THE REPRODUCTIVE BIOLOGY AND HABITAT REQUIREMENTS OF *ALOE PEGLERAE*, A MONTANE ENDEMIC ALOE OF THE MAGALIESBERG MOUNTAIN RANGE, SOUTH AFRICA

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DECLARATION

I declare that this Dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

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ABSTRACT

In this study I investigated the reproductive biology and pollination ecology of *Aloe peglerae*, an endangered endemic succulent species of the Magaliesberg Mountain Range in South Africa. The aim was to determine the pollination system of *A. peglerae*, the effects of flowering plant density on plant reproduction and the suitable microhabitat conditions for this species.

Aloe peglerae possesses floral traits that typically conform to the bird-pollination syndrome. Pollinator exclusion experiments showed that reproduction is enhanced by opportunistic avian nectar-feeders, mainly the Cape Rock-Thrush (*Monticola rupestris*) and the Dark-capped Bulbul (*Pycnonotus tricolor*). Insect pollinators did not contribute significantly to reproductive output. Small-mammals were observed visiting flowers at night, however, the importance of these visitors as pollinators was not quantified in this study.

Interannual variation in flowering patterns dictated annual flowering plant densities in the population. The first flowering season represented a typical mass flowering event resulting in high seed production, followed by a second low flowering year of low seed production. Reproductive success was significantly related to flowering plant densities at a spatial scale of 30.0-35.0m in 2011, corresponding to the scale at which flowering plant density influenced diurnal (bird) visitation rates (25.0-40.0m). In the second flowering season, neither plant reproduction nor diurnal visitation rates were related to flowering plant densities. Nocturnal (small-mammal) visitation rates were not related to flowering plant densities in both years and this is perceived to be a reflection of the smaller home/forage range of small-mammals. However, further investigation is required to confirm the hypothesis that small-mammal visitations contribute to reproductive success in *A. peglerae*.

Aloe occupancy was generally associated with “safe site” microhabitat conditions (i.e. high rock cover, low grass cover and biomass). This is expected to be a fire-survival strategy of *A. peglerae*, where the persistence of individual plants in less vulnerable microsites over time is favoured. However, plant morphology might also confer adaptations, such as vegetative recovery at the apical meristem, to tolerate the harsh effects of fire.

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PREFACE

The motivation behind this research was essentially the need to build the knowledge base about the reproductive biology of *Aloe peglerae.* Populations of this species are declining throughout its range in the Magaliesberg Mountain Range, and as a result are becoming increasingly threatened by extinction. It is my hope that any outcomes from this, and other studies, that would ultimately fill existing knowledge gaps, would be considered for further research and publication. There are five chapters in this dissertation: a general introduction, three "results" chapters, and a general concluding chapter. The general introduction gives a detailed review of the literature to set the scene for the structure of the dissertation. The three results chapters each address specific objectives outlined in the general introduction. These three chapters have also been presented and written in the format of journal articles with the intention of publication in peer-reviewed journals. Chapter two, which addresses the pollination aspect of the study, was presented at the South African Association of Botany (SAAB) conference in Pretoria, 15–18 January 2012, and was later published in the journal *Plant Ecology* (Arena, G., Symes, C.T., Witkowski, E.T.F. 2013. The birds and the seeds: opportunistic nectarivores enhance reproduction in an endemic montane aloe. *Plant Ecology,* vol. 214, pp. 35–47. DOI 10.1007/s11258-012-0144-z). However, I have changed the formatting of this chapter slightly to match the general formats of the other chapters. For example, the acknowledgements of the published article have been removed and incorporated into the general acknowledgments of the dissertation. Chapter three, which addresses the question of density-effects on plant reproduction, was presented at the SAAB conference in Drakensville, 19–24 January 2013. As a result of the formatting of these chapters in the form of journal articles, repetition of certain information between chapters, particularly the methods and materials, could not be avoided. Each results chapter has its own list of references, except the general introduction and concluding chapters which share a reference list at the end of the dissertation.

CHAPTER ONE

GENERAL INTRODUCTION

Pollination biologists have made considerable advancements in understanding the interactions between plants and their pollinators, and are continually contributing to the body of research (e.g. Proctor *et al.* 1996; Waser and Ollerton 2006). A relatively recent explosion of interest in the ecology and evolution of pollination has led to the publication of numerous papers that focus on pollination syndromes, pollinator-driven speciation, the origins, evolutionary diversification, adaptations, and the genetic and ecological associations of plant-pollinator interactions (e.g. Stiles 1978; Ollerton 1996; Waser *et al.* 1996; Johnson and Steiner 2000; Fenster *et al.* 2004; Waser and Ollerton 2006; Johnson and Nicolson 2008; Botes *et al.* 2009b; Mitchell *et al.* 2009; Hargreaves *et al.* 2012). In the past, most studies merely recorded the pollinator taxa of plant species. Now, pollination biologists are advancing the science through interdisciplinary research of ecological and evolutionary perspectives of the plant-pollinator relationship (Feinsinger 1978; Feinsinger *et al.* 1991; Lamont *et al.* 1993; Waser and Ollerton 2006; Mitchell *et al.* 2009).

The genus *Aloe* has received a considerable amount of attention in areas of pollination biology; however, many species are yet to be the focus of pollination studies. The majority of research on this genus has only focused on a few select species, investigating biochemistry, micropropagation, metabolism, commercial uses, fire survival and population demography (e.g. Kluge *et al.*1979; Bond 1983; Smith and Correia 1992; Cavallini 1993; Abrie and van Staden 2001; Liao *et al.* 2004; Duncan *et al.* 2006; He *et al.* 2007; Grace *et al.* 2009). Several published works have recently investigated pollination systems (discussed later) as well as the ecological context of the plant-pollination interactions within the genus (e.g. Melville 1988; Stokes and Yeaton 1995; Johnson *et al.* 2006; Botes *et al.* 2008; Johnson and Nicolson 2008; Symes and Nicolson 2008; Forbes *et al.* 2009; Wilson *et al.* 2009; Duffy and Johnson 2011; Wolf and Hatch 2011).

1.1 Background information

The genus *Aloe* (Asphodelaceae) is an Old World group that comprises approximately 550 species (Holland 1978; Smith and Van Wyk 2009). The distribution of *Aloe* extends from the southern tip of Africa to the Arabian Peninsula, and also occurs in Malagasy and Socotra in West Africa (Holland 1978). South Africa is home to approximately 125 species of *Aloe*, excluding variations and hybrids (Reynolds 1950; Van Wyk and Smith 2003).

Aloes were first described in the 1600s in South Africa (Reynolds 1950). The first documented evidence of cultivation for the aloe drug (*Aloe barbadensis* Mill.) was recorded in southern and eastern Africa (Cavallini *et al.* 1991). A detailed account of the different uses of *Aloe* throughout the world is given by Grace *et al.* (2009). The biggest bio-cultural importance of *Aloe* is suggested to be medicinal and pest control uses, but aloes are also important in horticultural, social, art, and environmental practices (Grace *et al.* 2009).

Each species differs considerably in size, ranging from a height of 18cm for *A. saundersiae,* to the height of a large tree aloe, *A. barberae,* at about 18m (Smith and Van Wyk 2009). Some species are arborescent, and others herbaceous, but all are succulents (Reynolds 1950). All tend to have shallow root systems, are perennial plants that generally flower in winter months and produce large numbers of wind-dispersed seeds, reproduce sexually and some are capable of vegetative reproduction (Reynolds 1950; Cousins and Witkowski 2012). Flowering phenology differs greatly between and within species (Reynolds 1950). Most of these differences in flowering times depend on the locality, altitude, and variable environmental conditions (Reynolds 1950; Van Wyk and Smith 2003). Reynolds (1950) describes how species with wide distributions will generally flower earlier in the warmer coastal belt, but later in the colder, higher altitude areas further inland.

Like many other plants and animals, an increasing number of *Aloe* species are becoming threatened due to anthropogenic activities such as habitat destruction and fragmentation, mining activities, domestic livestock overgrazing, unsustainable harvesting, and illegal plant-collecting from wild populations (Bornman and Hardy 1971; Van Wyk and Smith 2003). With a few exceptions, most species are protected by environmental legislation in all provinces (Van Wyk and Smith 2003), but despite this, illegal harvesting still remains unchecked in many areas throughout South Africa. Unsustainable harvesting from the wild for horticulture and natural products industries is one of the major concerns for *Aloe* that is regulated by CITES (Grace *et al.* 2009). Indeed, the sustainable harvesting level for *A.*

peglerae is so low (0.12%) that no harvesting of this species from the wild is recommended (Pfab and Scholes 2004).

Avenues of future research for this genus includes aspects of population structure, population monitoring of species threatened by unsustainable harvesting and illegal collecting, fire ecology of the different aloe groups, germination and seed storage of critically endangered species, and ecological studies in aloe-diverse regions in Africa and Madagascar, are vital to the conservation of certain species (Cousins and Witkowski 2012). The potential use of aloes for ecosystem restoration in areas which are particularly prone to erosion, desertification and degradation from land use practices is also suggested to be worth investigating (Smith and Correia 1992; Cousins and Witkowski 2012).

1.1.1 Pollination system

Of greater pertinence to this research project are the numerous studies in the last 20 years that have investigated the pollination systems of several species of *Aloe*. Many South African plants, particularly aloes, possess floral traits characteristic of a bird-pollination syndrome, and are frequently visited by a suite of specialist and generalist avian nectarivores (e.g. Oatley 1964; Oatley and Skead 1972; Stokes and Yeaton 1995; Botes *et al.* 2008, 2009a; Brown *et al.* 2009; Symes *et al.* 2009; Brown *et al.* 2010). Many species also receive abundant insect visitors (e.g. Hargreaves *et al.* 2008; Botes *et al.* 2009b). These studies have made significant contributions to investigating pollinator types and abundance, plant mating system, reproductive success, and the role that nectar properties play in selecting and influencing pollinator guilds and communities (Oatley and Skead 1972; Van Wyk *et al.* 1993; Colwell 1995; Nicolson 2002; Nicolson and Nepi 2005; Johnson *et al.* 2006; Nepi *et al.* 2006; Human and Nicolson 2008; Symes and Nicolson 2008; Coombs and Peter 2009; Wolf and Hatch 2011). A summarized list of pollination studies on South African *Aloe* species are listed in Table 1.1.

Based on the findings of many of these studies, the typical specialist-bird pollination syndrome (e.g. sunbirds) originally attributed to *Aloe* has been questioned. Several studies have provided evidence for ‘occasional’ or ‘opportunistic’ bird pollination (e.g. generalist nectar-feeding by passerines and non-passerines). In addition, many *Aloe* species receive abundant insect visitors, such as honeybees (Hoffman 1988; Botes *et al.* 2009a). However, in some cases, insect visitation does not always result in successful pollination due to floral morphology and inefficient transfer of pollen while foraging, e.g. *A. ferox,* (Botes *et al.*

2009a). The existence of mammal visitation and potential pollination in any particular *Aloe* species should be considered, particularly for species that are small, flowering close to the ground and that produce copious volume of nectar. No studies on *Aloe* to date appear to have addressed this in detail.

The major influence upon the pollination system of a species consists of a combination of floral visitor morphology and behaviour, and the genetic structure and spatial distribution of plant populations (Stiles 1978). Even while pollination will result in fruit and seed set, certain factors will influence the degree of success in reproducing viable offspring, such as plant population size and/or density, pollinator effectiveness, pollinator visitation and foraging behaviour, and microhabitat suitability and availability. It becomes necessary to investigate a host, if not all, these factors when studying the pollination ecology of a species.

1.1.2 Density-related reproductive success

Habitat fragmentation and degradation, and unsustainable harvesting are some important ecological perturbations that can contribute to the reduction in plant population size and density (Ashman *et al.* 2004; Knight *et al.* 2005). These perturbations are also known to alter plant-pollinator interactions further affecting the persistence of plant populations (Lamont *et al.* 1993; Stephens and Sutherland 1999; Stephens *et al.* 1999; Knowles and Witkowski 2000; Donaldson *et al.* 2002; Menz *et al.* 2010).

Pollinator abundance and diversity will change when habitat becomes fragmented because inter-patch forage distances become longer and isolated patches become less attractive to pollinators (e.g. Jennersten 1988; Lamont *et al.* 1993; Ågren 1996; Cunningham 2000; Aizen et al. 2002; Ward and Johnson 2005). Other pollinator requirements such as nesting sites or host larval dependency may also become unavailable, or predators and parasites of pollinators are also affected in increased fragmented populations. As a result, pollination efficiency and effectiveness becomes limited in fragmented patches (Rathcke and Jules 1993; Aizen et al. 2002). This ultimately leads to pollen limitation in plant species that rely heavily upon pollinator vectors, such as insects, birds, or mammals, further reducing plant reproductive output (Harper 1977; Lamont *et al.* 1993; Ågren 1996; Courchamp *et al.* 1999; Aizen et al. 2002; Knight *et al.* 2005; Kleizen *et al.* 2009). Reductions in plant population size and/or density may further lead to increased levels of inbreeding for species that are self-compatible, as well as for those that are naturally self-incompatible (Menges 1991; Busch 2005). Thus it is easy to understand how plant reproductive success within fragmented

populations can be significantly influenced by the local densities of conspecifics (Lamont *et al.* 1993; Roll *et al.* 1997; Ghazoul *et al.* 1998).

The concept of reduced plant reproduction as a result of pollinator and/or pollen limitation in small and/or sparse plant populations (Allee effect) has not been studied in great detail in *Aloe* (Allee 1931; Stephens *et al.* 1999; Wilcock and Neiland 2002; Ashman *et al*. 2004; Knight *et al.* 2005).This genus provides a good test model from which to study changes in plant reproduction in fragmented populations because the majority of species rely on pollinator vectors for successful pollination. Furthermore, many of the South African *Aloe* species have very localized distribution ranges and with an increase in urbanization and habitat destruction, it is predicted that many *Aloe* populations will suffer the effects of habitat fragmentation.

1.1.3 Microhabitat requirements

The interacting processes between climate, soils, topography, fire and grazing are some of the factors that can significantly affect the spatial patterns of plant distributions (e.g. Kephart and Paladino 1997; Bond *et al.* 2005; Sommers *et al.* 2011). In South Africa, most *Aloe* species occur in areas where fire is infrequent or absent (Smith and Van Wyk 2009), such as parts of the Great Karoo, Northern Cape, Eastern Cape and KwaZulu-Natal. However, many other species occur in fire-prone areas across South Africa, such as the fynbos, grassland, and savanna biomes. These regions frequently experience fire events which are an important determinant in shaping the vegetation patterns in these landscapes (e.g. Bond 1984; Clark *et al.* 2005). The spatial distribution of succulent plants within a patch of habitat depends on the degree of suitability and availability of microsites (Fowler 1988), and this is usually influenced by the effects of fire (Pfab and Witkowski 1999; Symes 2012; Cousins *et al.* in prep.).

Water storage is a characteristic physiological function in Crassulacean acid metabolism - photosynthesizing succulents enabling these plants to tolerate harsh, dry conditions. Many *Aloe* species, besides the grass aloes, colonize areas along steep slopes and rocky outcrops that enable good drainage and water runoff, thus reducing the incidence of rotting in waterlogged soils (Reynolds 1950). However, most succulents thrive in very similar microhabitat conditions which afford protection from fire and desiccation (Thomas and Goodson 1992).

Table 1.1 A summary of pollination biology research conducted on species (n=22) of the genus *Aloe.*

Species	**Research focus**	**Reference**
Aloe africana	Convergence with co-flowering species; floral traits; flowering phenology; bird, insect pollination	Botes 2007; Botes *et al.* 2008; Botes *et al.* 2009a,b
Aloe arborescens	Nectar properties; pollen theft; inefficient pollination; floral traits	Nicolson 2002; Hargreaves *et al.* 2012
Aloe boylei	Pollen theft; inefficient pollination; floral traits	Hargreaves *et al.* 2012
*Aloe candelabrum**	Pollination; pollinator attraction; seed dispersal; intraspecific competition	Stokes and Yeaton 1995
Aloe castanea	Nectar properties	Nicolson and Nepi 2005
Aloe ciliaris	Nectar properties	Nicolson and Nepi 2005
Aloe divaricata	Pollination; flowering phenology	Ratsirarson 1995
Aloe dominella	Pollen theft; inefficient pollination; floral traits	Hargreaves *et al.* 2012
Aloe ferox	Convergence with co-flowering species; floral traits; flowering; pollen theft; inefficient pollination; floral traits	Hoffman 1988; Botes 2007; Botes *et al.* 2008; Botes *et al.* 2009a; Hargreaves *et al.* 2012
Aloe greatheadii var. *davyana*	Nutritional content of pollen; bird/insect pollination	Human and Nicolson 2006; Symes *et al.* 2009
Aloe inconspicua	Insect pollination; pollen theft; inefficient pollination; floral traits	Hargreaves *et al.* 2008; Hargreaves *et al.* 2012
Aloe kraussii	Pollen theft; inefficient pollination; floral traits	Hargreaves *et al.* 2012
Aloe linearifolia	Insect pollination	Botes *et al.* 2009b
Aloe lineata var. *muirii*	Convergence with co-flowering species; floral traits; flowering	Botes 2007; Botes *et al.* 2008; Botes *et al.* 2009a
Aloe maculata	Pollen theft; inefficient pollination; floral traits; pollen reward	Hargreaves *et al.* 2010; Duffy and Johnson 2011; Hargreaves *et al.* 2012
Aloe marlothii	Flowering phenology; nectar production; bird nectarivory; niche partitioning; bird/insect pollination; nutritional content on nectar; pollen theft; inefficient pollination; floral traits	Symes and Nicolson 2008; Symes *et al.* 2008; Symes *et al.* 2009; Symes 2010; Symes *et al.* 2011; Hargreaves *et al.* 2012
Aloe minima	Insect pollination	Botes *et al.* 2009b
Aloe plicatilis	Insect pollination; seed dispersal; seed banks; germination	Cousins *et al.* 2013
Aloe pluridens	Convergence with co-flowering species; floral traits; flowering	Botes 2007; Botes *et al.* 2008; Botes *et al.* 2009a
Aloe pruinosa	Pollination; breeding system; pollen limitation	Wilson *et al.* 2009
Aloe speciosa	Nectar properties; convergence with co-flowering species; floral traits; flowering	Nicolson 2002; Botes 2007; Botes *et al.* 2008; Botes *et al.* 2009a
Aloe tenuior	Pollen theft; inefficient pollination; floral traits	Hargreaves *et al.* 2012
Aloe vryheidensis	Nectar palatability; Pollen theft; inefficient pollination; floral traits	Johnson *et al.* 2006; Hargreaves *et al.* 2012

* This species is synonymous with *A. ferox*, but has been placed on its own as used in the referenced publication by Stokes and Yeaton (1995).

Succulent plants are either tolerant or intolerant to fire (Thomas and Goodson 1992; Pfab and Witkowski 1999). Fire-intolerant species normally occur in regions where there is little or no fuel for fire (e.g. Witt and Nongogo 2011) or in local-scale "safe sites" (a site that is suitable for germination and establishment; Fowler 1988) such as rocky, sandy microhabitats with little surrounding fuel-carrying vegetation (Thomas and Goodson 1992; Pfab and Witkowski

1999; Cousins *et al.* in prep.). Fire-tolerant species, e.g. grass aloes such as *A. chortolirioides*, can survive frequent fires and/or high intensity burns due to morphological adaptations which allow them to cope in fire-prone environments (Thomas and Goodson 1992). Indeed, some grass aloes depend on the occurrence of fire for regulating growth and flowering (Cousins and Witkowski 2012). However, the evolution of well-developed meristems and rootstocks in *Aloe* has added to the vigour of many species (Smith and Van Wyk 2009; Cousins *et al.* in prep.). A 'skirt' of dead leaves which extends down the length of the stem of *A. ferox* and *A. marlothii* functions as a protective and insulating coat against fire (Bond 1983; Smith and Van Wyk 2009). The thick, corky bark of *A. plicatilis* is also suggested to provide protection from the heat of the fires characteristic of the Cape fynbos (Smith and Van Wyk 2009; Cousins *et al.* in prep.). Some *Aloe* species also resprout from the stem-base, e.g. *A. haemanthifolia, A. commixta* and *A. micracantha,* producing new leaves if above-ground parts are damaged in a fire (Smith and Van Wyk 2009).

Despite the various fire-survival strategies that have developed in *Aloe*, the effects of fire on aloes can be detrimental, sometimes resulting in death. Although there is a general understanding of the adaptation of aloes to fire, in-depth studies of the resprouting behaviour of different aloe groups is needed (Cousins and Witkowski 2012). Accompanying this, comparisons of the microhabitat conditions between different aloe groups and an improved understanding of the significance of microhabitat preference and fire occurrence can be explored further in this genus. These aspects of the fire ecology of different species of *Aloe* can aid in improving management and conservation practices.

1.2 Rationale

The focus of this research was dedicated to studying the pollination biology and the ecological interactions of the plant-pollinator relationship of the winter-flowering *A. peglerae*. *Aloe peglerae,* a montane endemic of the Magaliesberg and Witwatersberg mountain ranges in the Gauteng and North-West provinces in South Africa, is listed as Endangered (SANBI 2009). One of its greatest threats has been the over-zealous and illegal collecting by the public of whole plants, the attractive inflorescence, the leaves, or the fruits and the seeds, from wild populations (Scholes 1988; Pfab *et al.* 1998; Pfab and Scholes 2004). Pfab *et al.* (1998) suggested that hikers, along the many hiking trails in the Magaliesberg, were likely to have had more of a negative impact on populations of this species than the rest of the public that has only limited access to the wild. Populations of *A.*

peglerae are predicted to continue to decline if harvesting of this species is not controlled (Pfab and Scholes 2004; Phama 2012).

The floral traits of *A. peglerae* typically fit a bird pollination syndrome. However, based on the contrasting evidence for pollination systems in *Aloe*, it is important to formally identify the pollination system of *A. peglerae.* The decline in population size and/or density of *A. peglerae* is likely to cause shifts and/or declines in pollinator distribution and abundance. Hence, it is also essential to investigate how reproductive success is affected by varying local-scale flowering plant densities. Furthermore, any loss in suitable habitat, or any mismanagement of land use practices (such as burning), is expected to affect the distribution of subpopulations on a landscape scale. However, a deeper understanding of the local-scale microhabitat conditions that is suitable for *A. peglerae* germination, establishment and continued growth and survival will provide a better understanding of fire-survival strategies in this species.

Hence, factors that might limit successful reproduction or growth and survival for *A. peglerae* may include aspects of pollinator taxa, population size and density, local neighbourhood effects, and microhabitat requirements and availability. Efforts to conserve and manage this species rely heavily upon studies such as this that will provide vital information on these aspects.

1.3 Aims and objectives

The aim of the research project was to investigate the pollination and reproductive biology of *Aloe peglerae* and integrate this with an understanding of the ecology of plants in a fire-prone environment.

The first objective (Chapter Two) set out to determine the contributions of different pollinator guilds, i.e. birds and insects, to reproductive success, i.e. fruit set, seed set, and total plant seed production by conducting selective pollinator exclusion experiments. I also extended the interpretation of contributions by different pollinator guilds to reproductive success by determining seed viability, germination, and seedling emergence for each exclusion treatment. The most effective and frequent floral visitors were identified through field observations.

The second objective of the study (Chapter Three) set out to determine the effects of local conspecific flowering density at increasing spatial scales on the same measures of reproductive output as above, nectar volume standing crop, and pollinator visitation rates.

The final objective of the study (Chapter Four) was to compare differences in the microhabitat conditions of aloe-occupied and aloe-vacant microsites (defined as the site of measurement). I set out to determine what the best microhabitat predictors are for aloe presence as opposed to absence in summer and winter to determine seasonal differences.

CHAPTER TWO

THE BIRDS AND THE SEEDS: OPPORTUNISTIC AVIAN NECTARIVORES ENHANCE REPRODUCTION IN AN ENDEMIC MONTANE ALOE

2.1 Abstract

Opportunistic bird pollination has become more evident in studies that confirm distinct differences in floral adaptations that attract opportunistic, rather than specialist, bird pollinators. Pollination syndrome studies investigating the effectiveness of different pollinator guilds on reproduction seldom do so by measuring seed viability. We studied pollination in *Aloe peglerae*, a high altitude endemic succulent of the Magaliesberg mountain range, previously thought to be largely sunbird (specialist) pollinated. Using field observation and pollinator exclusion treatments, i.e. (i) open to all visitors, (ii) bird excluded, and (iii) all visitors excluded, we established that birds contributed significantly more to fruit (2.3–5.6 times) and seed (1.3–1.4 times) set than insect or self-pollination, respectively. Overall, pollination by opportunistic avian nectarivores amplified seed production per aloe ~7 and 10 times compared to insects and self-pollination, respectively. One of three opportunistic nectarivores, the Cape Rock-Thrush (*Monticola rupestris*), played a significant role in pollination, contributing ~60 % of all probes at inflorescences. The difference in reproductive output between insect visited and visitor excluded flowers was not significant and suggests possible self-pollination in *A. peglerae* which is particularly unusual in *Aloe* species. Breeding system experiments would help clarify this. In assessing the effectiveness of pollinator guild on seed viability, we found no differences in percentage seed viability, seed germination or seedling emergence between exclusion treatments. Seed viability and germination were low and variable; however, ~19 % seedling emergence was observed across the treatments. Practically, the net effect of bird pollination may result in 8–12 times more potential seedlings compared to insect and self-pollination respectively. These findings highlight the importance of pollination by opportunistic avian nectarivores in *Aloe*.

Keywords: *Aloe peglerae*, Bird pollination, Cape Rock-Thrush, Opportunistic nectarivore, Seed viability

2.2 Introduction

Pollination syndromes have arisen from the specialization in plants to attract particular groups of pollinators to carry out efficient pollination between plant conspecifics (Faegri and Van der Pijl 1979). The bird-pollination syndrome is traditionally characterized by interactions between specialist true nectarivores (e.g. sunbirds) and plants that possess 'ornithophilous' traits consisting of sturdy perches, odourless orange-red flowers, thickly clustered racemes, exserted anthers and stigmas and/or copious amounts of dilute nectar (Skead 1967; Cruden and Toledo 1977; Stiles 1978; Johnsgard 1983; Nilsson 1988; Ratsirarson 1995; Cronk and Ojeda 2008; Symes and Nicolson 2008).

However, specializations within plant-pollinator interactions receive increasing attention as the reliability to predict pollination syndromes based on floral adaptations alone has been questioned (Ollerton 1996; Waser *et al.* 1996; Johnson and Steiner 2000; Fenster *et al.* 2004). Numerous studies (e.g. Cruden and Toledo 1977; Motten *et al.* 1981; Olesen 1985; Karron 1987; Hargreaves *et al.* 2004) have, in fact, shown that pollination systems are often more generalized than expected.

Certain floral traits, such as nectar properties, were recently discovered to filter specialist and opportunistic avian nectarivores (e.g. Cruden and Toledo 1977; Waser *et al.* 1996; Johnson and Steiner 2000; Johnson and Nicolson 2008; Rodriguez-Rodriguez and Valido 2008; Botes *et al.* 2009a; Brown *et al.* 2009, 2010, 2011; Symes *et al.* 2009). Species typically pollinated by specialist long-billed nectarivores possess long tubular flowers that produce small volumes of concentrated nectar (10–30 µL; 15–25 % w/w; Johnson and Nicolson 2008), e.g. *Aloe pluridens* (Botes *et al.* 2008) and *A. greatheadii* var. *davyana* (Symes *et al.* 2009), whilst species typically pollinated by opportunistic short-billed birds possess shorter tubular flowers that produce larger volumes of dilute nectar (40–100 µL; 8–12 % w/w; Johnson and Nicolson 2008), e.g. *A. marlothii* (Symes *et al.* 2009) and *A. ferox* (Botes *et al.* 2008). The genera *Aloe*, *Erythrina* and *Kniphofia* have provided considerable evidence for the differences between nectar properties, corolla length and bird-pollinator type (Cruden and Toledo 1977; Jacot Guillarmod *et al.* 1979; Johnson *et al.* 2006; Botes *et al.* 2008; Symes and Nicolson 2008; Brown *et al.* 2009, 2010, 2011; Symes *et al.* 2009).

Both specialist and opportunistic nectarivores typically take advantage of the available nectar rewards of many winter-flowering *Aloe* species when resources are low, and in turn act as efficient pollinators (Oatley and Skead 1972; Cruden and Toledo 1977; Botes *et al.* 2008,

2009a, b; Rodriguez-Rodriguez and Valido 2008; Symes *et al.* 2008; Brown *et al.* 2009). Furthermore, whilst the majority of *Aloe* species have floral adaptations consistent with bird pollination and are visited by numerous nectar-feeding birds (Oatley 1964; Oatley and Skead 1972; Botes *et al.* 2008; Symes *et al.* 2008), pollen and nectar-collecting bees are also frequent visitors to some of these species (Williams 2002; Hargreaves *et al.* 2004, 2010, 2012; Botes *et al.* 2009a, b; Symes *et al.* 2009; Duffy and Johnson 2011). However, the degree to which different pollinators contribute to reproduction differs between species, with some plants receiving significant contributions to fruit and/or seed production from either specialist or opportunistic bird pollinators (Botes *et al.* 2008; Brown *et al.* 2009, 2010; Symes *et al.* 2008, 2009) or from both (Motten *et al.* 1981; Botes *et al.* 2008; Symes *et al.* 2008). Similarly, contributions by insects, particularly bees, can be equal to or more significant than bird pollination to any particular species, e.g. *Aloe greatheadii* var. *davyana* (Symes *et al.* 2009) and *A. maculata* (Duffy and Johnson 2011).

The pollination systems of many species of *Aloe* remain unstudied. Hence, documenting pollination systems, the effectiveness of different pollinators to reproduction and consequently the next generation, is important in unravelling the diversity and success of plant-pollinator interactions within *Aloe*.

Aloe peglerae Schönland (Asphodelaceae) is a montane endemic of the Magaliesberg mountain range (MMR) of the Gauteng and North-West Provinces in South Africa. It has a restricted distribution (~3,445 km^2) (Victor and Pfab 2005) and only grows above an altitude of 1,500 m a.s.l. (Glen and Hardy 2000). It is currently listed as Endangered due to illegal collecting from the wild (Pfab and Scholes 2004; Victor and Pfab 2005) and its pollination system has not been studied. The floral traits of *A. peglerae* strongly suggest bird pollination. We therefore measured the contributions by birds and insects to fruit set, seed set and total seed production. We also identified the most effective floral visitors through field observations. Lastly, to our knowledge, no studies on any *Aloe* species have assessed germination or seedling emergence success as a representation of pollinator guild (i.e. birds and/or mammals, insects) contributions to reproductive success. Therefore, we also assessed the influence of pollinator guild on seed viability, germination and seedling emergence.

2.3 Methods and Materials

2.3.1 Study site and species

The study was conducted in the Peglerae Conservancy (25°41'20" S, 27°59'05" E) on the north-facing slopes of the MMR, ~25 km west of Pretoria, South Africa. The region receives ~625–750 mm of rainfall per annum (Zucchini and Nenadić 2006). The vegetation is classified as Gold Reef Mountain Bushveld consisting of a tree/shrub and grassy layer on rocky hills and ridges, with more densely wooded vegetation on the lower slopes, dominated by *Protea caffra* and *Acacia caffra* (Rutherford *et al.* 2006). *Aloe peglerae* grows on the north-facing, rocky quartzitic slopes with good drainage (Scholes 1988) and amongst sparsely scattered trees and woody shrubs. Soils are shallow with underlying rock at a depth of 5–20 cm (GA, *unpubl. data*). *Aloe peglerae* flowers annually in winter (July–August); however, flowering and seed set are highly variable between years (Scholes 1988). Plants form a single rosette of leaves (Glen and Hardy 2000; Van Wyk and Smith 2003; Fig. 2.1a) and may show coppicing, producing multiple rosettes, where the stem has been damaged (Cousins and Witkowski 2012). The grey–green, succulent leaves are inwardly curved and provide a suitable perch to avian or small-mammal floral visitors (GA, *pers. obs.*). Adult plants, ~25 cm high and 30 cm wide (GA, *unpubl. data*), usually develop a single, vertically unbranched inflorescence, but may occasionally produce a branched inflorescence consisting of two racemes (Van Wyk and Smith 2003).The densely packed flowers are bright red in the bud stage and pale greenish–yellow after anthesis (Van Wyk and Smith 2003; Fig. 2.1a). Both stigma (yellow in colour) and stamina (filaments dark purple in colour) are exserted for ~25 mm beyond the opening of the corolla after anthesis (Van Wyk and Smith 2003; Fig. 2.1a).

2.3.2 Pollinator effectiveness

Pollinator exclusion experiments were used to determine the contributions of birds and insects to fruit and seed set. Individual aloes were numbered with an engraved metal tag (Fig. 2.1b), coordinates recorded using a handheld GPS (Garmin, GPSMAP 60C9, USA) and randomly assigned to one of three treatments (20 per treatment). A control ('all visitors') allowed access by all floral visitors and was left uncovered; a bird exclusion treatment ('insects only'; Fig. 2.1d) allowed access by insect visitors and excluded birds and small mammals using a wire mesh cage (20 x 15 mm holes); and a total exclusion treatment ('no visitors'; Fig. 2.1c) excluded all floral visitors using a fine nylon mesh cage (1 x 1 mm holes).

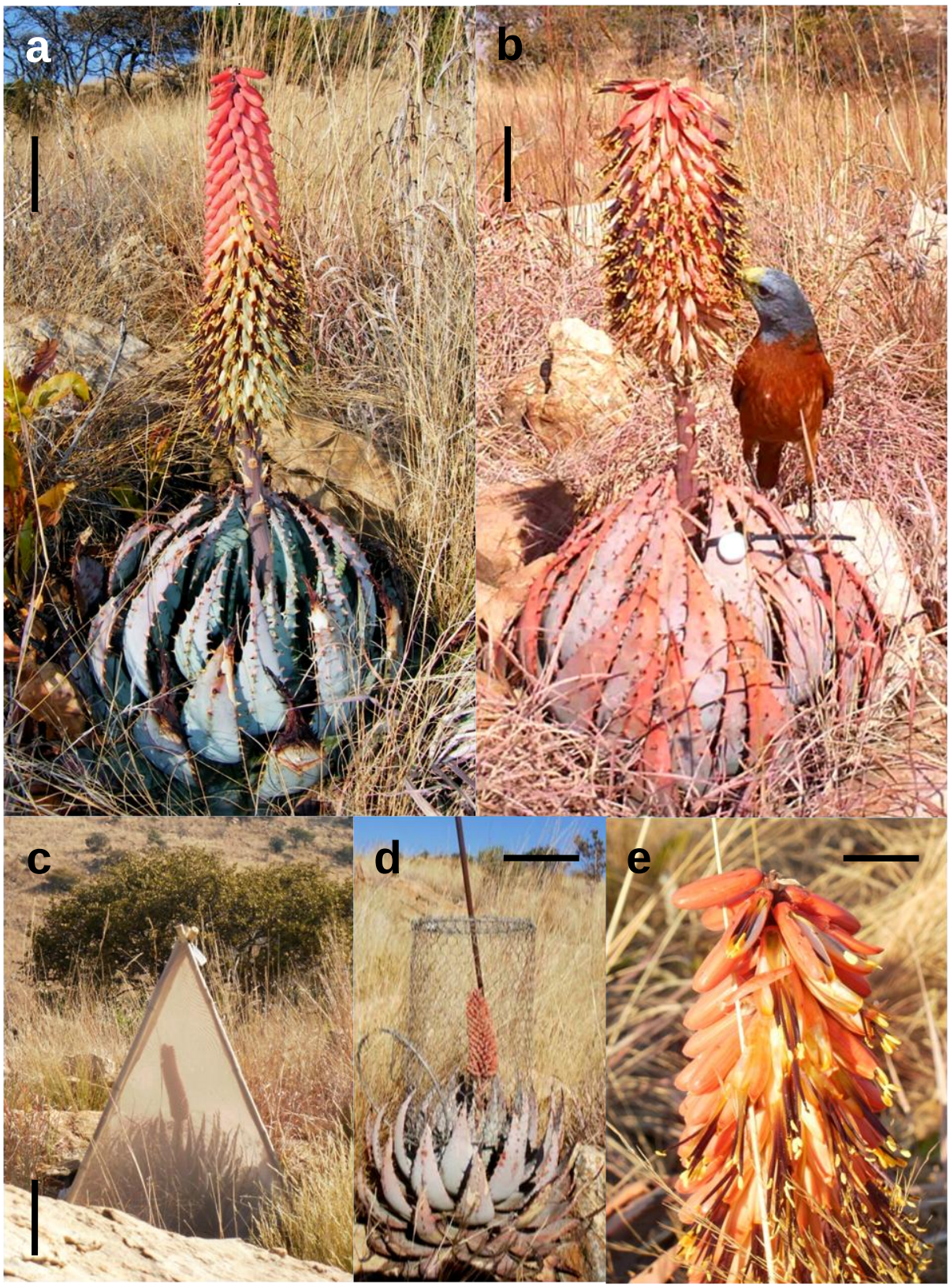

Figure 2.1 *Aloe peglerae* and its floral visitors: a) *Aloe peglerae*, a winter-flowering endemic succulent of the Magaliesberg Mountain Range (scale bar=5 cm); b) male Cape Rock-Thrush (*Monticola rupestris*), (scale bar=5 cm); c) a total exclusion cage, excluding all floral visitors (scale bar=30 cm); d) a bird exclusion cage, restricting access to insects only, while excluding birds and small-mammals (scale bar=10 cm); e) damage to flowers by Streaky-headed Seed-eater (*Serinus gularis*) while feeding on nectar and/or flower parts (scale bar=4 cm). Photographs: G. Arena

Inflorescences were covered before flowering (late June 2011) until the end of the flowering season (early September 2011) when the number of fruits on each replicate plant was counted. Fruit set was represented as the percentage of flowers per raceme (by counting the number of peduncles present after flowers had fallen off, see Symes *et al.* 2009) that produced fruit. A subsample of up to ten fruits per replicate per treatment was randomly collected. Each individual fruit was placed in a labelled envelope and allowed to air dry at room temperature in a laboratory. When the fruits had dehisced the number of seeds in each was counted to calculate the average number of seeds per fruit (seed set). Lastly, the average number of seeds per fruit was multiplied by fruit set to calculate total seed production per replicate plant. All remaining plant material not used for further tests was scattered at the study site to minimise the impact on the population.

2.3.3 Floral visitors

Observations of floral visitors were conducted during peak flowering to identify (1) the main floral visitors, (2) bird visitation rates, and (3) probe rates. Probe rates accounted only for the legitimate visits by bird pollinators that probed flowers, whereas visitation rates accounted for both probing and non-probing visits. Observations of bird and insect visitors were conducted in clear weather daily for two weeks at a varying number of flowering aloes for approximately 20-minute periods in the morning (06h00–10h00, total observation period = 18 h), midday (10h00–14h00, 13 h) and late afternoon (14h00–18h00, 8 h). Bird visitors were observed at a distance of ~30 m using binoculars to avoid observer presence influencing bird activity, whilst insects were observed closer to the flowering plants. The following were recorded for each visitor: (1) species, (2) time spent visiting, and in the case of birds, (3) number of probes and (4) pollen on the head. Six camera traps (2x Bushnell, 119456C, China; 2x ScoutGuard, SG550, China; 2x Cuddeback, C2000, Wisconsin) were set up at individual flowering aloes during the same period to supplement observations.

2.3.4 Seed viability

Standard tetrazolium staining protocol (see ISTA Tetrazolium Testing 2003) was used to assess the percentage of viable (live tissue) and non-viable (aborted/empty) seeds from subsamples per pollinator exclusion treatment. A random selection of ten seeds each from ten replicate aloes was pooled into a subsample of 100 seeds per treatment. Seeds were soaked in distilled water for 24 h at room temperature to soften the seed coat, and then longitudinally bisected to expose the embryo. One half was placed on two moist Whatman No. 2 filter paper

discs (90 mm) in a petri-dish. These seeds were saturated in a 1.0 % 2,3,5-triphenyltetrazolium chloride solution and a third moist filter paper used to cover them. The petri-dishes were stored in complete darkness at 25°C for 24 h. Viable seeds (stained dark red/light pink) and non-viable seeds (remained colourless) were counted.

2.3.5 Seed germination

Petri-dish germinations were conducted to determine the germination success of each exclusion treatment (ISTA 2003). Seeds from the same ten aloes per treatment were sampled. Seeds were randomly selected and pooled per treatment and the number varied (4–50) depending on the number of seeds produced by each plant. Seed surfaces were rinsed in 2% sodium hypochlorite for 20 min to decontaminate the seed coats and rinsed three times in distilled water (ISTA 2003). Seeds were placed in petri-dishes on two filter papers, saturated with new distilled water every 3–4 days and stored in an environmental control chamber at 25°C during the day and 15°C during the night (a dark/light cycle of 12 h/12 h) and at relative humidity of 50–60 %. Light was supplied by fluorescent bulbs at ~650 nm. All laboratory materials were sterilised in an autoclave prior to germination trials. Germination was determined by a protruded radical of ~2 mm and germinated seeds counted and removed each 'week' (4–7 days). The cumulative number of dead/empty seeds (characterized by seed softness, discolouration, mould and no signs of radical emergence) was determined (ISTA 2003).

2.3.6 Seedling emergence

Depending on the total number of seeds produced per plant, a subsample of between 4 and 20 seeds was randomly selected from the same ten aloes per treatment. Seeds were sown in seedling trays in germination mix potting soil (Culterra, South Africa) on a perlite substrate to help retain soil moisture. The seedling trays were stored in a growth chamber at the same conditions as above and watered every 3–4 days. The number of newly emerged seedlings per treatment was counted every 7 days (identified by the presence of a plumule). Total seedling emergence was expressed as a percentage of the total number of seeds sown per treatment. Potential seedling emergence was also calculated by multiplying total seed production per plant (per exclusion treatment) by the percent seedlings emerged, divided by 100.

2.3.7 Statistical analyses

All data from the (a) fruit set, (b) seed set, (c) total seed production per plant, (d) percentage of viable seeds, (e) percentage of germinated seeds and (f) percentage of emerged seedlings in each treatment were tested for normality using the Kolmogorov-Smirnov normality distribution. Data were either log or square-root transformed and percentage values arcsine transformed when not normal. One-way ANOVA, or the non-parametric equivalent, Kruskal-Wallis test, was used to compare differences between treatments for each of the above measures. Mann-Whitney U tests were used to compare differences between germination and emergence percentages between each treatment. Where possible, Tukey Post Hoc tests were applied to statistically significant results. All mean values are presented as mean ± SE. Analyses were conducted using Statistica 6.0 (StatSoft, Tulsa, OK, USA, 2002).

2.4 Results

2.4.1 Pollinator effectiveness

Fruit set differed between treatments (Fig. 2.2a; $F_{2, 54}$=15.67, P<0.0001), being higher in the all visitors (40 %) than the insects only (12 %) and no visitors (5 %) treatments. Seed set differed between treatments (Fig. 2.2b; $F_{2, 38}$=9.65, P=0.0004) and was higher in the all visitors (38.3 ± 3.1 seed/fruit) than the insects only (16.9 ± 3.2) and no visitors (15.7 ± 2.5) treatments. Total seed production per aloe differed between treatments (Fig. 2.2c; $F_{2, 38}$=19.5, P<0.0001), with a seven- and ten-fold greater seed production in the all visitors treatment (3869 ± 519) compared to the insects only (497 ± 139) and no visitors (323 ± 102) treatments, respectively.

2.4.2 Floral visitors

Combined field and camera trap observations gave a total of 1656 ‘aloe hours’ of observations. The term ‘aloe hour’ (equivalent to how many visitors were recorded at one aloe per hour) was calculated to account for the observations recorded at varying numbers of flowering aloes. Only three bird species were observed feeding on *A. peglerae* nectar, Cape Rock-Thrush (*Monticola rupestris*), Dark-capped Bulbul (*Pycnonotus tricolor*) and Streaky-headed Seedeater (*Serinus gularis*) (Table 2.1). The rock-thrushes (Fig. 2.1b) and the bulbuls had pollen visible on their heads after probing flowers, whilst the seedeaters did not. Visitors were observed feeding on nectar between 0630 h and 1700 h every day, where 53 % of all bird visits occurred in the morning, 22 % occurred midday and 25 % in the afternoon.

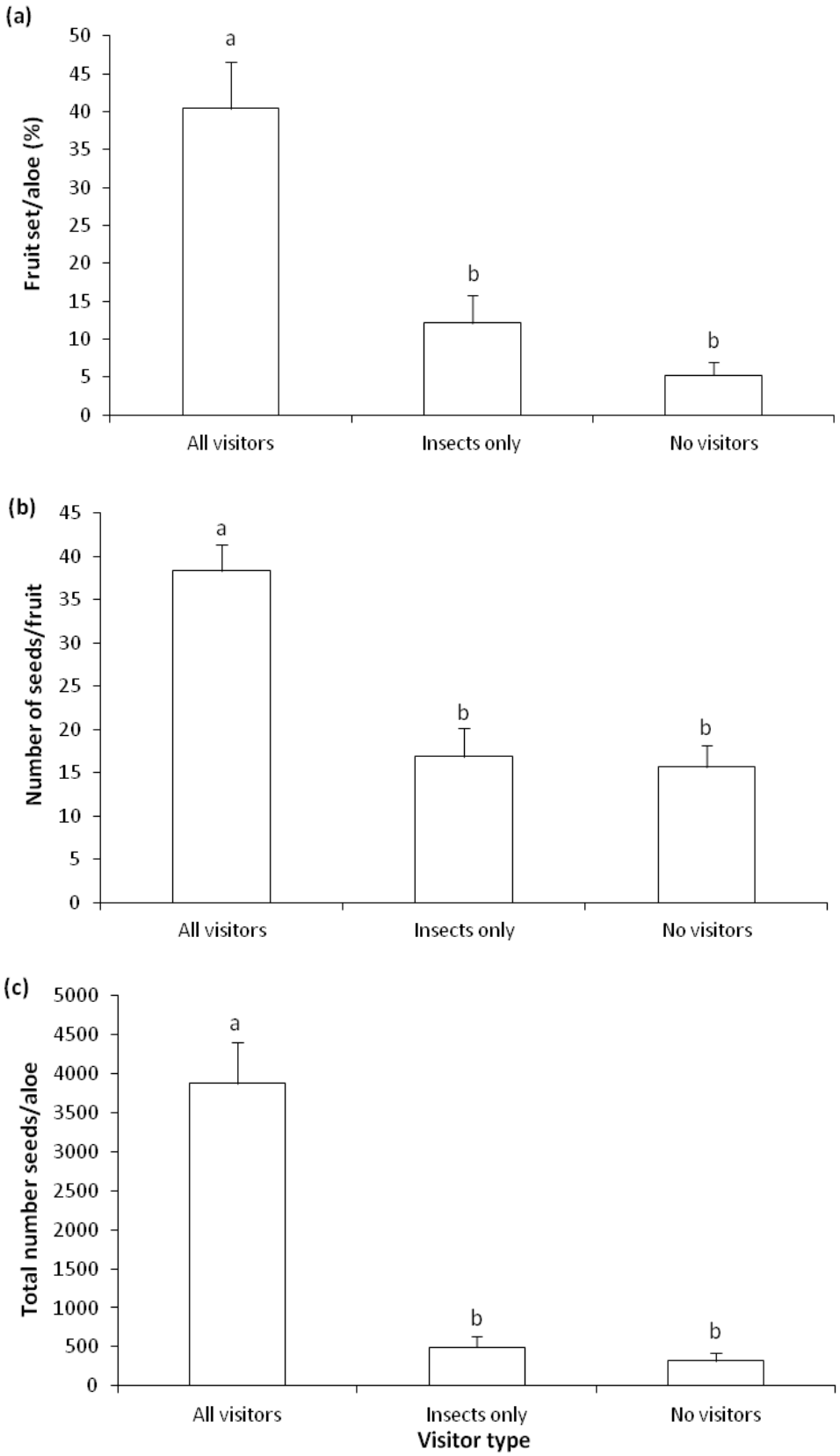

Figure 2.2 The contribution of different pollinator guilds (birds and/or small-mammals and insects) determined through pollinator exclusion treatments (n=19 for each treatment); a) fruit set per aloe, b) seed set per aloe, and c) total seed production per aloe. Different letters indicate significant differences between treatments (One-way ANOVA, Tukey HSD, $P<0.05$). Sample size per treatment changed from 20 to 19 due to baboon damage. Values are mean ± SE

Damage to flowers was evident after seedeaters had poked holes at the bases of flowers and ripped perianths to gain access to the nectar (Fig. 2.1e). The seedeaters did not enter from the opening of the flower, and therefore are less likely to contribute to pollen transfer as this is typical nectar-thieving behaviour. Birds usually fed on nectar by clinging to the inflorescence or by perching on the leaves of the aloe (Fig. 2.1b). An additional five bird species visited aloes, but were not observed probing flowers during these visits or displaying visible pollen on their heads (Table 2.1).

The average bird visitation rate was 0.14 birds/aloe/ hour (observations = 1,656 h). The average probe rate of all birds that visited inflorescences was 0.69 probes/bird/hour (observations = 39 h). The highest visitation rate was by the Cape White-eye which was not observed nectar feeding despite previous accounts of nectarivory in other *Aloe* species (Table 2.1). Insect visitors were seldom observed pollen collecting or nectar feeding. On only two occasions was a honeybee (*Apis mellifera*) seen pollen collecting and drinking nectar and a pollen wasp (*Tricarinodynerus* sp.) collecting pollen.

Camera trap photography further identified Chacma baboon (*Papio hamadryas ursinus*) and Smith's red rock rabbit (*Pronolagus rupestris*) near aloes, but no sign of nectar feeding was evident by either visitor. There is a possibility that small mammals feed on *A. peglerae* nectar during the dry winter months, and may contribute minimally to pollination. However, given that small mammals were not recorded from camera traps in 2011, small mammal pollination may be insignificant.

2.4.3 Seed viability

The percentage of viable seeds did not differ between treatments as determined by tetrazolium staining (Table 2.2; $H_{2, 9}$=0.62, P=0.73); however, a slightly lower percentage of viable seeds came from visitor excluded plants (Table 2.2).

Table 2.1 Visitation and probing rates of birds to *Aloe peglerae* flowers. Observation of visitors (n=1,656 h) and probing of flowers (n=39 h) represent averages (± SE) per aloe. Other *Aloe* species that are recorded being visited by the same species of birds are also given; references, 1=Oatley (1964); 2=Skead (1967); 3=Oatley and Skead (1972); 4=Hoffman (1988); 5=Johnson *et al.* (2006); 6=Botes *et al.* (2008); 7=Symes *et al.* (2008); 8=Forbes *et al.* (2009); 9=Hargreaves *et al.* (2010); 10=Symes (2010); 11=C.T. Symes (*pers. comm.*)

Species	Visitation rate (birds/plant/h)	Probe rate (probes/bird/h)	Other *Aloe* species visited	References
Cape Rock-Thrush *Monticola rupestris*	0.24 ± 0.09	3.31 ± 1.61	*ferox; marlothii; speciosa; vryheidensis*	3,5,6,7,8,10
Dark-capped Bulbul *Pycnonotus tricolor*	0.14 ± 0.07	1.13 ± 0.90	*arborescens; barberae; branddraaiensis; castanea; greatheadii* var. *dayvana; ferox; marlothii; spectabilis; spicata; vryheidensis; thraskii, wickensii*	1,3,5,8,11
Streaky-headed Seed-eater *Serinus gularis*	0.30 ± 0.16	1.08 ± 0.86	*africana; arborescens; castanea; greatheadii* var. *davyana; ferox; maculata; marlothii; speciosa; spectabilis*	1,3,4,5,6, 7,8,9,11
Cape White-Eye *Zosterops capensis*	0.30 ± 0.25	0	*marlothii; ferox; speciosa; barberae; vryheidensis*	2,3,6,7,8, 10,11
Amethyst Sunbird *Chalcomitra amethystina*	0.02 ± 0.02	0	*africana; ferox; lineata* var. *muirii; maculata; pluridens; speciosa; barberae; greatheadii* var. *davyana*	2,4,6,8,9,11
Cape Bunting *Emberiza capensis*	0.05 ± 0.05	0	*marlothii*	7,10
Familiar Chat *Cercomela familiaris*	0.04 ± 0.03	0	-	No record
Lazy Cisticola *Cisticola aberrans*	0.05 ± 0.03	0	-	No record

Table 2.2 Mean (± SE) percentage of viable and germinated seeds recorded from tetrazolium, petri-dish germination and soil seedling emergence trials. Values in parentheses represent total number of seeds used per treatment. No significant differences were found between treatments in any of the trials (Kruskal-Wallis, P>0.05).

	Pollinator exclusion treatment				
	All visitors	Insects only	No visitors	H	P
Viable seeds [a]	26.7 ± 2.3	26.7 ± 2.1	22.3 ± 2.1	0.62	0.73
Germinated seeds [b] min-max	17.2 ± 4.2 (500) 0–38	18.3 ± 7.3 (445) 0–58	27.0 ± 11.4 (327) 0–96	0.19	0.91
Seedling emergence [c] min-max	19.0 ± 4.2 (200) 0–40	21.0 ± 7.7 (176) 0–70	17.5 ± 7.1 (169) 0–60	0.53	0.77

[a] Tetrazolium staining tests on 100 seeds per replicate plant (n=10) per pollinator exclusion treatment
[b] Petri-dish germination tests on subsamples of seeds (n=4–50) per replicate plant (n=10) per pollinator exclusion treatment.
[c] Mean seedling emergence (%) from seedling tray trials of seeds (n=4–20) per replicate plant (n=10) per pollinator exclusion treatment.

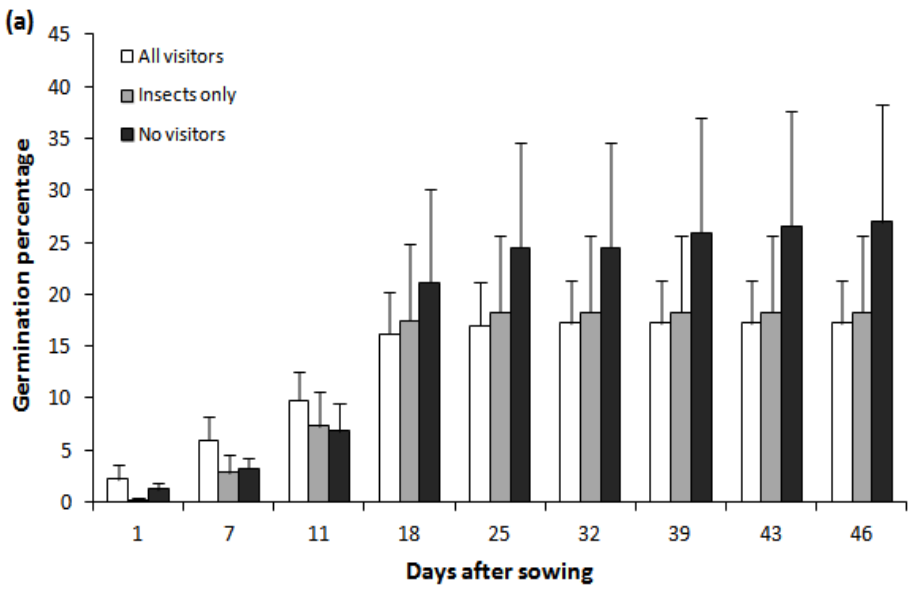

Figure 2.3 Cumulative mean percentage of germinated seeds per pollinator exclusion treatment (± SE), with no statistical difference between treatments (Kruskal-Wallis, *P*>0.05)

2.4.4 Seed germination

Despite sterilising seed coats, 90% of seeds were infected by fungus during the germination trials, which may have affected germination success. The percentage of seeds that germinated within the 7-week trial period was low, yet highly variable (ranging 0–96 % per replicate, Table 2.2) across all treatments (Fig. 2.3). Seeds began germinating after day 1 in all treatments and each treatment yielded the highest germination percentage at day 17 (Fig. 2.3). There was no significant difference in germination success between treatments (Table 2; $H_{2,30}$=0.19, P=0.91).

2.4.5 Seedling emergence

Seedling emergence did not differ between treatments (Table 2.2; $H_{2,\ 30}$=0.53, P=0.77). From each batch of sown seed, 19.0, 21.0 and 17.5% of seed emerged as seedlings from the all visitors, insects only and no visitors treatments, respectively. Potential seedling emergence per plant was not different between treatments ($H_{2,\ 57}$=4.44, P=0.11). However, mean potential seedling emergence for plants open to all visitors was the highest (467 seedlings) due to much higher seed production, compared to plants open to insects only (52) and plants excluding all visitors (36). Although emergence percentages were slightly higher than germination percentages (Table 2.2), when tested, we found no difference between seed

germination (%) and seedling emergence (%) between the all visitors (U=46.0, P=0.76), insects only (U=48.0, P=0.88) and no visitors (U=43.5, P=0.62) treatments.

2.5 Discussion

2.5.1 Effective pollinators

Based on the findings of this study, *A. peglerae* is bird pollinated, the main pollinators being the Cape Rock-Thrush and Dark-Capped Bulbul, both opportunistic nectarivores. The inferred contribution by birds to fruit set was ~2.5 times greater than that by insects, and birds contributed ~7 times more to total plant seed production than insects. Unlike other winter-flowering species that receive abundant bee visitors, such as *A. ferox* (Hoffman 1988), *A. greatheadii* var. *davyana* (Symes *et al.* 2009) or *A. maculata* (Hargreaves *et al.* 2010; Duffy and Johnson 2011), insect visitors to *A. peglerae* were minimal. As a result, insect pollination did not significantly enhance reproductive output in *A. peglerae*, similar to *A. ferox* and *A. marlothii* (Stokes and Yeaton 1995; Symes *et al.* 2009). Despite high insect visitation to *A. ferox* (Hoffman 1988), exclusion of birds from inflorescences resulted in zero fruit and seed set (Stokes and Yeaton 1995). Furthermore, when foraging for pollen, bee visitors were ineffective at transferring pollen to stigmas (Stokes and Yeaton 1995). The number of insect pollinators visiting *A. peglerae* may be low because of (1) cold and windy weather conditions at high altitude on the MMR leading to low insect pollinator abundance during this period (Cruden *et al.* 1976; Gu *et al.* 2010), (2) dilute nectar in mature flowers (10.3 ± 0.2 % w/w, 35.8 ± 2.9 µL, n=107; GA and CTS, *unpubl. data.*), (3) exserted stamina and stigma which block the opening of the corolla limiting access to insects (Botes *et al.* 2009a, b; Hargreaves *et al.* 2012) and/or (4) the inefficient pollen transfer by insects whilst foraging (Stokes and Yeaton 1995; Hargreaves *et al.* 2012).

Of the eight bird species that visited *A. peglerae* flowers, only three were observed feeding on nectar. Two of these, the rock-thrush and bulbul, effectively transferred pollen, similar to that observed in *A. pruinosa* (two nectar feeders) and *A. lineata* var. *muirii* (three nectar feeders) (Botes *et al.* 2008; Wilson *et al.* 2009). This plant-pollinator relationship is considered unique amongst *Aloe* species because generalized bird pollination is normally carried out by numerous bird-pollinating species (Johnson *et al.* 2006; Johnson and Nicolson 2008), such as *A. africana* and *A. pluridens* (5 species each), *A. ferox* (12), *A. marlothii* (~66), *A. speciosa* (10) and *A. vryheidensis* (8) (Johnson *et al.* 2006; Botes *et al.* 2008; Symes *et al.* 2008; Forbes *et al.* 2009; Symes 2010).

The Streaky-headed Seed-eater, commonly found in mixed woodland and scrub habitat (Hockey *et al.* 2005), feeds on both nectar and flower parts of *A. marlothii* and *A. ferox* (Hoffman 1988; Symes *et al.* 2009), but also robs nectar of *A. maculata*, *A. marlothii* (Hargreaves *et al.* 2010, 2012) and *A. ferox* (Oatley and Skead 1972; Hoffman 1988). The Dark-capped Bulbul feeds on a range of species, perhaps because it frequents various habitats, whilst the Cape Rock-Thrush, restricted to mountainous, rocky terrain, consumes nectar from fewer species (Oatley 1964; Oatley and Skead 1972; Hockey *et al.* 2005). This close association between the rock-thrush and *A. peglerae* is a unique, yet vulnerable, relationship should either pollinator or plant population experience future declines.

The White-bellied sunbird, the most commonly observed sunbird at the study site, was not observed nectar feeding (GA, *pers. obs.*). This is due to floral traits that are unattractive to sunbirds; i.e. dilute nectar and densely packed flowers. Sunbirds prefer concentrated nectar (Johnson and Nicolson 2008) like that found in *A. greatheadii* var. *davyana* (Symes *et al.* 2009) and *A. pruinosa* (Wilson *et al.* 2009). Additionally, *A. peglerae* is the only *Aloe* that grows at this altitude, whereas populations of *A. greatheadii* var. *davyana* are restricted to the lower slopes of the mountain (GA, *pers. obs.*). Thus, opportunistic nectarivores, more specifically the Cape Rock-Thrush, assume the role of 'specialist' pollinator for *A. peglerae*.

Bird visitation rates to *A. peglerae* (0.14 birds/aloe/hour) were lower compared to *A. marlothii* (0.27 birds/ raceme/hour; CTS, *unpubl. data*), but higher than to *Kniphofia caulescens* (0.04 birds/raceme/hour; Brown *et al.* 2009). A number of factors could explain this variation: plant population size, nectar availability and turnover, daily flowering rate, bird abundance or legitimate feeding. Although pollen loads were not measured from bird visitors, probing rates of species were used as an indicator of visitor effectiveness. The white-eyes had the highest visitation rates (along with the seedeaters) and although there was no evidence of nectar feeding, based on numerous reports of nectarivory on many other aloes, this species is a likely nectarivore of *A. peglerae*. The Cape Rock-Thrush accounted for the most probes per hour (3.31 probes) compared to the bulbuls (1.13). In the absence of the rock-thrush, the bulbuls may adequately attain pollination success in *A. peglerae*; however, it is not known if pollination is pollinator limited in this species. Therefore, this indicates that within the pollination cohort of three opportunistic nectarivores, the Cape Rock-Thrush accounts for ~60 % of probing visits contributing to pollination in *A. peglerae*.

2.5.2 Seed viability

Unlike the bird-pollinated *Isoplexis canariensis* where seed viability was greatly enhanced by bird visitation (Rodriguez-Rodriguez and Valido 2008), our results revealed no significant differences in either percent seed viability, germination or emergence success between treatments. All three measures of seed viability were relatively low and exhibited fairly high within-treatment variation. However, potential seedling emergence of plants open to all visitors is 8–12 times higher than the insect visited and no visitor treatments, respectively. Hence bird pollination not only greatly enhances seed production, but also enhances potential seedling emergence.

Seed viability was comparatively much lower than that of *A. ferox* (≥95 %; Bairu *et al.* 2009). *Aloe ferox* seedlings required high watering frequency (three times a week) for successful growth (Bairu *et al.* 2009). Low seedling emergence of *A. greatheadii* var. *davyana* was suggested to be related to a lack of soil moisture and insufficient shade (Smith and Correia 1992). In the natural environment, like *A. marlothii* (Symes 2012), germination and seedling establishment are likely to be even lower when subjected to unpredictable rainfall patterns and fire events. Newly germinated *A. peglerae* seedlings are likely to survive longer for the first 3–4 months if protected by the shade of nurse plants from the harsh effects of full sunlight and desiccation (Cousins and Witkowski 2012). Whilst some woody savanna tree species require frequent summer rainfall to ensure successful seed germination and early seedling establishment (Wilson and Witkowski 1998), aloes are different in that the fleshy leaves and stems store water to enable them to survive long dry periods, and thus are also more prone to rotting in water-logged soils (Cousins and Witkowski 2012). Due to relatively steep rocky/gravelly slopes in the field, in the event of high rainfall, relatively rapid water drainage means that rotting is unlikely. Therefore, *A. peglerae* seedlings may desiccate if exposed to full sunlight following a single rainfall event when the surface soil dries rapidly, emphasising the importance of shade from nurse plants.

A suitable microhabitat is likely to benefit the survival of *A. peglerae* in such unpredictable conditions if it avoids fire by growing in refugia, such as a rock crevice with low fuel biomass and suitable soil conditions (Witkowski and Liston 1997; Pfab and Witkowski 1999). Arson fire events are an annual occurrence at the site, usually during late August when flowering is complete, but before seeds are dispersed (J. Wesson *pers. comm.*). Burning releases the nutrients stored in the vegetation to the ground in the form of ash, which later

leaches into the soil after rainfall (e.g. Lamont *et al.* 1993b). Given that (a) *A. peglerae* seed germinated quickly in the trials and (b) seed does not appear to exhibit dormancy because the seeds are short lived, the increased soil nutrients are expected to aid the establishment and growth of *A. peglerae* seedlings (Cousins and Witkowski 2012; Symes 2012). However, survival under successive annual burns is not likely in individuals that are growing in unsuitable microsites (GA, *pers. obs.*). With high seed production through bird pollination, the probability of seed dispersal to suitable microsites might be improved, thus enhancing seedling establishment. Given the low and variable germination success observed in this study, more work on the germination ecology of *A. peglerae* (and aloes in general) is needed.

Another important aspect of seed viability is that related to the mating system. Breeding experiments were not conducted to determine the mating system of *A. peglerae*. The existence of either self-incompatibility or self-compatibility, and the subsequent expression of inbreeding depression, may have an effect on the fitness of selfed or outcrossed seed (e.g. Liu and Spira 2001; Busch 2005). Significant inbreeding depression existed in both self-incompatible and self-compatible populations of *Leavenworthia alabamica* (Busch 2005), but with fewer and smaller seeds produced from selfed compared to outcrossed individuals within the self-incompatible populations (Busch 2005).

In pollinator-limited environments, or based on the small pollinator cohort of the study population, self-compatibility might be favoured in populations of *A. peglerae*, potentially leading to the production of sterile seed. A self-pollination effect may even be enhanced at the edges of *A. peglerae* populations (e.g. Fishman 2001; Busch 2005) where pollinator visits may be less (Ågren 1996; Oostermeijer *et al.* 1998; Stephens *et al.* 1999; Duffy *et al.* 2009; Kameyama and Kudo 2009). This could result in a positive feedback on declining plant densities or population size, leading to the extinction of smaller populations (Allee effect, e.g. Lamont *et al.* 1993a).

Although self-pollination is particularly rare in *Aloe* species because a high degree of outcrossing is required for successful seed set (Hoffman 1988; Botes *et al.* 2008, 2009a; Symes *et al.* 2009; Hargreaves *et al.* 2010, 2012), the lack of any difference in reproductive output between insect visited and visitor excluded plants may suggest potential self-pollination in *A. peglerae* (Ratsirarson,1995). Considering these implications, it is important that future research determines the breeding system of several populations of *A. peglerae* to identify differences in mating system between different sized populations, potential

inbreeding effects and the implications that the mating system has on declining populations of *A. peglerae*. On the other hand, even though bird pollination did not enhance seed viability as expected, it did result in much greater seed production. Hence, progeny fitness through the production of more seeds may be enhanced in *A. peglerae* (Symes 2012).

2.6 Conclusion

Aloe peglerae is pollinated by a small cohort of opportunistic avian nectarivores, where the Cape Rock-Thrush is its major pollinator. Insect visitation, and subsequent pollination and reproduction, was negligible. Although pollinator guild did not significantly enhance seed viability, bird pollination did greatly increase overall seed production which, in turn, is expected to increase potential seedlings in the aloe population. Seed viability was low and it is not clear whether this is a function of the plant mating system which has led to the production of infertile seed as a result of inbreeding. Hence, further investigations of the breeding system in numerous populations in the MMR would contribute to a better understanding of the magnitude and the levels at which seed viability is influenced by both cross- and self-fertilization. Our understanding of seed biology and germination ecology would benefit from more studies that assess the different contributions of pollinators to progeny fitness in other *Aloe* species. Based on the growing body of literature on floral adaptations consistent with the opportunistic bird-pollination syndrome (copious, dilute nectar, strongly exserted reproductive parts and dense inflorescences), future *Aloe* pollination studies should be able to predict pollination syndrome.

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CHAPTER THREE

SPATIAL FLOWERING PLANT DENSITY INFLUENCES POLLINATOR VISITATION PATTERNS AND REPRODUCTIVE SUCCESS IN THE ENDANGERED *ALOE PEGLERAE*

3.1 Abstract

Multiple factors influence plant reproductive output in a population, and plant-pollinator interactions are altered if habitat becomes fragmented or if population size or density declines. The aim of this study was to investigate the effects of local conspecific flowering plant density on reproductive success i.e. total fruit per plant, seeds per fruit set, total seed production per plant, and germination success, in a population of the opportunistic nectarivore bird-pollinated *Aloe peglerae* over two flowering seasons (2011 and 2012). The effect of varying flowering plant densities at increasing spatial scales (up to 40m) on, (i) reproductive output in both years, (ii) pollinator visitation rate (2012) and, (iii) nectar standing crop (2012) was determined. The greatest effect on reproductive output was between years indicating that all measures of reproductive success, except germination success, were significantly higher in 2011. This reflected the highly variable flowering patterns between years. The percentage of flowering plants and flowering plant density in 2011 was significantly higher than in 2012, and may have represented a mast-seeding year. Absolute flowering plant density in the population did not have an effect on reproductive output. However, flowering plant densities at successively increasing spatial distances from focal aloes did significantly influence fruit set and total seed production at 30.0–35.0m in 2011. Furthermore, all relationships between density and reproductive output strengthened as the distance from focal aloes increased. Both diurnal bird (Cape Rock-Thrush and Dark-capped Bulbul) and nocturnal small-mammal (Namaqua Rock Mouse and Eastern Rock Elephant-Shrew) visitors were observed visiting inflorescences in 2012. Diurnal visitation rates in 2012 were significantly influenced by flowering plant densities between 25.0–30.0m, while no effect of density was detected on nocturnal visitation rates at increasing distances from focal aloes. The interaction between diurnal visitation rate and nectar standing crop was also significantly related to flowering densities at 25.0–40.0m. The significant effect of flowering plant density on reproduction by birds is only identified beyond 30m; the scale at which birds forage. No effect of flowering density on reproduction was detected by nocturnal small-

mammal visitors as predicted by their smaller home range sizes. However, further investigation is required to determine the contribution of small-mammal visitation to reproduction in *A. peglerae*. Hence, plant reproductive success in *A. peglerae* is clearly influenced by its major pollinators, the birds, at and beyond the spatial scale at which birds are capable of foraging within a subpopulation. Future research into the plant mating system, and nocturnal small-mammal visitation patterns, foraging behaviour, and their potential as pollinator agents will enhance our understanding of the different factors that ultimately influence reproductive success in *A. peglerae.*

3.2 Introduction

For many plant species, particularly those that are rare, threatened, or affected by habitat fragmentation and increased isolation, reproductive success may decline within and among small populations. Known as the Allee effect (Allee 1931), this is the positive relationship between increased conspecific presence and plant reproductive output. This is normally a result of increased attractiveness and floral rewards followed by increased pollinator activity, and pollination and fertilization success (Kunin 1992; Stokes and Yeaton 1995; Stephens *et al.* 1999). With declining population size or density, the effect of reduced plant conspecific presence can lead to reproductive failure as a result of increased pollinator and pollen limitation, increased inbreeding through the transfer of closely related pollen, genetic drift, or loss of integrity by hybridization (Bierzychudek 1981; Stephens *et al.* 1991; Lamont *et al.* 1993; Wilcock and Neiland 2002; Forsyth 2003; Knight *et al.* 2005).

Comparing the effect of different population sizes or densities on plant reproductive output between multiple populations is regularly favoured over studying the effect of varying densities at smaller spatial scales within one population due to the difficulty in interpreting local density effects (Stephens *et al.* 1991). However, the presence and strength of an Allee effect in any particular population may be detected at different spatial scales, so it becomes important to study density-related effects at a finer scale too (Stephens *et al.* 1999).

In very dense populations which will vary according to pollinator guild, i.e. insect vs. bird, and abundance, smaller interplant distances may indicate that foraging distances would generally be shorter and more energetically efficient (depending on the availability of nectar) for pollinators compared to larger interplant distances (Collevatti *et al.* 2000). Multiple studies that have shown a strong relationship between plant density or population size and

plant reproductive success are particularly, and easily, detectable in insect-pollinated species (Kunin 1992; Ågren 1996; Kearns and Inouye 1997; Roll *et al.* 1997; Allen-Wardell *et al.* 1998; Groom 1998; Morgan 1999; Donaldson *et al.* 2002), and the perceived spatial scale at which these effects are typically seen reflect the short foraging distances (0.5–3.0m; Zimmerman 1979; Roll *et al.* 1997; Collevatti *et al.* 2000) of insect pollinators.

However, few studies have attempted to identify the scale at which the effects of density on reproduction might be expressed for larger, more mobile animal pollinators (e.g. birds or mammals) (Stiles 1978; Wiens *et al.* 1983), and furthermore, how pollinator visitation patterns influence reproduction at varying spatial scales. Many plant species receive visits from more than one pollinator guild, and it is expected that the effectiveness of pollen transfer and receipt will vary according to the foraging behaviour of different pollinators. Studying the patterns of visitation and foraging habits by different pollinators, and how each influences overall plant reproductive success at varying densities and spatial scales, could contribute a greater understanding of the ecological processes that occur within diverse plant-pollinator interactions.

Aspects of reproductive effort in a plant population such as annual flowering phenology, flowering synchrony, nectar rewards, floral display, or flowering densities, each influence pollinator activity, foraging behaviour and patterns, and determine overall reproductive output. Furthermore, the patterns and processes observed in declining plant populations and the associated effects of reduced reproduction will shape the selection pressures on floral traits (e.g. flower size and shape, floral longevity, plant mating system, or pollination syndrome) and life history traits (e.g. asexual reproduction, number and frequency of reproductive episodes, large seed banks) (Kearns and Inouye 1997; Knight *et al.* 2005). For instance, self-incompatible, dioecious, or specialist-pollinated plant species are more commonly affected by low conspecific densities, and resultant pollen limitation due to their reliance upon pollinator visits and cross-pollen for successful seed set (Rathcke and Jules 1993; Ågren 1996; Kearns and Inouye 1997; Mitchell *et al.* 2009). In some instances, chronic pollen limitation within a population or among populations of the same species might favour the evolution of a self-compatible breeding system or self-pollination as a form of reproductive assurance (Wilcock and Neiland 2002; Knight *et al.* 2005). Even though genetic variability decreases, especially in instances where self-pollen usurps ovules in the absence of a late-acting self-incompatibility system, and ultimately reduces the adaptability of a species

under changing environments (Waser and Price 1991; Kearns and Inouye 1997; Allen-Wardell *et al.* 1998), some reproductive assurance is better than none.

Aloe peglerae, an endemic succulent of the Magaliesberg Mountain range in South Africa, is a suitable species to investigate the effects of density on reproduction at varying spatial scales. In 2011, experimental assessments of pollinator contributions to reproductive output were carried out, and Arena *et al.* (2013) demonstrated that successful reproduction relies on opportunistic nectarivore bird-pollination. However, in 2012, more intensive observations at flowering plants revealed two new nocturnal small-mammal species visitors. After this discovery it became important to identify, firstly, how reproductive success is affected by flowering plant density; and secondly, to determine any differences in pollinator visitation patterns based on flowering plant density in *A. peglerae.*

Hence, the aim of the study was to investigate the effects of local conspecific flowering plant density at varying spatial scales on reproductive success, i.e. fruit set (produced per plant), seed set (seeds per fruit), and total seed production (per plant), in a relatively undisturbed/natural subpopulation of *A. peglerae*. Varying flowering plant densities at different spatial scales are expected to affect each pollinator guilds' visitation patterns differently as influenced by one major factor – the availability of nectar at different densities at different scales. Hence, nectar standing crop and visitation rates to flowering plants were determined to help explain the patterns of reproductive output in *A. peglerae.*

3.3 Methods and Materials

3.3.1 Overall flowering plant density

The study site was situated in the Peglerae Conservancy in the Magaliesberg Mountain Range. The entire subpopulation was sampled over a two year period (2011–2012) and mapped using a handheld differential GPS device, accurate to the nearest 0.5m (Trimble GeoExplorer GeoXH 6000, USA). In the first flowering season (July–August 2011) 440 aloes were sampled, and in the second flowering season (July–August 2012) the sample size was increased by 110 aloes to 550 sampled individuals. Plants which produced inflorescences in 2011 and 2012 were marked. *Aloe peglerae* plants typically produce one inflorescence per plant, and rarely produce two (henceforth all references to flowering plants refer to plants with single inflorescences).

To determine the effects of overall flowering plant density on reproductive success in *Aloe peglerae*, "focal" flowering plants were selected in 2011 (n=19) and in 2012 (n=35). Absolute densities of flowering conspecifics were determined for each focal aloe in each year by measuring nearest neighbour distances using ArcGIS (ArcMap 10.0) between each focal plant and its conspecific neighbours within a 40m–radius boundary. Care was taken to avoid overlapping of circular plot areas. The number of flowering conspecifics within each 40 m radius circular plot (total area=5,027 m^2) was used to determine the density (plants/ha) surrounding each focal plant. The mean density was used as an estimate for total flowering plant density in the subpopulation per year. In 2011, pollinator exclusion experiments were also conducted in the population (Arena *et al.* 2013), so caged inflorescences (i.e. 20 bird exclusion and 20 total exclusion cages) that fell within the 40m radius of focal plants were treated as non-flowering plants.

3.3.2 Density at increasing spatial scales

While overall flowering plant density may or may not determine the overall plant reproductive success, it does not indicate the spatial scale that is most important for reproduction. This study therefore considers the relationship between varying flowering plant densities on reproductive success at successively increasing distances from focal plants. The number of conspecific flowering neighbours were counted within concentric "bands" of increasing area of equal distance intervals around each focal plant (method adapted from Roll *et al.* 1997) to calculate flowering plant density within each band, i.e. 0–4.99 m, 5.00–9.99 m, 10.00–14.99 m, 15.00–19.99 m, 20.00–24.99 m, 25.00–29.99 m, 30.00–34.99 m and 35.00–40.00 m.

3.3.3 Reproductive success

Fruit set (percentage of flowers that produced fruit), seed set (the average number of seeds per fruit), total seed production (the total number of seeds produced per plant) were determined for each focal plant at the end of each flowering season. Results for the reproductive output of the focal plants in 2011 are the same as that presented for the "open to all visitors" treatment plants sampled in section 2.4.1 (Arena *et al.* 2013). Seed set was determined from a randomly selected subsample of three fruits per focal plant in 2012.

The percentage of germinated seeds per focal plant, the fourth measure of reproductive success, was recorded from seeds collected from focal plants in each year. Seeds were germinated in petri-dishes following the same procedures used in section 2.3.5 (Arena *et al.*

2013). The 2011 germination results are those presented in section 2.4.4 for the "open to all visitors" treatment plants (Arena *et al.* 2013).

3.3.4 Visitor observations

Visitation rates of floral visitors were calculated from camera trap photography in 2012 at each focal plant over a 24-h period during the flowering season. Twelve camera traps were used (Bushnell, 119456C, China) to record visitors and foraging behaviour and to calculate visitation rate per focal plant (visits/hr). Each camera trap was programmed at the highest sensitivity setting to trigger three consecutive shots when movement was sensed. A one second interval between each 3-photograph series was set so all possible visitors could be recorded. Visits included both feeding and non-feeding visits. Other information captured from the photographs included time of day of each visit, presence and location of pollen on any part of the visitors body; and foraging/visiting behaviour i.e. nectar-feeding, foraging on flower parts, or no apparent foraging such as perching. Visitation rates were used to determine if flowering densities at increasing spatial scales had an effect on visitation rates to focal plants in 2012.

3.3.5 Nectar standing crop

Seasonal variation in the volume of nectar standing crop was determined to investigate its relationship to seasonal variation in pollinator visitation rates in 2012. Randomly selected flowering plants (excluding focal plants) in the population were sampled for nectar volume and concentration throughout the 2012 flowering season (n=37). Nectar sugar concentration (w/w, mass of sugar/mass of water %) was measured in three flowers per plant using a handheld refractometer (Eclipse 45-81, Bellingham and Stanley, Tunbridge Wells, Kent, UK), one in the morning (06h00–09h59), midday (10h00–13h59) and afternoon (14h00–18h00). To determine if visitation rates to focal plants were related to nectar standing crop, nectar volume was measured from one flower per focal plant in 2012 (n=35). Using these measures per focal plant, density-related effects at increasing spatial scales was determined for, 1) nectar standing crop, and, 2) the interaction between available nectar standing crop and total visitation rates to focal plants.

3.3.6 Proportion, onset and duration of flowering

Seasonal variation in flowering was assessed in both years by recording the proportion of inflorescences with open flowers every 2–12 days in 2011 and 1–6 days in 2012. These data

were converted to the percentage of plants with open flowers at a particular time period during the flowering season. Flowering duration per flowering plant was also recorded by visiting each individual and estimating the percentage of open flowers in the inflorescence until flowering was complete. Flowering data for each year only extends from the 20 June –1 August 2011 and 28 June – 29 July 2012 and does not include flowering data to the end of the flowering season (i.e. late-August). However, flowering in the population had already reached completion when fruits were collected in the first week of September of each year (GA, *pers. obs.*).

3.3.7 Statistical analyses

A Chi-Square test (with Yates correction) was used to compare the difference in the percentage of plants that flowered between 2011 and 2012. Non-parametric independent t-tests (Mann-Whitney *U* test) were used to compare the differences between years for, (i) the density of flowering plants/ha (calculated for different focal plants in each year), (ii) fruit set/plant (%), (iii) seed set/fruit, (iv) total seed production/plant, and (v) germinated seeds/plant (%).

Spearman Rank correlations were used to test the strength of the relationships between visitation rates per plant and, 1) flowering plant density, 2) fruit set, seed set, and total plant seed production, 3) nectar volume standing crop per plant, and, 4) the proportion of plants flowering. All density and reproductive output data were arcsine (percentage data), $\log_{10}$- or ln-transformed to normalize the homoscedasticity of residuals.

Hierarchical regression analysis was used to investigate the effects of varying absolute flowering plant densities on the reproductive success of each focal plant at successively increasing bands for 2011 and 2012. Flowering plant density was determined in each successive band, and not cumulatively with increasing areas, in order to ensure that all the densities varied independently from each other. The hierarchical regression analysis produced 8 models, model 1 for the first spatial band, model 2 for the second, up to model 8 for the final band between 35.0–40.0m. The variance explained (R^2) in models 2 to 8 were compared to that of model 1 where reproduction is affected by density at the lowest spatial scale of 0–4.99m. The *F* ratio test was used in the analysis to test for significance in the relationship between density and reproduction at each spatial scale (Cohen and Cohen 1983).

Hierarchical regression analysis was also used to assess the effects of varying flowering plant densities on nectar standing crop, as well as on the relationship between visitation rate per focal plant and nectar standing crop. The latter analysis is based on the assumption that visitation rate would depend on the nectar volume available in an inflorescence, and that at increasing spatial scales, the relationship between this interaction and flowering plant density would increase positively. Analyses were performed using Statistica 6.0 (StatSoft, Tulsa, OK, USA, 2002) and R Statistics (R Core Team 2012).

3.4 Results

3.4.1 Effect between years

The greatest difference in reproductive success was between years. In the 2-year study, the number of aloes flowering annually varied greatly among years, with the percentage of plants that flowered in 2011 being ~3.5 times higher (48%) than that in 2012 (14%). The percentage of adult plants that flowered in the population in 2011 was 2.8 times higher (66%) than in 2012 (23%) (2x2 contingency table; X^2=95.76; df=1, $P < 0.001$). Inflorescences took an average of 15 days from the onset of flowering to reach completion (range: 7–23 days, n=37). The timing of flowering varied between years (Fig. 3.1) with the onset of flowering in 2012 beginning ~17 days earlier than in 2011. Flowering was more synchronous in 2011 than in 2012, with a relatively low proportion of plants (mean=44%; range=20–60%) flowering at any one time in 2012, while in 2011, flowering showed a late but steep increase to over 90% by 1 August (Fig. 3.1).

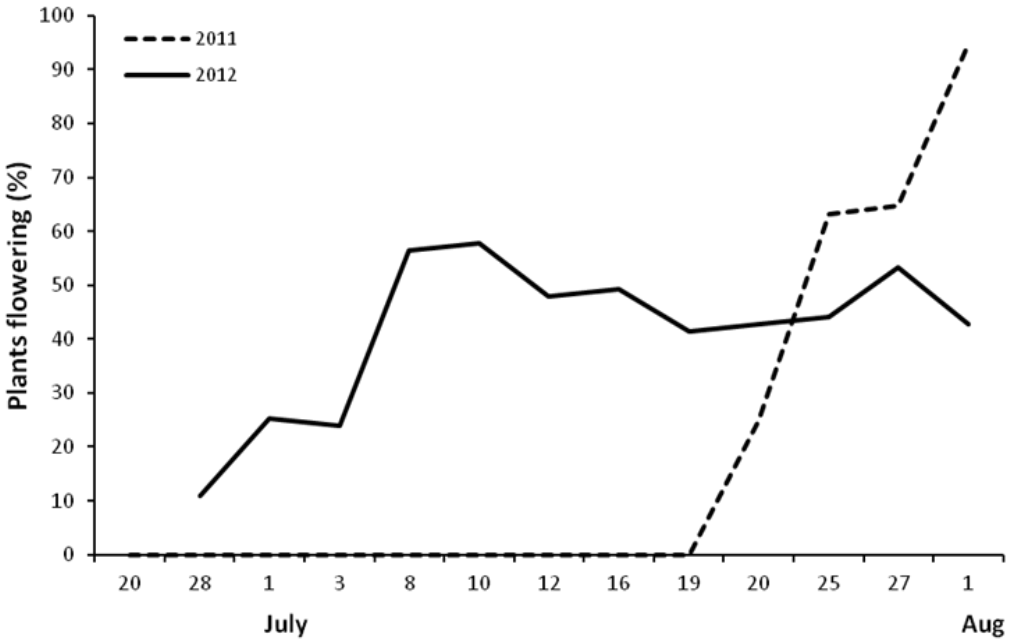

Figure 3.1 The proportion of *Aloe peglerae* plants flowering in the population in 2011 (n=440) and 2012 (n=550). Sampling commenced at different times in 2011 (20 June) and in 2012 (28 June). Flowering started earlier than anticipated in 2012 (before 28 June), which was significantly different to 2011 (~19 July).

The average density of flowering plants/ha per focal plant was significantly higher (2.8 times) in 2011 than in 2012 (Table 3.1). This is an exact match to the proportion of adult plants that flowered in 2011 (~2.8 times higher than in 2012). Furthermore, the overall flowering densities per focal plant had no significant effect on fruit set (Spearman Rank Correlation, 2011: R_s=0.21, df=17, *P*=0.38; 2012: R_s=-0.17, df=33, *P*=0.33), seed set (2011: R_s=0.06, df=17, *P*=0.79; 2012: R_s=-0.21, df=33, *P*=0.23), total seed production (2011: R_s=0.16, df=17, *P*=0.50; 2012: R_s=-0.16, df=33, *P*=0.36) or seed germination success (2011: R_s=0.32, df=8, *P*=0.36; 2012: R_s=0.42, df=12, *P*=0.14) in either year.

Associated with this tripling in flowering density in 2011, was an overall greater fruit set (~7 times higher), seed set (~2.5 times higher) and total plant seed production (~5 times higher) in the population in 2011 than in 2012 (Table 3.1). However, germination percentage in 2012 was ~5 times higher than in 2011 (Table 3.1).

Table 3.1 Flowering and reproductive success in focal *Aloe peglerae* plants in 2011 and 2012 (mean ± S.D.). Sample sizes for fruit set, average seed set per fruit and total seed production per plant for 2011 (n=19) and 2012 (n=35) represent the number of focal plants. Sample sizes for germinated seeds per plant depended on the number of plants that produced seed in 2011 (n=10) and 2012 (n=14). Mann-Whitney *U* tests were used for comparisons between years, except for the comparison between the percentages of plants that flowered in each year (Chi-Square test).[a] The percentage of flowering plants recorded in the population in 2011 (n=440 plants) and 2012 (n=550).

	2011 (n=19)	2012 (n=35)	P-value
Flowering plants (%)[a]	46	14	<0.001
No. flowering plants/ha	14 ± 10	5 ± 4	0.0004
Fruit set/plant (%)	40 ± 27	6 ± 11	<0.0001
Seeds/fruit	38 ± 19	15 ± 20	0.009
Total seeds/plant	3,869 ± 2,333	757 ± 1,294	0.0002
Germinated seeds/plant (%)	17 ± 13	82 ± 20	<0.0001

3.4.2 Flowering plant density at increasing spatial scales

While overall flowering plant density did not influence reproductive success in either year, the hierarchical regression analysis of flowering plant density at successively increasing spatial distances from focal plants in 2011 significantly influenced plant reproduction at a scale of 30.0–35.0m (Table 3.2). Fruit set and total seed production per focal plant at this scale was significantly related to flowering plant density (Table 3.2). In 2012, flowering plant density did not significantly affect reproductive success at any particular spatial scale measured (Table 3.2).

Overall, the strength of the relationships (R^2; Table 3.2) between flowering plant density and reproductive success (i.e. fruit set, seed set, total seed production, and percent germination) increased as the distance from the focal plant increased. This suggests that the density of flowering plants further away plays an important role in reproduction for *Aloe peglerae*.

3.4.3 Visitor observations

Camera trap photography from 2011 confirmed that the Cape Rock-Thrush (*Monticola rupestris*) and the Dark-capped Bulbul (*Pycnonotus tricolor*) were the main pollinators for *Aloe peglerae* (section 2.4.2; Arena *et al.* 2013). Although *A. peglerae* is bird-pollinated, I suspected that there were nocturnal visitors to the flowers in 2011, despite having not detected any on the camera traps (2011 total observations=1,656 h). In the second flowering season (2012 total observations=9,276 h) using twice as many camera traps at focal plants over a 24-hr period throughout the season, two new nocturnal visitors were detected. Two small-mammal species, the Namaqua Rock Mouse (*Micaelamys namaquensis*) and the

Eastern Rock Elephant-Shrew (*Elephantulus myurus*), were observed visiting the flowers normally during darkness. Based on the foraging behaviour observed from photographs, the rock mouse, an omnivore (Table 3.3; Skinner and Chimimba 2005) mainly foraged on flower parts. The elephant-shrew, an insectivore (Table 3.3; Skinner and Chimimba 2005) was observed probing flowers more often than foraging on flower parts. Pollen was clearly visible on the facial area of the birds; however, it was difficult to detect pollen on any body parts of the small-mammals. However, as observed from their foraging behaviour, there may be some degree of pollen deposition on the face and/or abdomen of the small-mammals.

The observations during 2012 also detected two new bird species visiting flowers for nectar, the Crested Barbet (*Trachyphonus vaillantii*) and the Cape White-eye (*Zosterops capensis*). Field observations of visitors from 2011 did not determine whether or not white-eyes were nectar-feeding, but increased sampling in 2012 clarified that they did occasionally feed on nectar. Other animals detected on the camera traps from 2012 but not observed nectar-feeding included the Chacma Baboon (*Papio hamadryas ursinus*), Large-spotted Genet (*Genetta maculata*) and Smith's Red Rock Rabbit (*Pronolagus rupestris*), Cape Bunting (*Emberiza capensis*) and Lazy Cisticola (*Cisticola aberrans*).

Table 3.2 The effect of absolute flowering plant density on four measures of reproductive success at successively increasing circular scales from focal plants (represented by the ratio test *F* vs. model 1) in 2011 (n=19) and 2012 (n=35) using hierarchical regression analysis. Bold values are significantly related at **P*<0.05; ***P*<0.01. Only Model 7 is significant for 2011 (up to 35.0m scale).

	Flowering plant density (2011)								Flowering plant density (2012)							
	Fruit set		Seed set		Total seeds/plant		Germination (%)		Fruit set		Seed set		Total seeds/plant		Germination (%)	
Model	R^2	*F* vs. model 1	R^2	*F* vs. model 1	R^2	*F* vs. model 1	R^2	*F* vs. model 1	R^2	*F* vs. model 1	R^2	*F* vs. model 1	R^2	*F* vs. model 1	R^2	*F* vs. model 1
1 (0–4.99m)	NA	-	NA	-	NA	-	NA	-	0.000	-	0.000	-	0.000	-	0.003	-
2 (5.0–9.99m)	0.01	0.23	0.03	0.42	0.01	0.26	0.04	0.12	0.06	1.88	0.08	2.58	0.08	2.44	0.36	5.56
3 (10.0–14.99m)	0.02	0.15	0.10	1.04	0.06	1.01	0.12	0.24	0.07	0.28	0.08	0.09	0.08	0.09	0.36	0.06
4 (15.0–19.99m)	0.08	1.40	0.10	0.02	0.07	0.06	0.13	0.04	0.07	0.08	0.13	1.36	0.13	1.35	0.36	0.00
5 (20.0–24.99m)	0.08	0.00	0.11	0.16	0.07	0.06	0.13	0.00	0.10	0.88	0.13	0.06	0.13	0.07	0.47	1.79
6 (25.0–29.99m)	0.13	1.27	0.11	0.02	0.08	0.17	0.18	0.16	0.14	1.17	0.16	0.91	0.16	0.94	0.48	0.17
7 (30.0–34.99m)	0.51	**8.59****	0.22	1.65	0.40	**6.47***	0.34	0.47	0.15	0.33	0.16	0.04	0.16	0.04	0.65	2.65
8 (35.0–40.0m)	0.52	0.05	0.26	0.64	0.46	1.32	0.34	0.01	0.15	0.03	0.16	0.03	0.16	0.04	0.68	0.54

Eight regression models which represent successively increasing circular scales from focal plants are used for flowering plant density for each reproductive measure:
Model 1: y = intercept + (# plants within 0–4.99m);
Model 2: y = intercept + (# plants within 0–4.99m + # plants within 5.00–9.99m);
Model 3: y = intercept + (# plants within 0–4.99m + # plants within 5.00–9.99m + # plants within 10.00–14.99m); etc.

Table 3.3 A detailed description of the biology of two small-mammal species observed visiting the flowers of *Aloe peglerae* (information from Skinner and Chimimba 2005).

	Namaqua Rock Mouse *Micaelamys namaquensis*	**Eastern Rock Elephant-Shrew** *Elephantulus myurus*
Order	Rodentia	Macroscelidea
Distribution	Angola, Zambia, Malawi, northern Mozambique, distributed widely throughout southern Africa (excluding parts of coastal KwaZulu-Natal, Eastern Cape, Mozambique)	Western & northern Mozambique; southern & eastern Zimbabwe; eastern Botswana; most provinces in South Africa excluding the Western Cape
Habitat	Occurs in various habitats, but prefer rocky koppies, outcrops, boulder-strewn hillsides; in central Kalahari found in open scrub, woodlands, pan fringes	Confine to rocky koppies or piles of boulders; takes refuge in holes, crannies, under overhanging ledges or vegetation
Habits	Nocturnal, communal, terrestrial, to some extent arboreal. Small colonies live in rock crevices, holes in trees, under fallen logs, piles of debris. Form huge piles of grass stems, fine and debris at shelter entrances. Well-adapted to hot arid environments.	Predominantly diurnal; activity peaks at dawn; also active at night; keep to shady/sheltered refuge; characteristic quick movements leaping from boulder to boulder; go into torpor when food-deprived or under low ambient temperatures
Feeding biology	Omnivorous - grass and plant seeds (*Protea, Restio, Willdenowia,* and *Hypodiscus* spp.). Seldom feeds on arthropods.	Insectivore - 90% invertebrate prey (isopterans, formicids, coleopterans, tenebrionids, curculionids, and other arthropods). Remainder of diet made up of plant material.
Reproduction	Gravid females September–May; gives birth to ~3 young.	One to two young are born September–March; gestation period 8 weeks; females become sexually mature 5–6 weeks of age

3.4.4 A spatial effect on nectar volume and visitation rates

While there was no significant effect of increasing flowering plant densities at increasing spatial scales on overall visitation rates, flowering plant densities at a distance of 5.0–10.0m from focal aloes significantly influenced the interaction between nectar volume and visitation rate per focal plant (Table 3.4). After discovering that *A. peglerae* is visited both by diurnal and nocturnal visitors, the individual influences of flowering plant densities on diurnal and nocturnal visitation rates at increasing spatial scales were investigated. Diurnal visitation rates were significantly affected by densities at 25.0–30.0m from focal plants (Table 3.4). Furthermore, densities also significantly affected the combined interaction of diurnal

visitation rates and the nectar standing crop of focal plants between 25.0 and 40.0m (Table 3.4). Nocturnal visitation rates and its interaction with nectar volume were not significantly related to flowering plant densities (Table 3.4). Once again, the strength (R^2; Table 3.4) of these relationships increased as the distance increases further away from focal plants, emphasising that flowering plant densities at increasing distances are important for visitors to flowers of *A. peglerae*.

3.4.5 Patterns in nectar volume and visitation rates

The relationships between visitation rate and i) overall flowering plant density (Spearman R_s=-0.18, df=27, *P*=0.34), ii) fruit set (Fig. 3.2a), iii) seed set (Fig. 3.2b), and iv) total seed production (Fig. 3.2c) for focal plants in 2012 were not significant. However, despite the lack of significance in these relationships, during the flowering season in 2012, visitation rate peaked when the percentage of plants flowering peaked (Fig. 3.3). As a response to the increase in visitation rate (visitors per plant.h^{-1}) at these flowering peaks, nectar standing crop significantly decreased (Fig.3.3; R_s=-0.67, df=9, *P*=0.02). It is noteworthy that the first peak (10 July 2012) in pollinator activity (Fig. 3.3) consisted mainly of the diurnal bird (rock-thrush and bulbul) pollinators (60%), compared to the nocturnal small-mammal (rock mouse and elephant-shrew) visitors (40%). The second peak (25 July 2012) in pollinator activity (Fig. 3.3) mainly represented nocturnal visitors (79%) compared to the diurnal pollinators (21%).

Many focal plants in 2012 produced no fruit and seed (Fig. 3.2) despite having a high visitation rate (2.5–42.0 visitors/plant.h^{-1}). There were slightly more observations of diurnal visitors (n=111) to focal plants than there were of nocturnal visitors (n=104) and a higher number of flowering plants were visited by diurnal visitors (43) than nocturnal visitors (32). Hence, this may indicate that diurnal bird visitation was generally higher than nocturnal small-mammal visitation; however, this does not indicate what the individual contributions by each to fruit and seed production were.

Partitioning in the visitation patterns of the diurnal visitors, the birds, and the nocturnal visitors, the small-mammals, was observed (Fig. 3.4). The omnivorous bulbuls were the first to visit flowers during the day (05h30–12h00), followed by the insectivorous rock-thrushes that mostly visited flowers in the afternoon. The visitation patterns observed for the nocturnal visitors were similar (during two peak visitation periods), but the omnivorous rock mouse visited flowering plants at double the rate of the insectivorous elephant-shrew (Fig. 3.4).

3.4.6 Seed germination

The percentage of seeds that germinated within the 7-week germination trial period from focal plants in 2011 (mean=17%, 0–38%; see section 2.4.4) was significantly lower (Mann-Whitney *U* test; $P<0.001$) than the percentage of germinated seeds in 2012 (82%; 40–100%). Germination trials in 2012 also yielded no zero germination percentages for any of the plants. Seed germination trials were started much sooner in 2012 (1 week after dehiscence) than in 2011 (5–6 weeks after dehiscence). Seeds from 2012 started germinating from day 1 after sowing and reached peak germination on day 15 which was the same as for 2011.

Table 3.4 The effect of flowering plant density on nectar volume standing crops and on the relationship between nectar volume and visitation rates to flowers of focal plants (n=35) in 2012. Diurnal visitation rates were influenced by flowering plant density at 25.0–35.0m, as well as the interaction between nectar volume and visitation rates between 25.0 and 40.0m. Statistical significance at **P*<0.05; ***P*<0.01; ****P*<0.001.

	Overall				Diurnal				Nocturnal			
	Visitation rate		Nectar*visitation		Visitation rate		Nectar*visitation		Visitation rate		Nectar*visitation	
Model	R^2	*F* vs. model 1	R^2	*F* vs. model 1	R^2	*F* vs. model 1	R^2	*F* vs. model 1	R^2	*F* vs. model 1	R^2	*F* vs. model 1
1 (0–4.99m)	0.00	-	0.16	-	0.00	-	0.13	-	0.00	-	0.14	-
2 (5.0–9.99m)	0.07	2.09	0.32	**5.71****	0.01	0.35	0.18	**3.50**	0.00	0.00	0.15	0.16
3 (10.0–14.99m)	0.09	0.79	0.33	0.21	0.15	0.11	0.19	0.08	0.04	0.51	0.15	0
4 (15.0–19.99m)	0.10	0.09	0.33	0.01	0.09	2.33	0.24	3.04	0.09	0.60	0.21	0.71
5 (20.0–24.99m)	0.11	0.19	0.33	0.06	0.09	0.01	0.24	0.13	0.15	0.80	0.21	0.04
6 (25.0–29.99m)	0.22	3.53	0.36	1.11	0.34	**8.13****	0.35	**6.70***	0.15	0.01	0.21	0.00
7 (30.0–34.99m)	0.26	1.06	0.45	3.38	0.41	2.24	0.62	**16.53*****	0.17	0.28	0.22	0.02
8 (35.0–40.0m)	0.33	2.14	0.48	1.28	0.47	1.73	0.73	**6.64***	0.24	0.90	0.22	0.02

Regression models as above (Table 3.2)

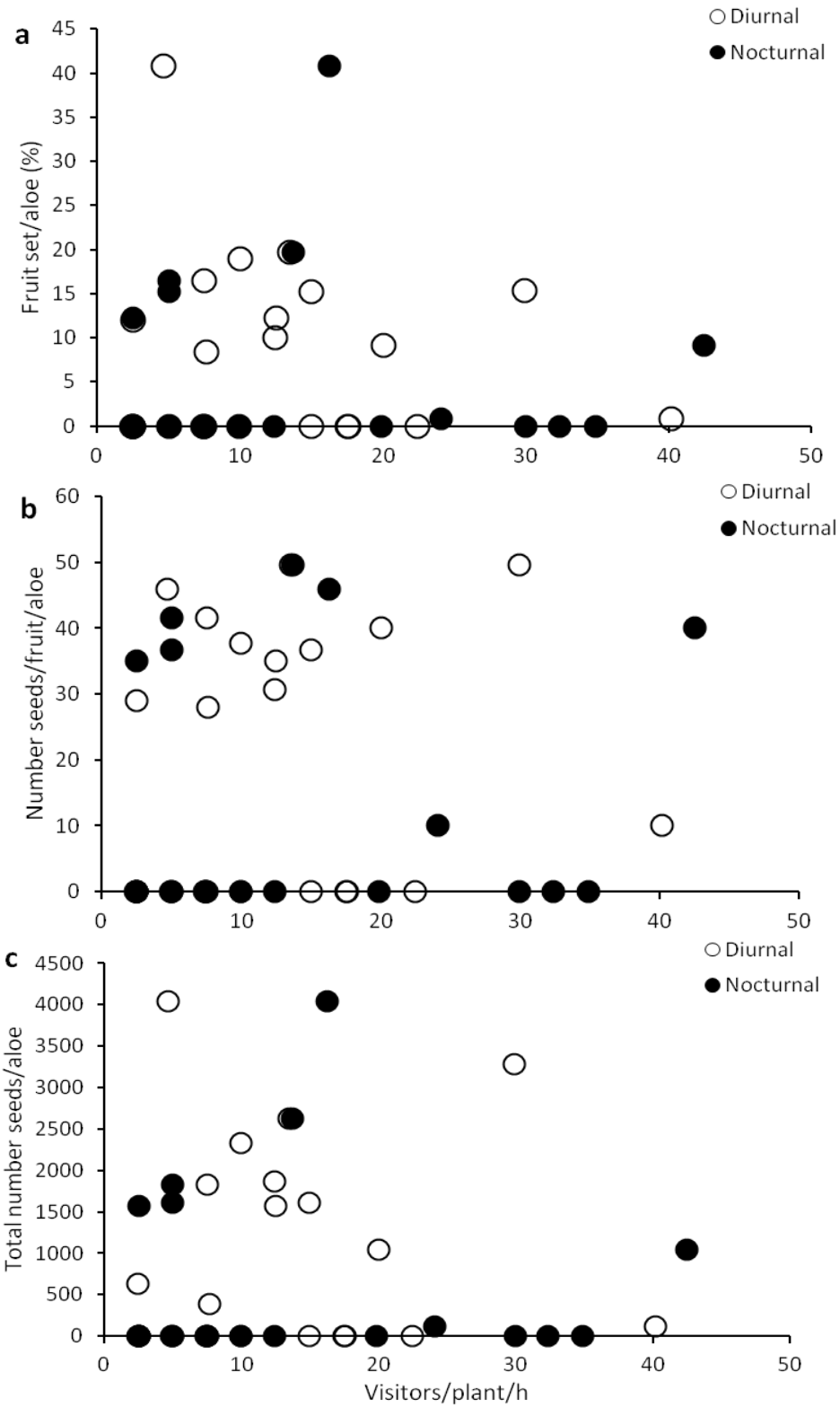

Figure 3.2 The effect of diurnal and nocturnal visitation rate per focal plant per hour in 2012 (n=30) on, a) fruit set (%), b) seed set and, c) total seed production per focal plant. Many focal plants received high visitation rates, yet produced zero fruit and seed. Linear relationships are not significant (Spearman Rank Order Correlation; $P>0.05$).

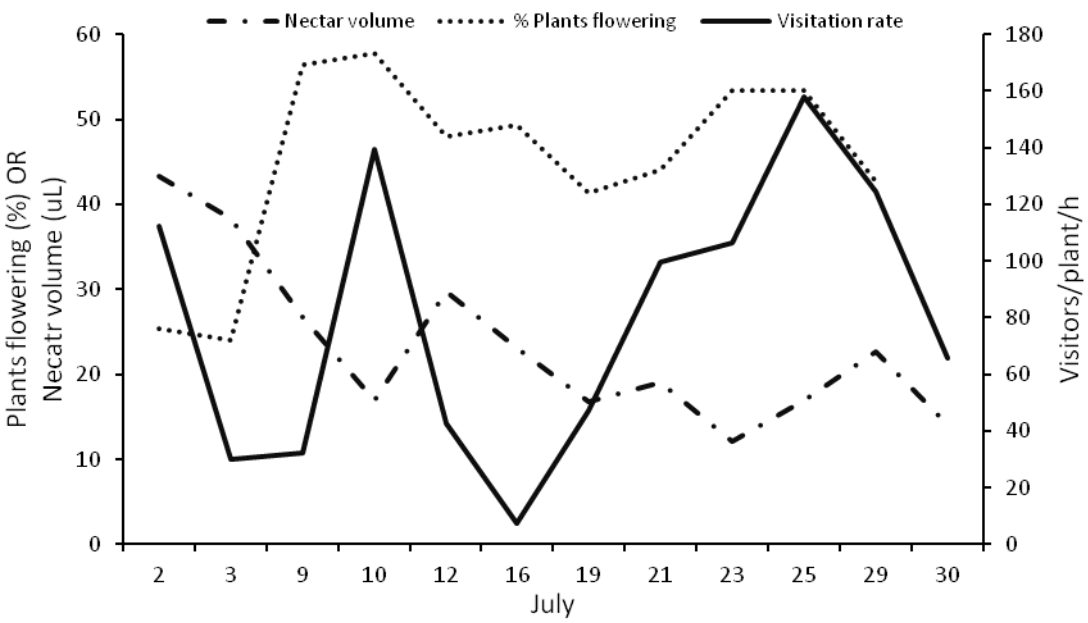

Figure 3.3 Daily variation in flowering of *Aloe peglerae*, nectar standing crop and visitation rates (all visitors) in the population in 2012 indicates that visitation rate peaked in response to two flowering peaks in the population, and an increase in the number of visitors to plants/h significantly reduced nectar standing crop (P<0.05).

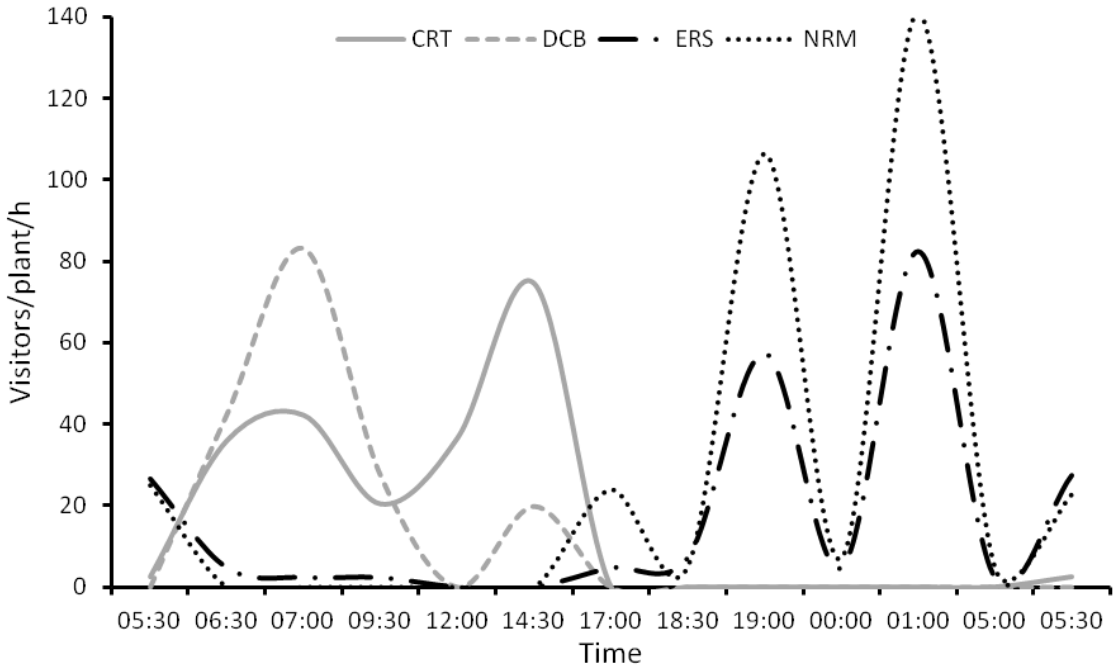

Figure 3.4 Daily patterns of visitation rates of diurnal (CRT = Cape Rock-Thrush, DCB = Dark-capped Bulbul) and nocturnal (ERS = Eastern Rock Elephant-Shrew, NRM = Namaqua Rock Mouse) visitors to focal plants in 2012. Each time interval used on the x-axis is different and represents a new period where a change in species occurred (i.e. 05:30 = dawn; 06:30 = sunrise etc.).

3.5 Discussion

3.5.1 General flowering patterns

The findings demonstrate that interannual flowering variation and subsequent flowering density can have direct and indirect effects on pollinator visitation and plant reproduction in *Aloe peglerae.* Flowering in *A. peglerae* is size-dependent (Fig. 4.5; Melville 1988; Scholes 1988) and highly variable between years (Scholes 1988). Scholes (1988) recorded highly variable annual flowering (mean=59%, range=30–82%) in a sample of one hundred *A. peglerae* plants within another subpopulation over an 11-year period. Flowering in *A. marlothii* was also variable over a three year period (9.2–45.4%) with taller plants flowering more frequently than shorter plants which either flowered only once or never (Symes and Nicolson 2008).The timing of flowering between years also differed substantially for *A. peglerae*, with the first onset of flowering starting two and a half weeks earlier in 2012. This difference in the onset of flowering may be a response to different environmental conditions between the years, such as poor rainfall in the previous growing season or very cold winter months prior to flowering (Smith and Van Wyk 2009; Forrest and Miller-Rushing 2010); however, these factors were not measured in this study.

Variation in rainfall over the last two growing seasons (~658mm from June 2010 – July 2011 and ~555mm from July 2011 – June 2012) may have been an important determinant for mast-seeding in 2011 and the subsequent drop in flowering and reproduction in the following year. However, annual flowering in *A. peglerae* was not significantly related to the previous growing season's rainfall (Scholes 1988; Kelly 1994), and further investigation into the effect of environmental variation on annual flowering in *A. peglerae* is needed.

Episodic mass flowering in perennial plants (Herrera *et al.* 1994; Forrest and Miller-Rushing 2010), such as *A. peglerae*, is a common phenomenon. The production of massive seed crops (known as mast-seeding) in some years normally follows a mass flowering pulse in the population (Herrera *et al.* 1998). Comparatively low flowering and/or poor seed set in subsequent years is common after a mast-seeding event (Herrera *et al.* 1998; Smith and Van Wyk 2009). It is suggested that long-lived species have adopted this life history strategy to either cope with the effects of seed predators, to enhance pollination efficiency, or is linked to the temporal patterns in resource availability, acquisition, accumulation, and allocation by plants (Herrera *et al.* 1994; 1998; Kelly 1994; Forsyth 2003; Crone *et al.* 2009; Smith and Van Wyk 2009).

The high and synchronous burst in flowering in 2011 followed by the much lower and prolonged flowering in 2012 may have been advantageous to satiate flower/seed predators in the first study year. Although there were no significant relationships between reproduction and pollinator visitation, high pollination efficiency may be linked to mast-seeding events during high flowering years, followed by pollen-limited reproduction in low flowering years (Crone *et al.* 2009). The result of low reproduction in 2012 compared to 2011 may likely have been caused by lower pollinator abundance and visitation leading to pollen-limitation.

While many plants did receive pollinator visits from birds in 2012 the absence of fruit after flowering might imply that reproduction is potentially resource limited (Stephens *et al.* 1999; Symes and Nicolson 2008). The other consideration is that during a mast-seeding event, pollination is increased and after reproduction plants become resource depleted and in following years may not flower (Crone *et al.* 2009). In subsequent low-flowering years, reproduction is low, allowing many plants with greater stored resources to flower the following year, resulting in these irregular masting events (Clotfelter *et al.* 2007; Crone *et al.* 2009). The causes for irregular flowering patterns in *A. peglerae* deserve further investigation in future studies, emphasizing the importance of investigating the fate of fruit and/or seed set, as well as the costs on reproduction due to patterns in resource allocation.

3.5.2 Broad-scale density effect

In individual years, overall flowering plant densities in the subpopulation (despite the significant difference between years) had no significant effect on plant reproductive success in *A. peglerae,* implying that an Allee effect is either not expressed within the subpopulation or it cannot be detected at the spatial scale that was measured. Despite this however, all measures of reproductive output tended to increase with an increase in flowering density in 2011. This may have been as a result of increased attractiveness and greater nectar rewards available to birds. In contrast, reproductive output declined as flowering density increased in 2012. One can only speculate on these differences that may be due to insufficient pollinators attracted to the area in 2012, due to the reduced flowering density in the population. Perhaps there may also have been other more attractive subpopulations of *A. peglerae* of higher flowering densities in 2012 at other sites along the mountain range. Furthermore, although not significant, all measures of reproductive output tended to increase as the number of pollinators at flowering plants increased in 2012. Therefore, these findings might suggest that increased pollinator visitation potentially enhances plant reproduction in *A. peglerae*, and that

in years of very low flowering densities (as in 2012), reproduction would be pollinator/pollen limited (Bierzychudek 1981; Ågren 1996; Wilcock and Neiland 2002).

Although fruit production is strongly related to inflorescence height (which is related to plant size), Melville (1988) suggested that fruit production was instead controlled by factors other than plant size, those being a self-incompatible mating system or patterns of resource allocation (Melville 1988). In the current study, while many plants received visits from both diurnal and nocturnal visitors, some plants still did not produce fruit. The suggested explanation for this could be that reproduction in those individuals was either resource- or pollen-limited (Stokes and Yeaton 1995).

Germination success was higher in the low-flowering year (2012). The mechanisms involved in seed viability for *Aloe* is largely unknown, except that seeds are orthodox and have relatively short periods of viability (Giddy 1973; Cousins and Witkowski 2012). As a result, seed banks are mainly transient e.g. *A. marlothii* (Symes 2012) and *A. plicatilis* (Cousins *et al.* in prep.). The only known exceptions for prolonged seed viability and dormancy in *Aloe* occurs in *A. dichotoma* and *A. asperifolia* whose seed remain viable for up to three years after dispersal; an adaptation to the arid, erratic rainfall regions in which they occur (Giddy 1973).

There may be two explanations for the higher germination success in the lower flowering year in this study. During mast-seeding years where massive seed crops are produced, as in 2011, there may not be sufficient resources to allocate to all seeds produced, leading to high numbers of non-viable seed (sections 2.4.3–2.4.5; Kelly 1994). Therefore, in years of low seed production, most, if not all, seed produced will be viable when resources are not limiting. By taking into account both the number of seeds produced and the percentage of germinated seeds each year, the net effect is very similar. The average number of germinated seeds/plant in 2011 (658 germinated seeds/plant) was similar to that in 2012 (621). This might imply that despite the mast-seeding event of 2011, if resources limit the overall number of viable offspring produced each year, then mast-seeding events is rather likely to be a strategy to satiate seed predators.

The second explanation may be linked to differences in pollen flow between years. In the high flowering year, flight distances for birds may have been shorter (due to a higher density of flowering plants) and overall greater nectar abundance compared to 2012. This may have allowed the birds to forage within smaller spatial areas in the population, leading to reduced pollen flow. In comparison, birds would have been forced to travel greater distances to gain

access to the next nectar source in 2012, thereby increasing pollen flow between plants (Wilcock and Neiland 2002). This may have resulted in higher seed viability and hence higher germination success (40–100%) in seeds collected in 2012 (Feinsinger 1978). However plausible these explanations, aspects of interannual variation in germination success certainly warrants further research by incorporating pollination supplementation experiments to determine the degree of self-compatibility for *A. peglerae.*

3.5.3 Local-scale density effect

Fruit set and total seed production per plant in 2011 were significantly related to flowering densities at a distance of 30.0–35.0m away from focal plants. However in 2012, when flowering density was lower, local flowering densities did not influence reproductive output at any of spatial scales measured. Due to the inconsistency in this finding for both years, further testing is required for multiple populations to detect an effect of density on reproduction. I predict that any notable effects would probably only be detected at a much broader spatial scale of over many kilometers for this bird-pollinated species.

Although few studies have investigated the spatial scale at which bird-pollinators move within or between patches of flowering plants, the potential foraging distance of bird-pollinators is likely to be much greater than that of small-mammals, foraging regularly over distances >1 km (Stiles 1978; Quesada *et al.* 2004). Therefore, the effect on reproduction observed at this distance (30.0–35.0m) might typically reflect the scale of movement at which birds will forage within a patch when flowering plant density is relatively high and interplant distances are short, as in 2011.

Flowering plant densities at increasing spatial scales also did not influence the germination success of seeds sampled from focal plants in either year (Fig. 3.5). The reason behind this may be related to plant mating system, which is currently unknown for *A. peglerae.* If *A. peglerae* is self-compatible, then it presumably would remain insensitive to any changes in flowering plant densities. If *A. peglerae* is self-incompatible (relying solely on cross-fertilization for viable seed production) then presumably seed viability would increase with flowering plant densities at greater distances from focal plants, as pollen flow, and gene flow, is enhanced. Another possible reason for there being no effect of density on germination success at different spatial scales is purely an indication that the effect of birds foraging over a wide distance helps facilitate efficient pollen flow between flowering plants at increasing

spatial scales. However, aspects of pollen quality and quantity were not investigated in this study.

The difference in the spatial scale observed at which flowering density would influence reproduction is largely dictated by annual flowering phenology. This further implies that interannual variation in flowering patterns in different density populations ultimately has diverse effects on pollination success (Méndez and Díaz 2001; Forsyth 2003; Quesada *et al.* 2004) both within local floral neighbourhoods and at larger spatial scales (Feinsinger *et al.* 1991).The fact that no effect of flowering plant density on reproduction was detected in 2012 may be a reflection of the low flowering plant density in the population. Due to the lower densities, the spatial flowering pattern in the study area was sparser and this might explain why an effect was not detected within the 40m measured. Hence, in years of low flowering plant density in this subpopulation of *A. peglerae,* events on a larger scale (>40.0m) might be important for overall reproductive output in the population (Mitchell and Ankeny 2001). This might be because bird pollinators are forced to forage over a larger range (>40m) between flowering plants when flowering densities are sparse. Under lower flowering plant densities in 2012, reproductive output was generally lower, and this potentially may be a reflection of pollen limitation due to reduced pollinator visitation (Kunin 1993; Lamont *et al.* 1993; Ågren 1996).

In 2012, flowering plant density significantly influenced diurnal (birds) visitation rates at 25.0m from focal plants (Table 3.4). Furthermore, the interaction between diurnal visitation rates and nectar standing crop was significantly related to flowering plant densities between 25.0–40.0m (Table 3.4). Despite flowering plant density having had no effect on plant reproduction in 2012, it did influence the foraging distance of birds at ≥25.0m. This confirms our hypothesis that in 2012 birds would have travelled greater distances between flowering plants due to the lower and sparser density of flowering plants. Although birds are capable of visiting widely scattered flowering plants, studies should be undertaken to identify the minimum density threshold at which the reproduction of isolated individuals will be negatively affected.

Flowering plant densities in 2012 did not significantly influence nocturnal visitation rates. Furthermore, the interaction between nocturnal visitation rate and nectar standing crop of focal plants was not related to flowering plant density at increasing spatial scales (Table 3.4). It is possible that the small-mammal population was sufficient to visit all flowering plants in

the area despite the difference in spatial flowering and density between years (Kleizen *et al.* 2009). I originally expected the range of movement of small-mammals to be within a smaller defined area than the birds (Wiens *et al.* 1983; Carthew and Goldingay 1997; Hackett and Goldingay 2001), mainly based on the home range, or forage range, size of small-mammals. Foraging distances by several small-mammal species on *Protea* nectar demonstrated that interplant nocturnal visitation occurs up to 15m from the source (Wiens *et al.* 1983). However, the home range lengths of *Micaelamys namaquensis* (Namaqua Rock Mouse) and a different species of elephant-shrew, *Elephantulus edwardii*, ranged from 27.5–58.0m and 27.5–44.2m, respectively (Wiens *et al.* 1983). The average home range sizes of *E. myurus* calculated for males (51–66m) and females (~25m) showed considerable overlap in home ranges between sexes (Ribble and Perrin 2005). Based on the small home ranges for these small-mammals, a positive effect of flowering plant density on plant reproduction would be expected, and yet was not detected at the scale of focal plots (5,026 m^2). This implies that small-mammals potentially do not influence reproduction in *A. peglerae.*

This further indicates that under relatively low flowering plant density in *A. peglerae*, and depending on small-mammal abundance, nocturnal visitors would have to travel within or beyond the area of their home ranges to access floral and nectar resources. Furthermore, the foraging behaviour of small-mammals is also likely to be influenced by factors other than flowering plant density. This may include body size, mobility, territory size, threats from predators, or spatial patchiness in the nectar reward which is shaped by the foraging patterns of both diurnal and nocturnal visitors (Pleasants and Zimmerman 1979; Carthew and Goldingay 1997; Skinner and Chimimba 2005). Despite there being no effect of density on nocturnal visitation rates, small-mammals may be important agents for outcrossing in *A. peglerae*. Hence it is important to determine the contribution of small-mammals to fecundity in *A. peglerae.*

3.5.4 Flowering, nectar availability and pollinator visitation

During the 2012 flowering season, two peaks in flowering were accompanied by peaks in pollinator activity at flowering plants. As a response to these peaks in visitation rates, nectar standing crop was significantly reduced, reflecting nectar depletion by increased visitation by nectar feeders (Waser and Mitchell 1990; Duffy and Stout 2008). This increase in pollinator activity and subsequent decrease in nectar standing crop at the two flowering peaks suggests that nectar-feeding increased during the peaks in flowering. It also suggests that successful

reproduction would likely depend on higher pollinator activity on the basis that reproduction is pollen-limited. However, this deserves more attention in future studies to differentiate between resource-limiting and pollen-limiting costs on reproduction.

The first peak in pollinator visitation rate predominantly represented the diurnal bird visitors, while the second peak mainly represented the nocturnal small-mammal visitors. If this phenomenon is a regular pattern observed between years or populations, then it may have an evolutionary significance in the flowering patterns of *A. peglerae*. Small-mammal visitation was not detected from camera traps in 2011, and yet there was sufficient evidence of herbivory on inflorescences in this year (GA, *pers. obs.*). Although we do not know if small-mammals are effective pollinators, the loss of potential fruit set through floral herbivory by small-mammals was not considered in the study. Video recordings, as well as photographic, evidence in the following year (2012) then showed small-mammals foraging on whole flowers (as well as accessing nectar) effectively removing flowers from inflorescences. Many focal plants produced zero fruit and seed, despite high visitation rates at those plants, and perhaps this may be a result of floral-removal by small-mammals. This type of destructive foraging will lead to a reduction in overall fruit production in a population. Hence, herbivory in *A. peglerae* is of importance to the overall plant reproductive success.

Daily spatial and temporal partitioning in the visitation rates of the different visitor taxa to flowering plants in 2012 was identified. The daily visitation patterns of the rock-thrushes mirror that of the bulbuls, except that there is temporal partitioning between the species. Nocturnal visitation patterns between the rock mouse and elephant-shrew are similar indicating that they rather partition resources spatially. Nocturnal visits to 50 plants showed that 19 (38%) were exclusively by the elephant-shrew and 22 (44%) by the rock mouse. Visits to the aloes by both nocturnal species were recorded at 9 (18%) plants. This might tentatively suggest some level of spatial partitioning between nocturnal species which possibly depends either on their limited movements which are territory-bound, or on abundance. The alternating periods in small-mammal activity for both species might indicate depressed metabolic rates during drops in ambient temperature (Merritt and Vessey 2000; Mzilikazi *et al.* 2002). Two nocturnal peaks in foraging in other shrew species has also been observed (Merritt and Vessey 2000), and the interaction of temperature and photoperiod on the availability of invertebrate food might also influence the activity of small-mammals, specifically the elephant-shrew (Merritt and Vessey 2000).

3.6 Conclusion

Several factors will influence pollinator abundance, visitation and foraging behaviour in a population of a plant species (Wolf *et al.* 1976; Feinsinger 1978; Schmitt 1983; Feinsinger *et al.* 1991; Quesada *et al.* 2004; Duffy and Stout 2008). One of the most obvious might be spatial and temporal changes in flowering densities between flowering seasons. *Aloe peglerae,* a predominantly bird-pollinated species (Arena *et al.* 2013), is unlikely to experience an Allee effect in a large-sized population, as in this study. However, it is crucial to emphasize that Allee effects are very difficult to detect on smaller scales of a bird-pollinated species and is usually easier to detect at broader scales when comparing between multiple populations of varying sizes or densities. Unlike many insect-pollinated plant species, reproductive output in individual plants of *A. peglerae* was not affected by overall flowering plant density in each year. Rather, any effects on reproduction are attributed to interannual flowering phenologies and density in the population.

The local floral neighbourhood during the day is more strongly defined and influenced by the foraging patterns of birds operating at a scale of ≥25.0m which is dictated by the flowering plant density, spatial flowering pattern, and nectar standing crop between 25.0–40.0m. Nocturnal small-mammal visitors to flowers did not appear to be influenced by flowering plant densities at increasing spatial scales.

If pollination depended solely on the nocturnal visitors, an Allee effect might exist in much lower flowering plant densities where interplant forage distances for small-mammals have been determined at ~15.0m (Wiens *et al.* 1983), and this would ultimately reduce outcrossing and genetic viability. However, if nectar and flowers are an important resource for nocturnal visitors at this time of year, then larger interplant distances might force small-mammals to move greater distances to access the nectar resource. This would ensure effective cross-pollination between flowering plants in a population (Carthew and Goldingay 1997). Small-mammal pollination in *A. peglerae* may therefore be more important than previously predicted (Arena *et al.* 2013) and future studies investigating this aspect of *A. peglerae* pollination biology are required.

Other factors that may have limited plant reproduction in 2012 may include resource limitation, herbivory of flower parts, or environmental conditions (Cruden *et al.* 1976; Pfab and Witkowski 1999; Suttle 2003; Crone *et al.* 2009; Steenhuisen and Johnson 2012). Hence, it becomes important to determine as many factors as possible that might influence seed

production in a plant species when investigating the effects of habitat fragmentation on plant reproductive success.

3.7 References

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CHAPTER FOUR

GROWING ON ROCKY GROUND: MICROHABITAT PREDICTORS OF SITE-OCCUPANCY OF A MONTANE SUCCULENT PLANT, *ALOE PEGLERAE*

4.1 Abstract

Most aloe succulents are either fire-adapted or fire-intolerant species and require particular microhabitat conditions in order to grow, reproduce and survive. However, due to the heterogeneous nature of grasslands and savannas, it is not clear what type of local-scale microhabitat is required for an aloe to persist. This study compared the microhabitat of aloe-occupied (n=100) and aloe-vacant microsites (n=100) of *Aloe peglerae,* a threatened endemic species of the Magaliesberg Mountain Range, in summer (February 2012) and winter (June 2012). The aim was to determine important microhabitat predictors of aloe persistence in a fire-mediated savanna. Twelve physical microhabitat variables, i.e. above-ground biomass ($kg.ha^{-1}$); percent grass, forbs, woody plants, rock, bare ground, plant litter cover; grass, forb and woody plant height (cm); overstorey shade (%); and soil depth (cm), were measured within circular plots (0.5m radius) replicated each season. Aloe-occupied microsites were strongly associated with typical 'safe site' microhabitat conditions consisting of higher rock cover (56%; aloe-vacant site=42%), lower bare ground cover (12%; 21%), lower grass cover (25%; 32%), lower above-ground biomass (714 $kg.ha^{-1}$; 1,373 $kg.ha^{-1}$) and higher forb cover (4.8%; 3.3%). Discriminant function analysis shows that rock, grass, and bare ground cover in summer, and bare ground, plant litter, and above-ground biomass in winter, contributed the most to defining differences between microhabitat conditions. *Aloe peglerae* persists in rocky safe sites of low surrounding vegetation, providing increased fire-protection. Such findings are applicable for *in situ* cultivation and *ex situ* re-introduction projects to protect remaining *A. peglerae* populations.

4.2 Introduction

Plant population distributions are influenced by spatiotemporal variation in the environmental conditions and structure of a landscape (Bazzaz 1991; Kephart and Paladino 1997; Münzbergová and Herben 2005; Spiesman and Cumming 2008). Successful plant dispersion at a regional scale thus depends on the availability of suitable patches of habitat for

colonisation within a matrix of unsuitable patches (Münzbergová and Herben 2005). Local-scale plant distribution within a suitable patch will thus depend on the heterogeneous nature of the patch itself (Lamont *et al.*1993; Witkowski and Liston 1997; Münzbergová and Herben 2005; Phama 2012). However, many plant species may not be present in all suitable habitats as a result of fine or broad-scale barriers to dispersal (Witkowski and Liston 1997; Witkowski and Lamont 2006). Therefore, defining the structure of the landscape, as well as the local-scale "microhabitat" (the structural conditions of the habitat at a plant-level) that provides suitable "safe sites" (defined as microsites in an environment that are suitable for germination and establishment; Harper 1961) for individual plant occupancy, is important in understanding plant community organisation and population biology (Spiesman and Cumming 2008; Sommers *et al.* 2011). This knowledge is especially beneficial for the conservation and management of rare, endemic or threatened species.

In ecosystems that experience frequent fire and changes in local conditions, a plant species would be exposed to a continuously changing environment (Bazzaz 1991; Lamont *et al.* 1993). Due to their immobility, plants have evolved several attributes to withstand the, sometimes unpredictable, changes in the local surroundings in order to survive, grow and reproduce (Bazzaz 1991). Harper (1977) suggested that each plant species has adapted to a unique set of microhabitat conditions that define its safe site and that these sites will vary in the degree of suitability for successful establishment. Furthermore, it can be seen that within a patch of suitable habitat for a particular species, some sites for plant occupancy can be considered safer, or more suitable, than other sites (Fowler 1988). For instance, wind-dispersed seeds are more likely to land in a wide range of possible habitats which will affect the likelihood of successful germination and establishment (Bazzaz 1991; Symes 2012).

Depending on the site of occupancy, under frequent fires of varying intensities, succulent plants will either tolerate fire through morphological adaptations, such as resprouting or vegetative recovery, or they will be intolerant to fire and die in unfavourable microhabitat (Thomas and Goodson 1992; Pfab and Witkowski 1999; Cousins *et al.* in prep.). Hence, considering these factors, as well as the spatial heterogeneity within a patch of habitat, what type of microhabitat conditions at an individual plant-level would collectively define an area more suitable for plant occupancy than other areas? I set out to answer this question by studying a threatened endemic succulent plant, *Aloe peglerae*.

Many studies have investigated the influence of microhabitat on seed germination, seedling emergence and establishment (Fowler 1988; Enright and Lamont 1989; Franco and Nobel 1989; Facelli and Pickett 1991; Lamont *et al.* 1993; Oswald and Neuenschwander 1993; Allison and Ehrenfeld 1999; De Villiers *et al.* 2001; Ibáñez and Schupp 2002; Moore and Elmendorf 2006), plant demographic change (Enright and Lamont 1989; Hawkes and Menges 1995, Hilton and Boyd 1996; Kephart and Paladino 1997), plant reproduction (Enright and Lamont 1989; Facelli and Pickett 1991; Hawkes and Menges 1995; Hilton and Boyd 1996), and resprouting (Bellingham and Sparrow 2000; Clarke 2002). However, few studies have attempted to define the predictors of suitable and/or unsuitable microhabitat for any particular species (Oswald and Neuenschwander 1993; Witkowski and Liston 1997; Sommers *et al.* 2011).

Knowledge of the optimal microhabitat conditions that make up a safe site for *A. peglerae* will contribute a better understanding of plant community dynamics and population biology for this endangered species, and hence management of land where these plants occur. The fire regime of the study site has primarily been in the form of annual anthropogenic fires for at least the past seven years (J. Wesson *pers. comm.*). Fires normally occur during the windy dry season in late-winter (August–October) prior to the first spring rains, however may also occur in May–June before flowering commences. Due to the frequency of fires at the site; the intensity of burns is expected to be low as fuel loads are replaced annually. Hence, the aim of the study was to compare the microhabitats of aloe-occupied and aloe-vacant "microsites" (defined as the sites of measurement in this study) within the area defined by the population (extent of occurrence). Three key questions were raised: 1) what are the key microhabitat differences between aloe-occupied and aloe-vacant microsites, 2) to what degree does each microhabitat variable contribute to the presence or absence of an aloe within a specific microhabitat, and, 3) what are the best microhabitat predictors of aloe presence?

4.3 Methods and materials

4.3.1 Species and site description

The study population represented a relatively undisturbed site inaccessible to the public. The estimated density of *Aloe peglerae* at the site is 142 plants/ha within ~56 ha. Compared to the range of density estimates of nine subpopulations in the Magaliesberg (31.5–99.1 plants/ha in 2010; Phama 2012), the study population is considered fairly dense and may represent a relatively 'natural' subpopulation with relatively limited human impacts.

Aloe peglerae is a slow-growing species and adults can survive up to ±60 years (Pfab and Scholes 2004). Recruitment is very low and it is estimated that seedlings only reach juvenile size after 4 years, and take an additional 6 years to reach 0.1m in diameter (Scholes 1988; Pfab and Scholes 2004). Reproductively mature adult plants (>0.2m in diameter; Pfab and Scholes 2004) may produce an inflorescence annually, but this is fairly uncommon and most attempts lead to premature abortion of buds (Melville 1988). Flowering is highly variable between years (Chapter 3; Scholes 1988). Seeds are small (~3–5mm), angled and winged, suitable for wind-dispersal after fruit capsules dehisce between September and October (Melville 1988; Smith and Van Wyk 2009). *Aloe peglerae* is confined to growing along the rocky north-facing ridges of the Magaliesberg Mountains in the Gauteng and North West provinces of South Africa.

Sampling of the population took place over a one year period (February 2011–June 2012) (Fig. 4.2). Transects, randomly selected in the field and spanning the length of the mountain ridge, were 100 x 20m. Each plot was searched thoroughly for all aloes and each was assigned a numbered metal tag and its waypoint marked using a handheld Garmin (Garmin, GPSMAP 60C9, USA) and differential Trimble GPS unit (Trimble GeoExplorer GeoXH 6000, USA) accurate to 0.5 m.

The vegetation type along the mountain ridge is classified as Gold Reef Mountain Bushveld (Rutherford *et al.* 2006; Fig. 4.1a) at an altitude of ≥1,500m a.s.l. and becomes more heavily wooded at lower altitudes. The soils on the ridge are shallow (5–20cm deep) and accommodates a grassy layer with intermittent clumps of trees and shrubs such as *Burkea africana, Englerophytum magaliesmontanum, Lannea discolor, Searsia magaliesmontana*, and *Strychnos pungens,* and succulent plants such as *Kalanchoe luciae, K. thrysiflora, Cotyledon orbiculata*, and *Euphorbia schinzii* (Van Wyk and Malan, 1998; Van Wyk and Van Wyk, 1997).

Figure 4.1 Visual comparisons between burnt and unburnt *Aloe peglerae* habitat, at (a) study site pre-fire; (b) study site post-fire exposing the characteristic rocky and bare ground; (c) suitable microhabitat for *A. peglerae* plants of low surrounding grass cover, with high rock and forb cover (scale bar=5cm); (d) a suitable microsite post-burn shows damage to the infructescence but the plant has survived (scale bar = 5cm); (e) a flower bud at the apical centre of the rosette (pre-flowering) where it is protected by the outer leaves. Photographs by G. Arena.

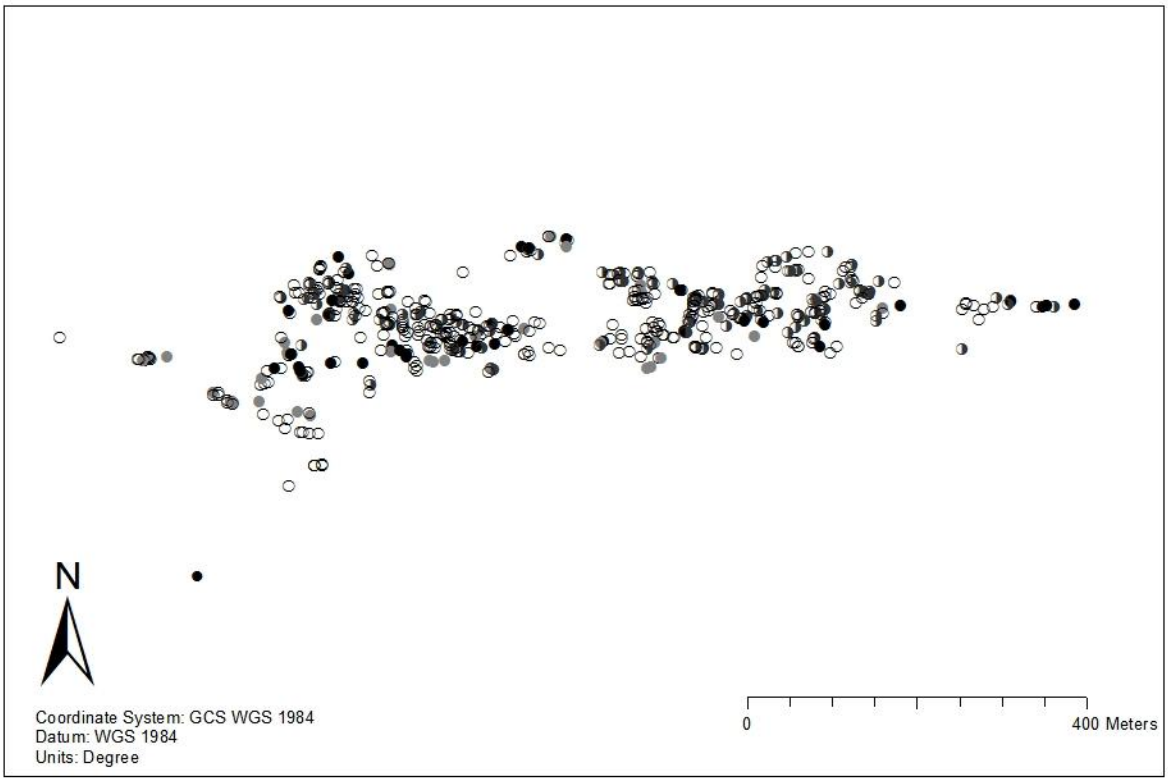

Figure 4.2 A map of the study population sampled in 2011 and 2012, each point representing an individual plant (white circles = plants that did not flower in either year; half-filled circles = plants that flowered in 2011; grey circles = plants that flowered in 2012; black circles = plants that flowered in 2011 and 2012).

Aloe peglerae plants were normally found scattered randomly, but often clumped within rocky outcrops where other forbs and trees grow. Individuals were also usually found growing close to *E. schinzii* plants (GA, *pers. obs.*). Along the mountain ridge, the soils appear to be deeper, and the landscape changes into a continuous grassy layer with a reduction in the woody component. Here the terrain is considerably less rocky with shallower slopes, and fewer aloes are found (GA, *pers. obs.*). Rainfall between July 2011–June 2012 was lower (~555mm) than in the previous year (July 2010 – June 2011; ~658mm) (South African Weather Service).

4.3.2 Plant size, age class and flowering

The mean diameter of each plant was measured by taking two readings, one diameter perpendicular to the other. The size of each plant was classified into seedling (<0.1m), juvenile (0.1–0.2m) and adult (>0.2m) (Pfab and Scholes 2004). The numbers of individuals that flowered successfully were counted during the flowering season (July–August) in 2012. The frequency of flowering and non-flowering individuals per size class in each year (2011

and 2012) was calculated. The correlation of plant size with each microhabitat variable was also tested for each season.

4.3.3 Microhabitat structure

One hundred aloes in the population were randomly selected to represent aloe-occupied sites. At a distance of 3m from the edge of each aloe-occupied site, a site where no aloe was growing was identified to represent an aloe-vacant site. Sampling took place in February 2012, generally regarded as a high summer-rainfall month in this region of South Africa, and again in winter (June) 2012, before any fires occurred. Replicate sampling in summer and winter was carried out to account for seasonal changes in the microhabitat variables that would change, and thereby influence the degree of suitability of safe sites, with fire, e.g. above-ground biomass.

A 0.5m radius circular quadrat centred on the aloe, or the defined point for aloe-vacant sites, was sampled. Each quadrat was partitioned into four quarters (NE, SE, SW, and NW). Twelve variables, each representing structural microhabitat feature either directly or indirectly associated with the occurrence and effect of fire, were measured for each aloe-occupied and aloe-vacant site. The aerial cover of grass, forbs, woody plants, rock, bare ground, plant litter cover, and overstorey shade were estimated (%); the minimum and maximum heights of grass, forbs and woody plants; and soil depths, were measured (cm) and all measures then averaged. Above-ground biomass in each aloe-occupied and aloe-vacant site was measured using a disk pasture meter, which uses the settling height of an aluminium disc dropped from a set height onto the grass sward (see Harmoney *et al.* 1997; Trollope *et al.* 2000), with two replicates per site. The disc settling heights were used to calculate grass biomass (fuel loads) using Equation 1. This is a calibration equation for the relationship between the settling height and the standing crop of grass/herbaceous vegetation that was determined for grassland and savanna systems in southern and east Africa (Trollope *et al.* 2000). It is normally used for estimating grass fuel loads for management purposes (Trollope *et al.* 2000).

$$\text{Mean fuel load } \left(\frac{\text{kg}}{\text{ha}}\right) = -3019 + 2260\sqrt{\text{mean disc height (cm)}}\,[\text{of 100 readings}]$$

Equation 1

4.3.4 Fire damage

Signs of fire damage were recorded from each sampled aloe and were estimated as the percentage of leaves that had evidence of fire damage (such as a charred stem base and/or charred leaf margins). Most stemless *Aloe* species are tolerant of mild to warm fires and may resprout, but some species are likely to produce multiple heads at the stem apex by vegetative recovery (Cousins and Witkowski 2012). *Aloe peglerae* plants typically grow a single rosette of leaves (Smith and Van Wyk, 2009); however, I expect that the rare occurrence of multiple leaf rosettes could be a result of crown damage from a relatively severe fire event(s). Hence, the number of rosettes per plant were also recorded, which was used as an index of crown damage from previous years. The percentage of fire damage to plants was then plotted against rosette number using linear regression.

4.3.5 Statistical analyses

Fire damage was plotted against aloe size class as well as the number of rosettes produced per plant using regression. To assess the individual differences in each microhabitat variable between sites excluding season as an independent factor, I used a Mann-Whitney *U* test. Spearman Rank correlations were used to investigate the relationships between the twelve microhabitat variables. Classification of the microhabitat variables between sites was undertaken to determine the discrimination between sites using discriminant function analysis. This analysis provides standardized discriminant function coefficients that represent the contribution of each continuous variable (i.e. microhabitat predictors) to the discriminate between aloe-occupied and aloe-vacant sites. Therefore, the largest coefficients would be associated with greater contributions of the respective variable to the discrimination between sites. However, the coefficients do not indicate between which sites the respective functions are more heavily discriminated towards, i.e. they do not indicate whether the functions best predict presence or absence of an aloe. Hence, to determine the best microhabitat predictors of aloe presence we determined the means for each variable between sites and used factor analysis and factor loadings to determine the contributions of the discriminants to each site. Microhabitat variables that were strongly correlated were excluded from the discriminant function analyses to avoid redundancy when interpreting the models. Lastly, using Spearman rank correlation, the relationship between plant size and each microhabitat variable was assessed. The microhabitat data were either log, square-root or ln-transformed and percentage values arcsine transformed prior to analysis to meet the assumption for normality. Means are

presented with standard errors. Analyses were conducted using Statistica 6.0 (StatSoft, Tulsa, OK, USA, 2002).

4.4 Results

4.4.1 Population size structure

The percentage of adult plants that flowered in the population in 2011 was significantly higher (66%), than in 2012 (23%) (2x2 contingency table; X^2=95.76; df=1, *P*<0.001). In 2012, the population was bell-shaped (Kolmogorov-Smirnov test; D=0.09, *P*<0.01, n=550 plants), consisting of many adults in the 20.0–25.0cm diameter size class (Fig. 4.3a). The population size structure from 2012 was not different from 2011, except that with increased sampling effort in 2012 more seedlings (< 10.0cm plant diameter) and juveniles (10.0–20.0cm) were detected (Fig. 4.3a).

Generally, *A. peglerae* plants reach reproductive maturity at ≥20.0cm in diameter (Fig. 4.3b; Melville 1988). However, individuals as small as 10.0–15.0cm in diameter (juvenile size class) also flowered in both 2011 (10% flowered, Fig. 4.3b) and 2012 (6%, Fig. 4.3b). The size class categories for *A. peglerae* were proposed by Pfab and Scholes (2004) and used for consistency, but do not exactly match the stage classes presented here. The percentage of plants that produced inflorescences was strongly related to plant diameter in 2011, but not significantly so in 2012 (Pearson's Correlation Coefficient; 2011: R^2=0.97; *P*=0.005; 2012: R^2=0.54; *P*=0.63). Furthermore, 100% of plants between 40.0 and 45.0cm flowered in both years, which emphasises the positive relationship between plant size and flowering frequency.

Although relationships were not strong, plant size was significantly correlated with grass cover (Spearman correlation; R_s=0.33; *P*=0.0009) and height (R_s=0.45; *P*=0.000003), above-ground biomass (R_s=0.34; *P*=0.0005), bare ground (R_s=0.24; *P*=0.018), rock cover (R_s=-0.41; *P*=0.0002), soil depth (R_s=0.25; *P*=0.01), and plant litter (R_s=0.32; *P*=0.001).

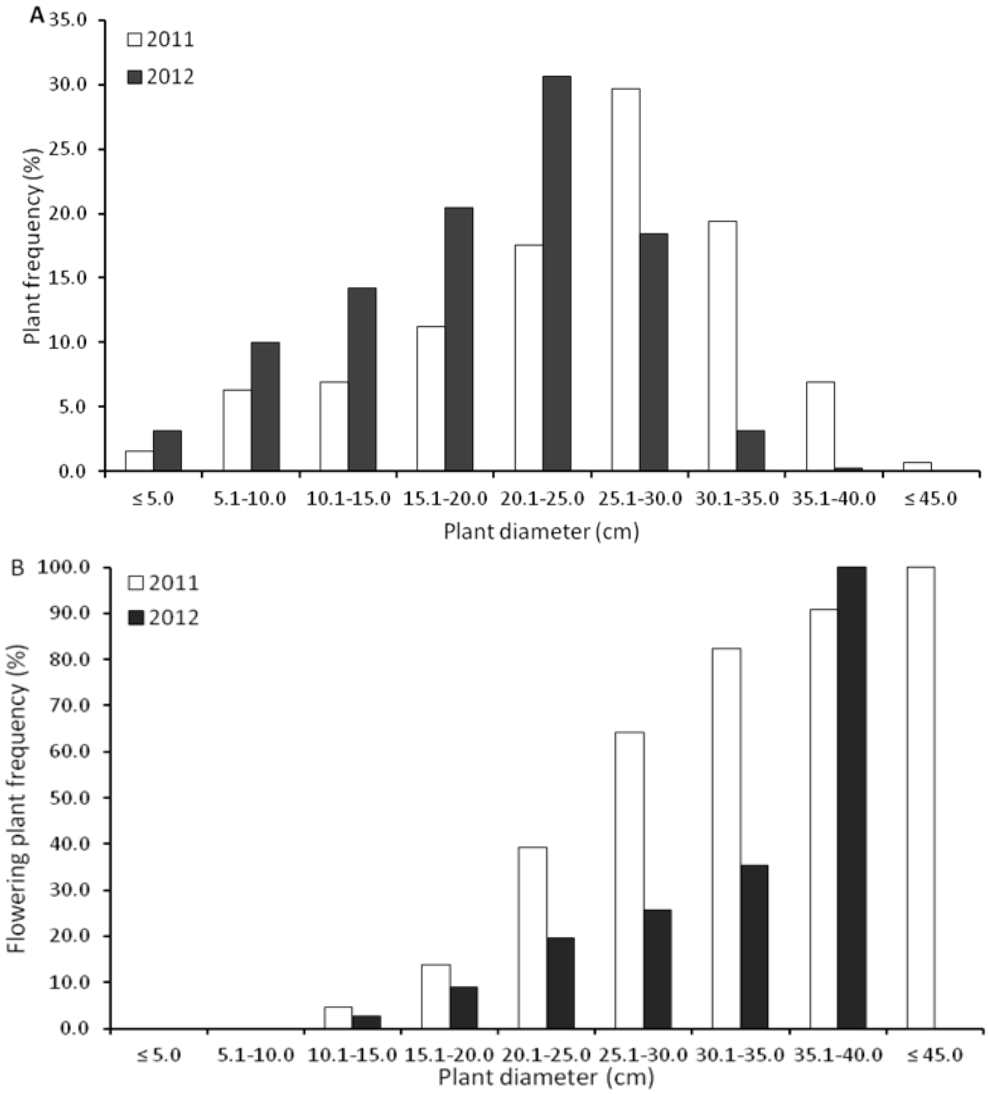

Figure 4.3 The population size structure of *Aloe peglerae* at the study site in a) 2011 (n=440) and 2012 (n=550); indicating that the change in size structure in the population between years was a result of increased sampling effort in 2012; and b) indicating the positive increase in the proportions of plants that flowered as plant size increased (Pearson's Correlation Coefficient; R^2=0.94; P<0.05).

4.4.2 Seasonal and site differences

Generally, aloe presence was positively correlated with high rock cover (Fig. 4.4g), low grass, above-ground biomass and bare ground cover (Fig. 4.4a, c and f), and higher forb cover and height (Fig. 4.4d and e) (Table 4.1). Grass height was the same between microsites in summer, but lower in aloe-occupied sites in winter (Fig. 4.4b). Plant litter cover was higher in winter in aloe-vacant sites, but was not significantly different between sites (Mann-Whitney *U* Test; Fig. 4.4h).

In summer, grass cover, biomass, and bare ground were higher, and rock cover, forb cover and height were lower in aloe-vacant sites. In winter, grass cover and height, biomass, and

bare ground were also higher and rock cover lower in aloe-vacant sites (Mann-Whitney *U* Test; Fig. 4.4).

Both forb and woody plant height were strongly correlated with forb cover (Spearman rank order correlation: summer R_s=0.89, winter; R_s=0.97; P<0.05) and woody cover (summer R_s=0.87, winter; R_s=0.60; P<0.05), respectively. Hence, forb and woody height were both removed from the summer and winter data before performing the discriminant analysis to avoid redundancy. The summer discriminant function model indicated that rock cover was the strongest microhabitat predictor (standardized discriminant function coefficient=3.70), followed by grass cover (3.15) and bare ground (2.62) (Table 4.1). The winter model indicated that the strongest predictor was bare ground (0.85), followed by litter cover (-0.73) and above-ground biomass (0.52) (Table 4.1).

Table 4.1 Discriminant function analysis used to determine the best predictors (continuous microhabitat variables) of aloe presence or absence in summer and winter, respectively. An asterisk indicates the variable with the greatest contributions to the discrimination between aloe-occupied and aloe-vacant sites. Dashes indicate variables not incorporated in either the summer or winter models as they did not significantly contribute to discriminating between aloe-occupied versus aloe-vacant sites.

	Standardized discriminant function coefficients	
Physical variable	Summer	Winter
Bare ground	2.62*	0.85***
Grass cover	3.15**	-
Rock	3.70***	-
Litter	0.53	-0.73**
Soil depth	-0.29	-
Grass height	-0.22	-
Woody cover	0.47	0.21
Forb cover	0.51	-
Shade	0.15	0.17
Biomass ($kg.ha^{-1}$)	-	0.52*

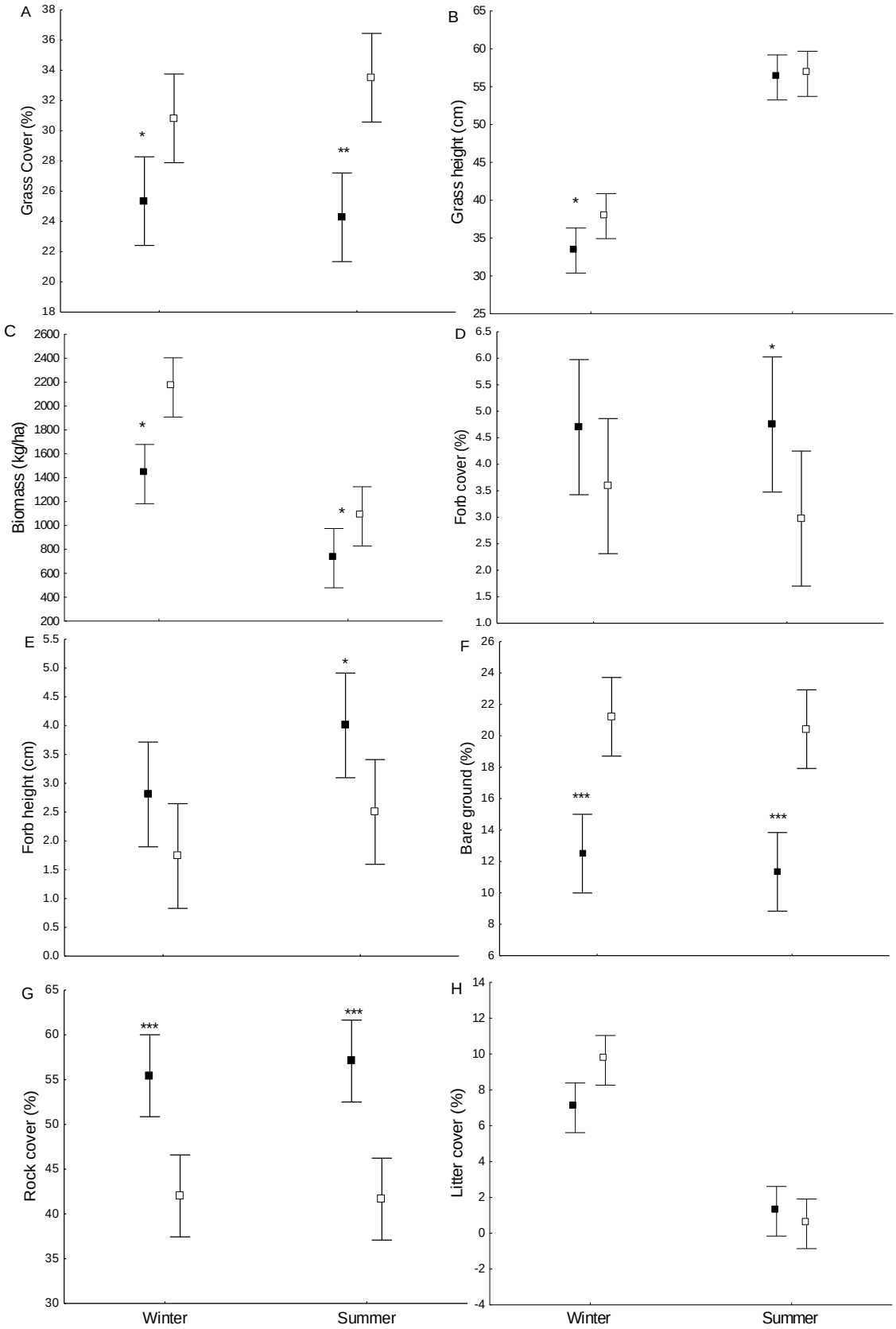

Figure 4.4 Comparison of microhabitat variables (mean ± SE) between aloe-occupied (n=100) and aloe-vacant (n=100) sites in summer and winter (2012). Asterisks only represent differences between aloe-occupied (black boxes) and aloe-vacant sites (white boxes) for each season (Mann-Whitney *U* test). * Statistical significance at *P*<0.05, ** *P*<0.001 and *** *P*<0.0001.

4.4.2 Factor loadings

Factor analysis was used to assess the variation among the correlated microhabitat variables and to determine the factors which account for most of the variance in the set of microhabitat variables. The loadings on each variable are weighted (≥0.7 for heavy loading) according to their respective contributions to explaining the variance in the data.

Two principal components were significant for each of summer and winter (Fig. 4.5). Factor 1 loadings for summer were strongly and positively correlated to those of factor 1 for winter (Pearson's Correlation Coefficient, R^2=0.94). Factor 1 accounted for 27% of the variance and factor 2 for 14%.Grass cover (Factor loading +0.84, positive) and rock cover (-0.90) were both heavily loaded for factor 1, while forb cover and forb height were equally heavily loaded (-0.80) for factor 2 (Fig. 4.5). Litter cover (-0.04) and rock cover (-0.03) had the smallest loadings for the first and second principal components, respectively (Fig. 4.5).

For winter, factor 1 accounted for 26% of the variance and factor 2 for 16%. Again, grass cover (+0.78) and rock cover (-0.91) were heavily loaded for factor 1 (Fig. 4.5). Woody cover (-0.09) and height (+0.10) had different contributions between seasons, having the smallest loadings for factor 1 in winter compared to summer, while grass height (-0.01) had the smallest loading for factor 2 (Fig. 4.5).Woody height (+0.73) and litter cover (-0.70) were heavily loaded for factor 2 (Fig. 4.5).

4.4.3 Principal components analysis

The first two principal components were plotted for both summer and winter (graph not displayed) showing the close association of microhabitat variables between seasons. Factor 1 and factor 2 accounted for 32% and 13% of the variance, respectively. Rock cover clearly separated out from the other vegetation cover (grass and forb cover and the respective heights) variables for both seasons. This spacing is due to the inverse relationship between aloe-occupied and aloe-vacant sites based on these microhabitat variables (e.g. rock cover is highest at aloe-occupied sites). Woody cover, plant litter, and overstorey shade also separated out from these variables, and were further spaced out between summer and winter, indicating that in winter, woody plants drop their leaves, providing more plant litter to the ground and less overstorey shade. Grass and forb cover and biomass were grouped together for both seasons indicating very minor changes between sites and seasons.

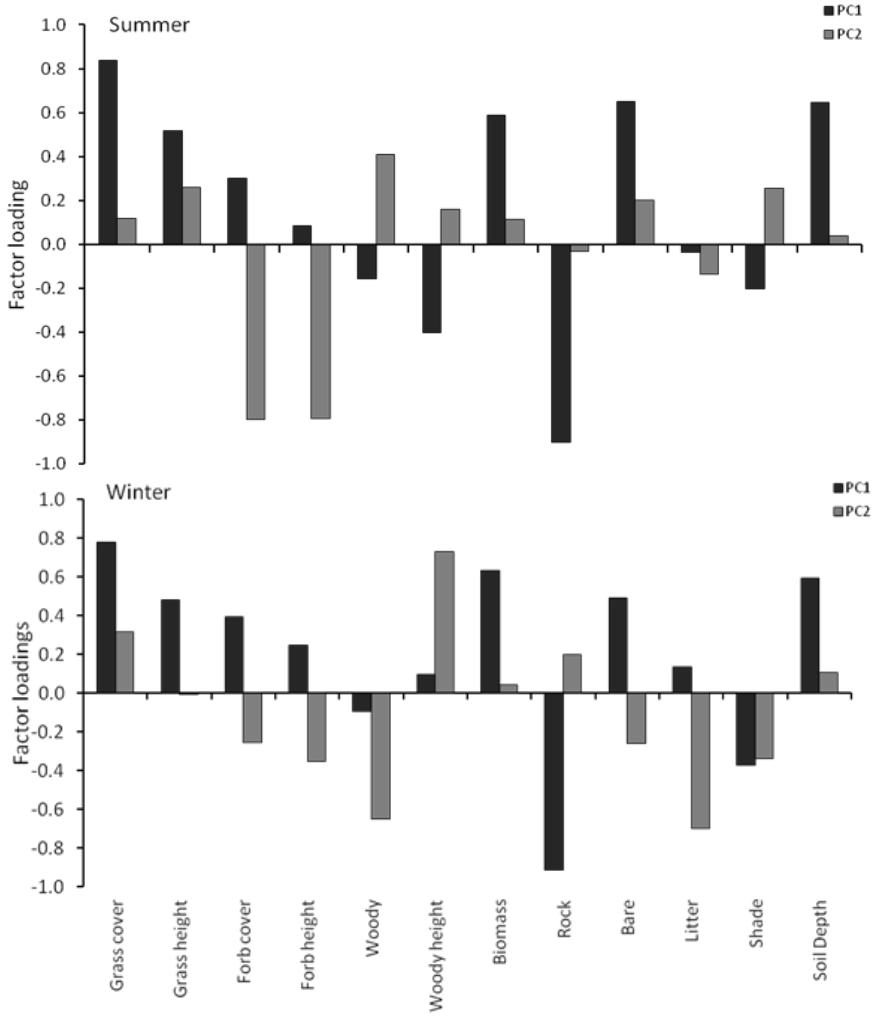

Figure 4.5 Factor loadings on microhabitat variables of *Aloe peglerae* from principal component analyses for summer and winter.

4.4.5 Fire damage

All size classes showed signs of fire damage, with the least damage to leaves and/or stems of seedlings (13% fire damage) and the most to juveniles (23%) and adults (19%). The outer leaves largely sustained the damage, with the younger inner leaves and growing point well protected. The percentage of fire damage to plants in aloe-occupied sites was not related to number of rosette-heads/plant (Spearman correlation; R_s=0.17; *P*=0.09; Table 4.2). After the population had been sampled in 2012, a census of all sampled plants (n=550) indicated five aloes died after a fire in August 2011. Four of the dead plants were adults (ranged from 23–35cm in diameter), and one a seedling (6 cm). This seedling was located in a microsite that consisted of a mosaic of grass clumps on a sheet of rock, indicating that not all 'safe sites' necessarily escape fire (Fig. 4.1d). There is no record of dead individuals after the August 2010 fire because the population was only sampled from February 2011 onwards.

Table 4.2 Comparison between the number of rosette heads per plant (aloe-occupied sites) and the mean percentage (variation) of damage to leaves and/or stems from fire (n=100)

# rosettes per plant	Average % fire damage	N
1	19	91
2	23	4
3	25	3
>4	25	2

4.5 Discussion

4.5.1 Microhabitat requirements

This study provides empirical evidence that local-scale microhabitat structure in both summer and winter is important in defining the persistence of the succulent, *A. peglerae*, in a fire driven savanna/grassland. The microhabitat conditions associated with aloe-occupancy are typically characteristic of a safe site for a succulent plant in a habitat with frequent (annual) fires (Fowler 1988; Thomas and Goodson 1992). Aloe presence was significantly associated with high proportions of rock cover and forb cover; and lower proportions of grass cover, above-ground biomass ($kg.ha^{-1}$), and bare ground cover (Fig. 4.4). In comparison, aloe absence was significantly related to lower proportions of rock cover, and higher proportions of grass cover, above-ground biomass, and bare ground cover.

Flowering frequency was positively related to plant size confirming similar findings by Melville (1988). Plant size was also significantly and positively related to grass cover and height, above-ground biomass, bare ground, rock cover, soil depth, and plant litter. Seedlings are strongly related to high rock cover and low grass (and height); low above-ground biomass and low bare ground cover. This indicates that seed germination and seedling establishment in *A. peglerae* depends heavily on these characteristic safe site microhabitat conditions, and indeed that the regeneration niche (Grubb 1977) will largely govern the local plant distribution. In other species, at the population level, *Aloe plicatilis* depends on high rock cover which aids in protection from fire. However, local-scale distribution of *A. plicatilis* does not seem to depend on rocky microsites *per se*, but rather a higher regional rock cover (Cousins *et al.* in prep.). In *Haworthia koelmaniorum* (Asphodelaceae), a closely related succulent, occupancy was also strongly associated with rock cover (Witkowski and Liston 1997).

4.5.2 Effects of fire

Fire is a major driver in shaping the vegetation patterns in many different ecosystems such as, the Cape fynbos, and other fire-prone environments like grasslands and savannas (e.g. Wilson and Shay 1990; Conedera *et al.* 2009; Penman *et al.* 2011). Fire reduces ground plant litter and above-ground biomass that has built up over time, either in the absence of, or between fire events, and which has resulted in increased overstorey cover and shade and increased surrounding vegetative cover (Wilson and Shay 1990). During the wet season, the surrounding vegetation cover increases and, depending on the location of an aloe, may provide shade, producing a slightly light-limited environment for *A. peglerae* (seedling emergence experiments showed improved leaf colour and vigour when shaded; GA, *pers. obs.*). Approaching the dry season, overstorey woody plants drop their leaves and the aloes become more exposed to sunlight. Leaf-fall produces more surface plant litter, and coupled with the build-up of dry grass and plant cover produces fuel for fire (Trollope *et al.* 2002). The occurrence of aloes in these local microsites means that they are more vulnerable during a fire compared to those found in the more suitable/favourable safe sites described above.

Due to the height of this species (~35cm adult plant, including the inflorescence) compared to other taller tree aloes such as *A. ferox, A. marlothii* and *A. plicatilis,* it does not have the opportunity of growing taller to escape the fire trap and it does not produce a thick protective corky bark (as in *A. plicatilis*) or 'skirt' of dry, dead leaves around the stem (as in *A. ferox* and *A. marlothii*) (Bond 1983; Smith and Van Wyk 2009; Cousins and Witkowski 2012). Furthermore, while some individuals were found growing in sites safer than others, few of these individuals were completely isolated from any surrounding vegetation cover (as observed by the five individuals that died post-fire in 2011). Hence, it is uncertain if fire would entirely miss these areas in the future. A number of factors that might explain post-fire mortality of succulents could be poor pre-fire plant vigour; high intensity and frequent burns; small plant size; or plant moisture content (Worthington and Corral 1981; Thomas and Goodson 1992; Witkowski and Liston 1997; Pfab and Witkowski 1999; Cousins *et al.* in prep.).

However, *A. peglerae* has developed morphological adaptations to cope under a hostile environment of high temperatures and resulting desiccation, which may further protect the plant from fire. While the impact of fires on succulents is suggested to be largely dependent on plant size and species (Bunting *et al.* 1980), succulents all possess similar morphologies

that aid in fire protection. The strongly, inwardly curved fleshy leaves of *A. peglerae* provide protection of the inner apical centre of the plant where new young leaves grow (Thomas and Goodson 1992). The outer leaves, similar to the persistent coat of dead leaves on the stems of *A. marlothii* or *A. ferox*, might further act as a shield to fire and insulate the inner leaves of the plant (Bond 1983), however these leaves invariably get burnt. Should a fire occur any time leading up to the flowering season, the newly formed flower buds which grow at the apical centre (Fig. 4.1e) will be protected. Most fires at the site, both prior to (J. Wesson *pers. comm.*) and during the study, have occurred after flowering and those individuals that produce an infructescence are more vulnerable at this time and may be severely burnt (Fig. 4.1d). However, post-fire evidence of collected fruits shows them to be well-protected from fire by the thick-walled fruit capsules in which the seeds are encased. Indeed, further evidence indicated that in microsites of low rock cover and high vegetation cover, canopy and flower damage occurred after veld fires in other populations in the Magaliesberg (Phama 2012). However, Phama (2012) noted that despite the occurrence of fire during the flowering and fruiting period, plants continued to produce fruit and seed.

Evidence of vegetative regeneration in *A. peglerae* occurs at the apical centre where multiple rosettes are formed. This is suggested to be a response to top-kill from fire (Worthington and Corral 1981; Thomas and Goodson 1992; Pfab and Witkowski 1999; Smith and Van Wyk 2009; Ne'eman *et al.* 2009; Keeley *et al.* 2011; Cousins and Witkowski 2012). However, our understanding of the evolutionary history of selection pressures on this specific adaptive trait for *A. peglerae* is not known, and warrants further research. None of the individuals that have grown multiple rosettes produced inflorescences in either flowering season, which indicates that most, if not all, their energy may have been invested in recovery and repair of the current generation for at least 2-3 years following a fire (Bellingham and Sparrow 2000). The availability and suitability of safe sites will afford a plant further protection from fire (Thomas and Goodson 1992), especially in an environment where fire is frequent.

Alternatively, when surrounding moribund vegetation is burnt, nutrients are released from the ash onto the ground. This injection of nutrients aids in further growth and/or repair (Wilson and Shay 1990), and may potentially be allocated to reproduction in the following flowering season. Scholes (1988) found that flowering percentage in a sample (n=100) of *A. peglerae* in another subpopulation increased in the year following a fire event, but was not related to annual rainfall (September–August). It was also noted that flowering percentage (range: 30–82%) and the proportion of inflorescences that set seed (4–97%) was highly variable between

years; some drier years (~524mm rain) yielding the highest (~79%) percentage of flowering plants in the population (Scholes 1988). A similar trend was found in this study, with a higher percentage of plants flowering in 2011 (49%), as well as the highest fruit per plant (40%) and number of seeds/fruit (38), compared to 2012 (14%; 6%, and 15, respectively). In this study, rainfall between July 2011 – June 2012 was lower (~555mm, by ~15%) than in the previous year (July 2010 – June 2011; ~658mm) (nearest weather station at Pretoria ~30km from the site at 1300m a.s.l.; South African Weather Service). This reduction in rainfall may have caused a reduction in the proportion of flowering individuals in 2012; however, based on the work of Scholes (1988), flowering in *A. peglerae* increased as rainfall decreased between years.

Vegetation cover and composition may change in response to annual fires, particularly around those aloes that are located in less favourable microhabitats that are more vulnerable to fire. Due to the importance of safe sites for *A. peglerae,* it is suggested that safe sites, i.e. those with high rock cover, low biomass and grass cover, also afford protection to other succulents, forbs and/or woody plants. In this instance, competition for water, space, nutrients, and light may occur between *A. peglerae* plants and co-occurring species, such as *E. schinzii.* Indeed, the occurrence of other species in fire refugia around aloes effectively removes or at least reduces the amount of surrounding grass fuel, therefore enhancing the suitability of the microsite. However, the spatial and temporal changes in the surrounding vegetation cover, particularly the short-lived and high-fuel carrying forbs, grasses and woody plants, regularly make the microhabitat conditions for an aloe unpredictable and vulnerable. The establishment of woody plant species near aloes would result in a shady microenvironment, with limited light for the aloe, at least during summer. Alternatively, this could create a favourable nurse plant environment for future seed germination and seedling establishment. Nonetheless, in the event of a fire, these woody plants may be burnt down or killed and either negatively affect the neighbouring aloe directly or indirectly by making available space for grasses to establish, or effectively open up the overstorey canopy producing more light and providing nutrients to the soil. Sommers *et al.* (2011) suggested that if suitable sites for lily-occupancy became unsuitable in a short space of time, i.e. if an open sunny area became closed and shaded by trees, then using predictive modeling would not be an appropriate approach. Our study is similar to that of Sommers *et al.* (2011) as the research was limited in studying the effect of microhabitat conditions on mature plants, and not the characteristics of a safe site for seed germination and seedling establishment, even though

these processes had occurred at all the aloe-occupied sites. However, it is assumed that similar habitat conditions are likely to be suitable for seedlings, juveniles and adult plants (Sommers *et al.* 2011).

4.5.3 Significance of nurse objects

Germination trials in 2011 were started 5–6 weeks later (15 November) than those in 2012 (5 October) and overall germination success was much higher in 2012 (40–100%) than in 2011 (0–38%). Germination occurred soon after seed planting, taking place one week after first watering in petri-dishes (see section 2.4.4 and section 3.4.6), but when planted in soil germination was slower (~2–3 weeks). Therefore, if conditions are not suitable to sustain seedling emergence and growth, i.e. insufficient rainfall, shade, excessive sun exposure etc. then seedlings are not likely to survive for longer than a month or two. Symes (2012) further suggested that *A. marlothii* seed also relies on optimal climatic conditions for germination due to the limited longevity of the seeds. In contrast, *A. greatheadii* var. *dayvana* seed is able to survive *ex situ* for more than two seasons (Smith and Correia 1992). *Aloe plicatilis ex situ* seed germination percentage was highest at 18 months (86%) and 24 months (80%) compared to 3 months which was three times lower (Cousins *et al.* 2013). Where seed germination success appears to be highest in unnatural conditions (e.g. growth chamber/greenhouse), germination success under harsher natural conditions in the wild is predicted to be much lower for *A. peglerae,* and other *Aloe* species. Therefore, replacement of dead aloes by germinated seed each year is largely dependent on the site of seed dispersal and the degree of suitability of the microsite for germination. Furthermore, shelter from nurse plants is reduced by fire (Thomas and Goodson 1992). The importance of nurse plants or nurse rocks for *A. peglerae* seedling establishment and growth is currently unknown.

The benefit of growing under or close to nurse plants on plant growth and survival has been discussed at length in the literature (Giddy 1973; Franco and Nobel 1989; Thomas and Goodson 1992; Smith and Van Wyk 2009; Cousins and Witkowski 2012). Nurse plants provide shade to newly germinated seeds and established seedlings growing under or near the canopies of nurse plants, creating an ideal microhabitat for growth. Cool soil surface temperatures, nutrient-rich and moist soils, and humid air, and particularly in some *Aloe* species, protection from harsh heat, radiation, desiccation, frost, and herbivores, are a few of the ideal conditions associated with nurse plant shade documented to benefit the seedlings of some succulent species (Giddy 1973; Franco and Nobel 1989; Smith and Van Wyk 2009;

Cousins and Witkowski 2012). One study in particular, showed that all seedlings of a cactus species, *Carnegiea gigantean*, were found only in sheltered microhabitats under the canopies of nurse plants because of reduced soil temperatures and higher soil nitrogen levels (Franco and Nobel 1989). However, they also found that seedling growth was reduced by increased shading and competition for soil water (Franco and Nobel 1989). The roots of an *A. peglerae* seedling are shallow and hence would only gain access to surface soil water (Smith and Van Wyk 2009). Neighbouring plants that also have shallow and overlapping root systems might compete for soil water with *A. peglerae* seedlings (Franco and Nobel 1989). However, the ability of this succulent to store large amounts of water in its leaves is an advantage under stressful conditions. In fact, *A. peglerae* seedlings in greenhouse conditions that were over-watered showed signs of rotting, especially in the upper surface layers of the soil where water was not draining properly (GA, *pers. obs.*). Hence, competition for surface soil water may not become a growth limiting factor for seedlings of this species. Under frequent and successive thunderstorms, the typical rainfall pattern at the site, seedlings may not survive under the shade of nurse plants because soil water will take longer to evaporate, leading to rotting.

The significance and importance of nurse rocks for seedling establishment and continued growth has recently become of interest, particularly for *A. plicatilis* and *A. peglerae.* While nurse plants have proven to be quite beneficial to seedlings of many species, this association also reduces the amount of sunlight that reaches a seedling. It has been demonstrated in eight *Mammillaria* species (Cactaceae), and many other cacti (Peters *et al.* 2008 and references therein), that rocks indeed provide a favourable microenvironment for seedlings mainly due to lower soil surface temperature, shade and moisture without reducing sunlight (Nobel *et al.* 1992). Thus, while safe sites of high rock cover protect *A. peglerae* plants from the harsh effects of fire, rocks may also act as nurse objects, specifically to young seedlings (GA, *pers. obs.*). *Aloe peglerae* seedlings that were sown in potting soil and river sand after germination trials, showed physical signs of stress when the leaves turned a green-brown colour and became slightly wilted (GA, *pers. obs*.). After placing a ~50% shade cloth over the seedlings (light intensity=650nm), they regained a healthy green colour and retained water more efficiently. Seedlings of a fynbos aloe, *A. plicatilis*, also showed signs of stress from over-exposure to light, but when covered by 50% shade cloth, regained vigour (S. Cousins *pers. comm.*). So it is likely that newly emerged aloe seedlings in field conditions require some form of shading for a portion of the day to protect against total desiccation, whether it is from the presence of a nurse plant or a nurse rock (Peters *et al.* 2008; Haussmann *et al.* 2010).

4.6 Recommendations

As a result of the morphological adaptations which are suggested to additionally aid in protection and tolerance of fire, coupled with the availability and occupancy of suitable safe sites, one might assume that a negligible number of aloes died post-fire (1% of the population) in comparison to other succulent species where post-fire mortality can be variable and sometimes very high (Worthington and Corral 1981; Pfab and Witkowski 1999). However, according to Pfab and Scholes (2004), *A. peglerae* is highly sensitive to harvesting of adult plants and is only sustainable at extremely low levels of only 0.12%/annum. This implies that in a population of 1000 plants, the harvesting of only one individual would be sustainable on an annual basis (Pfab and Scholes 2004). In three populations of *A. plicatilis,* plant density decreased after a fire (Cousins *et al.* in prep.); further emphasising the important role fire plays in the survival of *Aloe* succulents. The number of *A. peglerae* plants that died post-fire exceeds the level of sustainable harvesting permitted to collectors (Pfab and Scholes 2004), and the effect of frequent planned or unplanned burns may pose a serious threat to the population. Therefore, it is imperative that post-fire mortality, especially of adult plants, after planned and unplanned burns needs to be taken into account when planning conservation efforts for *A. peglerae*.

Currently there are ten known extant subpopulations within the distribution range of *A. peglerae* (Phama 2012). Population trends for *A. peglerae* have shown an overall decline of 43% in mean population density over an eleven year period (1999–2010; Phama 2012). This decline in population density has been linked to the illegal removal of plants by collectors from the wild (Pfab and Scholes 2004; Victor and Pfab 2005), however Phama (2012) found no evidence of removal of plants in any of the nine subpopulations (but given their small size, evidence of removal would quickly disappear). Other potential threats to the persistence of populations included structural damage to plants by browsing antelope, fire, erosion, and the white scale insect pest (Phama 2012). In addition to these potential threats, land management or lack thereof, e.g. planned burns or overgrazing by livestock, in the Magaliesberg is also expected to have detrimental effects on persisting populations if these findings are not considered in conservation management planning.

Management and conservation practices for threatened species are encouraged to apply the knowledge gained from studies like this. Due to the persistent decline in the size of multiple populations of *A. peglerae* (Pfab and Scholes 2004; Phama 2012), understanding the local-

scale microhabitat requirements and suitability for this species is critical for both *in situ* and *ex situ* cultivation programmes, managing and conserving extant populations and implementing translocations or reintroductions of new *A. peglerae* plants back into the wild.

Re-introducing seedlings back into the wild will benefit from these findings and will ensure that suitable safe sites are chosen when translocating or planting individuals in natural habitat. Microsites of high rock cover, low grass cover, low above-ground biomass, and sufficient access to sunlight for at least parts of the day are predicted to provide ideal microhabitat conditions for an *A. peglerae* individual. Seedlings (5–10cm in diameter) are predicted to only survive fires if planted within rock fissures or between rocks where surrounding ground temperatures are low and little or no grass cover surrounds it, and where there is enough soil for roots to access water and nutrients. Areas of deeper soils, high grass cover, and high woody plant biomass will likely not provide an ideal microenvironment for a succulent plant. Indeed, knowledge of the fire regime of any site prior to re-planting is crucial information that is needed before any decisions regarding re-introductions into the wild are implemented.

Long-term monitoring of all extant populations is highly recommended, to monitor individual plants, as well as population size and density, under different fire regimes. One hundred *A. peglerae* seedlings were planted at the Kgaswane Mountain Reserve in 2012 by a botanical group, and long-term monitoring of this new population is required. The planting of these seedlings was undertaken before the findings of this paper were identified, and it would be interesting to monitor the survival of these seedlings over the long-term based on the microhabitat characteristics of each aloe-occupied microsite.

For future studies we recommend investigating the importance of habitat suitability regarding the fire ecology of *A. peglerae* and other *Aloe* species (seed dispersal, seed biology and ecology, plant lifespan and vegetative reproduction, understanding spatial and temporal variability in microhabitat factors, factors responsible for seedling and adult mortality, etc.). It is also important to identify these microhabitat requirements for different stages of growth and not only for seed germination, seedling establishment or mature adult plants. The short-term and long-term effects of fire (of different intensities and frequencies) on the growth habit of all size classes of *A. peglerae* are also important in understanding the life history of this species.

4.7 Conclusion

This study has identified the characteristics of a safe microsite for *A. peglerae.* Although morphological adaptations such as protection of the inner apical meristem by the outer leaves suggests that this species is relatively fire-tolerant, if a plant is located in an unfavourable microsite, evidence suggests it is less likely to survive under annual burns. As a result, the findings show that this succulent primarily survives in refugia associated with a typical safe site microhabitat, growing in very rocky areas of little surrounding grass cover and fire-carrying biomass. This is specifically true in smaller individuals (seedlings) of *A. peglerae.* In reality, many of these safe sites will not always be completely void of any vegetation cover, especially grass cover, however the rockiness, lower grass cover, and presence of other succulents and forbs, also prone to establishing in sites that are relatively safe from winter fires, improves its degree of protection from fire. This may suggest that this species is largely fire-intolerant occurring in microsites that are safer from fire impacts. Whether or not *A. peglerae* can tolerate fire, warrants further investigation, using experimental techniques. However, that being said, *A. peglerae* can vegetatively recover from crown damage, despite the cause of damage being largely unknown and assumed to originate from a long history of annual fire events. As our knowledge of microhabitat suitability improves, and through the use of predictive modeling, we can apply this knowledge to *in situ* and *ex situ* conservation programmes for *A. peglerae*. For other threatened and/or rare endemic species managers can select sites suitable for growth, protection, or re-introduction, when attempting to conserve this endemic aloe.

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CHAPTER FIVE

GENERAL CONCLUSION

In this thesis I set out to study the pollination biology of *A. peglerae.* Prior to this study, not much was known about the factors that influence the reproductive success of *A. peglerae* in terms of its pollination system, population dynamics and microhabitat structure. A slow-growing species, it is under threat from illegal and unsustainable collecting of the adult, reproductive plants, as well as of fruit and/or seed, from the wild (Pfab and Scholes 2004). I attempted to identify other factors of its general biology and the interactions of population dynamics and community structure that might also influence the populations negatively. More specifically, I investigated if plant population density and local plant neighbour density of a single subpopulation influences plant reproduction. I also identified the type of suitable microhabitat conditions for plant growth, reproduction and survival, and related the effects of frequent burning on the survival of *A. peglerae* plants. A synthesis of the interlinking processes between the results from each chapter can be found in Figure 5.1.

Pollination exclusion experiments determined that *A. peglerae* is successfully pollinated by opportunistic avian nectarivores (Chapter 2, Arena *et. al.* 2013). While many *Aloe* species also receive important contributions to reproductive output through insect visitation, insect pollination contributed negligibly to reproductive output in *A. peglerae.* I attributed this difference to, 1) the floral traits of *A. peglerae* which conform to a bird-pollination syndrome, and 2) reduced insect abundance at the study site during the cold winter-flowering period. A vital aspect of this study which arose was the potential existence of a self-pollination mechanism in *A. peglerae.* As time did not allow, breeding system experiments were not conducted to determine the level of compatibility for *A. peglerae.* Although self-compatibility is unusual, or has not been reported, for *Aloe* species, it is recommended that supplemental hand pollination experiments be carried out in future pollination studies to determine plant mating system for *A. peglerae* and other species. However, it is recommended that future studies test different techniques to perfect a method of carrying out successful hand pollinations that will result in seed set. Due to the densely packed inflorescence, conducting hand pollinations efficiently, without contaminating flowers with self/cross-pollen, must be carried out with care. One way perhaps in which to do this would

be to perform artificial emasculation of plants by removing the anthers (self-pollen) so that any pollen that arrives at the stigmas would only be cross-pollen. Resulting seed set of emasculated plants would then be compared to intact control plants to assess whether or not self-pollen interferes with seed production.

At the start of the study (2011 flowering season), small-mammals were not detected on camera traps. Small-mammal visitation was reported as a possibility (Arena *et al.* 2013) and in the 2012 flowering season small-mammals were recorded visiting flowers using motion sensitive camera traps. Two small-mammal species were found visiting the flowers, potentially for the nectar (for moisture and/or energy), but also to forage on flower parts. Bird pollinators would potentially be affected by low flowering densities in some years and it is expected that so would small-mammals provided they are potential pollinators of *A. peglerae* too. Therefore, I investigated if local flowering plant density at successively increasing spatial scales had an effect on plant reproduction (Chapter 3). I then further examined if visitation rates of the different pollinator taxa, i.e. birds and small-mammals, contributed to plant reproduction based on the densities of flowering aloes at increasing spatial scales. Nectar standing crop was also measured in focal plants and correlated to pollinator visitation rates.

The only positive effect of flowering density on fruit set and total seed production was detected at 30-35m. Furthermore, a positive effect of flowering density on diurnal (bird) visitation rates was also detected at 25-40m. This confirmed that the bird visitors indeed have a greater influence on plant reproduction than the nocturnal (small-mammal) visitors. This would be particularly evident in years of high flowering density in the subpopulation. Camera trap evidence showed small-mammals often foraged on flower parts, sometimes removing whole flowers from inflorescences, and this may have contributed to the very low or zero fruit and seed production in some plants. However, pollen limitation and/or resource limitation may also likely be the cause for low reproductive output in 2012 due to the typical mast-seeding phenomenon in this species and deserves further testing. It is necessary to determine the contributions of the nocturnal visitors to reproductive output by conducting selective (day/night exposure) exclusion experiments, in order to identify whether or not they are effective pollinators of *A. peglerae.* It is also recommended that studies attempt to identify the impact of small-mammal herbivory on reproductive output in *A. peglerae,* and how these top-down effects on reproduction is related to masting events. This part of the thesis successfully showed that higher flowering densities have some effect on reproduction

within the subpopulation. However, future studies should investigate the effect of differing population sizes and densities on reproductive output in several subpopulations of *A. peglerae* to assess if an Allee effect is expressed at the population level.

Once again the importance of determining plant mating system is highlighted by a number of questions that have risen from this study. The foraging behaviour of the birds and small-mammals is likely to facilitate both cross- and geitonogamous (within-plant) self-pollination, and hence, may either result in high or low seed set, depending on its level of self-compatibility (Schmitt 1983; Lamont *et al.* 1993; Forsyth 2003; Quesada *et al.* 2004; Karron *et al.* 2012). Identifying plant mating system will help better interpret and understand the mechanisms involved in seed set under cross or self-pollination, as well as the effects of different pollinator taxa visitation patterns and foraging behaviour on plant reproduction in *A. peglerae.* Other factors that may limit plant reproduction in natural populations may include resource limitation, herbivory or predation on flower parts or environmental conditions (Cruden *et al.* 1976; Herrera *et al.* 1998; Pfab and Witkowski 1999; Suttle 2003; Crone *et al.* 2009; Steenhuisen and Johnson 2012). Hence, it becomes important to determine the factors that influence seed production in a plant species when investigating the effects of habitat fragmentation on plant reproductive success.

Lastly, I determined what type of microhabitat conditions would predict 'favourable' microsites for an *A. peglerae* plant (Chapter 4). Safe sites of high rock cover and low surrounding vegetation cover and biomass is an important element of the growth and survival of *A. peglerae* plants. Microsites consisting of high fuel-burning biomass and low rock cover are relatively unfavourable for individual plants that experience frequent burns. If subjected to high intensity fires, or frequent (annual) burns, some individuals might coppice at the apical meristem and produce multiple rosettes; however, this aspect of morphological adaptations to disturbances such as fire requires formal investigations. Some individuals died after a fire, equating to ~1% of the population, which is ~8.3 times higher than the allowable harvesting levels (0.12%; Pfab and Scholes 2004). Either the 2011 fire event was an unusually high intensity burn killing a few plants or the effects of frequent fire on *A. peglerae* individuals should be monitored and reported on. I predict that if fire is too frequent, or too intense, an aloe that is growing in unfavourable microhabitat has poor survival chances after successive burns over a few years. The effects of frequent fire on the growth, survival and flowering of this species must be taken into consideration when the sustainable harvesting recommendations are revised.

The effects of fire on *A. peglerae* growth, reproduction and survival, has not previously been a cause for concern. However, I strongly recommend that future studies focus on this aspect in order to facilitate the conservation of *A. peglerae.* The origin of most fires along the Magaliesberg mountain ridge is anthropogenic. Efforts to conserve this endangered species should take into account the findings of the microhabitat study when implementing *in situ* and *ex situ* cultivation programmes in the wild and in greenhouse, or nurseries studies. Re-introduction projects are also encouraged to make use of these findings when planting *A. peglerae* seedlings into natural habitat, as well as back into currently extinct or extant subpopulations in the Magaliesberg. It is vital that future projects like these have some knowledge of the fire regimes of study sites. Long-term monitoring of extant subpopulations and individual plants is important in understanding the life history strategy to survive under different fire regimes. I recommend that future studies also investigate the role that microhabitat plays regarding seed biology and ecology, fire ecology, life history strategies, and population dynamics under varying spatial and temporal changes in microhabitat factors.

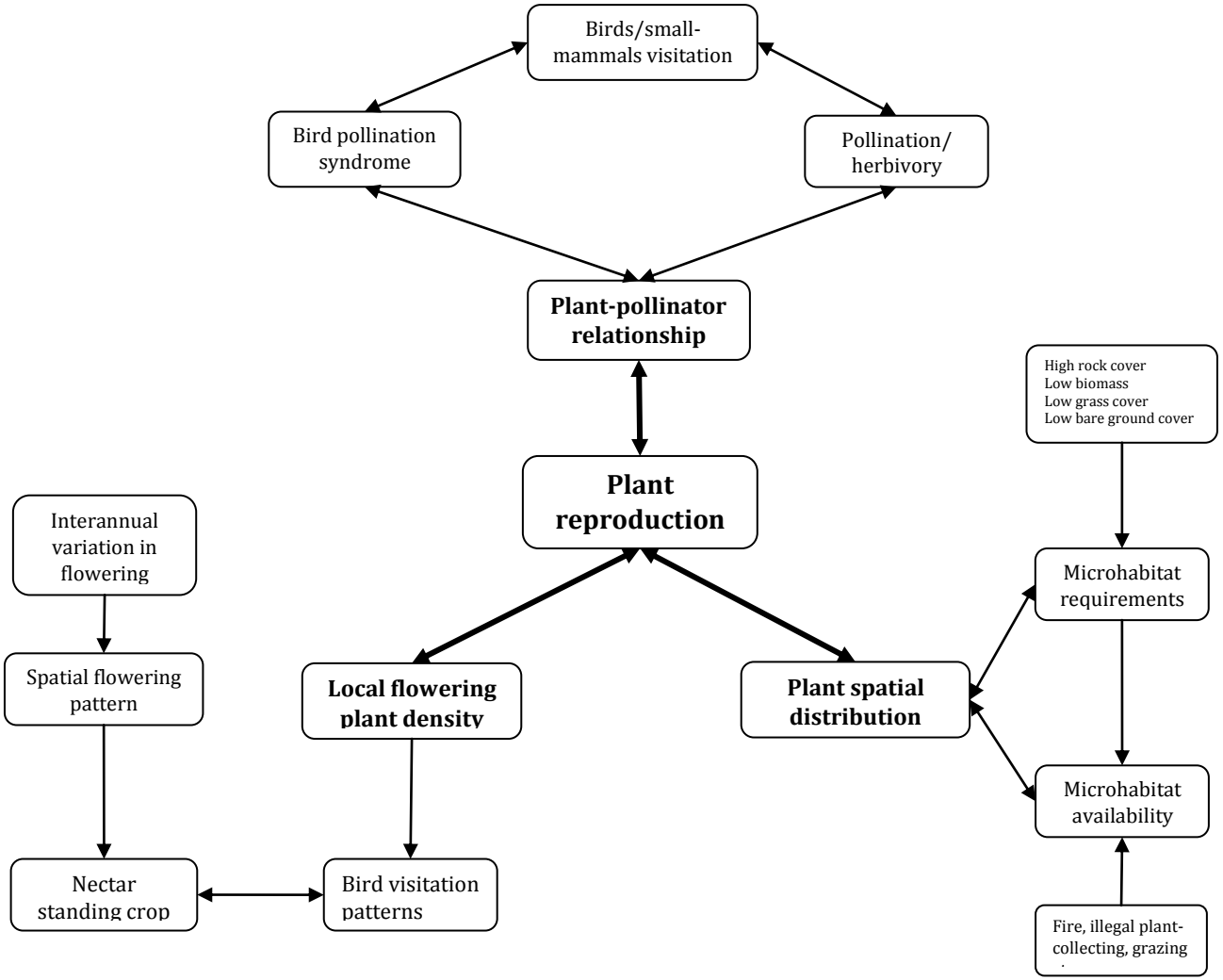

Figure 5.1 A synthesis of the interlinking processes between the different results obtained in the study and the factors (not comprehensive) that contribute to each component of the study.

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