing the importance of the role of reserve pollinators observed in this study.

Pollination was found to be more successful at receptor sites compared to the control population, despite fewer flowers. This may be due to the effects of flower density and available pollinators. G was the only population to show seedling percentages similar to that of the control population.

CHAPTER 5

FECUNDITY OF TRANSLOCATED ***FRITHIA HUMILIS*** POPULATIONS

5.1 Introduction

Frithia humilis Burgoyne is and endangered edaphic succulent species of the Aizoaceae (Burgoyne *et al.*, 2000). The species is endemic to the Highveld grasslands between the towns of Middelburg, eMalahleni and Bronkhorstspruit. The specific habitat comprises flat sandstone outcrops of the Dwyka and Ecca Groups of the Karoo Supergroup (Burgoyne *et al.*, 2000b; Burgoyne & Hoffman, 2011). Desirable coal deposits occur under these sandstones, and for this reason three of the 11 known *F. humilis* subpopulations have already been destroyed through mining activity (Cairncross, 2001; McCleland, 2014).

In 2008, a subpopulation of *F. humilis* was discovered and confirmed at Exxaro's Inyanda Coal Mine outside eMalahleni in Mpumalanga (McCleland, 2009). In an effort to rescue the population from imminent destruction, Exxaro initiated a collaboration with the South African National Biodiversity Institute (SANBI) and Mpumalanga Tourism and Parks Agency (MPTA) to translocate as much of the population as possible during July 2009. The population was translocated to one typical habitat and two a-typical habitats for experimental trials (Burgoyne & Hoffman, 2011) to determine whether other geologies could also be considered for future rescue endeavours.

For a translocation to be regarded as successful, such populations have to achieve certain measures of success as suggested by Pavlik (1996), Armstrong and Seddon (2008), Menges (2008) and Godefroid *et al.* (2011). Success can be measured in terms of survival, growth, fecundity, recruitment and establishment and is gauged against the abundance, extent, resilience and persistence of the translocated population.

To determine whether translocation of *F. humilis* was successful, a monitoring programme was requested by the Inyanda Coal Mine. This monitoring programme was initiated in 2010 with the purpose of establishing a methodology for long term monitoring and mitigation that could facilitate the exchange of participants as the project continued, and importantly, to identify the short term measures of success (Harris, 2014).

The first phase of the project, conducted by Harris (2014), concluded that *F. humilis* could survive the translocation process and that despite an initial decline in numbers the population in the typical habitat was stabilising in 2012. The a-typical receptor sites were, however, not as successful. This was indicated by poor survival and declining seedling numbers. The translocation to a-typical habitats was not an entire failure since the translocated plants continued their lifecycle even though this was at an inhibited or diminishing rate. Thus, Harris (2014) assessed the first two goals of a successful translocation, namely establishment and expansion of the translocated populations and found that *F. humilis* could survive the translocation process and that flowering, fruit production and seedling emergence continued at all translocation sites, though at a reduced rate on the a-typical geologies.

The purpose of the current study was to extend the monitoring of the translocated populations with the aim of assessing the next milestones in the translocation process, namely resilience and persistence. These milestones are important as they will indicate whether the populations have become self-sustaining or whether they require further human intervention. Usually these milestones can only be accessed at a later stage of a translocation, however considering the resources available to this project, attempts to identify these milestones have been made earlier than recommended, considering the minimum recommended monitoring period of ten years (Godefroid *et al.*, 2011).

Resilience refers to a translocated population's ability to survive environmental disturbances. These abilities include seed and plant dormancy, genetic diversity, high seed production or being able to resprout after a fire or herbivory (Guerrant & Kaye, 2007). Persistence considers all three of the previous milestones, implying that a population must be capable of completing its lifecycle, survive disturbances and function within the community. In other words, evidence needs to be gathered that translocated populations of *F. humilis* are performing adequately or even improving, in spite of environmental challenges.

Pollinator limitation in self-incompatible species, such as *F. humilis*, can result in reduced seed production, particularly for individuals in small or fragmented habitats (Costin *et al.*, 2001). The seed production phase is critical for population development and as such, low seed production can have serious implications for the population age structure and long term genetic health of a population (Brys *et al.*, 2003; Lande, 1988). Thus establishing the long-term fecundity of the reproductive system of *F. humilis* is crucial.

Seed germination and establishment is a requirement to achieve long-term persistence and is mainly affected by habitat quality (Bontrager *et al.*, 2014; Godefroid *et al.*, 2011). Rare and endangered species make the identification of suitable habitats particularly troublesome since they are habitat specialists and their requirements are often more specific than that of more common species. Bottin *et al.* (2007) brings particular attention to the suitability of habitats for long-term project success. Even microsites that are very close together can result in significant differences in germination, establishment and survival (Jusaitus, 2005). A microsite refers to small competitor-free spaces where conditions that are favourable for seedling development prevail (Eriksson and Ehrlén, 2008). For this reason it is important to understand the factors that inhibit the success of a species in a receptor habitat and its subsequent recruitment rates which affect the overall population dynamics (Eriksson and Ehrlén, 1992; Maschinski *et al.*, 2004). Identifying these factors can help to more accurately identify habitats for translocation while also helping to protect rare edaphic species *in situ*. To achieve this Jusaitus *et al.* (2004) suggested the use of multiple translocation sites to compare between results at different sites. This is also holds the advantage of spreading the risk of a catastrophic event happening, in other words having backup populations. This approach was followed during this collaborative project that addressed the translocation of *F. humilis*.

Long term establishment of a translocated population can only occur when the population has become self-sustaining (Guerrant & Kaye, 2007; Menges, 2008) and is able to complete its ecological processes such as interaction with other species (Bullock, 1998). However, long term persistence is not necessarily indicated by self-sustainability (Seddon, 1999). Population decline may occur even after the translocation was declared successful, since classifying the population as such, frequently results in a discontinuation of the monitoring process. Establishing whether long term establishment has actually occurred is often hindered by a lack of long term monitoring (Bullock, 1998; Fahselt, 2007). To maximise the chances of translocation success by achieving long term establishment, habitat quality has to be suitable and of an adequate size, the population has to be of a viable size with enough individuals for cross pollination, symbiotes and mutualists have to be present, any necessary natural disturbances have to occur in a balanced manner and recruitment should be robust enough to withstand influences which may negatively affect population numbers (Milton *et al.*, 1999). To further maximise the chance of including all of these requirements, translocations should be made to several receptor sites, as it has been proven that this is an effective way of identifying site suitability and increasing the chances of long-term persistence (Jusaitis *et al.*, 2004; Kirchner *et al.*, 2006).

The additional knowledge gained from this phase of the project will determine the feasibility of translocation as a conservation method for this species. It will further inform planning and resource allocation, by providing a reference and timeline for translocations of similar plant species.

5.2 Methods

A monitoring programme was designed to address immediate concerns regarding the disturbance caused by translocation in terms of survival, growth and reproduction (Armstrong & Seddon, 2008; Harris, 2014; Menges, 2008). The sampling design followed during this study also allowed for later questions to be addressed about the translocation success in terms of resilience, persistence, recruitment and establishment. Thus, the same sampling method, but different data analyses will be used in this chapter.

Monitoring commenced six months after translocation during February 2010 and continued until April 2016. The years of 2013 and 2014 experienced a lack of monitoring due to an exchange of project participants and lack of funding. Monitoring was done according to the relative size class classification system described in Chapter 3.

An initial total count of all translocated plants at all sites provided baseline data in the form of a population census on existing and emerging plants. Yearly population growth could be determined through changes in population size. However, monitoring the growth of individual plants was problematic due to the small size of the species, leaf retraction, exposed habitat and leaf senescence which made identification of individuals and marking difficult. The factors preventing individual plant monitoring also required the use of relative size classes (Chapter 3) to investigate population growth instead of relative age classes, since smaller but older individuals could erroneously be placed in 'younger' age groups based on the number of leaves. Thus, collective growth had to be deduced from increases or decreases in relative size classes. However, since the abovementioned factors could influence an individual's inclusion or exclusion in a relative size class, growth was deduced from an increase in one relative size group without a decrease in succeeding groups.

5.2.1 Habitat types

Two changes in methodology had to be affected for this follow-up study. The first concerned the numbering of translocation patches. Previously, receptor sites were named using a letter which corresponded with the site e.g. G, Goedvertrouwdt; E, Eagle's Rock; W, Witbank Nature reserve; Z, Ezemvelo Nature Reserve, and various patches were included under a single letter. In the case of the Goedvertrouwdt (G) site a second letter was used to indicate the different groups of patches e.g. GL (5). Subsequently, for the purpose of this study, receptor sites have maintained the first letter, G, E, W or Z to indicate the site and each translocation patch has been given an individual number for easier reference e.g. GW (2) is now G1.2. See Table 3.2 in Chapter 3 for all changes.

The second change in approach was aimed at providing additional information concerning the success of the translocation. Notable habitat differences occurred between the patches at the Goedvertrouwdt receptor site about two years after translocation. This was supported by the observations made by Harris (2014) concerning rainfall effects which showed that despite some similarities, size classes between the different receptor sites varied in their responses to rainfall.

This suggests that habitat characteristics had an effect on population numbers in terms of moisture retention and drainage.

Considering that *F. humilis* plants prefers fast draining and shallow soils that can dry out quickly (Burgoyne *et al.*, 2000a), it can be deduced that a typical *F. humilis* habitat has a limit to the amount of useful rainfall it can receive since the excess would immediately flow out of the habitat. This suggests that the frequency of rainfall events may be more important to *F. humilis* rather than the amount of rainfall received during any one event. Burgoyne and Hoffman (2011) also mentioned the potential effects of intense rainfall events on the health of *F. humilis* populations in the form of excessive habitat erosion caused by more intense rainfall events cause by climate change. To verify this, rainfall frequencies and amounts would have had to be recorded at each site for the duration of the study. Unfortunately, no site specific rainfall is available to facilitate further investigation of the relationships between rainfall parameters and *F. humilis* population health status.

Subsequently, in an attempt to address habitat characteristics, numerous habitat variables were identified and scored on a scale of one to ten. These were based on photos take at fixed points for every year of the study as well as soil chemical and physical analyses performed in the previous study (Harris, 2014). Several variables were scored subjectively and others objectively. A score of 1 was considered favourable and 10 unfavourable (Table 5.1). Photos were taken using a Canon EOS 500D and a Sony Xperia Z1 Compact Android Smartphone.

Habitat data were analysed using cluster analysis and non-metric multidimensional scaling (NMDS) with PRIMER software version 6 (Clarke, 1993) to group patches based on their habitat similarities. Habitat data were also analysed using Principal Component Analysis (PCA) in Canoco version 5 (Ter Braak & Šmilauer, 2012) to indicate which variables corresponded with which patches.

Table 5.1 Habitat characteristics, descriptions and evaluation methods. Photo points were taken annually throughout the study period.

	Description	Evaluation method		Data recordings
		Subjective	Objective	
Patch surface (Plot_Sur)	Shape of rock plate under each patch; flat or concave	*		Photo points
Up-slope run-off (Upslope)	Are above patches allowing for water accumulation within patches	*		Photo points
Drainage	Time for water to drain from patches based on plant growth within and bordering on patches	*		Photo points
Gravel	Accumulation or dissipation within patches	*		Photo points
Erosion	Amount of soil remaining in patches	*		Photo points
Selaginella dregei (Sel_dre)	Degree of presence of this species within patches	*		Photo points
Other plants				
(Otherpl)	Presence of other species within patches	*		Photo points
Remaining habitable space (RHS)	Remaining space preferred by *F. humilis*	*		Photo points
Size	Compared to 1 x 1m control patch size		*	Measurements
Border or Rock (B_rock)	Patch bordering directly against rocks		*	Measurements
Border on plate (B_plate)	Patch bordering directly against rock plates		*	Measurements
Border on Vegetation (B_veg)	Patch bordering directly against surrounding vegetation		*	Measurements
Sandy	Sand contents of the soil within patches		*	Soil physical analysis
Nutrients	Nutrient contents of the soil within patches		*	Soil chemical analysis
Exchangeable cations (ExiC)	Exchangeable cation contents of soil within patches		*	Soil Chemical analysis
Quartz content (Quartz)	Quartz contents of soil within patches		*	Soil physical analysis
Rock porosity (Rock_por)	Receptor site rock plate porosity		*	Soil physical analysis

5.2.2 Statistical analysis

Data were collected during each growing season for each of the three receptor sites as well as the control population. These data were analysed in terms of numbers of fruits, flowers, seedlings, sub-adults and adults to investigate whether the numbers of these important life stages have increased, decreased or stabilised and how they compare to the life stages of the control population.

Where data shows dependence or relation and does not display normal distribution, such as data gathered by counting, a linear mixed model (LMM) is applied (Bolker *et al.*, 2009; Noël *et al.*, 2011). Statistical tests, such as analysis of variance (ANOVA) can usually compensate for non-normality (Bolker *et al.*, 2009), but, due to zero values, it could not transform the count data of this study. Thus, due to zero values in the count data and the subsequent necessity for transformation, LMM analysis was used.

Population numbers in this data were dependant on the population numbers of previous years, making an LMM applicable, since grouping of individuals may change between size classes from season to season due to leaf growth or senescence. Furthermore, collection of data from fixed points over time has created a dependence of data (covariance), which a linear mixed model can account for. Since multiple measurements per translocation patch were conducted annually over six years, i.e. sampling was replicated over time and across populations, correlated data arose in the statistical analysis.

As data sets increase in size, statistical significance tests are likely to display smaller p-values. For this reason this study also reports effect size (*d*-values), which relates to the distribution of the data and makes the difference between two means independent of units and sample size, thereby reporting practically significant relationships between variables (Ellis & Steyn, 2003).

A linear mixed model in SPSS version 21 (IBM, 2012) was applied to the raw data, allowing for a variety of correlation patterns to be modelled with the aim of investigating two questions:

1. How do the five life stage variables of flowers fruits, seedlings, sub-adults and adults change over time at the different translocation sites?

2. How do translocation sites compare to each other over the study period with regards to the five life stage variables (flowers, fruits, seedlings, sub-adults and adults)?

5.3 Results

5.3.1 Classification of habitat types

Analyses revealed similar habitats within the translocation patches of the Witbank Nature Reserve (W) receptor site and the Ezemvelo Nature Reserve control population (Z) (Figure 5.1). Translocation patches at Eagle's Rock (E) were less similar in habitat attributes, while the translocation patches at the Goedvertrouwdt receptor site showed the greatest variation. Four groups were identified based on these results. W and E were grouped separately due to the differences in geology at these sites. For Goedvertrouwdt two groups were distinguished, independent of geology, based on a 90% similarity in habitat variables. These two groups are henceforth referred to as Goedvertrouwdt A and Goedvertrouwd B (GA and GB) (Figure 5.1). Even though geology of this receptor site is uniform, other habitat features have created this overriding division.

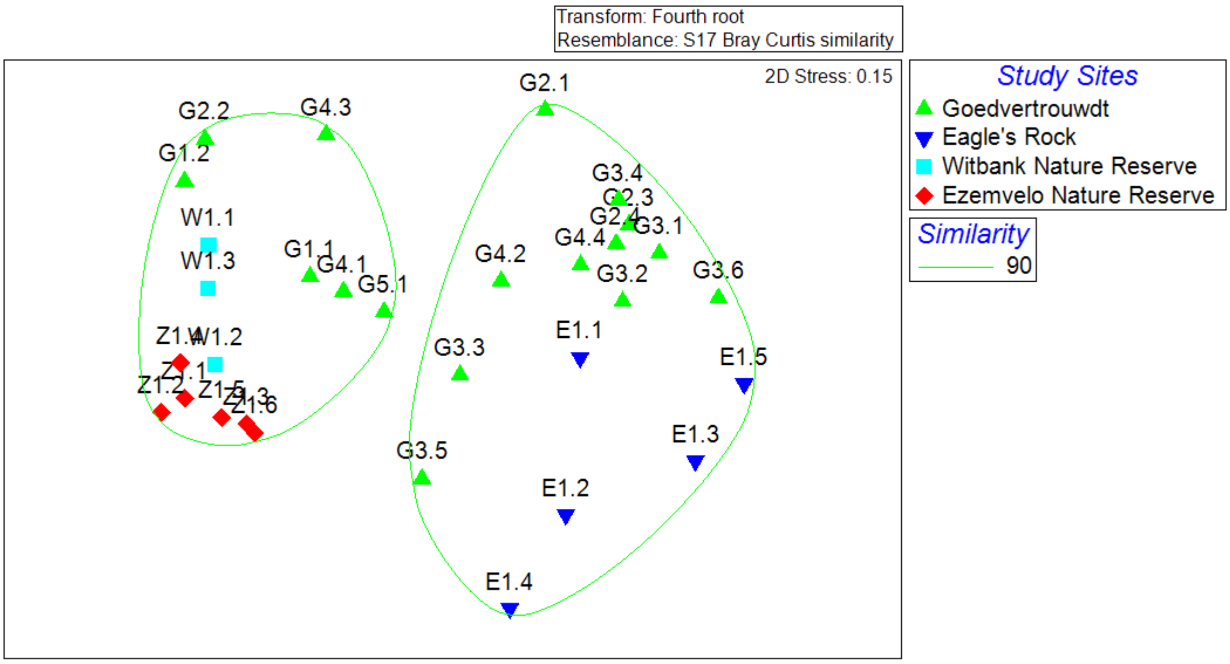

Figure 5.1 Grouping of translocation patches of *Frithia humilis* at receptor sites based on non-metric multidimensional scaling based on the similarities between in-habitat attributes.

The PCA results (Figure 5.2) revealed that the control population at Z was strongly associated with larger patch sizes and that most of the other habitat variables did not have such a compelling effect on the positioning of the control population. Similarly, the W receptor site also showed little association with the majority of habitat variables, apart from high nutrient values and having the patches bordering on rocky surroundings. G and E showed more diverse associations with habitat variables. GA patches grouped separately from the majority of GB patches and, based on larger patch sizes and higher nutrient values, were placed towards the patches of the control population. Conversely, the majority of GB patches were positioned close together and were strongly associated with prominent habitat variables of drainage, plot surface, up-slope run-off, other plants as well as the occurrence of *Selaginella dregei* (C. Presl) Hieron. (a mat forming, moss-like plant, which grows close to, but not usually in *F. humilis* habitats), all of which occurred to a high degree at the various GB patches and consequently received high scores.

However, inconsistencies occurred at the individual patches of G2.2, G3.3 and G3.5 when plant performance was considered. The former, G2.2, was grouped in GA by the NMDS analysis, yet all plants in this patch had died out by 2014. This is inconsistent with the other patches in GA which showed good performance. Similarly, the NMDS analysis placed G3.3 and G3.5 in GB while their performance was in fact similar to the good performance of patches in GA. This is also corroborated by the position of these patches in the PCA analysis where these two patches are closer to the GA patches than the majority of GB patches. These inconsistencies indicate that the habitat analysis results should be interpreted with caution and certain tendencies may be a result of the subjective nature of the scoring system.

An evaluation of photos taken at fixed points during each year of the study indicated that habitat change or degradation occurred since the translocation took place. Particularly noticeable habitat changes were erosion, increased amounts of gravel, increased numbers of *S. dregei* and/or other plants encroaching in various patches at GA, GB as well as E (Figure 5.3).

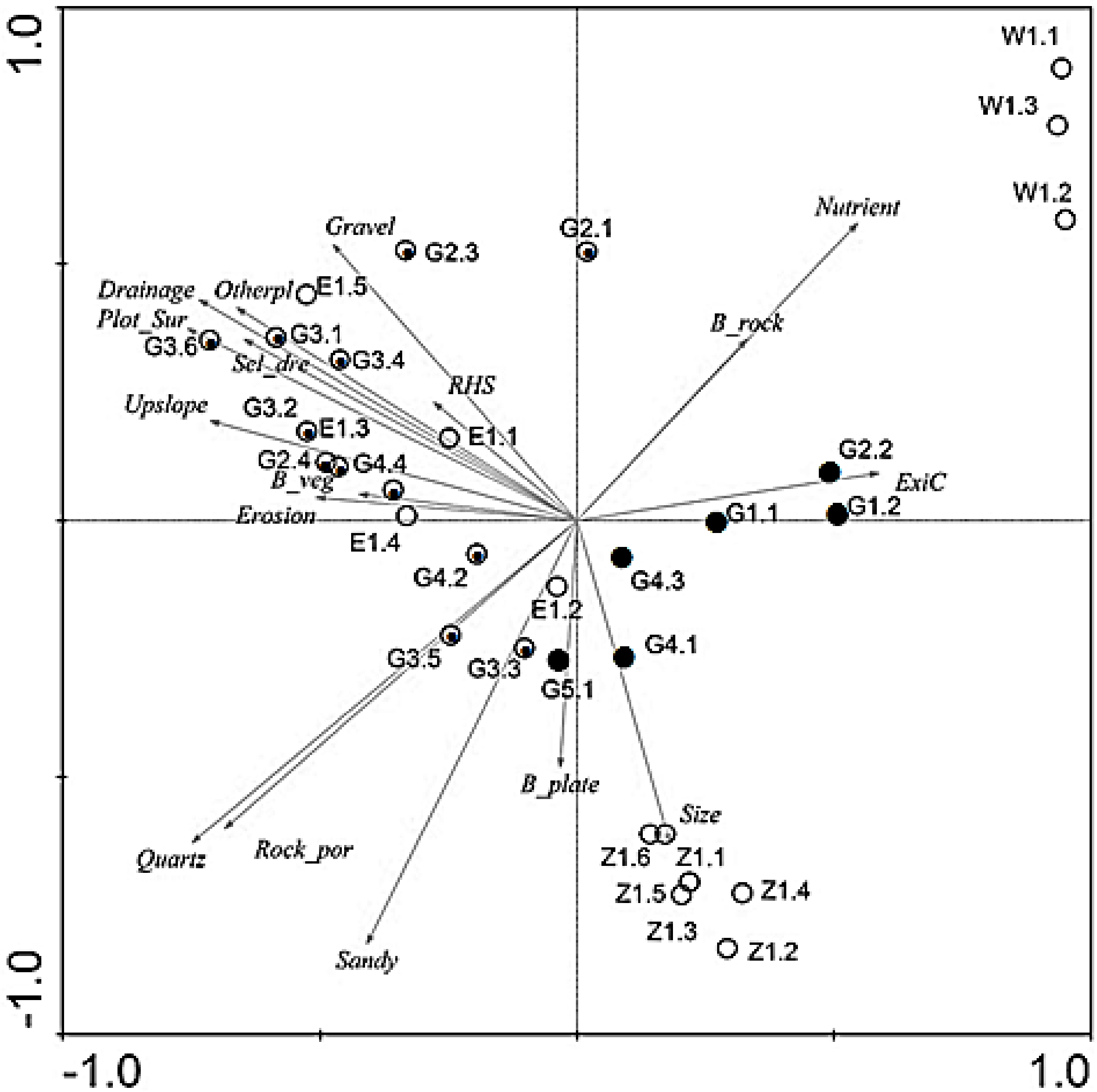

Figure 5.2 **Principal components analysis grouping patches of *Frithia humilis* from all receptor sites, and indicating the association with habitat variables. Dots indicate GA while circles containing dots indicate GB patches. (GA, Goedvertrouwdt A; GB, Goedvertrouwdt; E, Eagle's Rock; W, Witbank Nature Reserve; Z, Ezemvelo Nature Reserve, control population).**

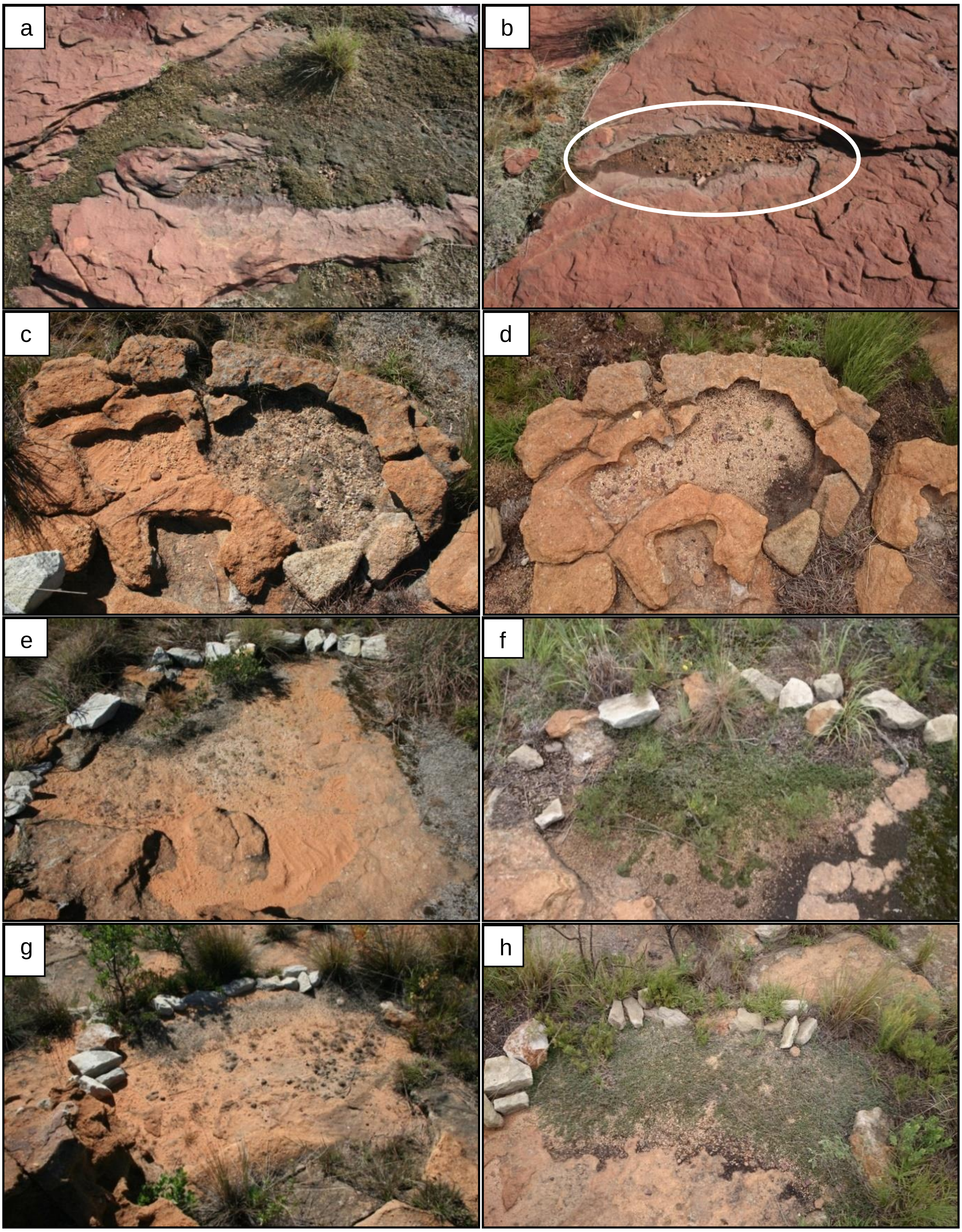

Figure 5.3 **Encroachment of mosses and algae in patch E1.3 (a), severe erosion in patch E1.4 (b), indicated by the 'water line' showing the previous soil level within the patch. Patch G2.2 in 2010 (c) and 2016 (d) where soil has washed away almost completely and large amounts of gravel have accumulated. Patch G2.4 in 2010 (e) and 2016 (f) show encroachment of *Selaginella dregei* and other plants. Patch G3.2 in 2010 (g) and 2016 (f) show erosion of soil and encroachment of *Selaginella dregei*.**

5.3.2 Flowers

The mean number of flowers fluctuated at all the translocation sites over the study period (Table 5.2). All populations showed initial increases in the mean number of flowers. However, all these increases were followed by significant declines for all populations, except the control population.

E showed a significant initial increase from 2009 to 2010 (d=1.71), followed by a significant decrease from 2010 in 2011 (d=1.97). The mean number of flowers increased slightly from 2012 to 2014 and again significantly from 2014 to 2015 (d=0.71). However, compared to the initial number of flowers, no significant change occurred by the end of the study period (2009 mean = 13.26, 2015 mean = 12.39, d=0.06). Compared to the Ezemvelo control population E had a significantly lower mean number of flowers per $1m^2$ over the study period with statistically significant differences for each year (2010, d=0.72; 2011, d=2.91; 2012, d=1.36; 2015, d= 2.75).

GA showed a significant general increase in mean flower numbers over the study period (d=1.94). However, this population showed the greatest fluctuations for the study period amongst all the receptor sites. The population experienced a significant increase in numbers during 2010 (d=2.38) followed by a significant decrease in 2011 (d=0.76) and a slight increase in 2012. Another significant decrease occurred in 2014 (d=1.58) followed by a significant increase during 2015 (d=1.63). Compared to the control population, GA presented significantly fewer flowers per $1m^2$ during 2010, 2011 and 2015 (d=2.18; d=3.16; d=2.87).

GB also experienced a significant initial increase in mean flower numbers during 2010 (d=2.43). However, this was followed by a significant decrease in 2011 (d=2.02), a slight increase in 2012 and another significant decline in 2014 (d=1.01). During 2015 another significant increase was observed (d=1.02). However, the increase over the study period was not highly significant (d=0.73). Compared to the control population, significantly fewer flowers per $1m^2$ was recorded at GB throughout the study period (2010, d=1.74; 2011, d=3.97; 2012, d=1.34; 2015, d= 3.70).

W also displayed a significant initial increase in mean numbers of flowers (d=2.43) during 2010. This was followed by a significant decline in 2011 and a slight decline during 2012 (d=0.76; d=0.46). However, a significant increase occurred again in 2015 (d=2.02). The increase in mean numbers of flowers was the most significant at any of the receptor sites (2009 mean=0.26; 2015 mean=33.27, d=3.23). Despite the significant increase in mean numbers of flowers over time, the lower numbers per $1m^2$ observed at W, compared to the control population, were also significant for every year of the study period (2010, d=2.38; 2011, d=3.35; 2012, d=1.48; 2015, d= 1.83).

Table 5.2 Linear mixed model (LMM) results for mean numbers of *Frithia humilis* flowers per translocation patch (1 m^2) at all receptor sites. Bold numbers indicate significant effect sizes (*d*-values). Shaded cells indicate the values considered most important to assess the flowering trend at each of the receptor sites over time.

Locality	**Year**	**Geometric Mean**	**Std. Error**	***d*-values**					
				2009	2010	2011	2012	2014	Control
Eagle's Rock	2009	13.26	0.58						
	2010	80.96	0.58	**1.71**					**0.72**
	2011	9.90	0.58	0.26	**1.97**				**2.91**
	2012	3.92	0.58	**1.04**	**2.75**	0.78			**1.36**
	2014	5.49	0.58	0.77	**2.48**	0.51	0.27		
	2015	12.39	0.58	0.06	**1.77**	0.20	**0.98**	0.71	**2.75**
Goedvertrouwdt Habitat A	2009	0.62	0.52						
	2010	17.41	0.52	**2.38**					**2.18**
	2011	7.45	0.52	**1.62**	0.76				**3.16**
	2012	10.14	0.52	**1.89**	0.49	0.27			**0.56**
	2014	1.22	0.52	0.31	**2.07**	**1.31**	**1.58**		
	2015	10.78	0.52	**1.94**	0.44	0.32	0.05	**1.63**	**2.87**
Goedvertrouwdt Habitat B	2009	1.40	0.37						
	2010	27.87	0.37	**2.43**					**1.74**
	2011	2.67	0.37	0.42	**2.02**				**3.97**
	2012	3.99	0.37	0.72	**1.72**	0.30			**1.34**
	2014	0.78	0.37	0.29	**2.73**	0.71	**1.01**		
	2015	4.06	0.37	0.73	**1.70**	0.31	0.01	**1.02**	**3.70**
Witbank	2009	0.26	0.80						
	2010	14.08	0.80	**2.43**					**2.38**
	2011	5.91	0.80	**1.66**	0.76				**3.35**
	2012	3.33	0.80	**1.21**	**1.22**	0.46			**1.48**
	2015	33.27	0.80	**3.23**	**0.80**	**1.57**	**2.02**		**1.83**
Ezemvelo	2010	170.75	0.52						
	2011	212.01	0.52		0.21				
	2012	18.69	0.52		**2.12**	**2.33**			
	2015	220.66	0.52		0.25	0.04	**2.37**		

Small effect: *d*=0.2, Medium effect: *d*=0.5, Large effect: *d*=0.8.
LMM significant interaction effects: Locality*Year (*p*<0.001)
LMM significant within effects: Locality (*p*<0.001); Year (*p*<0.001)
Mean Square Error = 0.74 and Variance plot = 0.305

5.3.3 Fruits

The mean number of fruits per 1m^2 for all study sites varied over the study period, though not to the same degree as the variation in flowers numbers (Table 5.3). While increases did occur over the study period, not all were statistically significant compared to the control population.

E showed a significant initial increase in mean numbers of fruits shortly after translocation in 2010 (d=1.01). However, in subsequent years a decrease occurred (2011, d=0.86) followed by significant fluctuations between 2011 and 2014 (2011, d=0.86; 2012, d=1.50; 2014, d=1.56). The change in the mean number of fruits during 2015 was not significant compared to the previous year or to 2009 when numbers were similar to those recorded during the initial survey (2009, mean=1.95; 2015, mean=2.02; d=0.03). Compared to the control population the mean number of fruits were significantly fewer at E, particularly during 2015 (2010, d=0.77; 2011, d=2.77; 2012, d=0.95; 2015, d= 4.37).

GA also showed a significant initial increase in mean number of fruits during 2010 (d=1.98). This was followed by a relatively significant decrease in 2011 (d=0.77) and further significant decreases during 2012 (d=0.92) and 2015 (d=1.22). The difference in the increase in mean numbers of fruits between 2009 and 2015 was also significant (d=2.12). Compared to the control population, significantly lower mean number of fruits were recorded per 1m^2 during most of the years (2010, d=1.15; 2011, d=3.06; 2012, d=0.66; 2015, d= 3.64), despite the increase in numbers of fruits within the receptor site.

GB also showed a mean increase in mean numbers of fruits produced during 2010 (d=1.94), followed by a significant decrease in 2011 (d=1.66). The fluctuations in mean numbers of fruits per 1m^2 from 2012 to 2015 were statistically insignificant. The increase from 2009 to 2015 was only of medium significance (d=0.60). Compared to the control population, the mean numbers of flowers were lower at GB throughout the study period (2010, d=0.78; 2011, d=3.58; 2012, d=0.53; 2015, d= 4.75).

At W a decrease in numbers of fruit per 1m^2 was recorded during 2011, following on an increase recorded between 2009 and 2010 (d=0.96). By 2015 a significant increase in fruit numbers occurred (d=1.97) with a significant overall increase during the study period (d=2.72). Compared to the control population, only during 2012 did mean numbers of fruits not differ with those at W (2010, d=2.17; 2011, d=3.69; 2015, d=3.03).

Table 5.3 Linear mixed model (LMM) results for mean number of *Frithia humilis* fruits per translocation patch (1 m^2) at all receptor sites. Bold numbers indicate significant effective sizes (*d*-values). Shaded cells indicate the values considered most important to assess the fruiting trend at each of the receptor sites over time.

Locality	Year	Geometric Mean	Std. Error	*d*-values					
				2009	2010	2011	2012	2014	Control
Eagle's Rock	2009	1.95	0.43						
	2010	5.60	0.43	**1.01**					**0.77**
	2011	2.31	0.43	0.15	**0.86**				**2.77**
	2012	<0.001	0.43	**1.35**	**2.36**	**1.50**			**0.95**
	2014	2.47	0.43	0.20	**0.80**	0.06	**1.56**		
	2015	2.02	0.43	0.03	**0.98**	0.12	**1.38**	0.17	**4.37**
Goedvertrouwdt Habitat A	2009	<0.001	0.39						
	2010	3.86	0.39	**1.98**					**1.15**
	2011	1.62	0.39	**1.21**	0.77				**3.06**
	2012	0.26	0.39	0.29	**1.69**	**0.92**			**0.66**
	2014	1.04	0.39	**0.89**	**1.09**	0.31	0.60		
	2015	4.43	0.39	**2.12**	0.14	**0.91**	**1.83**	**1.22**	**3.64**
Goedvertrouwdt Habitat B	2009	0.39	0.27						
	2010	5.55	0.27	**1.94**					**0.78**
	2011	0.74	0.27	0.28	**1.66**				**3.58**
	2012	0.40	0.27	0.01	**1.93**	0.27			**0.53**
	2014	0.80	0.27	0.33	**1.62**	0.04	0.31		
	2015	1.23	0.27	0.60	**1.35**	0.31	0.58	0.27	**4.75**
Witbank	2009	<0.001	0.59						
	2010	1.15	0.59	**0.96**					**2.17**
	2011	0.59	0.59	0.58	0.38				**3.69**
	2012	0.82	0.59	0.75	0.21	0.17			**0.20**
	2015	7.79	0.59	**2.72**	**1.76**	**2.14**	**1.97**		**3.03**
Ezemvelo	2010	11.17	0.39						
	2011	29.31	0.39		**1.14**				
	2012	1.14	0.39		**2.17**	**3.32**			
	2015	98.34	0.39		**2.63**	**1.48**	**4.80**		

Small effect: *d*=0.2, Medium effect: *d*=0.5, Large effect: *d*=0.8.
LMM significant interaction effects: Locality*Year (*p*<0.001)
LMM significant within effects: Locality (*p*<0.001); Year (*p*<0.001)
Mean Square Error = 0.74 and Variance plot = 0.305

5.3.4 Seedlings

The number of seedlings per $1m^2$ for most sites showed fluctuation throughout the study period (Table 5.4). Only one translocation site showed an increase in seedling numbers.

At E the mean number of seedlings declined consistently over the entire study period. The declines from year to year were only around medium to low significance. However, the general decline in mean numbers of seedlings from 2009 to 2015 was significant (*d*=1.29). Compared to the control population, significantly fewer seedlings per $1m^2$ were observed over time (2011, *d*=2.07; 2012, *d*=1.98; 2015, *d*=2.63).

At GA the mean number of seedlings fluctuated over the study period, though not significantly. Only during 2014 was a decrease of medium significance (*d*=0.63) observed. However, in 2015 a significant increase occurred (*d*=0.83). Overall, this study site showed an increase in mean seedling numbers, though this was only of medium significance (*d*=0.55). Compared to Z, GA had significantly fewer seedlings during 2011, 2012 and 2015 (*d*=0.93; *d*=0.85, *d*=0.89).

GB showed fluctuation in mean number of seedlings over the study period, with a general tendency of long term declined. Only the overall decline between 2009 and 2015 was of medium significance (*d*=0.59). Again, as in the case of GA, was the mean numbers of seedlings per $1m^2$ recorded during 2011, 2012 and 2015 significantly lower compared to the control population (*d*=0.97; *d*=1.24, *d*=1.49).

W also showed a decline in seedling numbers from 2009 to 2010 and were of medium significance (2010, *d*=0.36). A medium increase in seedlings occurred during 2015 (*d*=0.68) and between 2009 and 2015 the number of seedlings declined significantly (2009, mean=16.41; 2015, mean=4.52; *d*=0.83). Compared to the control population significantly fewer seedlings were recorded at W throughout the study (2010, *d*=0.78; 2011, *d*=2.61; 2012, *d*=3.03; 2015, *d*=2.60).

Table 5.4 Linear mixed model (LMM) results for mean numbers of *Frithia humilis* seedlings per translocation patch (1 m^2) at all receptor sites. Bold numbers indicate significant effective sizes (*d*-values). Shaded cells indicate the values considered most important to assess the seedling trend at each of the receptor sites over time.

Locality	Year	Geometric Mean	Std. Error	*d*-values					
				2009	2010	2011	2012	2014	Control
Eagle's Rock	2009	31.06	0.86						
	2010	18.52	0.86	0.36					**0.34**
	2011	9.57	0.86	**0.80**	0.44				**2.07**
	2012	8.26	0.86	**0.90**	0.54	0.10			**1.98**
	2014	4.53	0.86	**1.27**	**0.91**	0.47	0.37		
	2015	4.33	0.86	**1.29**	**0.94**	0.49	0.40	0.03	**2.63**
Goedvertrouwdt Habitat A	2009	26.47	0.76						
	2010	35.75	0.76	0.21					**0.11**
	2011	50.50	0.76	0.45	0.24				**0.93**
	2012	43.74	0.76	0.35	0.14	0.10			**0.85**
	2014	17.64	0.76	0.28	0.49	0.73	0.63		
	2015	58.01	0.76	0.55	0.34	0.10	0.20	**0.83**	**0.89**
Goedvertrouwdt Habitat B	2009	57.64	0.52						
	2010	36.13	0.52	0.33					**0.12**
	2011	47.77	0.52	0.13	0.20				**0.97**
	2012	24.77	0.52	0.59	0.26	0.46			**1.24**
	2014	12.48	0.52	**1.06**	0.73	**0.93**	0.47		
	2015	24.93	0.52	0.59	0.26	0.46	<0.005	0.47	**1.49**
Witbank	2009	16.41	1.23						
	2010	9.63	1.23	0.36					**0.78**
	2011	4.04	1.23	**0.89**	0.54				**2.61**
	2012	1.15	1.23	**1.51**	**1.15**	**0.61**			**3.03**
	2015	4.52	1.23	**0.83**	0.47	0.07	0.68		**2.60**
Ezemvelo	2010	30.50	0.76						
	2011	186.88	0.76		**1.29**				
	2012	143.48	0.76		**1.10**	0.19			
	2015	202.49	0.76		**1.34**	0.06	0.25		

Small effect: *d*=0.2, Medium effect: *d*=0.5, Large effect: *d*=0.8.
LMM significant interaction effects: Locality*Year (*p*<0.001)
LMM significant within effects: Locality (*p*<0.001); Year (*p*<0.001)
Mean Square Error = 0.74 and Variance plot = 0.305

5.3.5 Sub-adults

All study sites showed initial increases or stability in mean sub-adult numbers per $1m^2$ after translocation, followed by fluctuations and declines (Table 5.5). No population showed an increase in numbers of sub-adults.

By 2011, a decline of medium significance had occurred (*d*=0.55) at E, followed by a significant overall decline from 2011 to 2015 (*d*=1.58). There were significantly higher numbers of sub-adults per 1 m^2 at the control population than at E during the study period (2010, *d*=0.58; 2011, *d*=1.92; 2012, *d*=1.88; 2015, *d*=2.60).

At GA the mean number of sub-adults fluctuated over the study period. Changes from year to year were hardly of any significance. The general decline over the study period was not significant (*d*=0.37). GA had significantly fewer sub-adult plants per $1m^2$ compared to the control population during 2011, 2012 and 2015 (*d*=2.01, *d*=1.24, *d*=1.67).

GB showed a general decline in mean numbers per 1 m^2 over the study period. A medium decline occurred during 2011 (*d*=0.56), and a significant decline again occurred between 2012 and 2014 (*d*=0.99). The overall decline from 2009 to 2015 was medium to significant (*d*=0.75). The control population, again, had medium to significantly more plants than GB per year (2010, *d*=0.58; 2011, *d*=1.92; 2012, *d*=1.23; 2015, *d*=1.86).

W showed a significant initial increase in sub-adult numbers during 2010 (*d*=0.90), but a steady decline of low significance continued after this. Between 2012 and 2015 a significant decline occurred (*d*=1.03). The general decline at this site was significant (*d*=0.96). For all years of the study W had significantly fewer sub-adults than the control population (2010, *d*=1.00; 2011, *d*=2.29; 2012, *d*=2.30; 2015; *d*=3.41).

Table 5.5 Linear mixed model (LMM) results for mean numbers of *Frithia humilis* sub-adults per translocation patch (1 m^2) at all receptor sites. Bold numbers indicate significant effective sizes (*d*-values). Shaded cells indicate the values considered most important to assess the sub-adult trend at each of the receptor sites.

Locality	Year	Geometric Mean	Std. Error	*d*-values					
				2009	2010	2011	2012	2014	Control
Eagle's Rock	2009	45.18	0.71						
	2010	39.13	0.71	0.12					**0.58**
	2011	19.77	0.71	0.67	0.55				**1.92**
	2012	13.93	0.71	**0.94**	**0.82**	0.28			**1.88**
	2014	7.83	0.71	**1.38**	**1.26**	0.71	0.44		
	2015	5.98	0.71	**1.58**	**1.46**	**0.91**	0.63	0.20	**2.60**
Goedvertrouwdt Habitat A	2009	32.01	0.63						
	2010	46.86	0.63	0.31					**0.44**
	2011	17.72	0.63	0.47	0.78				**2.01**
	2012	30.88	0.63	0.03	0.34	0.44			**1.24**
	2014	18.09	0.63	0.46	0.77	0.02	0.43		
	2015	20.09	0.63	0.37	0.68	0.10	0.34	0.08	**1.67**
Goedvertrouwdt Habitat B	2009	39.80	0.44						
	2010	39.18	0.44	0.01					**0.58**
	2011	19.81	0.44	0.56	0.55				**1.92**
	2012	31.42	0.44	0.19	0.18	0.37			**1.23**
	2014	8.85	0.44	**1.19**	**1.17**	0.62	**0.99**		
	2015	15.59	0.44	0.75	0.74	0.19	0.56	0.43	**1.87**
Witbank	2009	7.32	1.00						
	2010	23.33	1.00	**0.90**					**1.00**
	2011	12.28	1.00	0.39	0.51				**2.29**
	2012	7.96	1.00	0.06	**0.83**	0.33			**2.30**
	2015	1.62	1.00	**0.96**	**1.86**	**1.35**	**1.03**		**3.41**
Ezemvelo	2010	79.80	0.63						
	2011	206.81	0.63		0.79				
	2012	140.70	0.63		0.47	0.32			
	2015	155.92	0.63		0.55	0.23	0.09		

Small effect: *d*=0.2, Medium effect: *d*=0.5, Large effect: *d*=0.8.
LMM significant interaction effects: Locality*Year (p<0.001)
LMM significant within effects: Locality (p<0.001); Year (p<0.001)
Mean Square Error = 0.74 and Variance plot = 0.305

5.3.6 Adults

The mean number of adult plants per 1 m^2 decreased at all receptor sites except W (Table 5.6). Some significant fluctuations were present during some years at all of the monitoring sites.

At E the initial increase in numbers of adults during 2010 was insignificant, followed by further declines over the next two years. In 2014, another decline of medium significance occurred (d=0.64), followed by further decline. The overall decline for the study period was significant (d=0.99). For all years of the study the control population had significantly higher numbers of adult plants than E (2010, d=1.08; 2011, d=1.37; 2012, d=1.51; 2015, d=2.12).

GA showed an initial increase in numbers of adults in 2010, though this was only of medium significance (d=0.56). This was followed by major fluctuations of low significance and then a decline of medium significance during 2014 (d=0.71). An insignificant increase occurred in 2015. The decrease in mean number of adult plants per 1 m^2 over the study period (since 2009) was not significant. Compared to the control population GA had significantly fewer adult plants during each year of the study (2010, d=2.08; 2011, d=2.53; 2012, d=2.08; 2015, d=2.60).

At GB a medium significant decrease in mean number of adults occurred in 2011 (d=0.84). This was followed by a slight increase during 2012 and another significant decline in 2014 for the short (d=0.90) and the long-term (d=1.06). This was followed by a slight increase in 2015. The overall decrease in numbers of adult plants per 1m^2 since 2009 at GB was significant (d=0.86). Once again the control population maintained significantly higher numbers of adult plants compared to GB (2010, d=1.98; 2011, d=2.93; 2012, d=2.31; 2015, d=2.95).

The mean number of adult plants at W increased over the study period, though mostly by insignificant increments over years. The most significant (d=1.22) increase in numbers of plants per year was observed at W during 2010. This was sustained and the increase over the entire study period was significant (d=1.63). Despite this increase the mean number of adults at the control population site was still significantly higher for each year (2010, d=2.43; 2011, d=2.40; 2012, d=1.97; 2015, d=1.86).

Table 5.6 Linear mixed model (LMM) results for mean adults of *Frithia humilis* per translocation patch (1 m^2) at all receptor sites. Bold numbers indicate significant effective sizes (*d*-values). Shaded cells indicate the values considered most important to assess the adult trend at each of the receptor sites over time.

Locality	Year	Geometric Mean	Std. Error	*d*-values					
				2009	2010	2011	2012	2014	Control
Eagle's Rock	2009	57.19	0.62						
	2010	71.64	0.62	0.21					**1.08**
	2011	58.42	0.62	0.02	0.19				**1.37**
	2012	40.29	0.62	0.32	0.52	0.34			**1.51**
	2014	19.72	0.62	**0.96**	**1.16**	**0.97**	0.64		
	2015	19.00	0.62	**0.99**	**1.19**	**1.01**	0.67	0.03	**2.12**
Goedvertrouwdt Habitat A	2009	12.39	0.55						
	2010	23.66	0.55	0.56					**2.08**
	2011	16.04	0.55	0.22	0.34				**2.53**
	2012	21.37	0.55	0.47	0.09	0.25			**2.08**
	2014	9.39	0.55	0.24	**0.80**	0.46	0.71		
	2015	10.98	0.55	0.10	0.67	0.33	0.58	0.13	**2.60**
Goedvertrouwdt Habitat B	2009	19.72	0.39						
	2010	26.30	0.39	0.26					**1.98**
	2011	10.03	0.39	0.58	**0.84**				**2.93**
	2012	16.46	0.39	0.16	0.41	0.42			**2.31**
	2014	5.61	0.39	**1.06**	**1.31**	0.47	**0.90**		
	2015	7.19	0.39	**0.86**	**1.11**	0.28	0.70	0.20	**2.95**
Witbank	2009	3.48	0.87						
	2010	15.81	0.87	**1.22**					**2.43**
	2011	18.62	0.87	**1.37**	0.14				**2.40**
	2012	24.11	0.87	**1.59**	0.37	0.23			**1.97**
	2015	25.23	0.87	**1.63**	0.41	0.27	0.04		**1.87**
Ezemvelo	2010	231.91	0.55						
	2011	261.59	0.55		0.11				
	2012	211.13	0.55		0.09	0.20			
	2015	197.66	0.55		0.15	0.26	0.06		

Small effect: *d*=0.2, Medium effect: *d*=0.5, Large effect: *d*=0.8.
LMM significant interaction effects: Locality*Year (p<0.001)
LMM significant within effects: Locality (p<0.001); Year (p<0.001)
Mean Square Error = 0.74 and Variance plot = 0.305

5.3.7 Summary of results

A summary of the performance of each patch group is given in Table 5.7.

Table 5.7 Summary of NMDS, LMM and PCA analyses results for translocated *F. humilis* populations at the various study sites.

Patch group (NMDS)	Life stages	Performance (LMM) (2009-2016)	Common habitat variables (PCA & observations)
W	Flowers	Significant increase (*d*=3.23)	• Flat surface • Bordering on rocks • Low gravel content • Larger, flat felsic pebbles • Good drainage
	Fruits	Significant increase (*d*=2.72)	
	Seedlings	Significant decline (*d*=0.83)	
	Sub-adults	Significant decline (*d*=0.96)	
	Adults	Significant increase (1.63)	
GA	Flowers	Significant increase (*d*=1.94)	• Bordering on rock plate • High gravel content • More exchangeable cations • Somewhat larger patch size
	Fruits	Significant increase (*d*=2.12)	
	Seedlings	Medium increase (*d*=0.55)	
	Sub-adults	Insignificant decline (*d*=0.37	
	Adults	Insignificant decline (*d*=0.10)	
GB	Flowers	Medium increase (*d*=0.73)	• Concave patch surface • Poor drainage • Competition from other plants and *S. dregei* • High up-slope run-off • High erosion
	Fruits	Medium increase (*d*=0.60)	
	Seedlings	Medium decline (*d*=0.59)	
	Sub-adults	Medium decline (*d*=0.75)	
	Adults	Significant decline (*d*=0.86)	
E	Flowers	No significant change (*d*=0.06)	• High erosion • Competition from *S. dregei* • Lack of quartz pebbles
	Fruits	No significant change (*d*=0.03)	
	Seedlings	Significant decline (*d*=1.29)	
	Sub-adults	Significant decline (*d*=1.58)	
	Adults	Significant decline (*d*=0.99)	

5.4 Discussion

5.4.1 Habitat types

NMDS analysis revealed two distinct groups within the translocation patches at a 90% level of similarity. Receptor sites exhibited much variability in terms of habitat characteristics. This 90% similarity serves to indicate how specific the habitat requirements of *F. humilis* are, even within a supposedly suitable receptor habitat (compare GA and GB).

In a survey of 249 plant reintroductions, Godefroid *et al.* (2011) found that the most important reasons identified for failure were the selection of unsuitable habitats, changing habitats and predation. Unfortunately, the placement of patches in this study was subject to the same constraints, with the PCA analysis (Figure 5.2) indicating that the majority of receptor sites exhibited habitat variables that did not conform to those of the control population. When the PCA results are compared in terms of GA, GB and the population performance of each, similarities are noticed. Generally, based on LMM results, GA patches performed better than GB patches. This was confirmed by their grouping in proximity to the Z patches. However, even though these GA patches performed the best of all translocation sites, these still underperformed compared to the control population. Again their position on the PCA explains this since, even though they are closer to the Z patches, GA receptor patches are still not identical to the control habitat. GB patches have, on the other hand, shown strong association with various receptor patch characteristics of the a-typical habitat at E, such as patch surface, up-slope run-off, other plants and the occurrence of *S. dregei,* which do not feature in the habitat descriptions by Burgoyne *et al.* (2000a, 2000b). Up-slope run-off most likely resulted in high levels of erosion, while concave patch surfaces and poor drainage may have encouraged the growth of other plants including *S. dregei*. These undesirable habitat features are reflected in the mean numbers of the LMM results.

Concerning the aforementioned inconsistencies of the positions of G2.2, G3.3 and G3.5, some desirable habitat characteristics may have produced adverse effects on translocated *F. humilis* plants. In patch G2.2 all *F. humilis* plants died off by 2014, yet the NMDS Analysis placed this patch in GA. One possible explanation for this may be the excessive gravel content within the patch, since in 2015 and 2016 it was observed that this patch consisted almost solely of gravel and no soil, making plant growth impossible. An excess of gravel was also believed to be a contributing factor to the poor conditions at G1.1 and G1.2, where relatively high seedling but low adult plant numbers were observed. It therefore seems that an excess of gravel may allow seed germination but a lack of soil hinders seedling establishment, indicating that too much gravel may be as detrimental as too little.

As for G3.3 and G3.5, their grouping into GB may be as a result of their patches containing several other plant species and *S. dregei*. However, counts for these patches revealed better performance, which may be a result of the patches' large sizes which can accommodate both *F. humilis* as well as other plants. This would also explain the position of G3.3 and G3.5 on the PCA, which of all the patches within the GB population, tended the most towards GA and the control population. This indicates the possibility that some habitat characteristics may be able to offset other undesirable characteristics.

5.4.2 Flowers

All translocated populations of *F. humilis* showed significant increases in flower numbers shortly after translocation. This increase was reflected to varying degrees in the other life stages of fruits, seedlings, sub-adults and adults. This can be viewed as an example of the initial favourable results often seen directly after translocation, but which is subsequently followed by declines (Fahselt, 2007). *Amsinckia grandiflora,* is an example of such a case in which populations introduced to several sites initially increased, but then declined suddenly after two to three years (Pavlik, 1996). Similarly, *Abronia umbellata* subs. *breviflora* was translocated to its area of occurrence into cautiously selected sites. Populations were vigorous and reproductive during the first year but after four years started to fluctuate significantly and eventually the receptor sites contained only one or two individuals or none at all (Kaye, 2002).

Members of the family Aizoaceae are known to be generally mass flowering (Ihlenfeldt, 1994). Understandably, flowering is affected by weather conditions, often the vigour and duration of flowering is influenced by the amount of rain received during and before the flowering period (Struck, 1995). However, rainfall is not the determining factor for flowering since it may be erratic. A more reliable trigger for flowering is temperature (Struck, 1995). This is supported by Hartmann (1991), who has also noted that flowering may still occur in spite of less precipitation than normal. This may provide an explanation for the sustained flowering of *F. humilis* despite potentially adverse conditions at the translocation sites. Succulents are after all adapted to function in spite of low rainfall and *F. humilis* is a prime example, since moisture that is received evaporates quicker from their shallow soil compared to deeper soil. The fluctuations in mean numbers of flowers over the years may be the influence of the aforementioned effects of precipitation, the fluctuations in mean number of flowering-sized plants as well as the timing of monitoring (Elzinga *et al.*, 1998).

Flowering indicates the health of plants within the population and, along with fruiting, is a measure of population viability in terms of reproductive output (Drayton and Primack, 2012; Godefroid *et al.*, 2011). Flowering is therefore used as one of the main criteria when the success of a translocation project is assessed. While flowering is certainly an important milestone, it is only a short term objective and must be seen as a step towards the long term objectives of self-sustaining and persistent populations (Krauss *et al.*, 2002). Despite its importance as an initial milestone, flowering and fruiting rates in reintroductions have proven to be relatively low and continually decreasing (Godefroid *et al.*, 2011). Hence, plant survival often receives more attention than flowering due to fluctuations of the latter. Mean numbers of flowers per 1 m^2 in this study was low and showed large scale fluctuations. Over the short term, increases in flower production were often as significant as the decreases, which could provide unreliable indicators of success or failure. This study has shown that flowering should be considered over the long term (>5 years), so as to allow rapid increases and decreases to stabilize. One should consider flowering trends in founding years for comparison with flowering trends in later years to assess the true extent of increases or decreases. As such, this study has therefore shown that the general flowering trend is not one of catastrophic decline, but generally of underperformance compared to the control.

However, even though a population may be flowering well under certain conditions, this does not necessarily mean that the population is healthy. In Australia three endangered plant species showed that although their reproductive systems allowed restoration of their populations, population increases did not occur (Jusaitis, 2005). The challenge of establishing healthy populations is situated elsewhere in the form of appropriate microsites, herbivory and competition with weeds. This may be the situation for *F. humilis* as well since numbers of seedlings, sub-adults and adults, despite fluctuations and some increases, have generally been declining since the beginning of the study and have not increased as significantly as the numbers of flowers have in the most recent years of the study.

5.4.3 Fruits

Successful fruit production and subsequent seed set is crucial for the self-sustained continuation of a population (Bontrager *et al.*, 2014). Ovule numbers for *F. humilis* are not known, however since one of the closest relatives of *F. humilis* is *Conophytum* (Burgoyne *et al.*, 2000a), the number of ovules may be inferred from this genus being between 77 and 335 (Jürgens & Witt, 2004). Even though the production of fruits at all the translocation sites was significantly lower than the control population, this may not necessarily be problematic. Seed counts from ten fruit capsules indicated that a single *F. humilis* fruit may produce between 90 and 200 seeds (unpublished data). It is therefore expect that enough seeds are produced to contribute to population growth, as well as to supplement the seed bank even at a germination rate of 10% (a mean of 1.96 capsules per 1 m^2 across all the translocation sites translates to a mean value of 280 seeds per 1 m^2).

It is known that many species vary their seed production from year to year (Young *et al.*, 2005). Apart from this annual variation, seed production may be limited by a number of factors including ovule production, pollen limitation (referring to quality and quantity), resource limitation (referring to nutrients available for seed production), granivores, herbivores, diseases and environmental conditions (Lee, 1988). Even though fruits may be present at receptor sites, it does not therefore necessarily mean that an adequate number of seeds are being produced.

Pollination of *F. humilis* has recently been addressed by Harris *et al.* (2016) and in Chapter 4 of this study. While quality and quantity could not be verified, numerous potential pollinators from different genera were identified. Little is known regarding granivores and diseases of *F. humilis*. Herbivory, primarily by rodents (Harris, 2014), may however affect fruit production since they destroy an entire plant by digging it up and consuming the rootstock. The occurrence of rodent damage at translocation sites diminished by 2014 and was not observed at all during the 2015 monitoring period. Resource limitation and environmental effects will be discussed later in this chapter.

Even though pollination does occur at the translocation sites, seed quality may be an increasing point of concern. As a member of Aizoaceae, *F. humilis* is most likely self-sterile and requires xenogamous pollination (Ihlenfeldt, 1994). Therefore, in small populations, successful cross pollination may not occur or pollination may occur between near-related plants resulting in genetic assimilation through inbreeding (Brys *et al.*, 2003; Ellstrand & Elam, 1993). A reduction in seed production has often been connected to small population size (Hendrix & Khyl, 2000) and may in turn weaken offspring and shift the population age structure (Brys *et al.*, 2003). This was the case for the endangered Australian plant, *Grevilliea scapigera*, where four out of nine clones produced 85% of the next generation due to inbreeding (Krauss *et al.*, 2002). Similarly, Brys *et al.* (2003) found negative reproductive success to be linked to small population size and demographic structure of populations of *Primula veris*. Another example is that of a grassland daisy, *Rutidosis leptorrhynchoides*, which also showed a positive link between population size and seed production per plant (Morgan, 2000). However, though the reintroduced populations of this daisy species were not as large as remaining natural populations, they were able to produce similar amounts of seed. A possible explanation for this was that the reintroduced populations occurred at a higher density, thus improving pollination success (Morgan, 2000).

Frithia humilis plants are naturally clumped together by a restrictive habitat, which may explain successful pollination and fruit production despite a low number of plants of reproductive age. Another factor which may contribute to successful pollination and fruit production is the condition of the surrounding habitat. In the case of *Leucochrysum albicans*, the diversity of flowering plants, and consequently pollinators, in its species rich grassland habitat, was suggested to compensate for populations size (Costin *et al.*, 2001). Similarly, the grassland habitat around the *F. humilis* translocation sites has remained relatively undisturbed and may be supporting healthy pollinator diversity. Consequently, it may be that *F. humilis*, due to its limited specialist habitat, is adapted to surviving in small populations, but that it also relies on a healthy surrounding habitat for adequate generalist pollinators. Three observed natural populations were smaller than the control population at Ezemvelo Nature Reserve, though still not as small as most of the translocation patches.

Even though the fruit production of all translocated populations remained significantly lower than that the control population for the majority of years over the study period, fruits are still being produced. Only two of the translocated populations showed significant increases but a positive observation is that the populations which did not increase (GB & E) did not fall into absolute decline either. While these observations are positive, reliance on such low numbers (<1 fruit per year) to sustain a population and seed bank is of concern. Furthermore, in some species recruitment is determined more by their germination biology than the number of seeds produced by previous generations (Crawley & Ross, 1990). However, fruit production at translocation sites could be considered stable for the following reasons: all translocation sites maintained some *Frithia* individuals until 2016, including seedlings and sub-adults which must have originated from recently produced seeds. Despite significant decreases at some sites, the *Frithia* populations also showed the capacity to increase in numbers. This was particularly evident during 2015, when increases in mean numbers of fruits produced at GA and W exceeded production during any of the other years during the study period.

5.4.4 Seedlings

If seed limitation does not occur within a population, the problem of poor recruitment has to lie elsewhere in terms of microsite availability, inter- and intraspecific competition or herbivores (Crawley & Ross, 1990; Eriksson & Ehrlén, 1992). Seed germination and establishment are the weakest links in any plant’s lifecycle since seedlings are more vulnerable to unfavourable environmental factors and habitat conditions than any other life stage (Galatowitsch, 2008; Jusaitis, 2005). Successful translocation relies on adequate research and knowledge concerning the required habitats for the species in question (Bottin *et al.*, 2007; Hodder & Bullock, 1997). For example, habitats that seemed similar to the original was chosen for the brown bog rush, *Schoenus ferrugineus*, a threatened perennial in Scotland, but turned out to be unsuitable and may have been the reason for the failed transplantation of this species (Hodder & Bullock, 1997).

Identification of suitable translocation habitats is not easy and it may take a significant amount of research to determine the environmental requirements of a species (Hodder & Bullock, 1997).

To complicate matters further, Maunders (1992) suggested that knowledge of the original habitat may not even be helpful since the requirement for early phases of growth may be different compared to the requirements of mature plants. Even though *F. humilis* population at the translocation sites were not natural, the habitat conditions at W served as an example of different requirements in different life stages since it seems to sustain conditions which maintain adult numbers, but are not increasing seedling numbers.

Microsite refers to small competitor-free spaces where the quality of the site is favourable for seedling development (Eriksson and Ehrlén, 2008). The most important factors influencing seedling survival are the following microsite characteristics: moisture availability, temperature regimes, herbivory, nurse plants and diseases (Kollmann, 2008). The effects of microsite on *F. humilis* seed germination were evident in the translocated populations. Quartz pebbles are known to be necessary for seedling germination and protection from desiccation and excessive sun exposure (Burgoyne *et al.*, 2000b). However, it has been observed that an over-accumulation of pebbles may have a negative effect on *Frithia* populations. For example, at GA, where some patches seemed to be suitable, there was high seed germination but few adult plants were present. Presumably an excess of quartz pebbles allows for good germination but not establishment of *F. humilis* seedlings.

Conversely, a deficiency of pebbles may also inhibit seed germination. This was particularly evident at E and W receptor sites. At the latter, translocated quartz pebbles were washed away or replaced by larger felsic pebbles, reducing the availability of microsites for germination. However, at the Ezemvelo control population, it was noticed that one of the control patches contained absolutely no quartz pebbles while still maintaining high numbers of seedlings and adult plants. Presumably, other habitat features must be compensating for the lack of pebbles. At the abovementioned control patch, soil could be described as almost muddy when moist, suggesting that soil composition could most likely compensate for the gravel layer which usually provides retention of soil moisture. This in turn alludes to the opportunistic nature of *F. humilis,* despite its narrow habitat requirements, and also to the importance of soil moisture in *F. humilis* habitats. Bontrager *et al.* (2004), in their study on the habitat and interactive effects of the wetland herb, *Arenaria paludicula*, concluded that for a single rare species an optimal management regime may not apply to all habitats and it would depend on the context of their use. With this in mind, and the ability of *F. humilis* to colonise unusual micro-habitats, within limits, it would seem that any future translocations should identify margins within which translocations of this species would be feasible and adapt management strategies according to specific situations.

Proximity to other plants as competitors and nurse plants may also influence *F. humilis* seedling emergence. Several studies have shown that seedling establishment tends to be better in open microsites (Kollmann, 2008). This is applicable to *F. humilis* since in its natural habitat germination and establishment necessarily have to be out in the open. Anderson *et al.* (2004) corroborated this when he reported that mesemb species with small seeds germinated more successfully and in open areas at a distance from adult plants due to reduced competition for moisture. However, *F. humilis* seedlings were also observed under nurse plants which provide shade and protected seedlings from herbivory and desiccation. The soil under nurse plants often has a higher nutrient content, is somewhat cooler and maintains moisture for slightly longer periods than soil in open spaces (Armas & Pugnaire, 2005). In arid environments nurse plants often provide necessary protection in this form (Maschinski *et al.*, 2004). However, this is the exception rather than the norm for *F. humilis*. At two of the translocation patches at Goedvertrouwdt numerous seedlings occurred under individuals of the suffrutex *Searsia magalismontana*. This was also observed by Harris (2014). Occasionally, tufts of grass also acted as suitable nurse plants, with the shading effect being reflected in the leaves of *F. humilis* plants which were larger and more turgid in these protected environments.

In terms of the effect of competition on seedling emergence, Harris (2014) stated that the presence of *Selaginella dregei* facilitated seedling emergence by acting as seed traps since a thick layer of quartz pebbles was present at these colonies. However, it was also noted that there were fewer sub-adults amongst the colonies of *S. dregei*. In the most recent monitoring period few to no seedling or sub-adults were counted amongst the *S. dregei* colonies. A similar situation was reported for the Japanese beech, *Fagus crenata,* with disturbances from typhoons creating significant patches of trees of similar age and large portions of forest floor covered by a dwarf bamboo species. Consequently, the majority of resulting age structure diagrams showed a lack of recruiting age classes, due, partially, to intraspecific competition but especially due to competition with the dwarf bamboo (Nakashizuka, 1987). Thus, even though *F. humilis* seeds may germinate within *S. dregei* colonies, conditions may not be conducive for recruitment. Competition between species in a translocation project, be it weeds or naturally occurring species, often turns out to be a significant influencing factor for seedling and adult survival of target species (Jusaitus, 2005; Lofflin & Kephart, 2005; Navarro & Guitián, 2003; Schmidt, 2002).

It was also observed in several receptor plots that dense moss and soil algae bound the soil which would otherwise have been loose and sandy, making it difficult for *F. humilis* seedlings to become established after germination. This is contrary to other species where mosses often facilitates establishment (Riba *et al.*, 2002).

It is important to note, once again, that weather conditions may be the reason for significant increases and decreases in seedling numbers. However, it would seem that in cases where general declines occur, the quality of translocation patches also deteriorated compared to other patches experiencing similar weather patterns. This was observed at receptor patches at GA, GB and E where gravel content or *S. dregei* growth within patches increased too much or where soil and gravel eroded away, subsequently reducing habitable space (Table 5.7).

5.4.5 Sub-adults and adults

While it is true that requirements for early phases of seedling growth may be different to that of mature plants (Maunders, 1992), several factors which influenced *F. humilis* seedling germination also affected the mean numbers of sub-adult and adults. . However, considering translocated populations were established with sub-adult and adult plants, the reported decrease in mean numbers is problematic considering that flowering, fruiting and seedlings have been produced since 2010. Various factors may have had an effect on sub-adult and adult numbers including herbivory, unsuitable or changing habitats, insufficient species knowledge and competition (Goedfroid *et al.*, 2011; Menges, 2008).

Harris (2014) noted that predation was a problem at the Goedvertrouwdt receptor site. However, this was not observed to be a significant problem during the latter years of the study. Factors which persisted throughout the translocation and monitoring programme, particularly in the latter half, were the changing habitats which could easily be identified in the aforementioned photo points.

While these other factors are naturally context dependant, Crawley and Ross (1990) ranked interspecific competition as the most important factor influencing population dynamics. For *Brachycome muelleri,* a small herbaceous perennial in Australia, it was found that populations were most threatened by competition from weeds for moisture, nutrients and light (Jusaitis *et al.*, 2004). For the rare annual forb, *Amsinckia grandiflora*, it was shown that competition for space in high density grasslands in California, created by exotic grasses, was greater than competition for other resources such as moisture. The latter study suggested that even in restored grassland, intermediate densities of native species would have higher habitat value for *A. grandiflora* than grasslands restored to a denser composition of species (Carlsen *et al.*, 2000).

Competition for space between *F. humilis* and *S. dregei* within the translocation plots seems to significantly influence the recruitment of young plants as well as the survival of adults. In various G and E patches, *S. dregei* has found the conditions of the translocation patches favourable for its own increase. In a natural habitat setting, while the two species do occur within close proximity to each other, there is either enough space for both to coexist or conditions are not as favourable for dense mats of *S. dregei* to form (Figure 5.4). This is not an unusual occurrence for rare plants since in Australia the recruitment of 71% rare plants studied was influenced by competition from weeds (Jusaitis *et al.*, 2004).

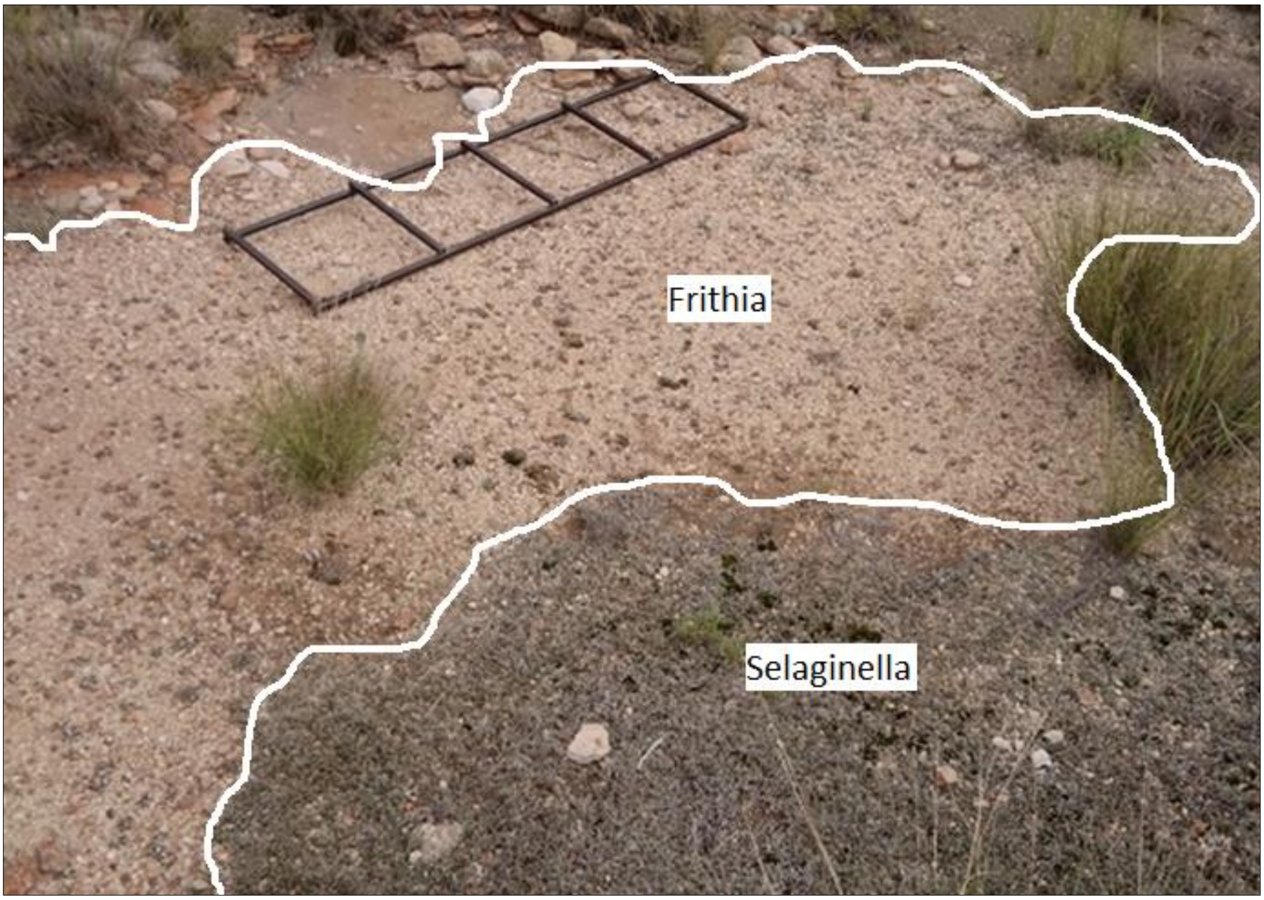

Figure 5.4 A patch at the control population (Z) where *Frithia humilis* and *Selaginella dregei* are able to co-exist in close proximity without competing for space.

An explanation for the competition within receptor patches may be that receptor sites which may initially have seemed suitable for the target species may have become overly productive, in other words, supporting excessive growth of co-existing species after the intermediate disturbance. In the case of Purple needlegrass, *Nassella pulchra*, performance was better in marginal and less productive sites, suggesting that this species is more adapted to marginal habitats with less competition (Lombardo *et al.*, 2007). The situation is similar for *F. humilis* since its preferred habitat is naturally marginal, with seedlings, sub-adults and adults at translocation sites preferring open soil surrounding the colonies of *S. dregei*. This is supported by the hypothesis presented by Bertness and Callaway (1994) which suggested that competition increases in productive environments, whereas facilitation, from nurse plants for example, is more significant under harsher conditions. This explains the aforementioned occurrence of *F. humilis* under *S. magalismontana* and occasionally under tufts of grass. It further alludes to the fine line between suitable and unsuitable habitats for *F. humilis.* Jusaitis (2004) also mentioned the high variability of conditions across a translocation site and the influence it may have on successful establishment. This is particularly true for the G receptor sites where a variety of conditions have been expressed within and between receptor patches over the course of the study.

Another consequence of the invading *S. dregei* colonies were that, apart from increasing in density, colonies in several receptor patches at G have either been elevated through soil accumulation or, more likely, soil surrounding these colonies have eroded away to leave islands of *S. dregei*. This was caused either by too much up-slope run-off, combined with a too concave plot surface, or that *S. dregei* acted like an obstacle causing water to flow around it rather than over it, thereby eroding away any remaining habitat suitable for *F. humilis* colonisation. The lack, or erosion, of remaining suitable habitats also occurred at one of the receptor patches at E, were erosion from up-slope run-off was particularly severe. This directly affected the plants in this patch as was evident from exposed rootstocks where soil has been washed away. Soil "water lines" were visible, indicating the initial soil depth. Furthermore, the recovery of such eroded patches through seedling recruitment is unlikely since the majority of seeds have probably been washed away. Furthermore, quartz pebbles or suitable naturally occurring pebbles for seedling establishment were also lacking. The supply of soil and pebbles can also not be replaced by accumulation of up-slope erosion material, as is the case at the control population, since the E patch is on top of a rocky outcrop.

Little research has been done on the effects of receptor site erosion and the consequences thereof for translocation projects. However, much more research has been done on micro-topography formation through biotic and abiotic factors on slopes around shrubs (Bergkamp, 1998; Bochet *et al.*, 2000). The reduction in habitat for *F. humilis* colonisation is clear when comparing photo points of patches at the G receptor site over subsequent years of the study. The effects of this on the remaining populations of *F. humilis* were clearly reflected in the reduction in mean numbers at several patches at the G and E receptor sites (Table 5.7).

Conversely, at the W receptor site there are no other large plants or *S. dregei* with which *F. humilis* plants had to compete. Despite this, seedlings and sub-adult numbers declined while the mean number of adult plants increased significantly, contrary to the other receptor sites. A conspicuous feature of W is the lack of a quartz gravel layer. Soil is completely exposed with fewer larger, flat, felsic pebbles. This suggests a lack of suitable microsites for seeds to germinate and that the quartz pebble layer at the other receptor sites is necessary for seedling establishment. However, the few seedlings that survived at W seem to have a better chance of survival to adulthood than is evident at other receptor sites where adult numbers are declining. Presumably, seeds only germinate if they are strong and can survive the harsher condition or if they happen to end up in a suitable microsite amongst felsic pebbles. However, the conditions at W may also be inducing a genetic shift by selecting only the most adaptable individuals for that particular environment (Fahselt, 2007; Lesica & Allendorf; 1999; Menges, 2008).

5.5 Conclusion

F. humilis is a threatened species in need of conservation due to various threats, notably coal mining. This study assessed the feasibility of translocation as a conservation method to save populations from destruction caused by mining activities. The four measures of success are abundance, extent, resilience and persistence. The previous study by Harris (2014) confirmed the first two measures of success, namely that *F. humilis* could survive the translocation process and successfully complete its lifecycle. The other two measures of success, resilience and persistence, were measured in this study in terms of the life stages of flowering, fruiting, seedling, sub-adult and adult plants.

In general the mean number of flowers increased or returned to original numbers since the start of the study across all the receptor sites. The only receptor site to show a steady decline in flowering over time is GB. In comparison, W showed a significant increase in the mean number of flowers. At most receptor sites the mean numbers of flowers were significantly lower than that of the control population.

All receptor sites showed increases to some extent in their mean number of fruits. At E this was not significant though. However, for the other three sites the increase in fruit numbers was of medium to high significance, with GA and W showing the most substantial increases. For most years, at all the receptor sites the mean number of fruits per 1 m^2 was significantly lower than that of the control population.

Overall, every receptor site showed a medium to significant decrease in numbers of seedlings, apart from GA which showed a medium increase. All sites had mean seedling numbers which were, in most years, significantly lower than those of the control population.

Mean sub-adult numbers declined over the study period at all translocation sites. The only receptor site where this was non-significant was GA. The control population also continuously had significantly greater mean numbers of sub-adults in the majority of years when compared with the translocated populations.

E and GB showed a significant decline in mean adult numbers. These numbers also declined at GA, but not as significantly. W showed a significant increase in numbers of adult plants over the study period. The translocated populations performed significantly worse than the control population in all instances.

Results from this study indicate that the reproduction biology of *F. humilis* is stable and functional, despite low rates compared to the control population. Numbers of seedlings, sub-adults and adult have generally declined for all populations. GA was the only population where declines were not as rapid. The number of adults increased significantly over the study period for W. The mean numbers of all life stages generally remained significantly lower than those of the control population.

Yearly fluctuations in mean numbers may be ascribed to variability in rainfall. However, this study has shown that deteriorating habitat quality was the most important factor that influenced population declines over the study period. The most significant constraints to seedling establishment were a lack of suitable microsites for their establishment, erosion of soil due to up-slope run-off, and competition from encroaching *S. dregei* and other plants due to concave patch surfaces and poor drainage.

While the translocation of *F. humilis* initially seemed favourable, these latest findings indicated that translocation, using current methods, is not a feasible long-term conservation tool. Translocation as a strategy to save endangered populations of *F. humilis* is strongly discouraged until more research is done on its habitat requirements and identification of suitable receptor habitats that will remain stable over the long term.

CHAPTER 6

POPULATION DYNAMICS OF ***FRITHIA HUMILIS***

6.1 Introduction

In many conservation studies of rare species, causes for decline have been associated with problems with pollination, germination and establishment of seedlings (Pfab, 1997). Generally, translocated populations often show population variability and decline at only the later stages of a project (Fahselt, 2007; Goedefroid *et al.*, 2001). Results from the previous chapters have indicated that pollination does occur (Chapter 4) and that the reproduction biology of translocated populations of *F. humilis* is stable and functional (Chapter 5). Despite this, population numbers tended to fluctuate and most populations have declined since the beginning of the study (Chapter 5). Only at the Witbank Nature Reserve receptor site did the number of adult plants increase. However, here the number of seedlings significantly decreased. These results merited further investigation into the population dynamics of translocated *F. humilis* populations.

Analysing the size-class distribution (SCD) of a population is a useful tool in population dynamics as it reveals the proportion of non-reproductive and reproductive plants within a population and thus provides indicators of the persistence or decline of a population (Marom, 2006; Pfab, 1997). SCD can also provide insight into individual plant vigour, regenerative capability, availability of space and resources within a habitat, as well as whether SCD is influenced by genetics alone or by inter and intra-specific competition (Bonan, 1988). When a species' optimal environmental requirements are compromised, these stress factors are often reflected in the demography of a population (Cousins, 2013).

The analysis of SCD has been used for various succulent species in the past. For *Euphorbia clivicola*, SCD analysis was used to determine the conservation status of the species and investigate potential reasons for population decline (Pfab, 1997). In another example, analysis of SCD was used for *Adenium swazicum* populations to investigate various habitat effects, which revealed that SCD for the species varied between populations, which correlated with habitat variables (Van der Walt, 2016). In the case of *Kumara plicatilis*, SCD analysis revealed bell-shaped SCD for half of the populations surveyed, supporting the hypothesis of an adult-persistence survival strategy for this species (Cousins, 2013). For other aloe species SCD was also used to investigate reasons for decline. *Aloe pilansii*, for example, showed a bimodal height class distribution, which was accredited to poor recruitment in the past or significant seed removal in the mid-twentieth century (Duncan *et al.*, 2006). For *A. dichotomum*, demographic analysis was used to identify the effects of climate change, rather than direct human effects, as the driver of population instability (Foden *et al.*, 2007; Powell, 2005). A population structure characterised by adult persistence was also identified for *A. peglerae* and was used in population modelling to determine the sustainability of harvesting adult specimens of the species (Pfab & Scholes, 2004). For *Mammillaria gaumeri* (Cactaceae) analysis of population dynamics revealed that stable size distribution was skewed toward small plants and that the population growth rate relied on individual plant survival (Ferrer-Cervantes *et al.*, 2012). Another example of where size class distribution was used in formulation of conservation strategies for a species is that of *Harworthia koelmaniorum* (Witkowski & Liston, 1997).

In SCD studies concerning other mesemb species, population dynamics of two species, *Ruschia robusta* and *Cheiridopsis denticulata,* was studied along a grazing gradient to investigate the changes in vegetation composition close to a long-term stock pen, the survival of succulent shrubs under heavy grazing and to determine distance effects of a stock pen on population dynamics (Riginos & Hoffman, 2003). The study concluded that reproductive output was mostly affected by heavy grazing for both species. In another study concerning threatened *Delospema* species in Gauteng, population analysis was used to study various aspects of species biology for populations and sub-populations to recommend a conservation plan for the threatened species. All species in the latter study showed healthy regeneration through inverse J-curves, except *Delosperma leendertziae,* which was identified as a habitat specialist and hence required special conservation attention (Marom, 2006).

Considering the value of population dynamics studies as highlighted above, similar analyses on *Frithia humilis* populations of the various translocated sites are expected to assist in the evaluation of translocated population health. In this chapter, results obtained from various measures of population health will be explored to evaluate whether translocated populations are stable and whether the demography is within acceptable deviations from those of the control population.

6.2 Methods

Population structure was analysed using a linear regression of SCD parameters. Size-class midpoints (mi) were used as the independent variables, while the number of individuals per size-class was used as the dependant variables (N_i). Since several size-classes had zero individuals N_i was transformed by $\ln(N_i+1)$ (Obiri *et al.*, 2002). Final analysis was performed between $\ln(N_i+1)$ and ln(mi) using XLSTAT Evaluation 18.06 (Addinsoft, 2016). The shape of the population structure was interpreted following Sop *et al.* (2011), Shackleton (1993), Everard *et al.* (1995) and Obiri *et al.* (2002). While a negative slope indicates good regeneration and recruitment since it represents fewer individuals in larger size-classes. A flat distribution indicates a similar number of plants in small and large size-classes. Positive slopes indicate poor recruitment since it suggests that plant abundances were higher in the larger size-classes than in the smaller size-classes. Slope steepness is also indicative of recruitment success where steep negative slopes illustrate better recruitment than shallow slopes (Lykke, 1998; Mwavu and Witkowski, 2009; Obiri *et al.*, 2002).

To examine the stability of translocated populations, the quotients between successive size-classes were calculated (Meyer, 1952). Quotients were graphically displayed and interpreted according to Botha *et al.* (2004) and Shackleton (1993), in which constant quotients represented stable populations, whereas fluctuating quotients were interpreted as instability within the particular population.

Simpson's index of dominance (SDI) (Equation 6.1) was used to investigate the likelihood that any two plants within the population originate from the same size-class (Botha *et al.*, 2004). Where a decline is steeper than expected for an exponentially declining population, values are expected to be higher, while less steep declines will be closer to zero, suggesting size-classes to be more evenly distributed (0-0.1).

$$C = \frac{1}{N(N-1)} \sum_{i=1}^{k} N_i \,(N_i - 1)$$

Equation 6.1

N is the total number of plants per year, *Ni* is the number of plants in class *i*, and k is the number of size-classes (Botha *et al.,* 2004).

Wiegand *et al.* (2000) reported that various indices did not sufficiently describe asymmetrical size distributions. Consequently they developed the Permutation Index (PI) (Equation 2), which calculates the degree of deviation from a monotonic decline or from an inverse-J curve SCD. The combination of SDI and PI could better describe size distribution within a population and was based on the assumption that an 'ideal' undisturbed population should represent a monotonic decline. The Permutation Index is applicable to short lived perennial species since they also tend to show variability in recruitment frequencies (Crawley & Ross, 1990; Picó *et al.*, 2003; Verkaar *et al.*, 1984)

For Equation 6.2, rank is equivalent to enumerating size-classes from the smallest class, which should show the highest frequency, to the largest class, which should show the lowest frequency. In a monotonically declining population PI is equal to zero (*P*=0), while in a discontinuous population, where larger individuals show a higher frequency than a previous size-class, the rank of that size-class has deviated from enumeration and PI is greater than zero (P>0) (Botha *et al.*, 2004; Venter & Witkowski, 2010).

$$P = \sum_{i=1}^{k} |J_i - i| \; ; \; J_i = 1, 2, \ldots \ldots, k$$

Equation 6.2

Ji is the rank of size-class i (i = 1 for the plants occupying the smallest size-class), with the highest rank (Ji = 1) given to the most frequent size-class, and k is the number of size-classes (Wiegand *et al.*, 2000).

Similar size-classes, and also the habitat divisions for Goedvertrouwdt (GA and GB), were used as reported in Chapter 3. Each population was divided into seven size-classes based on reproductive potential (Harris, 2014) and number of leaves (Chapter 3).

6.3 Results

6.3.1 Control population (Ezemvelo)

The results of the size-class distribution (SCD) for the Ezemvelo control population (Z) revealed significant negative SCD slopes for most of the sampling years, which indicated healthy recruitment for 2009-2012 and 2015-2016. Similarly, Permutation Index (PI) values indicated stability for 2011-2012 and 2015-2015 (PI=0) periods but deviations from a monotonic decline during 2010-2011 and 2012-2013 (PI=6; PI=4). This was also supported by the data for Ezemvelo (Z) (Figure 6.1a), which did not show a suitable inverse-J curve since the smallest size-class during the period of 2009 to 2012 and 2012 to 2016 both had fewer individuals than the subsequent larger size-class. These patterns are further supported by the quotients for Z (Figure 6.1b), which suggest a relatively unstable population. However, when the control population average was measured for the entire study period, overall stability with healthy recruitment was evident. This was further confirmed when average size-class frequency distributions were compared to the translocated sites (Z, Figure 6.2a & b). SDI values for Z range between 0.2 and 0.3, which indicated slightly uneven size-class frequency distributions.

When all SCD measures are considered (Table 6.1), the Z population trends theoretically indicate the healthiest regeneration during the latest study period, i.e. 2015-2016. Since the Ezemvelo site represents the control site, it was expected to show a stable population throughout the study period (2010-2016). However, results suggested relative instability between size-classes for these natural *F. humilis* populations. For this reason, and since no similar study exists for comparison, translocated populations will be measured against the results of the control population. Thus, populations with quotient variability similar to Z (Figure 6.1b), significant negative slopes (R^2 values ≥0.5) and with PI values of <6 will, for the purpose of this study, be considered as acceptable deviations from a monotonic population decline. Similarly, SDI values less than 0.3 could be considered healthy when compared to a natural *F. humilis* population. Despite the instability of the control population, averages (Figure 6.1a) for the smaller size-classes for 2010-2012 and 2012-2016 were identical, suggesting that habitat conditions remained largely stable but improved in favour of seed germination and seedling development. Furthermore, the larger size-classes have not shown significant quotient variation, especially when compared to the translocated populations.

6.3.2 Goedvertrouwdt A

The SCD for Goedvertrouwdt A (GA) revealed significant negative slopes during the entire study period, indicating healthy recruitment in every year (Table 6.1). R^2 values close to one indicated that the number of individuals in GA was adequately explained by the size-classes. PI values compared to Z indicated an acceptable monotonic decline for the population over the study period since the highest PI value in the study period was PI=2. This was supported by the data for GA (Figure 6.1d) which showed suitable inverse-J curves for the 2009-2012, 2012-2016 and 2009-2016 population averages. Quotients, however, indicated weaker population stability at GA than at Z (Figure 6.1d). SDI values indicated several years within the study period where unevenness exceeded SDI=0.3. Overall, when comparing average size-class frequency distributions of GA to Z and the other translocated populations (Figure 6.2a & b), stability and recruitment was evident, despite lower average population numbers compared to the control population (Z).

6.3.3 Goedvertrouwdt B

The SCD for Goedvertrouwdt B (GB) revealed significant negative slopes for the entire study period, indicating healthy recruitment in every year (Table 6.1). R^2 values >0.8 indicated that the number of individuals in GB was generally adequately explained by the size-classes. PI values indicated stability through healthy monotonic declines for GB during the entire study period. The data for GB (Figure 6.1e), showed a suitable inverse-J curve for the 2009-2012 population average, while the 2012-2016 population curve illustrated skewed SCD. For the overall population average, a suitable inverse-J curve was revealed (Figure 6.1e). Quotients for GB, however, indicated population instability (Figure 6.1f). SDI values support this observation since values in every year of the study period indicated unevenness by exceeding SDI>0.3. As for GA, the GB population was considered to be more stable with healthier recruitment numbers compared to other translocated populations despite its apparent instability and unevenness (Figure 6.2a & b).

6.3.4 Witbank Nature Reserve

The SCD for Witbank Nature Reserve (W) revealed negative slopes for the entire study period (Table 6.1). However, this was not significant for the periods of 2011-2012, 2012-2013 and 2015-2016, indicating poor recruitment. R^2 values indicated that the numbers of individuals were increasingly inadequately explained by the size-classes for the study period. PI values indicated increasing deviation form a monotonic decline throughout the study period. The data for W (Figure 6.1g) showed that population averages for 2009-2012, 2012-2016 and 2009-2016 did not reveal inverse-J curves. SDI values indicated increasing population evenness within W. Quotients for W (Figure 6.1h), suggest that it has the lowest instability of all the translocated populations. However, the latest population survey (2015-2016) revealed overall instability and unhealthy recruitment when all SCD measures were considered (Table 6.1). Comparison of average size-class frequency distributions to Z and the other population indicated poor stability and recruitment (Figure 6.2a & b).

6.3.5 Eagle's Rock

SCD for Eagle's Rock (E) revealed negative slopes for all years of the study period (Table 6.1). However, these were not significant for the 2011-2012, 2014-2015 and 2015-2016 periods, indicating poor recruitment. R^2 values indicated that the number of individuals was inadequately explained by the size-classes for the study period, especially towards the later surveys. PI values were high (>PI=6) in 2010-2011 and 2012-2013 indicating deviation from a monotonic decline in these years. The data for E (Figure 6.1i) did not show inverse-J curves in the population averages for 2009-2012, 2012-2016 or 2009-2016. Quotients for E (Figure 6.1j), indicated instability for this population. SDI values for E did not exceed 0.3 during any of the study years, which indicate an even SCD. Average size-class frequency distributions revealed overall population instability with poor recruitment for the population at E, compared to Z and other translocated populations (Figure 6.2a &b).

Table 6.1 Summary of size-class distributions for *Frithia humilis* populations at the various study sites. Ordinary least square regression slopes (and p-values), Permutation Index (PI) and Simpson's Index of Dominance (SDI) results are presented per year interval. * indicates significance (p<0.05)

	Year	Slope	SE Slope	R^2	*p*	PI	SDI
Ezemvelo	2010-2011	-0.561	0.257	0.487	0.081	6	0.21
	2011-2012	-0.107	0.212	0.836	0.004*	0	0.25
	2012-2013	-0.937	0.227	0.772	0.009*	4	0.24
	2015-2016	-1.211	0.162	0.918	0.001*	0	0.30
GA	2009-2010	-1.966	0.278	0.909	0.001*	0	0.40
	2010-2011	-1.394	0.209	0.899	0.001*	2	0.34
	2011-2012	-1.836	0.174	0.957	<0.001*	2	0.66
	2012-2013	-1.573	0.267	0.874	0.002*	0	0.32
	2014-2015	-1.942	0.327	0.876	0.002*	1	0.36
	2015-2016	-2.174	0.238	0.944	<0.001*	0	0.52
GB	2009-2010	-1.557	0.163	0.948	<0.001*	0	0.39
	2010-2011	-1.274	0.177	0.912	0.001*	0	0.31
	2011-2012	-1.97	0.228	0.937	<0.001*	0	0.45
	2012-2013	-1.908	0.362	0.848	0.003*	2	0.34
	2014-2015	-2.089	0.367	0.867	0.002*	0	0.36
	2015-2016	-1.952	0.268	0.914	0.001*	0	0.39
Witbank	2009-2010	-1.394	0.123	0.963	<0.001*	0	0.42
	2010-2011	-0.908	0.254	0.719	0.016*	2	0.33
	2011-2012	-0.601	0.308	0.433	0.108	7	0.27
	2012-2013	-0.196	0.337	0.064	0.586	11	0.25
	2015-2016	0.014	0.107	0.003	0.9	15	0.14
Eagle's Rock	2009-2010	-0.626	0.154	0.767	0.01*	3	0.23
	2010-2011	-0.41	0.152	0.592	0.041*	8	0.19
	2011-2012	-0.392	0.21	0.412	0.12	6	0.20
	2012-2013	-0.51	0.152	0.693	0.02*	8	0.18
	2014-2015	-0.468	0.193	0.54	0.06	5	0.20
	2015-2016	-0.474	0.211	0.48	0.084	6	0.18

Slope: negative slopes indicate healthy decline/regeneration/distribution, SE Slope: Standard error of slope, R^2: closer to 1 = the number of individuals is best explained by the size-classes. Significant SCD when $p<0.05$. PI: Permutation Index of 0 = monotonic decline in SCD. SDI: Simpsons Dominance Index >0.1 = uneven SCD.

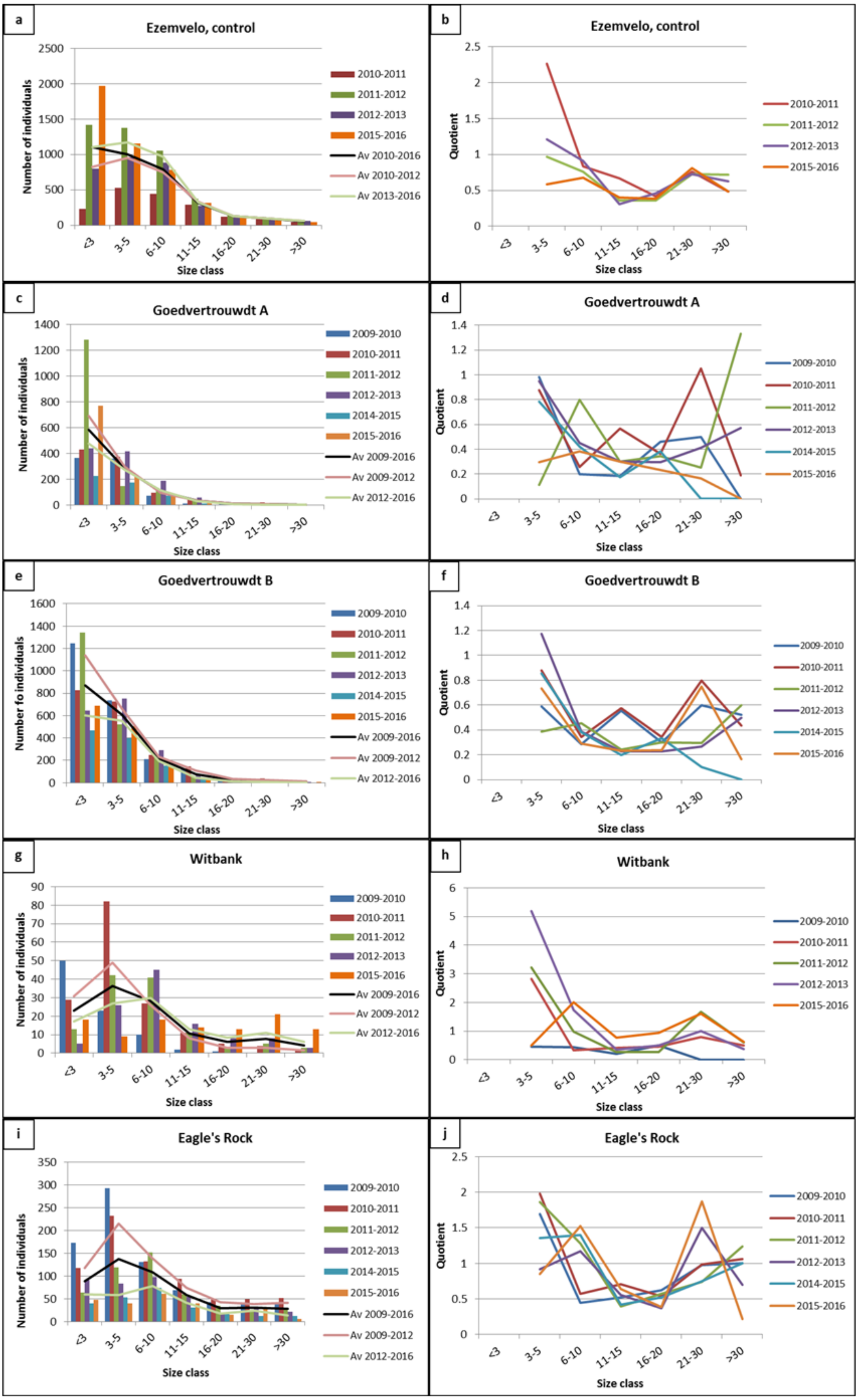

Figure 6.1 Size-class distributions, population means and their respective quotients (b, d, f, h, j) for the periods 2009-2016, 2009-2012 and 2012-2016 for each study site (a, c, e, g, i).

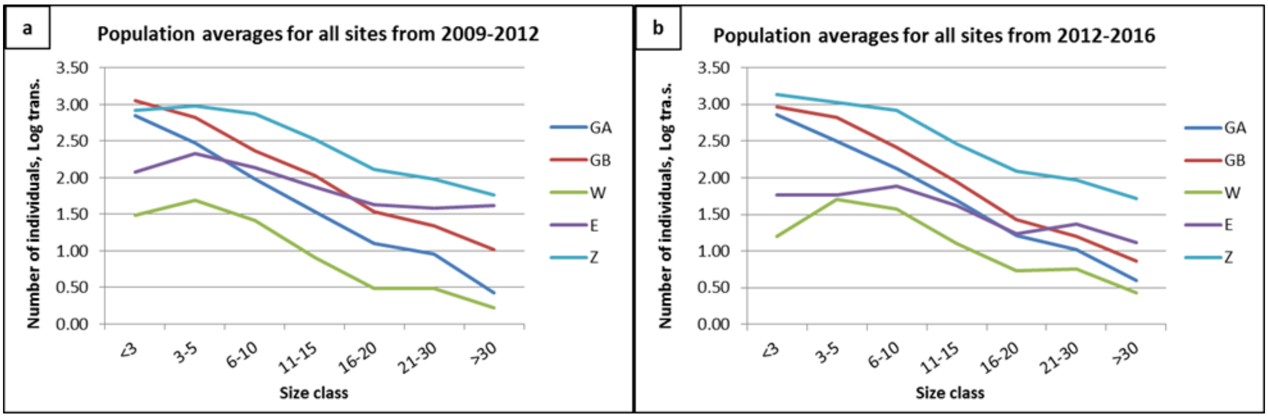

Figure 6.2 Log-transformed averages per size class for all populations between 2009-2012 (a) and 2012-2016 (b).

6.4 Discussion

Demographic analyses revealed significant differences in population performance between the four receptor sites and the control population. Unexpected variability in population dynamics of the control population of *F. humilis* at Ezemvelo (Z) suggests that stability measures are limited in explaining population dynamics of short-lived perennial plant species with a unique growth form and which occupy ephemeral micro-habitat types (Burgoyne & Hoffman, 2011). Measures of population stability were designed primarily for long-lived perennial species, since a longer life span usually has a positive relationship with stable population dynamics (García *et al.*, 2008). Short lived perennial species show greater temporal variability in population demography because survival is less variable than fecundity, which is relatively more important for healthy population dynamics of such species (García *et al.*, 2008). These differences in population variability correspond with the theory of r- and K-selection, which suggests that organisms can develop short or long lifespans based on variation in ecological factors such as availability of resources (Reznick *et al.*, 2002).

F. humilis is considered a short lived perennial based on two observations. Firstly, *F. humilis* has a high juvenile to adult ratio of 1.25:1 (80:64) per square meter (Burgoyne *et al.*, 2000a) and 1.25:1 (35:23) at Z in this study. Secondly, it occupies an ephemeral habitat which is subject to erosion, a phenomenon observed by Burgoyne and Hoffman (2011) and during this study.

Studies on population dynamics of mesemb species are limited and comparisons of results are difficult due to different study objectives. However, a study on the population structure of six threatened perennial *Delosperma* species in Gauteng, South Africa, revealed population variability amongst sub-populations (Marom, 2006). Such observations, although limited for short-lived perennials, infer that some instability and variability may be expected for mesembs and that monotonic decline does not necessarily occur every year. Therefore, the temporal variations observed within populations of translocated *F. humilis* should not necessarily be of concern. However, variations in monotonic decline, evenness and stability which exceed those of the control populations are of concern since they indicate decreasing population health. This is particularly true for the translocated Witbank Nature Reserve (W) and Eagle's Rock (E) receptor sites which are increasingly deviating from a monotonic decline or becoming increasingly even.

GA sowed a suitable SCD but with a sharper decline than Z. GB also showed a suitable SCD but deviated from the monotonic decline toward the end of the study and also showed a sharper decline than Z. The relatively good performance of GA and GB, compared to the other populations, may be attributed to these sites being on the geology preferred by *F. humilis.* Seddon (2010) specified that donor and receptor sites should be as similar as possible to improve the performance of translocated plants. Several studies confirmed that populations translocated to habitats as similar as possible to the donor site have an improved survival advantage compared to less similar receptor habitats which generally reduce population fitness (Fahselt, 2007; Lawrence & Kaye, 2011; Montalvo & Ellstrand, 2000). However, even those receptor sites that seem most similar to a donor population may differ to some extent (Fahselt, 2007). These seemingly minor or undetectable differences at the time of translocation may be the reason for the poor micro-habitat conditions and performance of GB by the end of the study.

Few demographic studies investigated the response of plant populations to local habitat variability, even though these factors are important for understanding population stability and may significantly affect seedling and adult survival, growth and clonal development (Kephart & Paladino, 1997; Menges, 1990). Studies, did however, address individual factors that may possibly affect various plant life stages (Blignaut & Milton, 2005; Bullock 1998; Riba *et al.*, 2002). Several factors relating to micro-habitat characteristics have been reported to influence seedling germination. These include space and moisture availability, heat, nurse plants, herbivory and disease (Kollmann, 2008). At Goedvertrouwdt (both GA and GB) a lack of suitable micro-habitats for germination was observed, caused either by too much gravel or competition from *Selaginella dregei* and various mosses and algae. At W and E a prominent lack of gravel appeared to be the most limiting factor for seed germination. In terms of adult plants the greatest factor suggested to affect population dynamics was interspecific competition (Crawley & Ross, 1990). Within numerous translocation patches conditions seem have been favourable for the establishment and increase of *S. dregei* colonies. The dense mats created by this species have presumably increased competition for adult *F. humilis* plants in combination with reduced habitable space. For at least one herbaceous grassland endemic, *Silene douglasii* var. *oraria*, in Oregon, micro-habitat factors of light, soil depth, and features of surrounding vegetation significantly influenced survival and abundance (Kephart & Paladino, 1997). Habitat quality and its potential effects on *F. humilis* are discussed in Chapter 5.

GA and GB have shown the best performance of the four translocated populations. This can be attributed to the typical geology preferred by *F. humilis* which is the same as that of the control population (Z). The literature impresses the importance of translocation projects using receptor habitats as similar as possible to the donor population (Lawrence & Kay, 2009; Montalvo & Ellstrand, 2000; Seddon, 2010). The importance of similar habitats is confirmed by successful translocations such as that of *Brachycome muelleri,* similar to *F. humilis* in that it is also an edaphic species. Jusaitus *et al.* (2004) paid particular attention to identifying receptor sites of similar geology, edaphic characteristics, topography, aspect and vegetation. For *F. humilis* results from this chapter and Chapter 5 confirm that translocation to geology and habitats as similar as possible to a natural population provide the best results.

When looking at the populations showing better performance on typical geologies (GA & GB), a further division between these two populations can be made based on micro-habitat conditions. The literature also mentions the importance of these factors for translocation success (Eriksson & Ehrlén, 1992; Seddon, 2010), which is confirmed by the abovementioned consideration for *B. muellerii*, ensuring its translocation success. Results from this study support this since habitats that correspond most closely with those of Z have shown the best performance. Thus, it can be inferred that GA has performed the best in terms of SCD since geological and micro-habitat conditions show the greatest association with that of Z.

6.5 Conclusion

Analysing the size class distribution of plants is useful since it helps to determine the health of a population in terms of the proportions of reproductive and non-reproductive individuals, as well as the stability and evenness of these proportions, thereby indicating whether the population is persisting or declining. Analysis of the slope, size class distribution, permutation index and Simpson's dominance index for the control population (Z) suggested that some instability and deviation from a monotonic decline might be normal for healthy populations of *F. humilis*. This was supported by the link between lifespan and population dynamics of other herbaceous perennials and the probability that *F. humilis* is a short-lived perennial which has inherent variability in its population structure.

Based on this it can be concluded GA has performed the best amongst the translocated populations due to suitable geology and micro-habitat which reflect those of Z most closely. GB, W and E have all shown increasingly unhealthy SCD due to various forms of habitat deterioration including erosion, competition due to encroachment and over accumulation of gravel, which have not only reduced suitable micro-habitats but have also reduced habitable space in general for *F. humilis* within receptor patches.

Detailed habitat assessments are needed to explain population instabilities at these translocation sites since habitat deterioration is suggested as an important driver of population instability and reduction in numbers observed in the translocated populations. Furthermore, it is recommended that long term studies of the dynamics of other natural *F. humilis* populations be conducted to verify to what extent instability and deviation from a monotonic decline can be considered normal. In addition to this, it may be useful to also investigate the population dynamics of *Frithia pulchra* since it is the closest relative of *F. humilis*, and may provide useful information regarding the population dynamics of this species.

CHAPTER 7

CONCLUSION

7.1 Introduction

Translocation is a last-resort conservation method for many threatened species (Bontrager *et al.*, 2004; Hewitt *et al.*, 2011; Heywood & Iriondo, 2003). It is still experimental and highly debated due its potential effects on the species in question, the receptor habitat and its species, as well as the high levels of failure (Fahselt, 2007; Hewitt *et al.*, 2011; Riccaiardi & Simberloff, 2009a). Despite this, translocation is becoming an increasingly important conservation strategy (Godefroid *et al.*, 2011; Griffith, 1989; Hodder & Bullock, 1997). In South Africa the use and study of translocation is still poorly understood (Burgoyne & Hoffman, 2011; Milton *et al.*, 1999), thus the translocation of an endangered *Frithia humilis* population can be seen as having a dual purpose of saving a population as well as evaluating the use of translocation as a conservation method.

The previous study by Harris (2014) concluded that *F. humilis* could survive the translocation process and successfully reproduce in receptor habitats. The continued monitoring of the translocated *F. humilis* populations in this study aimed to quantify and compare pollination, fecundity and population structure over time and between populations at receptor and control sites to establish whether translocation is a feasible long term conservation tool for this species. Below a detailed assessment is provided based on the major findings of the study.

7.2 Chapter 4. Pollination biology of *Frithia humilis*

- Six new records were made of insect species carrying *Frithia* pollen in varying degrees. The current list of containing five potential pollinators of *F. humilis* has now been expanded to 11 species.
- The newly observed species support the standing Melittophilous pollination syndrome, however, the observations made of wasp pollinators supports the Anemophilous pollination syndrome and the possibility of reserve pollinators being present at receptor sites.
- Based on the distribution and number of observations among study sites, several primary pollinators could be suggested and the probability of reserve pollinators was reinforced.

- Pollination was found to be more efficient at the receptor sites than the control population, based on the percentage of fruits produced per number of flowers. The reason for this is density related.
- The high percentage of fruits at receptor sites suggest that one or more effective pollinator are present at these sites and is confirmed among the pollinators observed thus far.

Based on these results the first hypothesis, which proposes that primary pollinators will be present at the receptor sites and will be carrying *F. humilis* pollen, is partially accepted since insects visiting *F. humilis* flowers could be confirmed to be pollen carriers. Further studies are required to confirm which of these pollen carriers are the most effective pollinators.

7.3 Chapter 5. Fecundity of translocated *Frithia humilis* populations

- Significant micro-habitat differences were observed for the Goedvertrouwdt receptor site and led to its division into two habitat types GA and GB.
- Flower production for most translocated populations increased or returned to original numbers since the start of the study. Only GB showed a steady decline in flowers over time. Conversely, W showed a significant increase in the mean number of flowers. The mean number of flowers at all receptor sites was significantly lower than that of the control population.
- All receptor sites showed increases in their mean number of fruits. GA and W showed the greatest increases, while E showed the least increase. The mean number of fruits was, however, lower than that of the control population.
- Overall, every receptor site showed a medium to significant decrease in numbers of seedlings, apart from GA which showed a medium increase. Seedling numbers for all receptor sites were also lower than that of the control population.
- Sub-adult numbers declined for all study sites over the study period. Only at GA was the decline not as significant. Sub-adult numbers were significantly lower that the control population for all receptor sites.
- E and GB showed the greatest declines in adult numbers, while GA showed less of a decline. W, however, showed a significant increase in adult number overs the study period. Adult numbers for all receptor sites were significantly lower than the control population.
- Yearly fluctuations in mean numbers may be ascribed to variability in rainfall, however, deterioration of habitat quality has had the greatest effect on population health over the study period.

- A lack of suitable micro-habitat was the greatest restraining factor for seedling establishment.
- Sub-adults and adults were most affected by patch erosion due to up-slope run-off and competition from encroaching *S. dregei* and other plants due to the concave patch surfaces and poor drainage in certain habitats.

Based on these results the second hypothesis, which proposes that fruit set will increase over time and subsequently the seedling numbers, is partially rejected since fruit set did not increase significantly for all populations, and all except one population showed declines in seedling number. The populations that showed the best performance occurred on the typical geology at Goedvertrouwdt in habitat GA. Further monitoring is required to find reasons for the dwindling population numbers over time, since fruit set is satisfactory, but seedling establishment is weak.

7.4 Chapter 6. Population dynamics of *Frithia humilis*

- Size class distribution of a natural *F. humilis* population suggested that some instability and deviation from a monotonic decline might be normal, even for healthy, undisturbed populations.
- GA showed the best performance due to a suitable size class distribution (SCD), however, the population slope was somewhat more uneven than that of the control population.
- GB showed similar results to GA, however from 2012-2016 the SCD deviated from a monotonic decline with fewer individuals in the younger size-classes for this period.
- W and E have all shown increasingly unhealthy SCD. W has increasingly deviated from a monotonic decline and shown increasing evenness in SCD, while E has shown constant evenness in SCD over the study period.
- Factors similar to those discussed in Chapter 5 are believed to be influencing these populations.

Based on these results the third hypothesis which proposes that, over time, the population structure of the receptor sites will become comparable to that of the control population, is partially accepted since only two populations on the typical geology showed population structures comparable to the control population, while the other two populations showed dissimilar populations structures dominated either by seedlings or adults. A decision regarding the success of the translocation needs to be made in 2019 (10 years). The SCD for the populations of each of the receptor sites will be compared to the control to assess whether the population structures are approaching that of natural populations, which will then indicate the health of the translocated populations.

7.5 Final proposition

This monitoring programme of the translocated *F. humilis* population, revealed population health and structure problems, six years after translocation took place, despite healthy reproductive systems at all translocation sites. Populations translocated to the typical Dwyka and Ecca sandstones performed better than populations translocated to a-typical geologies. At receptor patches where micro-habitats on the typical geologies were similar to those of the natural control population, *Frithia* populations performed best, though still at rates well below that of the control population. This highlights the need for conservation ecology studies prior to any translocation, to ensure that the edaphic needs of edaphic specialists, such as *F. humilis*, are met and to gain a complete understanding of habitat requirements and accurate selection before a translocation project is attempted.

While the translocation of *F. humilis* initially seemed favourable, these latest findings indicated that translocation, using current methods, is not a feasible long-term conservation tool. Translocation as a strategy to save endangered populations of *F. humilis* is strongly discouraged until more research is done on its habitat requirements and the identification of suitable receptor habitats that will remain stable and protected over the long term.

7.6 Practical outcomes from this study

In Chapter 3 it was mentioned that preliminary mining activities seemed to be taking place in mid-2015 at the Goedvertrouwdt receptor site. This was confirmed in mid-2016 when an application for a mining permit was submitted by Ixolox (Pty) Ltd. Mining Company to the Department of Mineral Resources to extract remaining coal from the site. In response to the threat posed to the *Frithia* populations on this site, a letter of concern was submitted to the Environmental Assessment Practitioners, Eco Elementum (Pty) Ltd., as part of the public participation process. Consequently, Ixolox withdrew the mining permit on the 20th of October 2016. The reasons for the withdrawal were a petition from interested and affected parties as well as the occurrence of *F. humilis* on the site, which occur as natural and translocated populations and could not be moved based on the conservation status of the species and findings from this study.

7.7 Recommendations

7.7.1 Pollination biology

Other aspects of *F. humilis* pollination biology which still require investigation are pollen ovule ratios, the presence of nectaries and the amount of nectar. Furthermore, it is possible that *F. humilis* has a more complex method of visual attraction than initially thought. *Bergeranthus multiceps* for example appears completely yellow under normal light, however, under ultra-violet light petals reflect UV light while the anthers absorb it, making the centre of the flower more prominent to UV sensitive eyes (Peter *et al.*, 2004). Such occurrences of patterns under UV light have been shown to influence visitation and foraging behaviour of pollinators (Koski & Ashman, 2014). The colour change of pollinated *F. humilis* flowers, which may act as an indicator as suggested by Burgoyne *et al.* (2000a), could be apparent in the UV reflectivity of the flowers. These details may indicate another way by which *F. humilis* communicates with pollinators. This information could in turn reinforce the pollination syndrome of the species.

Zietsman (2013), also mentioned the timing of the male and female flowering phases and showed that *Stomatum bolusiae* is not protandrous as described for most melitophilous flowers (Hartmann, 1991), and that floral traits in fact indicate homogamy and self-incompatibility. Since this is the closest related study, further investigation into the timing of floral phases for *F. humilis* may also be suggested.

In terms of pollinator species, specifically designed experiments would be needed to verify which insects actually perform this task. While this may provide useful information, if a variety of visiting insect are capable of pollination, the significance of any such results may be reduced since Mayer and Pufal (2007) and Welsford and Johnson (2012) have suggested that even a single visit to a flower may be sufficient or even preferable for pollination to occur, making it possible that even the most unsuitable visitor may successfully pollinate a flower. Confirming pollinator species will determine whether the pollination biology *F. humilis* will be secure or a point of concern for future conservation efforts. In addition to this, further field observations are required at various *F. humilis* populations to expand the list of potential pollinators and more accurately identify species which are important to the pollination biology of *F. humilis*.

7.7.2 Translocation

Based on the findings described in this chapter it is suggested that further translocation projects of *F. humilis* are not to be initiated until studies have been conducted and conclusions drawn as to the best methods for accurately selecting suitable receptor habitats where populations may persist without human intervention.

This study identified erosion as a noteworthy problem for *F. humilis* translocations. For this reason future studies should include erosion monitoring and mitigation measures. In this project the mining company has withdrawn all support and subsequently the translocated populations were unable to persist sufficiently at all the translocation sites. Encroachment and competition were also identified as constraints and should also be considered in future monitoring programs and experimental studies

7.7.3 Population dynamics

This study found a general lack of other studies on the population structure and dynamics of other species of Aizoaceae. Thus, it is suggested that more studies of a similar nature are conducted on other species of Aizoaceae to improve the understanding of population dynamics within this family and to provide a reference for future conservation projects and research.

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