Population structure and ecology of *Aloe lettyae*, an endangered Woodbush Granite Grassland endemic

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Sylvie Kremer-Köhne

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DEDICATION

Artist: C. Letty

This dissertation is dedicated to Cythna Letty (1895–1985) and the grassland remnants to which *Aloe lettyae* is restricted.

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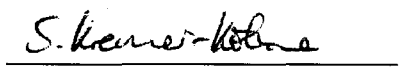
DECLARATION

I declare that this dissertation is my own, unaided work. It has been submitted in fulfilment of the requirements of a Master of Science at the University of the Witwatersrand. It has not been submitted before for any degree or examination to another university or similar institution.



Sylvie Kremer-Köhne

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Supervisors:

Prof. E.T.F Witkowski

Dr. D.I. Thompson

PREFACE

Aloe lettyae is an endangered aloe, endemic to the critically endangered Woodbush Granite Grassland in the Limpopo Province in South Africa. This study elucidates the basic ecology of the previously poorly known summer-flowering species and provides baseline data for long-term monitoring, urgent management and conservation decisions as well as future research. My dissertation consists of four chapters: an introduction, two data chapters and a conclusion with recommendations.

During 2016, the first year of this study, the focus was on locating *A. lettyae* populations in the severely fragmented landscape, obtaining an initial estimate of their size and designing an appropriate sampling strategy. The bulk of the field work, assessing *A. lettyae* population structures, habitat profiles and reproductive outputs as well as investigating *A. lettyae*'s reproductive ecology (determining the pollinators through selective pollinator exclusion treatments and by means of camera traps) was undertaken during the second study year (2017), as was the seed germination trial. Chapter Two was presented at the South African Association of Botanists Conference in Pretoria during 9–12 January 2018. It is envisaged to publish this dissertation as original research articles. Therefore, Chapters Two and Three have been written in the general format of research articles, each with their own abstracts and reference sections, broadly following the guidelines of the *South African Journal of Botany* while keeping repetition between chapters to a minimum.

Good prerequisites for conducting this study were my in-depth knowledge of the Woodbush Granite Grassland remnants and my network in the area. Unaware of *A. lettyae*, I lived on the farm Westfalia Estate near Modjadjiskloof (1984–2004) where the aloe was first collected by Cythna Letty in the 1930s. After moving to the village of Haenertsburg in 2004, I became interested in the grassland flora, and Pieter Winter drew my attention to *A. lettyae*. Through submitting a research funding application to the Botanical Education Trust, I was introduced to Cythna Letty's son, Bruce Forssman, who shared memories of his mother with me.

ABSTRACT

This is the first study on the biogeography, population biology and reproductive ecology of the endangered *Aloe lettyae* endemic to the highly threatened Woodbush Granite Grassland (WGG) in Limpopo Province, South Africa. *A. lettyae*'s distribution has been severely reduced by habitat loss and fragmentation and most populations were clustered on the south western side of the WGG polygon, with all known localities less than 40 km apart in this fragmented vegetation type. In total, 19 *A. lettyae* populations were located and documented, and the total area of occupancy was calculated (17.5 ha). Population size varied from 10 to 4000 plants, with 8500 individuals estimated in total, and plants occurred in clumps. Plant size and stage demographics were determined in seven of the 19 currently known *A. lettyae* populations which included the two largest populations with ~4000 and ~3000 *A. lettyae* plants, respectively, as well as a high- and a low-lying population, and constituted a representative sample over the entire geographical range of *A. lettyae*. Based on number of leaf layers and leaf rosette diameter, across all surveyed populations, 15% of the plants were juveniles, 9% were subadults, 15% non-reproductive adults and 61% reproductive adults. There was a clear difference in population structure between the main population and the second largest population, with the main population having a higher proportion of juveniles (14%) than the second largest population (3%). The low percentages of juveniles and non-reproductive subadults indicated an apparent current and historic lack of recruitment. Most size class distributions (SCDs) (76%) were bell-shaped which included the two largest populations, 9% were J-shaped, 3% had an inverse J-shaped SCD and 12% had an irregular SCD. *Aloe lettyae* grows in tall, dense grassland vegetation. For all surveyed populations combined, habitat profile within 1 m of an *A. lettyae* individual and $\geq$ 5 m away from *A. lettyae* plants in adjacent non-populated areas varied very little, but habitat variables differed significantly between populations. Percentages aerial covers for grass, forbs and woody species were 50%, 37% and 5% for the largest population and 49%, 26% and 15% for the second largest population with the small remaining percentages being made up of bare soil and rock cover. The main threats to *A. lettyae* were identified as misapplication of fire, cattle grazing and invasive alien plants.

In the *A. lettyae* populations surveyed in 2016/7, approximately 80% of the adult individuals were reproductive. Reproductive success in terms of fruit set and seed production was monitored in the two largest *A. lettyae* populations with ~3000 plants each and four smaller populations with <350 plants each. Fruit set varied from 29% in the largest population to 3% in the smallest population. Total seed production per plant was high in the large populations (5200 and 2400), compared with the four smaller populations surveyed (130–920), which indicated that an Allee effect may be operating. Approximately 90% of the sampled intact fruit contained seed predators, a fly (*Apenthecia* cf. *crassiseta*, Drosophilidae) and/or a wasp (*Eurytoma* sp., Eurytomidae). During flowering, camera traps were used at four localities to identify the main floral visitors, bird visitation and probe rates. Three bird species were observed feeding on *A. lettyae* nectar, Amethyst sunbirds (*Chalcomitra amethystina*) as well as Southern and Greater Double-collared sunbirds (*Cinnyris chalybeus* and *Cinnyris afer*). Amethyst sunbirds, the most frequent visitors (554 visits/1000 plants/h), were observed at all four localities monitored, while Double-collared sunbirds only occurred at two localities and had a much lower visitation rate (46 visits/1000 plants/h). *Aloe lettyae* nectar volume in unscreened flowers was very variable (range 5–85 µl; mean 15.5 ± 1.2 µl) and nectar sugar concentration was high (range 11–25%; mean 18.6 ± 0.2%) confirming that *A. lettyae* flowers are adapted for specialist nectarivores. The contribution of different pollinator guilds (birds and insects) was determined through selective pollinator exclusion treatments which showed that birds contributed significantly more to *A. lettyae* fruit set (1.7 times), seed production per fruit (1.5 times) and seed production per plant (4.3 times) than insects. In a germination trial conducted under controlled conditions with seeds from the populations monitored for their reproductive success and seeds obtained from the pollinator exclusion trial, mean germination time was approximately 2.5 weeks and cumulative germination rates were high (approximately 90%), and did not differ between populations/treatments. In combination with baseline *A. lettyae* population data provided by this study, the understanding of *A. lettyae's* reproductive ecology will aid in designing an adaptive grassland management strategy. The history of habitat loss and fragmentation, coupled with the severe reproductive decline as *A. lettyae* population size declines (Allee effect), points to the necessity to conserve the remaining small extent of WGG.

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The following individuals and companies are thanked for locating *A. lettyae* populations and/or access to grasslands: Wiam Haddad, Lourens Deysel, David Deysel (ZZ2, Olyfberg); Braam Mostert (Merensky Timber); James Turner; Ian Hubble (Veekraal); Cliff Bosman (Silicon Smelters); Elaine Tribe (Lepelle Northern Water), Ken Strang (Reizigerrust); Edward Ramala (Serala Community Property Association). Thanks go to the Department of Agriculture, Forestry and Fisheries for granting me access to their WGG fragments. I thank Toban McMahon, Meshack Nethavhani, Wiam Haddad, Bright Ndavani, Stefan Köhne, Shimy Malatji and Pluto for assistance in the field. Further, I thank Joe Grosel (Tembele Ecological Services) and Quentin Hagens for assistance with bird identifications. Insect identifications by the following experts are gratefully acknowledged: Shane McEvey (Australian Museum, Sydney), Simon van Noort (Iziko Museums of South Africa, Cape Town), Corey Bazelet (Department of Conservation Ecology and Entomology, Stellenbosch University), James Harrison (Wits Life Sciences Museum, University of the Witwatersrand), Beth Grobbelaar and Riaan Stals (South African National Collection of Insects (SANC), ARC-Plant Protection Research Institute (ARC-PPRI)), Ashley Kirk-Spriggs (National Museum, Bloemfontein), Michael Stiller (Agricultural Research Council), Peter Hawkes (AfriBugs, Pretoria), André Coetzer (The Lepidopterists' Society of Africa). Sonia du Buisson (Merensky Timber) is thanked for making space available on a Jacobsen germination table for seed germination trials and Lettie Ramaphoko is thanked for assistance with these trials. Francois Steffens is thanked for statistical advice. Ronell Klopper is gratefully acknowledged for access to the *Aloe* collection at the National Herbarium in Pretoria and advice on the preparation of *Aloe* specimens. I thank Gavin Selfe, Quentin Hagens and Cilla Wildgoose for help with Arc GIS and Google Earth Pro, and appreciate Les Powrie's and Anisha Dayaram's (SANBI) inputs on the WGG vegetation

map. Pieter Winter (SANBI) is acknowledged for the use of an unpublished plant list. To my fellow students, Stephanie Payne and Blair Cowie, thanks for your help with the camera traps and installing Statistica. Weather data were provided by SAEON.

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GLOSSARY OF ABBREVIATIONS

AOO	Area of Occurrence
ARC	Agricultural Research Council
MGT	Mean Germination Time.
SANBI	South African National Biodiversity Institute
SAEON	South African Environmental Observation Network
SCD	Size class distribution
WGG	Woodbush Granite Grassland

CHAPTER ONE

ALOE LETTYAE - FLAGSHIP OF THE WOODBUSH GRANITE GRASSLAND

Species and vegetation type description

The Woodbush Granite Grassland (WGG) occurring in the south eastern part of the Limpopo Province (Mucina *et al.* 2006), boasts the multi-stemmed *Aloe arborescens* Mill., the grass aloe *A. ecklonis* Salm-Dyck (=*A. boylei*) as well as two maculate aloes, the winter-flowering *A. greatheadii* var. *davyana* (Schönland) Glen & D.S.Hardy (=*A. longibracteata*) and the Endangered summer-flowering *A. lettyae* Reynolds (P.J.D. Winter, unpublished data; Von Staden and Kremer-Köhne 2015).

Aloe lettyae plants occur in small groups (Figure 1.1a), are succulent and stemless (Reynolds 1937). The dense leaf rosettes consist of up to 20 erectly spreading bluish green leaves (Figure 1.1c). The leaves have dull cream spots on both surfaces (adaxial surface with numerous elongate spots; abaxial surface with larger, more obscure spots in undulating transverse bands), and are armed with small brown marginal teeth (Reynolds 1937, 1969). The inflorescence is up to 2 m tall (Figure 1.1b), branched from approximately the middle (Smith *et al.* 2012) and has distinctly widely upwards curved side branches (Figure 1.1d), the lower ones usually re-branched (Carter *et al.* 2011). The racemes are cylindrical and laxly flowered (Letty 1962; Carter *et al.* 2011). Flowers are long, tubular and rose-red, with paler margins (Reynolds 1937); however, the flower colour is more accurately described as orange-red according to the Royal Horticultural Society (RHS 2001) colour chart (Kremer-Köhne, pers. obs.). The perianth has a large, distinctly rounded basal swelling above which it is constricted and then widens towards the mouth. Stamens and style are scarcely exserted (Reynolds 1937). *Aloe lettyae* flowers in summer, at the same time as the sympatric *A. ecklonis*, and a rare natural hybrid between the two species has been observed (Jeppe 1969; Kremer-Köhne, pers. obs.). In a very small area on the south western boundary of its range, *A. lettyae* occurs alongside the summer-flowering *A. ammophila* growing in the adjacent Mamabolo Mountain Bushveld vegetation type and hybrids exist (Kremer-Köhne, pers. obs.).

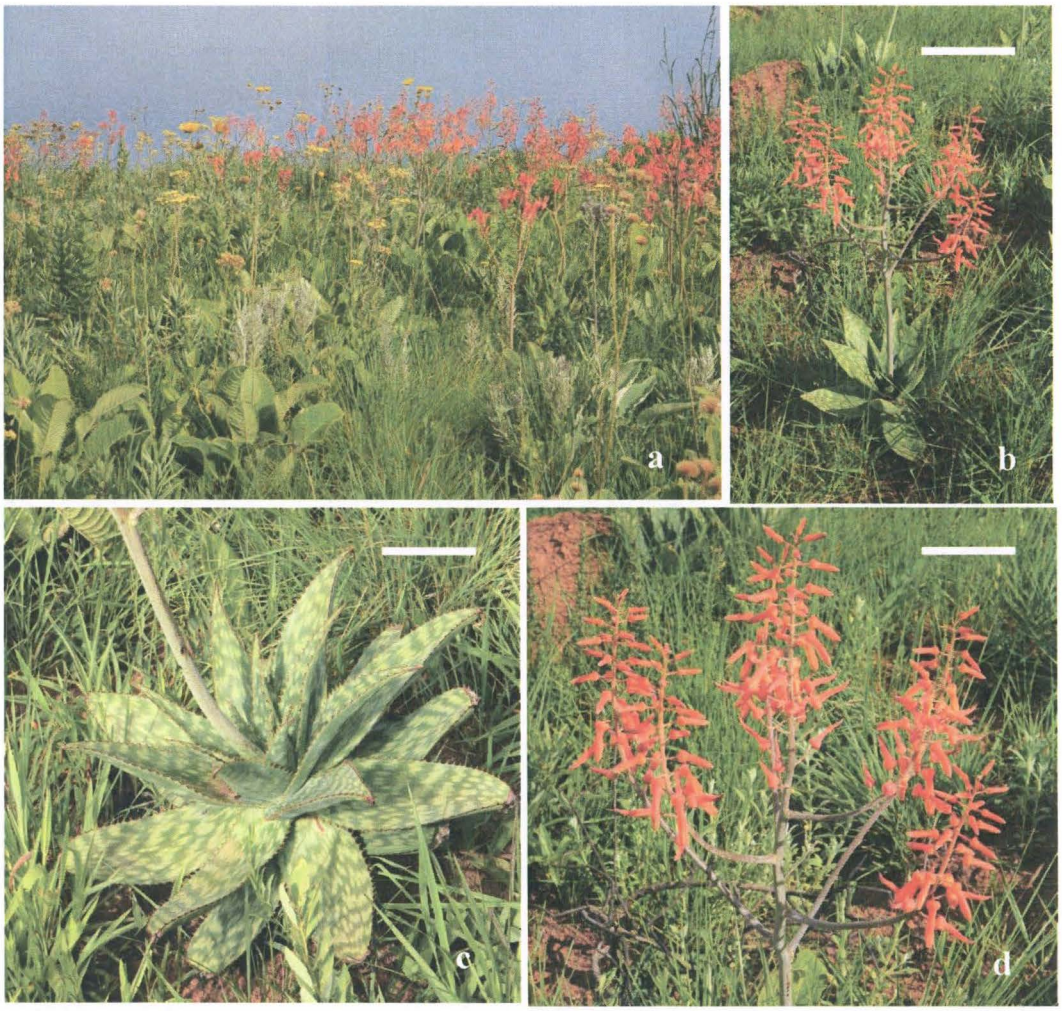

Figure 1.1. Summer-flowering *Aloe lettyae* (a) Colony in the Ebenezer Dam Nature Reserve, Haenertsburg. (b) Flowering adult (scale bar = 30 cm). (c) Dense leaf rosette of flowering adult (scale bar = 10 cm). (d) Inflorescence with curved side branches (scale bar = 10 cm). Photographs: S. Kremer-Köhne.

Aloe lettyae was first collected by well-known South African botanical artist Cythna Letty on the farm Westfalia near Duiwelskloof (now Modjadjiskloof, Limpopo Province) in March 1932 and subsequently named after her in 1937 (Reynolds 1937, 1940; Letty 1962). Taxonomically, the maculate aloes are one of the most difficult groups in the genus *Aloe* (Reynolds 1969) due to hybridisation and other probably continuing evolutionary processes (Glen and Hardy 2000). Most maculate aloes are very difficult to identify, especially in the vegetative phase (Van Wyk and Smith 2014). *Aloe lettyae*,

described by Reynolds in 1937, was included in the synonymy of *A. zebrina* Baker by Glen and Hardy (2000). The species *A. lettyae* has, however, been retained by many authors as a separate species (e.g. Van Wyk and Smith 2008; Carter *et al.* 2011) as it differed from *A. zebrina* in the following diagnostic characters: *A. lettyae* inflorescences are branched from about the middle, have widely curving branches and their flowers have a remarkably large globose base as opposed to *A. zebrina* inflorescences which are branched from above the middle, have branches at narrower angles and flowers with a smaller bulbous base (Smith *et al.* 2012). Therefore, the name *A. lettyae* was reinstated (Smith *et al.* 2012).

Reynolds (1937) observed the species in long grass and among bushes and trees on eastern slopes of the northern extremity of the Drakensberg near Duiwelskloof and southwards near the top of Magoebaskloof, and beyond. Further *A. lettyae* localities were subsequently found north-east of Modjadjiskloof for a few miles towards Queen Modjadji's kraal and the Cycad Forest (Reynolds 1969). On a typewritten, undated note kept in the National Herbarium in Pretoria, many specimens in bloom were recorded for a locality halfway up the slope of the Farm Stylkop in the Groot Letaba Valley stretching to the western escarpment of Wolkberg (Reynolds, unpublished data). According to Van Wyk and Smith (2014), *A. lettyae* occurs in the Limpopo Province in a fairly restricted area in the vicinity of Modjadjiskloof and Magoebaskloof (Haenertsburg), about 100 km east of Polokwane. This area coincides with the Woodbush Granite Grassland (Gm 25) defined by Mucina *et al.* (2006).

The grassland biome is one of the most threatened and under-protected biomes in South Africa (Carbutt *et al.* 2011). The Limpopo Province contains only a very small area of the South African grassland biome (2307 km^2 or 0.7% of the biome compared e.g. to the neighbouring Mpumalanga Province with 50730 km^2 or 15% of the biome) and was considered marginal to the biodiversity profile and priority assessment undertaken as part of the National Grassland Biodiversity Programme (Reyers *et al.* 2005). The mesic Woodbush Granite Grasslands (WGG) are situated at the northern limit of the Drakensberg Range which forms part of the Great Escarpment at the eastern edge of the interior plateau of southern Africa (Partridge and Maud 1987). WGG occur at an altitude of 1 080 to 1 800 m a.s.l. on the Woodbush Plateau and its outliers, to the north of the Wolkberg. Climatically the WGG are

characterised by summer rainfall with a mean annual precipitation of 1166 mm peaking in January. Some precipitation may occur in winter. Mist is common in both summer and winter. The mean annual temperature is 16.6°C, and frost is infrequent with an average of only 7 frost days per year (Mucina *et al.* 2006).

Grasslands are among the most diverse vegetation types on the planet (O'Connor and Bredenkamp 1997; Wilson *et al.* 2012). Grasses contribute the bulk of the plant biomass but only 13.5% of the species richness of mesic grassland. Forbs (dicotyledonous herb species, non-graminoid monocots and geophytes) constitute by far the largest component of the total plant biodiversity of grasslands (Scott-Shaw and Morris 2015). Most forbs have swollen roots which serve as underground storage organs (USOs) and can be very large, suggesting that the forbs may be very old (Bond and Parr 2010). Grasslands and their associated small patches of forest form part of the Afromontane Region, a centre of endemism in high altitude areas in many parts of Africa (White 1978). An assessment of the endemic flora of the Limpopo/Mpumalanga Escarpment (formerly north eastern Transvaal Escarpment) showed that nearly all the endemics are grassland species (Matthews *et al.* 1993; Van Wyk and Smith 2001). According to Matthews *et al.* (1993), a centre of endemism north of the Wolkberg Centre in the Tzaneen/Woodbush area (mainly granite) may exist, and a floristic analysis of this region in which patterns of endemism are assessed in relation to geology is warranted.

A checklist of 661 plant species compiled for the largest WGG fragment of 190 ha adjacent to the small town of Haenertsburg in 2007 (P.J.D. Winter, unpublished data; Dzerefos and Witkowski 2016) contains two taxa endemic to the WGG, *Aloe lettyae* (Endangered) and *Indigofera rehmannii* Baker f. (Endangered) (Von Staden *et al.* 2015; Dzerefos *et al.* 2017). Although difficult to assess due to the lack of historical WGG biodiversity data, Niemandt and Greve's study (2016) suggest that WGG biodiversity has been considerably compromised by the large-scale WGG habitat loss and fragmentation. Smaller WGG fragments have fewer species (Kremer-Köhne, pers. obs.) but may still contain rare species such as the WGG endemic, Critically Endangered (Possibly Extinct), *Kniphofia crassifolia* Baker (Winter and von Staden 2008) and the Critically Endangered *Chlorophytum radula*

(Baker) Nordal (Von Staden and Winter 2008b). Other WGG plant species of conservation concern include two Endangered species *Brachystelma gerrardii* Harv. (Styles and von Staden 2007) and *Afroaster nubimontis* (W.Lippert) J.C.Manning & Goldblatt (Von Staden and Winter 2008a), two near threatened species *Alepidea attenuata* Weim. (Von Staden and Winter 2007) and *Disa extinctoria* Rchb.f. (Victor *et al.* 2009) and one data deficient medicinal plant, *Xerophyta purpurascens* Behnke (Von Staden and Burrows 2014; Dzerefos *et al.* 2017).

Egoh *et al.* (2009) showed that grassland biomes provide five ecosystem services (surface water supply, water flow regulation, carbon storage, soil accumulation, and soil retention), and that there is a correlation between ecosystem services and species richness and vegetation diversity hotspots. Most of the natural grassland vegetation on the Limpopo/Mpumalanga Escarpment has been transformed by extensive afforestation, and large tracts of land have been severely invaded by alien plants causing a substantial loss of water through their high transpiration rates (Witkowski and Garner 2008; O'Connor and Kuyler 2009). This is in conflict with the necessity to protect mountain catchments, conserve ecosystems and preserve scenic landscapes for the tourism industry (Deall *et al.* 1989). Originally, about 340 km² of WGG existed (Mucina *et al.* 2006). The first eucalyptus and pine plantations were established in WGG circa 1910 to provide an alternative to indigenous timber (Dzerefos 2004). In 2006, it was estimated that there was only about 10% of WGG still in a natural state, and that the exceptionally high conservation target of 27% set for this vegetation type with a high level of plant species diversity could therefore not be met (Mucina *et al.* 2006). In a recent study, Niemandt and Greve (2016) established that less than 6% of WGG may be truly intact. The WGG are classified as a Critically Endangered ecosystem and consequently WGG, inclusive of associated and endemic species such as *A. lettyae*, carries the highest conservation index in the Limpopo Province (Mucina *et al.* 2006; RSA 2011). In 2011, 0% of the original WGG area was protected (RSA 2011). The provincial Haenertsburg Nature Reserve was declared in 2016, protecting a portion of the largest remaining WGG fragment, which constitutes just 0.4% of the original WGG area (Limpopo Provincial Government 2016).

Rationale for the study of *Aloe lettyae*

Aloe lettyae, a species protected by provincial legislation (LEMA 2003), has a very limited distribution range (extent of occurrence 123 km^2) and is endemic to the highly fragmented, Critically Endangered WGG. In 2015, only four *A. lettyae* populations, varying in size between 200 and 1000 mature individuals and occurring on grassland fragments, were known. A possible fifth site was known through historical records. Consequently *A. lettyae* was listed as Endangered (Von Staden and Kremer-Köhne 2015). As no accurate estimates of the population size for the species existed, possible past declines driven by habitat loss (*sensu lato*) and fragmentation were unknown. Furthermore, nothing was known about factors such as pollinator taxa and efficiency, population demography, local neighbourhood effects, and microhabitat requirements, all of which potentially limit successful reproduction and growth and survival of *A. lettyae*.

In 2016, less than 6%, or approximately 1700 ha, of the Woodbush range's original grasslands still existed intact (Niemandt and Greve 2016). This is a generous calculation based on 2008 aerial imagery that was not ground-truthed, and the actual value will be less as for example natural and secondary grasslands, such as abandoned croplands, could not always be distinguished on the images (Niemandt, pers. comm.). The largest, relatively well-preserved WGG remnant lies immediately adjacent to the small town of Haenertsburg and is one of several global change mountain observatories being monitored by the South African Environmental Observation Network (SAEON). Evidence suggests that inappropriate fire policies and possible effects of the conditions of global climate change are facilitating increasing shrub density and woody transformation in some remaining areas (Thompson and Swemmer 2014). Other threats to the grasslands, and therefore to *A. lettyae*, include agricultural as well as housing and infrastructure developments, land appropriation, driving on the grasslands with off-road vehicles and potential mining activities. Harvesting of *A. lettyae* from the wild for horticulture and natural products industries does not seem to be practised (Kremer-Köhne, pers. obs.), in contrast to the illegal collecting threatening e.g. the Critically Endangered *A. peglerae* (Phama *et al.* 2014). Currently, only 0.4% of the original WGG area is formally protected (Limpopo Provincial Government 2016), and

any further fragmentation, habitat loss and habitat transformation is likely to have deleterious effects on the remaining *A. lettyae* population.

An exhaustive search of the literature yielded no scientific studies on any aspect of the population biology, biogeography and ecology of *A. lettyae*, including the response of the species to fire and woody plant encroachment, and the effects of habitat isolation. Furthermore, no baseline *A. lettyae* population data were available to facilitate future research and long-term monitoring as well as to establish the time-trends and driver-response relationships necessary to inform urgent conservation and management decisions.

Aims and objectives

The primary aims of this study were (1) to establish species range and population demographics for *Aloe lettyae;* (2) to investigate the general ecology, including the reproductive biology, of *A. lettyae*; and (3) to identify and, where possible, quantify potential threats to populations of *A. lettyae.*

The objectives addressing the aims of the study were to:

a) Determine the distribution, size and densities of *A. lettyae* populations across its range (Chapter Two);
b) Determine the size/age demographics of all extant populations of *A. lettyae,* and of the national population; in addition, assess *A. lettyae* plants for damage, pests and diseases, and document the vegetative regeneration response of *A. lettyae* individuals following disturbance (Chapter Two);
c) Establish the flowering phenology and determine the contribution of different pollinator guilds as well as fruit and seed production for *A. lettyae* (Chapter Three);
d) Assess recruitment rates and seed germination for *A. lettyae* (Chapters Two and Three);
e) Define the habitat profile of *A. lettyae* across its restricted geographical range (including aspect, slope, altitudinal range, topography and fire regime) (Chapter Two);
f) Describe major threats to *A. lettyae* individuals and populations (Chapter Two).

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CHAPTER TWO

HANGING IN THERE: *ALOE LETTYAE* POPULATIONS IN CRITICALLY ENDANGERED GRASSLAND FRAGMENTS

Abstract

This is the first study on the biogeography, population biology and ecology of the endangered *Aloe lettyae* endemic to the highly threatened Woodbush Granite Grassland (WGG) in Limpopo Province, South Africa. Most populations were clustered on the south western side of the WGG polygon, with all known localities less than 40 km apart within this severely fragmented vegetation type. In total, 19 *A. lettyae* populations were located and documented, and the total area of occupancy was calculated at17.5 ha. Population size varied from 10 to 4000 plants, with 8500 individuals in total. In the two large populations with ~3000 plants each and one smaller population with <350 plants, the point centred quarter method (PCQ) was used in combination with the Nearest-neighbour method (NN) to estimate plant density. The two estimates obtained differed to some extent due to the fact that *A. lettyae* plants had an aggregated distribution as indicated by the Pielou index calculated and occurred in clumps within populations. Plant size and life history stage demographics were determined in seven of the 19 currently known *A. lettyae* populations, which included the two large populations as well as a high- and a low-lying population and constituted a representative sample over the entire geographical range of the species. Based on number of leaf layers and leaf rosette diameter, most size class distributions (SCDs) (76%) were bell-shaped suggesting stable populations, which included the two largest populations, 9% J-shaped, 3% had an inverse J-shaped SCD and 12% had an irregular SCD. For all surveyed populations combined, 15% of the plants were juveniles, 9% subadults, 15% non-reproductive adults and 61% reproductive adults. There was a clear difference in population structure between the main population and the second largest population, with the main population having a higher proportion of juveniles (14%) than the second largest population (3%). Although the SCDs suggested stable populations the low percentages of juveniles and non-reproductive subadults were of

concern as they indicated an apparent current and historic lack of recruitment. *Aloe lettyae* grows in tall, dense grassland vegetation. For all surveyed populations combined, habitat profile within 1 m of an *A. lettyae* individual and ≥ 5 m away from *A. lettyae* plants in adjacent non-populated areas varied very little, but habitat variables differed significantly between populations. Percentages aerial cover for grass, forbs and woody species were 50%, 37% and 5% for the main population and 49%, 26% and 15% for the second largest population respectively, with the small remaining percentages being made up of bare soil and rock cover. The main threats to *A. lettyae* were identified as misapplication of fire, cattle grazing and invasive alien plants. Routine future monitoring of *A. lettyae* populations by means of counting leaf layers and measuring the leaf rosette diameter is recommended, as these metrics most appropriately reflect life history stages. Baseline *A. lettyae* population data are now available for long-term monitoring, and to inform urgent management and conservation decisions in this threatened vegetation type.

Key words

Aloe lettyae, area of occupancy, population density, population structure, recruitment, Woodbush Granite Grassland (WGG).

Introduction

The escarpment grasslands in the Limpopo and Mpumalanga Provinces have been transformed by extensive afforestation which is in conflict with the protection of mountain catchments and the conservation of natural ecosystems (Deall *et al.* 1989; Dzerefos 2004). Of the original Woodbush Granite Grassland (WGG) vegetation type in Limpopo Province's Haenertsburg-Magoebaskloof area - estimated at 340 km^2 (Mucina *et al.* 2006), an approximated 6% remained in 2008 (Niemandt and Greve 2016). Consequently, the WGG is Critically Endangered and currently considered the most threatened vegetation type in the Limpopo Province (Mucina *et al.* 2006).

The summer-flowering, maculate *Aloe lettyae* is endemic to the WGG and listed as Endangered (Von Staden and Kremer-Köhne 2015). Plants are succulent, short-stemmed or stemless and have a

dense rosette of erectly spreading bluish green leaves with dull cream spots on both surfaces (Reynolds 1937, 1969). Especially in the vegetative phase, *A. lettyae* is difficult to identify, as are most maculate aloes (Van Wyk and Smith 2014). A conspicuous character, however, is its inflorescence with brightly coloured orange-red flowers which can be up to 2 m tall, branched from about the middle and bearing distinctly widely upwards curved side branches (Smith *et al.* 2012). Seed germinates easily and plants flower the 3rd or 4th year from seed (Jeppe 1969). *Aloe lettyae* occurs in tall grass and among bushes (Reynolds 1937, 1969; Scheepers 1978; Smith *et al.* 2012). Adult individuals appear to survive at least periodic grassland fires with scorched and charred leaves (Kremer-Köhne, pers. obs.), as their apical meristem is well protected by tightly overlapping, succulent leaf bases and their short stem insulated by a persistent skirt of dead leaves (Bond 1983; Clarke *et. al.* 2013). Small and/or exposed *A. lettyae* individuals on the other hand are likely to be killed by fire (Cousins and Witkowski 2012).

A number of studies have been undertaken on the population size structures of the tree aloes *A. pillansii* (Midgley *et al.* 1997; Bolus *et al.* 2004; Duncan *et al.* 2006), *A. dichotoma* (Midgley *et al.* 1997) and *Kumara plicatilis* (=*A. plicatilis*) (Cousins *et al.* 2014). Population size structures have also been investigated in the single-stemmed *A. ferox* (Stokes and Yeaton 1995) as well as in the stemless aloe *A. petricola* (Weisser and Deall 1989). Long-term population monitoring data, however, have only been published for the slow-growing, stemless aloe *A. peglerae* (Scholes 1988; Pfab and Scholes 2004; Phama *et al.* 2014). In *A. petricola* and *A. peglerae*, the population size structure was characterised by the dominance of individuals in the intermediate and the intermediate and large size classes, respectively and very few seedlings and juveniles were observed (Weisser and Deall 1989; Pfab and Scholes 2004). This size class distribution is considered typical of aloes, which generally reach reproductive maturity relatively quickly (± 5 years), and then continue growing slowly for many years before dying (Weisser and Deall 1989). It is also suggested that the low frequency of seedlings and juveniles may be attributed to fire damage, since smaller aloes tend to be more fire susceptible than larger ones (Weisser and Deall 1989; Cousins and Witkowski 2012). As no population studies have been conducted on maculate *Aloe* species, the demographics of the WGG endemic *A. lettyae* are poorly understood. The aim of this study was therefore (a) to establish *A. lettyae*'s range, (b) to determine size, area of occupancy, density, plant

size and stage demographics of selected *A. lettyae* populations, and (c) to investigate their habitat profile as well as disturbance factors to provide baseline data for long-term monitoring as well as for informing urgent management and conservation decisions.

Methods

Study area

The study area encompassed the entire distribution of *A. lettyae,* which is restricted to the areas immediately around Haenertsburg, Magoebaskloof and Modjadjiskloof (altitude 860–1800 m a.s.l.), on the Woodbush Plateau to the north of the Wolkberg mountain, Limpopo Province, South Africa. Climatically the area is characterised by summer rainfall with a mean annual precipitation of 1166 mm peaking in January. The region received 786 mm and 1192 mm of rain during the periods July 2015 to June 2016 and July 2016 to June 2017 respectively (SAEON 2017). Some precipitation may occur in winter, and frost is infrequent. Mist is common in both summer and winter. The mean annual temperature is 16.6°C (Mucina *et al.* 2006). The geological substrate is dominated by granite, gneiss and greenstone basement (Mucina *et al.* 2006). The original vegetation is classified as Woodbush Granite Grassland (WGG) which has undergone major transformation during the last century, mainly due to silviculture. WGG is a Critically Endangered vegetation unit (Mucina *et al.* 2006; RSA 2011; Desmet *et al.* 2013; SANBI 2013). Two sizeable WGG fragments are government-owned, the largest WGG fragment (192 ha) situated adjacent to Haenertsburg and a peninsula-shaped WGG patch of 60 ha protruding into Ebenezer dam; several smaller WGG remnants, ranging in size from about 5 to 85 ha, occur in the Haenertsburg, Magoebaskloof and Woodbush Forest areas on privately and community-owned farms or on land managed by the Department of Agriculture, Forestry and Fisheries (Dzerefos and Witkowski 2016; Dzerefos *et al.* 2017) (Figure 2.1 and Appendix 2.1).

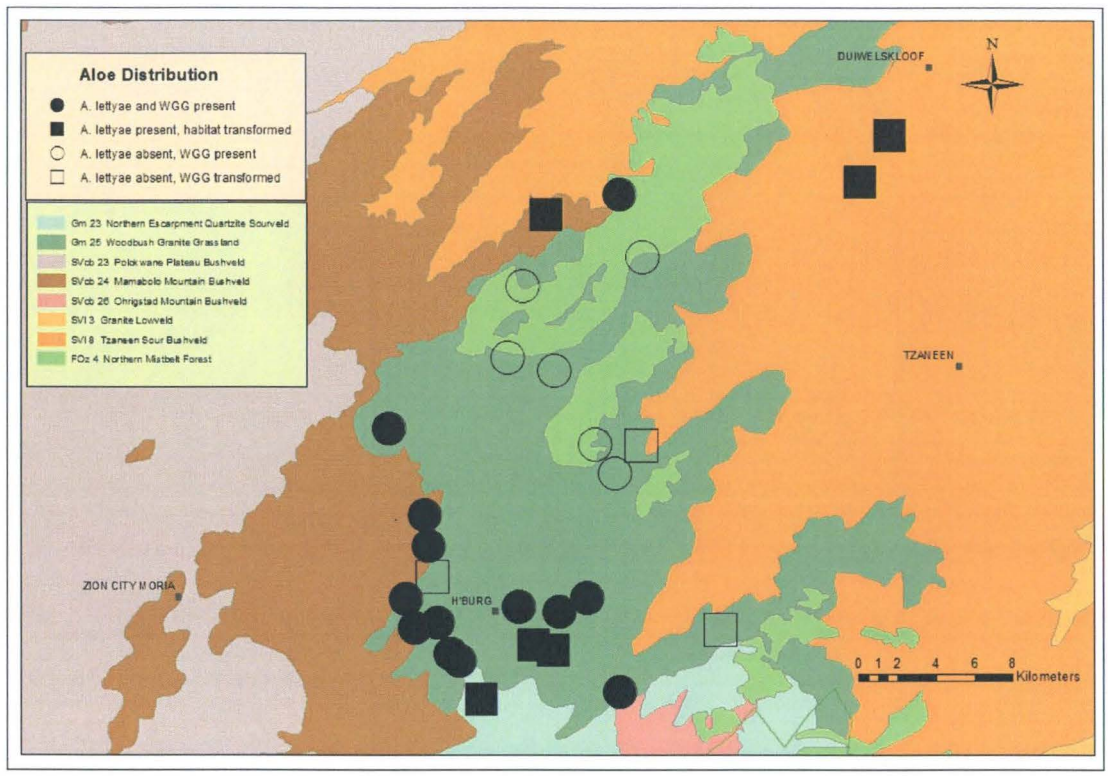

Figure 2.1. Geographical distribution of the 19 known *Aloe lettyae* populations and 28 current and historic Woodbush Granite Grassland (WGG) localities surveyed, Limpopo Province, South Africa. Small blue squares denote towns. Map from Mucina *et. al.* (2006).

Locating populations

Eight *A. lettyae* populations were known at the start of the study in January 2016, based on my knowledge of the flora in the Haenertsburg area (HB, EB, RE, DR, BL, BU, FG, FH; Appendix 2.1) which included the four populations mentioned in Chapter One. Another four populations were located after consulting the National Herbarium (O 1 and 2, W 1 and 2). Further searches, enhanced by networking with the local community, were undertaken in the Modjadjiskloof (previously Duiwelskloof) area and in WGG fragments around Haenertsburg and in the Woodbush Forest as well as in the Groot Letaba Valley during the *A. lettyae* flowering season (early January to late March 2016) as inflorescences are conspicuous, even in tall surrounding vegetation. These efforts yielded another seven small *A. lettyae* populations (SK, VE, LE, RR, PR, G, PA). GPS coordinates were taken

approximately at the centre of all known WGG fragments using a hand-held Garmin unit (Garmin, GPSmap 62sc, USA), and these data were used to produce an up-to-date distribution map for *A. lettyae*, as well as to establish its possible historic range (Figure 2.1). For each WGG fragment, owner, altitudinal range, topographic position, slope, predominant aspect, the year of the most recent burn, surrounding land use as well as threats were determined, and the habitat condition visually rated on a scale of good to poor based on the plant species richness observed. Further, an estimated number of flowering *A. lettyae* plants was recorded. Google Earth Pro was used to measure the size of the WGG polygons and distances to nearest neighbouring *A. lettyae* populations (Appendix 2.1). Duplicate voucher specimens of *A. lettyae* were collected from six newly documented populations (Kremer-Köhne 1163 – PR; Kremer-Köhne 1164 – DR; Kremer-Köhne 1165 – EB; Kremer-Köhne 1168 – SK; Kremer-Köhne 1169 – BL; Kremer-Köhne 1172 – W) and will be lodged in the National Herbarium in Pretoria and the Larry Leach Herbarium at the University of Limpopo.

Data collection

From January to March 2016 and 2017, as locating clumps of aloes progressed, GPS coordinates were taken of the peripheries of fairly continuous aggregations (clumps) of *A. lettyae* in all populations, and the flowering plants were counted. In September 2016, after fire had cleared very densely vegetated areas in populations HB and BL, *A. lettyae* clump periphery coordinates were recorded and the number of adult aloes was estimated in these areas (Appendix 2.1). *A. lettyae* polygons were measured using Google Earth Pro to determine the Area of Occupancy (AOO).

Plant density and the total number of individuals in populations HB, EB and FG were estimated in summer 2017 by placing transects across the larger *A. lettyae* clumps (0.1–0.7 ha) with sampling points spaced at 20 m intervals and using the point centred quarter method (PCQ) in combination with the Nearest-neighbour method (NN) (Cottam and Curtis 1956; Marom 2006). Placing transects across *A. lettyae* clumps with large variations in plant density, in tall vegetation, and sometimes undulating terrain, proved very challenging. As the dense vegetation also necessitated long, thorough searches for *A. lettyae* plants at each sampling point, a distance limit of 10 m perpendicular to the transect point was

set to increase the sampling efficiency. Plant densities were calculated using the following equations: For PCQ according to Mitchell (2007),

$$\text{Density (plants/m}^2) = 1 / l^2,$$

where 'l' is the mean distance from each PCQ point to the nearest *A. lettyae* plant. This density calculation was corrected for vacant quarters to avoid overestimating the true density (Mitchell 2007). For comparison, PCQ plant density was also calculated using the equation (Pollard 1971, cited in Krebs 2014):

$$N_p = (4\,(4\,n - 1)) / (\pi \sum(r_{ij}^2))$$

where 'N_p' = Point-quarter estimate of population density; 'n' = number of random points; $\pi = 3.14159$; 'r_{ij}' = distance from random point i to the nearest organism in quadrant j (j = 1,2,3,4; i = 1, ...n). This is considered the appropriate estimate of population density for the point-quarter method which is the least biased by typical plant clumping (Krebs 2014).

For NN,

$$\text{Density (plants/m}^2) = 0.3556 / (\text{sum of r} / \text{n})^2,$$

where 'r' is the distance from each sampled individual per quadrat to its nearest neighbour and 'n' is the total number of random points sampled. For both PCQ and NN: Number of individuals in the population = density × AOO. The results obtained from these calculations were compared by regression analyses.

Aggregation characteristics of the *A. lettyae* clumps were determined by calculating a non-randomness index according to the equation:

$$a = \pi\, D\, w,$$

where 'D' is the population density and 'w' is the mean of the squares of the point-to-plant distances (Pielou 1959). If 'a' is equal to, less than or greater than '(n – 1) / n' (where 'n' is the number of point-to-plant distances sampled) the distribution is random, regular or aggregated, respectively (Pielou 1959).

Plant size and stage demographics were determined in seven of the 19 currently known *A. lettyae* populations (Appendix 2.1) which included the two largest populations (HB and EB) as well as a high- (FG) and a low-lying population (W) and constituted a representative sample over the entire geographical range of *A. lettyae*. Three survey methods were used: (a) In summer 2016 (January–April), four smaller *A. lettyae* populations comprising about 150 flowering plants (initial count) each were selected to obtain preliminary data on size and stage classes. Eighty, 40, 60 and 95 plants were randomly selected and measured in populations LE, BL, RE and W, respectively. (b) From March to May 2017, transects were placed across the larger *A. lettyae* clumps in populations HB, EB and FG, as described above. In each PCQ 90° quarter, the size of the *A. lettyae* plant closest to the sampling point and that of its nearest neighbour was measured. (c) In April–May 2017, the size of all *A. lettyae* plants was measured in two small rocky (population HB clumps N and Ro1) and in three small non-rocky areas (population HB clumps F and Jo1 and population EB clump 20) to compare estimated with actual plant numbers. For each surveyed *A. lettyae* plant, the following size-related counts and measurements were recorded: the number of rosettes, leaves and leaf layers, the diameter and height of the leaf rosette as well as the height of the inflorescence. Further, the length and diameter of stems were measured with vernier callipers (Moore & Wright MW100-20B), and visually estimated percentages of herbivory, the fungal rust disease and aloe cancer caused by mites (Smith and Van Wyk 2008; Van Wyk and Smith 2014) recorded. All *A. lettyae* plants surveyed were assigned to a life history stage (juveniles which included seedlings, sub-adults, adults) to establish population demographics. Based on the variables recorded, size class frequencies were calculated for size class distribution (SCD) graphs and related to stages.

The habitat profile for each surveyed *A. lettyae* clump was established by recording the topographic position, determining aspect and slope with a compass and a clinometer (Suunto Tandem 360PC 360R DG), and measuring the altitudinal range with a hand-held Garmin unit. Vegetation structure immediately around *A. lettyae* plants was assessed by placing (1 × 1 m) quadrats at random within 1 m of an *A. lettyae* individual and visually estimating the percentage aerial cover of grass, forbs, woody plants, rock and bare soil; the maximum heights of grass, forbs and woody plants in the quadrat

were measured and their respective species recorded. Taxonomy for plants followed Retief and Herman (1997) and SANBI (2017). Two replicate disc pasture meter (DPM) readings were taken in and very near the quadrat to quantify the standing plant biomass. Standing plant biomass was calculated using the following calibration equation for the relationship between the disc settling height and the standing crop of grass/herbaceous vegetation (Little *et al.* 2015):

$$\text{Plant biomass (kg/ha)} = (358.7 \times \text{mean disc height (cm)}) - 746.4$$

This calibration equation was determined for Lydenburg Montane Grassland which is similar to WGG in terms of altitude, climate and vegetation type (Mucina *et al.* 2006). To elucidate the habitat preference of *A. lettyae*, the same number of quadrats was placed at random and DPM readings taken ≥ 5 m away from *A. lettyae* plants in adjacent non-populated areas (Witkowski and Liston 1997; Witkowski *et al.* 2001; Arena *et al.* 2015). The sampling method followed that described above for plant size and stage demographics: (a) In March 2016, the small populations LE, BL and RE were randomly sampled by placing 20 quadrats and taking 40 DPM readings each near and further away from *A. lettyae* individuals in each population. As population W was confined to a very small area on a fire break in a eucalyptus plantation between a dirt road and a railway, only one quadrat could be placed and two DPM readings taken near the aloes. (b) From March to May 2017, larger *A. lettyae* clumps in populations HB, EB and FG were sampled along transects by placing one quadrat and taking two DPM readings each near and further away from *A. lettyae* individuals in each quarter yielding eight and 16 replicates respectively per sampling point. (c) In April–May 2017, the small rocky and non-rocky areas in population HB were sampled by placing four quadrats and taking two DPM readings each near and further away from *A. lettyae* individuals in each area.

Statistical analyses

Regression analyses were used to investigate the relationships between clump size, aggregation and plant density as well as number of plant estimates; plant size-related counts and measurements for all *A. lettyae* plants (number of leaf layers and leaves, leaf rosette height and diameter) as well as the

relationships between the vegetation surrounding *A. lettyae* plants (percentage aerial cover of grass, forbs, woody plants, and bare soil) and standing plant biomass. The best fit curve was selected. The regression analyses were done in Microsoft Excel 2016. Data relating to the habitat profile (percentage aerial cover of bare soil, rock, grass, forbs and woody plants, maximum height (cm) of grass, forbs and woody plants and standing herbaceous biomass (kg/ha) were transformed (either log or square-root transformed and percentage values arcsine transformed) and tested for normality using the Kolmogorov-Smirnov normality distribution. Untransformed and transformed data were not normally distributed. Therefore, the Mann-Whitney U Test was used to test for differences between habitat variables near and further away from *A. lettyae* plants. The non-parametric Kruskal-Wallis test was used to test for differences between populations, and the Bonferroni inequality test was applied to statistically significant results ($P < 0.05$). Analyses were performed using Statistica 13.2 (StatSoft, Tulsa, OK, USA, 2016). Values are presented as mean ± SE.

Results

National distribution

In total, 19 *A. lettyae* populations were located and documented (Figure 2.1 and Appendix 2.1). Most populations (84%) occurred in remnants of WGG and were clustered on the south western side of the WGG polygon, with all known localities less than 40 km apart. The two largest populations, HB and EB, occur near Haenertsburg and are close together (1.4 km) while overall distances to nearest neighbouring *A. lettyae* populations range from 1 to 13 km (Figure 2.1 and Appendix 2.1). Populations VE, RE and DR to the west of Haenertsburg appear to straddle the WGG (Gm 25) – Mamabolo Mountain Bushveld (SVcb 24) boundary. Population O is located just outside the WGG within Mamabolo Mountain Bushveld while population W near Duiwelskloof is found in completely transformed Tzaneen Sour Bushveld (SVI 8), and population PR occurs in Northern Escarpment Quartzite Sourveld. A small *A. lettyae* population also existed in a privately owned 6 ha WGG fragment at locality RU (Kremer-Köhne, pers. obs.) before it was ploughed in 2012. At locality PA, a few remnant *A. lettyae* individuals were found growing in a pine plantation, indicating that the species was more

widely distributed in the past. These individuals looked healthy but had longer, narrower and greener leaves than non-shaded *A. lettyae* growing nearby.

Population size and plant density

Preliminary estimates of flowering *A. lettyae* plants which varied greatly from 1240 in population HB to 10 in population PR (Appendix 2.1), were useful for deciding on the sampling strategy. However, these preliminary numbers of *A. lettyae* plants underestimated populations as both non-flowering and flowering *A. lettyae* individuals were not detected in the surrounding tall, dense vegetation. For the small clumps in which all plants were ultimately sampled, the underestimation ranged from 24 to 86% (non-rocky HB F–24%, HB Jo1–58%, EB 20–50%; rocky HB N–46% and HB Ro1–86%). Initially, seemingly fairly continuous clumps displayed a large variation in plant density, and all *A. lettyae* clumps sampled along transects were aggregated ($a > (n - 1) / n$, Pielou's index) (Table 2.1). Therefore, the PCQ and NN estimates differed to some extent. For all populations combined, the total number of *A. lettyae* individuals was estimated at 8500, and the total AOO calculated as 17.5 ha.

For the large populations HB and EB, the total areas of occupancy amounted to 7.4 and 2.4 ha, of which 54 and 83% of the areas were sampled, respectively (Table 2.1). In population HB, the NN and PCQ (Mitchell 2007) methods resulted in very similar estimates of total numbers of plants (NN 3516 and PCQ 3230) and average plant densities (NN 882 and PCQ 1041 plants/ha). However, for eight of the 11 HB clumps (A1, A2, B, D1, D2, D3, G, R) the NN method gave higher estimates than the PCQ (Mitchell 2007) method while the reverse was found in clumps D4, L and O, as well as for population FG. In population EB, estimates obtained with the NN method were considerably higher than those obtained with the PCQ (Mitchell 2007) method for the total number of plants (NN 2683 and PCQ 1380) and the average plant densities (NN 1306 and PCQ 629 plants/ha) as well as for all clumps except clump EB 3 (Table 2.1). Regression analyses showed that there is a strong relationship between PCQ plant numbers and densities calculated according to Mitchell (2007) and those calculated according to Pollard (in Krebs 2014), and in both instances the PCQ plant numbers were significantly related to the NN plant numbers. Aggregation had a significant effect on PCQ (Mitchell) and NN plant

numbers and densities, but not on PCQ (Pollard). Clump size only had a significant effect on plant numbers calculated according to PCQ (Pollard) and NN, but not on densities (Appendix 2.2)

Table 2.1. Area of occupancy (AOO), number of *Aloe lettyae* plants and their density (plants/ha) as determined by Nearest Neighbour (NN) and the Point Centred Quarter (PCQ, Mitchell 2007) methods, as well as the aggregation index (Pielou 1959) of *A. lettyae* clumps sampled along transects in the large populations HB and EB and the smaller population FG. For all clumps a > (n – 1) / n, indicating an aggregated distribution. Total AOO and number of plants as well as mean plants/ha listed for populations HB and EB.

			NN		PCQ		
Population	Clump	AOO (ha)	Number of plants	Plants/ha	Number of plants	Plants/ha	Aggregation
HB	A1	0.40	228	575	137	347	2.87
HB	A2	0.66	433	654	121	183	2.22
HB	B	0.38	326	864	64	168	1.50
HB	D1	0.27	92	345	66	247	2.78
HB	D2	0.26	99	386	55	216	2.48
HB	D3	0.35	289	823	112	319	2.15
HB	D4	0.18	156	862	201	1109	2.87
HB	G	0.30	204	672	194	639	3.19
HB	L	0.59	570	959	796	1340	4.90
HB	O	0.15	263	1777	812	5487	4.84
HB	R	0.48	856	1780	672	1396	3.53
	Overall	4.02	3516	882	3230	1041	2.99
EB	1	0.63	983	1564	542	863	5.69
EB	3	0.11	91	821	99	900	2.62
EB	5	0.04	91	2104	28	649	3.61
EB	7	0.24	200	835	117	489	2.48
EB	9	0.44	544	1227	313	705	3.06
EB	10	0.20	339	1670	127	626	3.50
EB	14	0.21	314	1510	105	507	4.01
EB	16	0.17	121	709	49	287	2.23
	Overall	2.04	2683	1305	1380	628	3.55
FG	1	0.35	140	522	317	903	3.09

Plant size and stage demographics

Aloe lettyae seedlings and juveniles have a fan-shaped arrangement of leaves which changes to a distinctly rosette-shaped arrangement as the plants mature (Figure 2.2a and b). As it is difficult to

distinguish between seedlings and juveniles, the two stages were combined when relating size to stage structure. Subadults (non-reproductive individuals which are distinctly smaller than non-reproductive adults, Figure 2.4) (Figure 2.2c) and young adults (reproductively mature individuals) bear the leaf rosettes close to the ground (Figure 2.2d). As the plants grow older they develop a short stem (<20 cm long) covered with a persistent skirt of withered leaves (Figure 2.2e), and they fall over when they become top-heavy (Figure 2.2f), sometimes partially exposing stem and roots (Figure 2.2g). The exposed stem and roots may be damaged by fires, ultimately resulting in the death of the plant (Figure 2.2h and i). The average adult lifespan of an *A. lettyae* individual is not known.

There were strong positive correlations between numbers of leaf layers and leaves ($R^2 = 0.91$), leaf rosette diameter and height measurements ($R^2 = 0.70$) as well as between the number of leaves and rosette diameter ($R^2 = 0.68$) and height ($R^2 = 0.75$). These relationships are shown in Figure 2.3. *Aloe lettyae* size structure was related to life history stage structure based on the number of leaf layers and leaves, the leaf rosette diameter and height for all surveyed populations combined (Figure 2.4a). The strong correlations between numbers of leaf layers and leaves, and leaf rosette diameter and height measurements resulted in identical life stage percentages for the respective pairs. However, the proportion of juveniles was higher when considering leaf layers and leaf rosette diameter (15%) than when considering leaf numbers and leaf rosette height (9%), while there were only minor differences in the percentages of the other life history stages. Based on number of leaf layers and leaf rosette diameter, 15% were juveniles, 9% were subadults, 15% non-reproductive adults and 61% reproductive adults (Figure 2.4a). Between populations HB and EB there was a clear difference, with HB having a higher proportion of juveniles (14%) than EB (3%) (Figure 2.4b and c). Life history stage and size classes based on various metrics for *A. lettyae* were defined as shown in Table 2.2.

Figure 2.2. Life history stages and vegetative regeneration of *Aloe lettyae* (white scale bars=10 cm) (a) Seedlings (b) Juveniles. (c) Subadult. (d) Young, stemless adult. (e) Mature adult with stem. (f) Mature adult with stem, fallen over when rosette became top-heavy. (g) Top-heavy mature adults fallen over, partially exposing stem and roots. (h) Dead mature adult. (i) Roots of dead mature adult. (j) Apical sprouting and (k) Basal suckers following disturbance. (l) Plant with six rosettes, presumably originating as basal suckers around a previous central stem. Photographs: S. Kremer-Köhne.

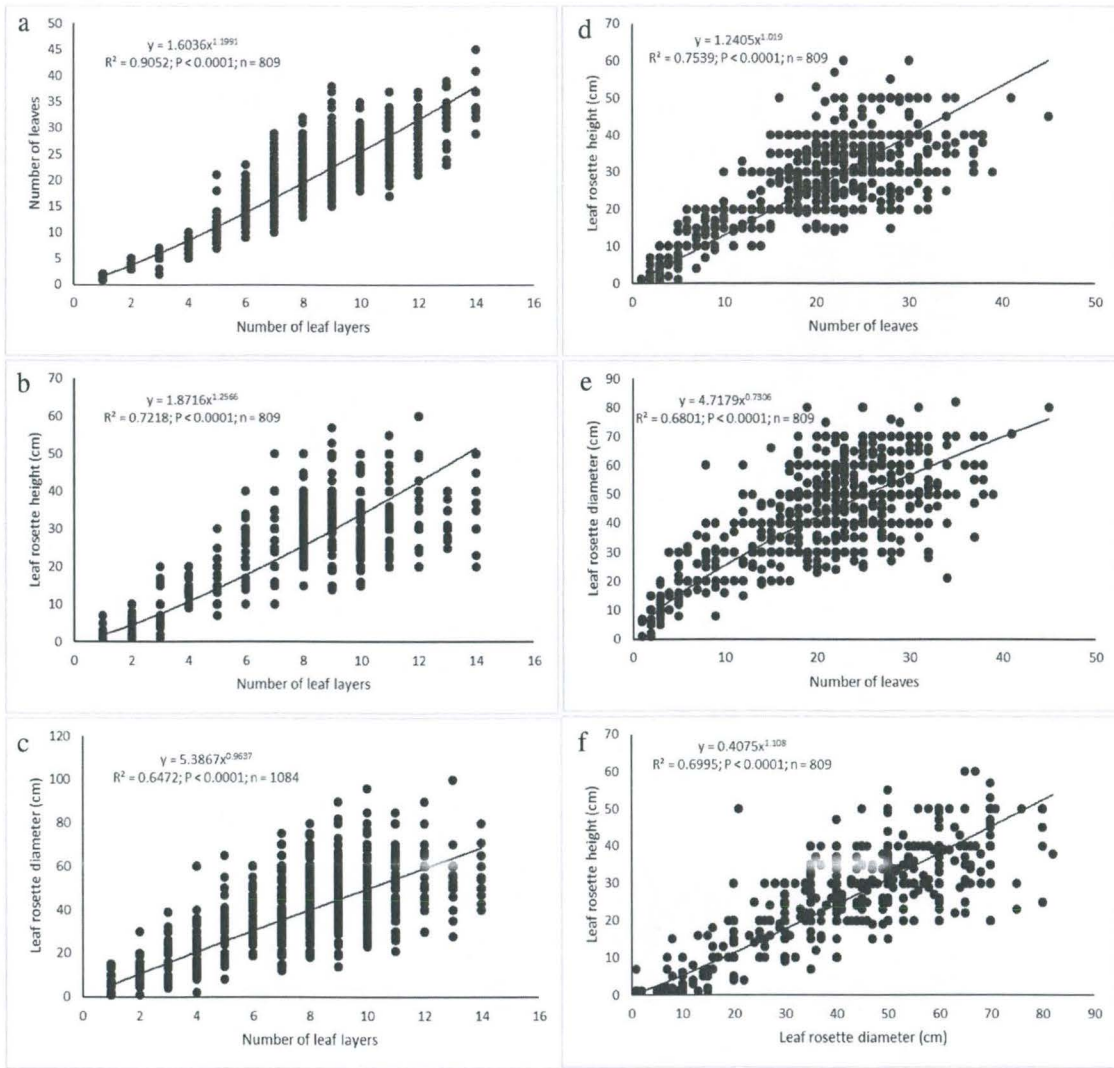

Figure 2.3. Relationships between plant size related counts and measurements for all *Aloe lettyae* plants surveyed. Regression relationships between number of leaf layers and (a) number of leaves (n = 809), (b) leaf rosette height (n = 809) and (c) leaf rosette diameter (n = 1084). Regression relationships between number of leaves and (d) leaf rosette height, (n = 809) and (e) leaf rosette diameter (n = 809); as well as (f) between leaf rosette diameter and height (n = 809). Equation of best fit line (power) and R^2 on graph.

Table 2.2. *Aloe lettyae* stage classes and their size limits in terms of numbers of leaf layers (LL) and leaves (L), as well as leaf rosette diameter (RD) and height (RH). Juveniles include seedlings.

Stage	Leaf layers (number)	Leaves (number)	Rosette diameter (cm)	Rosette height (cm)
Juveniles	≤ 4	≤ 5	≤ 20 cm	≤ 10 cm
Subadults	$4 < LL \leq 6$	$5 < L \leq 15$	20 cm $< RD \leq 30$ cm	10 cm $< RH \leq 20$ cm
Adults	> 6	> 15	> 30 cm	> 20 cm

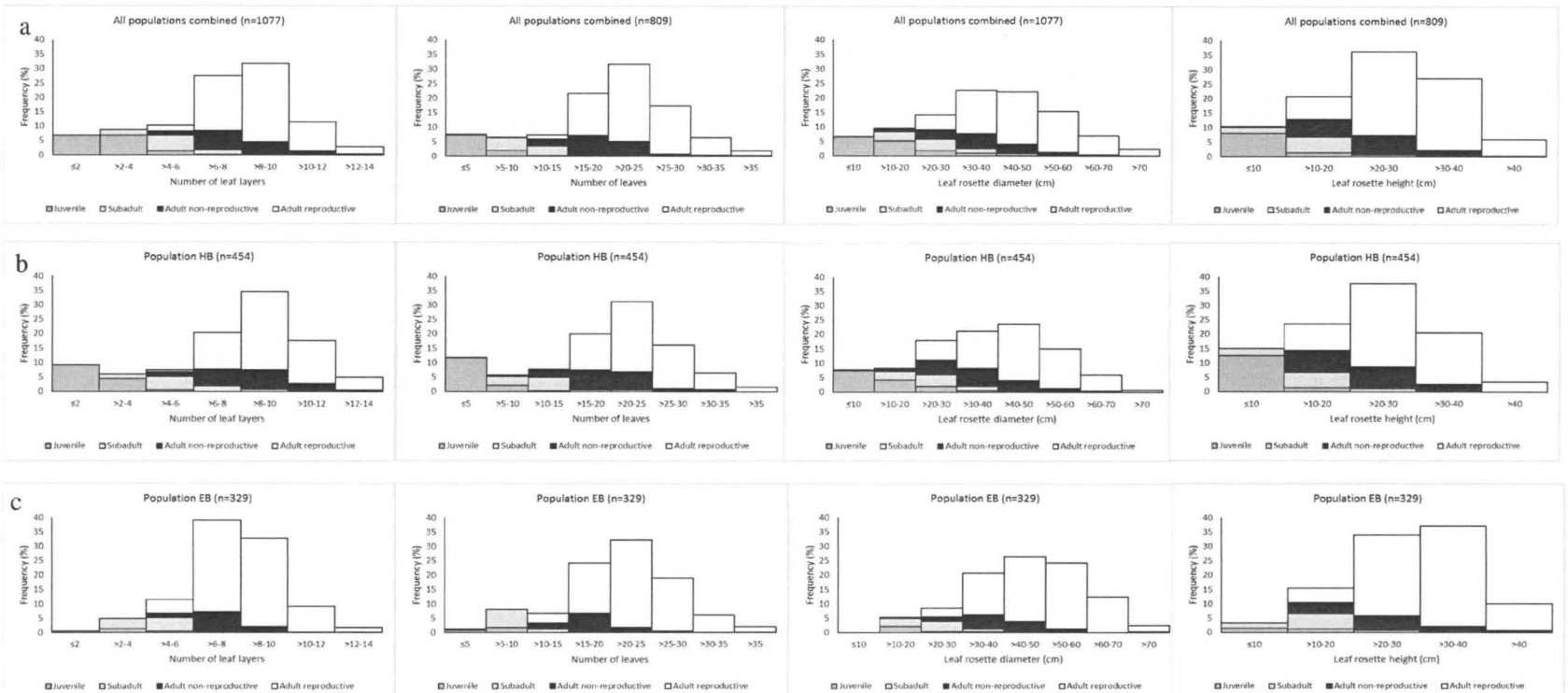

Figure 2.4. *Aloe lettyae* size class distributions (number of leaf layers and leaves, leaf rosette diameter and height) and life history stages (juvenile, subadult, non-reproductive and reproductive adult) for (a) all individuals sampled, and for populations (b) HB and (c) EB separately. Juveniles include seedlings.

Overall, size distribution frequencies for the number of leaf layers and leaves, as well as for leaf rosette diameter and height, within a clump or population appeared fairly consistent (Figure 2.4 and Appendix 2.3). Size structure did not vary greatly between populations, although population W (Appendix 2.3 (ah)) growing on a firebreak shaded by eucalyptus plantations had larger plants in terms of leaf rosette diameter than the other populations. The SCDs were separated into four groups. Most of the size distribution frequencies (76%) which included the large populations HB and EB, were bell-shaped, three (9%) were J-shaped (HB O, LE, BL2) (Appendix 2.3 (aa, ab, ac), four clumps (12%) had an irregular SCD (EB 5, HB F, HB Jo1, W) (Appendix 2.3 (ae, af, ag, ah)), and one clump (3%) had an inverse J-shaped SCD (HB Ro1) (Appendix 2.3 (ad)). The small clump HB Ro1 grew in a rocky area (17% rock), had a very high recruitment rate, and had a very high density estimate of 15000 plants/ha compared to a mean of 1041 plants/ha in population HB.

Physical damage and vegetative regeneration

The levels of herbivory (mainly insects, but also cattle and occasionally baboons) varied between populations and tended to be higher in WGG fragments frequented by cattle such as populations RE and FG than in those not accessed by cattle (EB, W) (Table 2.3). Disease (rust, aloe cancer) incidence and severity recorded on *A. lettyae* were generally low. Rust and aloe cancer incidences were highest in BL2, a population with low reproductive output (Chapter Three) (Table 2.3). The incidence of pests (white scale, aphids) was very low (<0.2%), and no evidence of plant harvesting was found. In terms of the regeneration potential following a disturbance, it was observed that damaged *A. lettyae* plants may sprout from the apical meristem (Figure 2.2j), i.e. grow onward from a surviving stem apex, or produce basal suckers (Figure 2.2k); the latter then developing into multiple leaf rosettes on a single *A. lettyae* plant (Figure 2.2l). Only 3% of all plants surveyed in this study had more than one rosette, likely due to crown damage which had occurred in previous years.

Table 2.3. *Aloe lettyae* herbivory and diseases (incidence (%) and visually estimated severity (%)) in surveyed populations.

		Herbivory (%)		Rust (%)		Aloe cancer (%)	
Population	n	Incidence	Severity	Incidence	Severity	Incidence	Severity
HB	454	37	4.0	6	0.7	1.5	0.4
EB	329	22	2.4	8	0.8	0	0
FG	26	46	4.4	8	1.5	0	0
RE	60	92	18.5	13	0.9	0	0
LE	80	15	2.3	3	0.1	3.8	0.3
BL1	20	60	7.5	5	0.3	0	0
BL2	20	10	0.8	15	1.5	10	0.8
W	95	14	1.4	0	0	0	0

Habitat profile

Elevation ranged from 800–1800 m above sea level. *Aloe lettyae* occurred on lower to upper slopes which were all gentle to moderate (5°–20°). Aspects were variable, although mostly northerly and westerly (Appendix 2.1). Regression analyses of the variables concerning the vegetation surrounding *A. lettyae* plants and standing plant biomass showed that significant but very weak positive relationships existed between grass height and standing plant biomass as well as between woody height and standing plant biomass (Table 2.4).

Table 2.4. Relationships between habitat variables estimated (percentage bare soil, grass, forb and woody cover) or measured (maximum height of grass, forbs and woody plants) and calculated standing plant biomass (kg/ha) for all populations surveyed (n = 820 plants). The best fit curve was selected. Significance of relationship *** ($P < 0.001$), ** ($P < 0.01$), * ($P < 0.05$), NS ($P > 0.05$). Bold values represent significant relationships.

Variable	R^2	Curve	P
Bare soil (%)	**0.0539** (negative)	Exponential	***
Grass cover (%)	**0.0085** (negative)	Linear	**
Forb cover (%)	0.0245 (negative)	Polynomial	NS
Woody cover (%)	**0.1150** (positive)	Linear	***
Grass height (cm)	**0.2828** (positive)	Polynomial	***
Forb height (cm)	**0.1267** (positive)	Linear	***
Woody height (cm)	**0.2025** (positive)	Linear	***

For all surveyed populations combined, habitat profile within 1 m of an *A. lettyae* individual (near) and ≥ 5 m away from *A. lettyae* plants in adjacent non-populated areas (away) only differed significantly with regard to the maximum height of woody plants which were slightly taller near aloes than further away (Mann-Whitney U test; $P < 0.05$; Table 2.5). The following variables differed in individual populations: percentage forbs (Mann-Whitney U test; $P < 0.05$) in population LE which was located in a disturbed habitat; forb (Mann-Whitney U test; $P < 0.05$) and grass height (Mann-Whitney U test; $P < 0.01$) as well as biomass (Mann-Whitney U test; $P < 0.001$) in population RE where grazing occurred and habitat differences were visible; and percentages of rock and forbs, forb height and biomass (Mann-Whitney U test; $P < 0.05$) in the small rocky clump BL1 (Table 2.6). However, in populations HB, EB, FG and BL2 (representing 90% of the observations), no difference was detected between the habitat profile near an aloe and that further away (Mann-Whitney U Test, $P > 0.05$). Therefore, the data on percentages cover, maximum plant heights and biomass were combined per population for further analysis of habitat profile.

Table 2.5. Habitat profile within 1 m of an *Aloe lettyae* individual (near) and ≥5 m away from plants in adjacent non-populated areas (away): visually estimated percentage aerial cover of bare soil, rock, grass, forbs and woody plants, maximum height (cm) of grass, forbs and woody plants and calculated standing plant biomass (kg/ha) for all surveyed populations combined. Mean ± SE; Mann-Whitney U Test, * for $P < 0.05$ and NS for $P > 0.05$). Bold values represent significant difference.

Variable	Near aloe		Away from aloe		
	Mean ± SE	n	Mean ± SE	n	P
Bare soil cover (%)	8.7 ± 0.4	378	8.1 ± 0.4	442	NS
Rock cover (%)	1.2 ± 0.4	378	0.6 ± 0.2	442	NS
Grass cover (%)	51.4 ± 1.0	378	51.1 ± 0.9	442	NS
Forb cover (%)	29.4 ± 0.9	378	31.1 ± 0.8	442	NS
Woody cover (%)	9.3 ± 0.7	378	9.1 ± 0.7	442	NS
Grass height (cm)	123.0 ± 2.0	377	120.1 ± 1.9	442	NS
Forb height (cm)	61.1 ± 1.4	364	61.1 ± 1.4	427	NS
Woody height (cm)	**73.1 ± 2.4**	193	**67.5 ± 2.45**	219	*
Standing biomass (kg/ha)	5678 ± 150	378	5685 ± 146	442	NS

All habitat profile variables differed highly significantly between populations (Table 2.6). The percentage bare soil cover was highest in the disturbed population LE (18%) and differed significantly between the two largest populations HB (7%) and EB (9%) ($H_{6,820}$ = 50.0331, P<0.0001). The percentage rock cover was generally very low (<4%), except in BL1 (17%) ($H_{6,820}$ = 266.8566, P < 0.0001). Grasses dominated the aerial cover with percentages ranging from 40% (BL1, rocky) to 74% (RE, grazed), and 50% in populations HB and EB ($H_{6,820}$ = 90.8201, P < 0.0001). Forb cover differed significantly between populations, ranging from 8% (RE, grazed) to 39% (BL2) and differed between the two largest populations HB (37%) and EB (26%) ($H_{6,820}$ = 217.1004, P < 0.0001). The woody cover percentages ranged from 5% (HB) to 15% (EB), which was a significant difference ($H_{6,820}$ = 81.3334, P < 0.0001). The maximum height of grass, forbs and woody plants was lowest in the grazed population RE and differed significantly between populations (grass: $H_{6,819}$ = 68.5062, P < 0.0001; forbs: $H_{6,791}$ = 77.4312, P < 0.0001; woody plants: $H_{6,411}$ = 30.5005, P < 0.0001). Standing plant biomass differed between populations ($H_{6,820}$ = 28.4679, P = 0.0001) and populations surveyed in the drier 2016 summer tended to have a lower standing plant biomass than those surveyed in 2017, although this was not significant (Table 2.6).

Across all populations, no differences were observed in the tallest plant species occurring within 1 m of an *A. lettyae* individual and ≥5 m away from *A. lettyae* plants, except for the woody species *Rabdosiella calycina* which was more abundant near aloes than further away. The most common (abundance >10%, Appendix 2.4) forb species were *Berkheya setifera* and *Helichrysum platypterum*, woody species were *Athrixia phylicoides* and *Fadogia homblei*, and grass species were *Cymbopogon nardus, Trachypogon spicatus* and *Loudetia simplex.*

Table 2.6. Habitat profile of *Aloe lettyae* populations surveyed. Visually estimated percentage aerial cover of bare soil, rock, grass, forbs and woody plants, maximum height (cm) of grass, forbs and woody plants, and calculated standing plant biomass (kg/ha). For each population, data sets (collected within 1 m of an *A. lettyae* individual (near) and ≥ 5 m away from *A. lettyae* plants in adjacent non-populated areas (away)) were combined. Mean ± SE; significant differences are indicated by different letters (Kruskal-Wallis Tests, $P \leq 0.0001$, Bonferroni inequality).

Variable	HB		EB		LE		RE		FG		BL2		BL1	
	Mean ± SE	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE	n
Bare soil cover (%)	6.9 ± 0.3^{be}	353	9.4 ± 0.5^{ad}	317	17.6 ± 2.6^{a}	40	7.7 ± 1.2^{cde}	40	7.2 ± 0.7^{bd}	30	3.9 ± 1.0^{b}	30	11.0 ± 3.3^{abc}	10
Rock cover (%)	1.0 ± 0.3^{b}	353	0.1 ± 0.1^{b}	317	0.1 ± 0.1^{b}	40	3.6 ± 0.6^{a}	40	0^{b}	30	0.1 ± 0.1^{b}	30	16.8 ± 9.9^{a}	10
Grass cover (%)	49.9 ± 0.9^{b}	353	49.4 ± 1.1^{b}	317	65.7 ± 3.3^{a}	40	74.1 ± 2.0^{a}	40	46.3 ± 1.9^{b}	30	45.8 ± 2.9^{b}	30	40.0 ± 6.0^{ab}	10
Forb cover (%)	37.4 ± 0.9^{a}	353	26.0 ± 0.9^{b}	317	13.3 ± 1.9^{bc}	40	7.8 ± 0.8^{c}	40	38.0 ± 1.7^{a}	30	39.2 ± 3.1^{a}	30	26.7 ± 6.5^{abc}	10
Woody cover (%)	4.8 ± 0.4^{ac}	353	15.1 ± 1.0^{d}	317	3.3 ± 0.6^{ab}	40	6.8 ± 1.1^{bef}	40	8.5 ± 1.6^{cde}	30	11.0 ± 3.1^{afd}	30	5.5 ± 3.2^{abcd}	10
Grass height (cm)	125.3 ± 2.0^{cef}	353	127.6 ± 2.3^{def}	317	107.1 ± 4.2^{abc}	40	84.6 ± 5.3^{ag}	40	112.3 ± 7.7^{bfg}	30	93.5 ± 4.9^{abd}	30	100.0 ± 12.3^{abcd}	9
Forb height (cm)	65.9 ± 1.4^{bf}	352	58.7 ± 1.4^{bc}	299	59.1 ± 5.6^{bd}	32	29.3 ± 3.1^{a}	39	66.3 ± 4.4^{bf}	30	61.9 ± 5.9^{bc}	30	79.4 ± 7.0^{acdef}	9
Woody height (cm)	73.2 ± 3.2^{bc}	130	72.1 ± 2.5^{bc}	192	59.5 ± 6.7^{ab}	21	46.6 ± 3.1^{a}	34	75.6 ± 6.2^{bc}	18	81.5 ± 5.6^{bc}	13	88.3 ± 17.0^{ac}	3
Standing biomass (kg/ha)	5665 ± 154^{bd}	353	6054 ± 188^{cd}	317	5338 ± 245^{bd}	40	4197 ± 294^{ab}	40	6218 ± 607^{ad}	30	4257 ± 227^{a}	30	4437 ± 836^{ad}	10
Year surveyed	2017		2017		2016		2016		2017		2016		2016	

Discussion

National distribution

Aloe lettyae has a very restricted geographical range, with almost all populations occurring in the WGG as outlined by Mucina *et al.* (2006), except the three small, seemingly outlying populations (*viz.* O2, PR and W) in transformed habitats (Figure 2.1). Historically, *A. lettyae* also occurred in several now transformed areas which were searched during this study (Figure 2.1). Letty collected the first *A. lettyae* specimen at "Duiwelskloof; Westfalia Farm" in 1932 (Letty 299). Reynolds then collected the *A. lettyae* type specimen at "Duiwelskloof, in long grass" in 1937 (Reynolds 2339). In 1960, Scheepers collected *A. lettyae* at Duiwelskloof; Westfalia Estate and provided a very detailed locality description (Scheepers 904), but the species does not occur there anymore. The lower part of Westfalia Estate has been farmed since 1900 and was probably transformed to a large extent by about 1930, leaving only seral communities (Scheepers 1978). Scheepers (1978) also stated that *A. lettyae*, a relic of the formerly widespread grassveld, is one of the pioneer species in seral communities e.g. on annually burned firebreaks, which may explain the occurrence of *A. lettyae* in disturbed areas (VE road side, W fire break and dam wall; HB edge of donga) and secondary grassland (O2) observed in this study. Further *Aloe lettyae* was observed at Stylkop (ST) in the Groot Letaba Valley (Reynolds, undated note at the National Herbarium) as well as north-east of Duiwelskloof (Reynolds 1969). In 1936, *A. lettyae* was collected in "Magoebaskloof" (MK) (van der Merwe 108) where WGG occurred according to photographic evidence dated 1917.

The WGG map obtained from Mucina *et al.* (2006) is part of a national map drawn up at a scale of 1:1 000 000 and small patches of vegetation (<20 ha) were not mapped (L. Powrie, pers. comm.). Desmet *et al.* (2013) revised the WGG polygon and increased the estimated original WGG extent from 340 km^2 (Mucina *et al.* 2006) to 408 km^2. A revision of the WGG boundaries on the national map is therefore underway for which an extension of WGG into Mamabolo Mountain Bushveld and into Northern Escarpment Quartzite Sourveld on the south western side has been proposed. On the north eastern side of the WGG (Westfalia Estate), WGG boundary adjustments may prove problematic as the

habitat has been completely transformed (A. Dayaram, pers. comm.). These boundary changes will result in the peripheral *A. lettyae* populations on the boundary with Mamabolo Mountain Bushveld (VE, RE, DR) and population PR (currently in Northern Escarpment Quartzite Sourveld) being located within WGG. Excluding the two small pioneer populations at Westfalia Estate in completely transformed habitat and population O2 which occurs in secondary grassland, this confirms *A. lettyae* as a WGG endemic.

Population size and plant density

Aloe lettyae populations are aggregated and occur in tall, dense grassland vegetation in which plants are difficult to detect. This impedes the ability to locate populations in the landscape as well as individuals within the population and slows down sampling. The PCQ method, developed for sampling tree composition in forests (Cottam and Curtis 1956), has been shown to underestimate forb and grass species densities in grassland populations which often exhibit aggregation (Risser and Zedler 1968; Good and Good 1971; Mitchell 2007). While the results obtained with the NN and PCQ (Mitchell 2007) methods were fairly consistent for the main population HB, the NN method gave a 50% higher value for the second biggest population EB, and discrepancies between estimates obtained with the two methods have been in observed in other studies (Marom 2006; Cousins *et al.* 2014). Comparing initial estimates of flowering *A. lettyae* plants with the actual plant counts in small clumps showed that actual abundance may be underestimated by 53%. Populations have also been underestimated in *A. peglerae* (Arena *et al.* 2015) and in the Asphodelaceae taxon *Haworthia koelmaniorum* (Witkowski and Liston 1997). In this study, the total number of *A. lettyae* individuals for all populations combined was estimated at 8500 which is likely an underestimate.

Plant size and stage demographics

Numbers of leaf layers and leaves, and leaf rosette diameter and height measurements resulted in identical life stage percentages for the respective pairs. However, counting leaf layers and measuring the leaf rosette diameter is not only easier and faster to do than counting leaves and measuring the leaf

rosette height, but the proportion of juveniles detected is higher. Based on number of leaf layers and leaf rosette diameter, most size class distributions (SCDs) (76%) are bell-shaped, which included the two largest populations and suggested that these populations were probably stable (Cousins *et al.* 2014), 9% J-shaped, 3% had an inverse J-shaped SCD and 12% had an irregular SCD. For all surveyed populations combined, 15% of the plants were juveniles, 9% subadults, 15% non-reproductive adults and 61% reproductive adults. There was a clear difference in population structure between the main population and the second largest population, with the main population having a higher proportion of juveniles (14%) than the second largest population (3%). Although bell-shaped SCDs for both populations suggested stable populations, the low percentages of juveniles and non-reproductive subadults are of concern as they indicate an apparent current and historic lack of recruitment. Very few *A. lettyae* juveniles were found in this study, although they may have been present, as they were difficult to detect in the dense vegetation. This may be due to low reproductive output (Chapter Three) and/or due to disturbances such as fire which causes mortality in the smallest size classes (Wilson and Witkowski 2003). This study has also shown that *A. lettyae* seedling survival was significantly lower in burned (26%) than in unburned areas (76%) (Chapter Three). Recruitment levels in the relatively long-lived (average adult lifespan of 60 years) *A. peglerae* were also found to be low, and on average, seedlings grew into juveniles after four years, and juveniles matured to adults within six years (Pfab and Scholes 2004). Monitoring a summer flowering *A. vossii* population in an area burnt annually for many years indicated that the plants were stunted and that there were very few seedlings. Seeds were produced but they did not germinate, or seedlings did not survive (Willis and Willis 1995). Further, successive annual fires over a long period are thought to impede recruitment in grass aloe populations (Craib 2005). For over 20 years, fire was applied annually to the largest remaining WGG fragment to protect timber plantations and infrastructure in the area. Due to concerns over *A. lettyae*'s recruitment and that of other slow-growing plants (P.J.D. Winter, pers. comm.), this fire policy was revised in 2014 (Dzerefos and Witkowski 2016; Dzerefos *et al.* 2017) and an adaptive fire management strategy adopted which was based on opinions rather than on empirical evidence. However, this study provided baseline

A. lettyae population data for routine monitoring of populations to observe changes and inform management decisions.

Habitat profile

The total area of WGG fragments, excluding the secondary grasslands, surveyed in this study was 613 ha (Appendix 2.1) which constitutes 1.8% of the original WGG extent. Visually rated condition was good, medium and poor in 53%, 27% and 20% of the total surveyed WGG area, respectively. Predominant land uses surrounding the WGG fragments were timber plantations (30% of the fragments) and indigenous forests (15%) (Appendix 2.1) which reflect the extensive WGG transformation to exotic plantations and the occurrence of natural forest patches in the original WGG area (Niemandt and Greve 2016). Overall, threats to the WGG fragments were identified as mostly fire related (42% of the fragments), followed by cattle grazing and the invasion of alien plants (16% each) (Appendix 2.1). In a long-term grassland burning and mowing experiment disturbance type, timing and frequency were shown to impact on grass and forb species richness (Fynn *et al.* 2004). Uys *et al.* (2004) found forbs to be relatively resilient to fire while dominant grasses were strongly influenced. Too frequent burning can lead to species loss (Drury et al. 2016). Anthropogenic fire suppression, however, also results in loss of plant species and affects small herbs the most, presumably because of their high requirement for light (Uys *et al.* 2004; Bond and Keeley 2005). Fire suppression further promotes bush encroachment (Bond *et al.* 2003; O'Connor *et al.* 2014) and a significant increase in woody abundance, cover and height following reduction in fire frequency has been found in the WGG (Thompson and Swemmer 2014; Mutileni 2016). In this study, the two largest populations HB and EB differed significantly in terms of forb and woody cover, with HB having a higher forb (37%) and lower woody cover (5%) than EB (26% and 15%, respectively) which may have been caused by differences in fire application in the past (Table 2.7) and is possibly the reason for the higher proportion of juvenile aloe plants found in HB (14%) than in EB (3%) (Figure 2.4b and c). The other important threat to WGG, grazing, has been shown to reduce or eliminate the highly sensitive forb flora (Witkowski *et al.* 2001; Scott-Shaw and

Morris 2015) which is evident in the extremely low percentage forb cover (8%) in the grazed WGG fragment RE.

For all surveyed populations combined, habitat profile variables only differed significantly with regard to the maximum height of woody plants which were slightly taller near an *A. lettyae* individual than further away and is supported by the higher abundance of the woody species *Rabdosiella calycina* observed near aloes (12%) than further away (7%), suggesting that *A. lettyae* is mostly unaffected by these variables. This is in contrast with *A. peglerae* which occurs in rocky areas associated with low surrounding vegetation (Arena *et al.* 2015). The percentage bare soil cover was highest in the disturbed population LE (18%) which had the second lowest forb cover (13%) in this study and a J-shaped SCD (Appendix 2.2 Figure ab) indicating a scarcity of juveniles and suggesting a declining *A. lettyae* population (Cousins *et al.* 2014). In contrast, the small clump (~50 m^2) HB Ro1 had an inverse J-shaped SCD (Appendix 2.2 Figure ad) and 28% rock cover around the aloes. The percentage rock cover was generally very low (<4%), when compared to sites occupied by e.g. *A. peglerae* which had a 56% rock cover (Arena *et al.* 2015). It is interesting to note that small *A. lettyae* clumps were discovered in confined, relatively recently disturbed areas (HB edge of donga in grassland; VE along road cutting; W on fire break between timber plantations with irregular SCD (Appendix 2.2 Figure ah)) which is consistent with Scheepers' (1978) observation that *A. lettyae* is one of the pioneer species in seral communities. Several succulent plant species e.g. *Euphorbia clivicola* and *Delosperma* spp. appear to be adapted to disturbances (Pfab and Witkowski 1999; Marom 2006). Young pioneer populations can act as nuclei from which plants spread slowly (Stokes and Yeaton 1995). Population O2, located in a secondary grassland (Appendix 2.1), which differs from primary grassland in term of species composition, vegetation structure and ecological functioning (SANBI 2013), may have established in this way.

Conclusion

While other populations might yet be located, the results from this study indicated that *Aloe lettyae* is restricted to 19 populations, 17 of which occur in Critically Endangered WGG remnants and

two small populations are located in transformed habitat. Low percentages of juveniles and non-reproductive subadults indicate an apparent current and historic lack of recruitment. Routine future monitoring of *A. lettyae* populations by means of counting leaf layers and measuring the leaf rosette diameter is recommended to observe changes in the population structure and to inform urgent management and conservation decisions in view of the fact that delays between the start of habitat change and local extinction of populations have been estimated at 50–100 years (Lindborg and Eriksson 2004; Krauss *et al.* 2010). This study has highlighted the need for a thorough assessment of WGG fragments, including a quantification of the species richness, to evaluate the effects of disturbances such as fires of different intensities and frequencies on biodiversity and adjust grassland management accordingly.

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Appendix 2.1. Woodbush Granite Grassland (WGG) fragments and occurrence of *Aloe lettyae* populations in Limpopo Province, South Africa.

	WGG fragment									*A. lettyae*	
Locality[a]	Size (ha)	Owner[b]	Altitudinal range (m)	Topographic position	Pre-dominant aspect	Condition[c]	Most recent burn	Threats[d]	Surrounding land use[e]	Initial estimate[f] (number of individuals)	Distance to nearest neighbouring population (km)
HB*	192	G/C	1368–1534	Upper–lower slope	N	Good	2017, part	C, F, W	H, P, W, R	1240	2.0
DR	86	P	1505–1618	Crest, upper slope	NW, NE	Medium	± 2011	C, W	I, P, R, S	35	2.3
SK	76	Com	1750–1874	Mountain top	NW	Poor	2017	C, F	I, S	40	6.0
EB1*	60	G/C	1365–1413	Crest slope	Various	Good	2016	F, OD	D, H, SG	925	1.4
EB2	20	G/C	1365–1400	Mid slope	W	Sec. grassland	2016	I	D, H, I	0	1.4
VE	40	P	1430–1520	Mid slope	NW	Medium	2014	F, I	P, S	150	1.5
WS	25	G	1770–1880	Crest	Various	Medium	2017	F	I, P	0	3.5
RE1¤	10	P	1470–1530	Mid slope	NW	Good	± 2007	C, F. I, W	I, P	120	1.5
RE2	15	P	1470–1530	Mid slope	NW	Poor	± 2007	C, F. I, W	I, P	0	1.5
FG*	14	G	1614–1746	Upper–mid slope	E	Good	2017	C	F, P	145	1.1
WO	11	G	1593–1736	Upper slope	NE	Poor	2017	B, F	F, P	0	5.5
ES1	7	G	1430–1471	Upper slope	N	Good	2014	F	F, H, P	0	7.8
ES2	4	G	1401–1430	Lower slope	E	Poor	2016	B, F	F, H, P	0	7.8
WP	10	G	1808–1831	Mountain top	Various	Good	2017	F, OD	F, P	0	3.4
WF	10	G	1662–1707	Upper slope	W	Good	2017	F	F, P	0	7.2
O1	9	P	1327–1398	Upper slope	NW	Good–medium	2016	F	P, S	20	3.4
O2	10	P	1356–1369	Lower slope	NW	Sec. grassland	± 2007	I, W	O, S	200	3.4
LE¤	9	G	1374–1402	Mid slope	NW	Poor	2017	I	H, I, O	195	1.4
BL¤	9	G	1490–1607	Lower slope	SE	Good	2017	F	F, P	150	0.9
BU	6	G	1611–1721	Upper slope	SE	Good	2017	C, I, F	F, I, P, S	15	0.9
FH	6	P	1616–1685	Upper slope	NE	Medium	± 2014	F, I	F, P, S	50	1.1
SB1	6	P	1396–1420	Upper slope	S	Poor	> 15 years	F, I, W	P, R, W	0	6.6
SB2	5	G	1390–1416	Upper slope	E	Good	2017	B, F	F, H, R	0	6.6
RR	< 1	G	1473	Mid slope	N	Medium	Unknown	Con	P, R	135	2.0
G	< 1	Com	1196	Lower slope	N	Medium	2015	C	O, D	140	4.7
PR	< 1	P	1555–1577	Mid slope	NE	Poor	2016	C, F	P, W	10	1.2
W¤	< 1	P	863	Lower slope	W	Transformed	2017	F	P, R, D	150	13.2

[a] ¤ population surveyed in 2016; * population surveyed in 2017.

[b] Com = community owned; G = government owned; G/C = part of government owned land in conservation area; P = privately owned.

[c] Condition rated visually on a scale of good–poor, or classified as secondary grassland.

[d] B = bracken; C = cattle; Con = construction; F = misapplication of fire (too frequent or infrequent); I = invasive alien plants; OD = off-road driving; W = woody indigenous plants encroaching.

[e] D = dam; F = forest; H = habitation; I = invasive alien plants; O = orchard; P = timber plantation; R = road/railway; S = scrub; SG = secondary grassland; W = watercourse.

[f] Estimate of reproductive plants at the start of field work in summer 2016.

Appendix 2.2

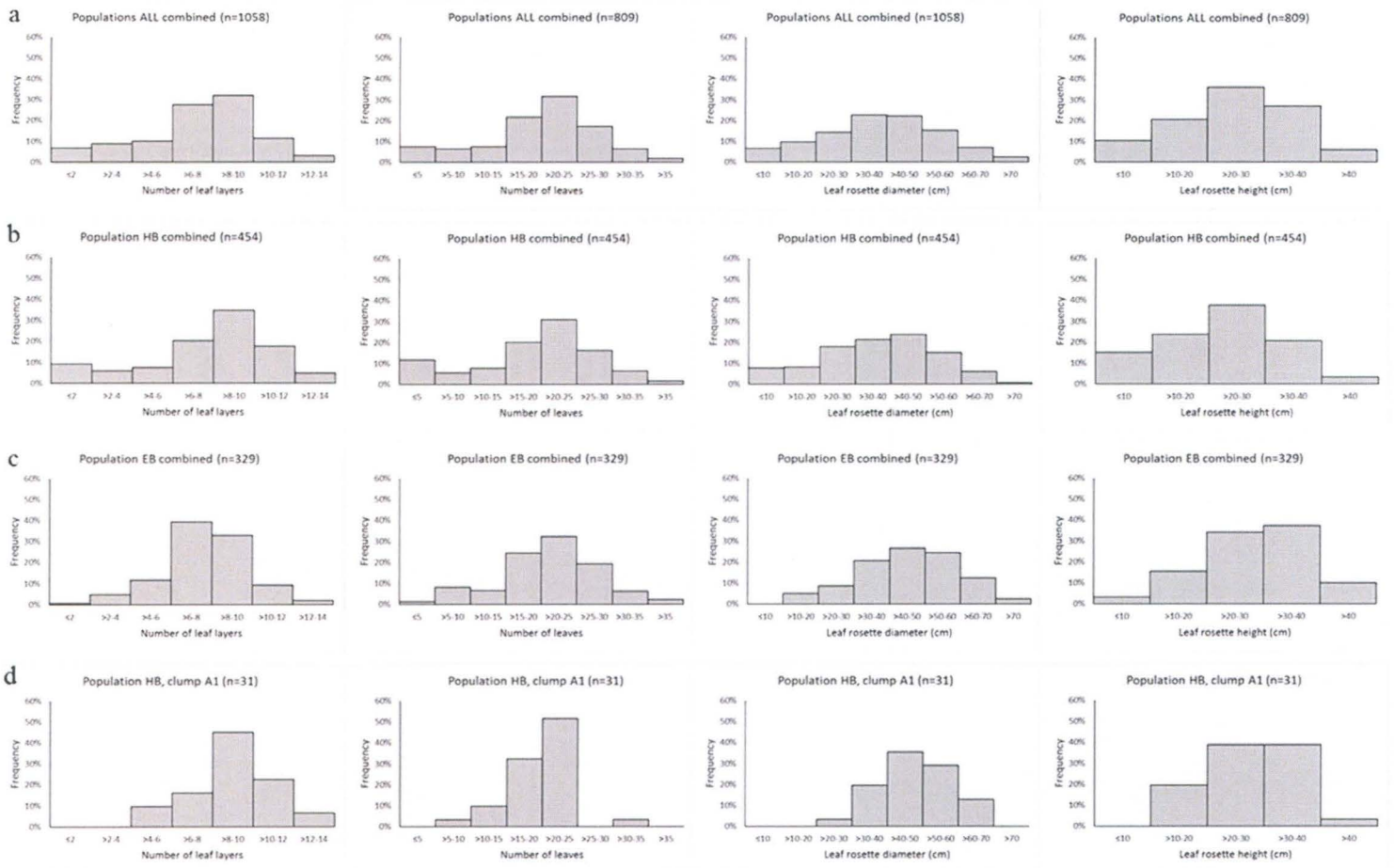
R^2 values and best fit curves of regression analyses of the relationships between clump size (m^2) and aggregation (Pielou index) and the number of *Aloe lettyae* plants and their density (plants/ha) as determined by the Nearest Neighbour (NN) and the Point Centred Quarter (PCQ) methods as well as the aggregation index (Pielou 1959) of *A. lettyae* clumps sampled along transects in populations HB, EB and FG. PCQ was calculated according to Mitchell (2007) and Pollard (cited by Krebs 2014). For all clumps a > (n – 1) / n, indicating an aggregated distribution. Significance of relationship *** (P < 0.001), ** (P < 0.01), * (P < 0.05), NS (P > 0.05); bold values represent significant relationships.

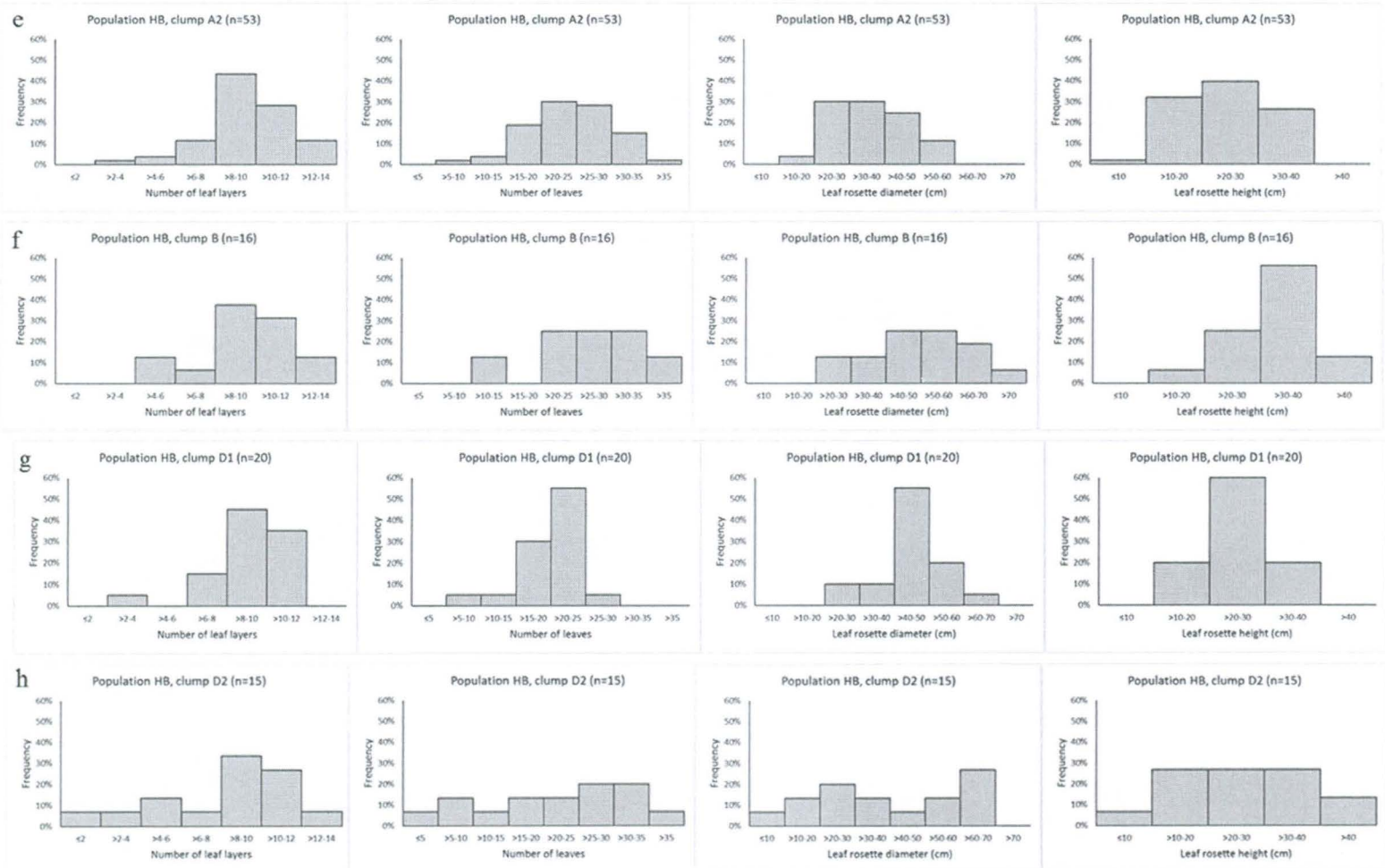
Variable 1	Variable 2	PCQ Mitchell			PCQ Pollard			NN		
		R^2	Curve	P	R^2	Curve	P	R^2	Curve	P
Clump size (m^2)	Plants	0.27	Power	NS	**0.52**	Power	*	**0.61**	Exponential	***
	Density	0.05	Polynomial	NS	0.05	Polynomial	NS	0.20	Polynomial	NS
Aggregation	Plants	**0.57**	Polynomial	***	0.21	Polynomial	NS	**0.42**	Polynomial	*
	Density	**0.37**	Power	*	0.12	Linear	NS	**0.38**	Polynomial	**
PCQ Mitchell	Plants				**0.84**	Polynomial	***	**0.58**	Polynomial	**
	Density				**0.96**	Polynomial	***	0.24	Power	NS
PCQ Pollard	Plants							**0.53**	Exponential	***
	Density							0.15	Power	NS

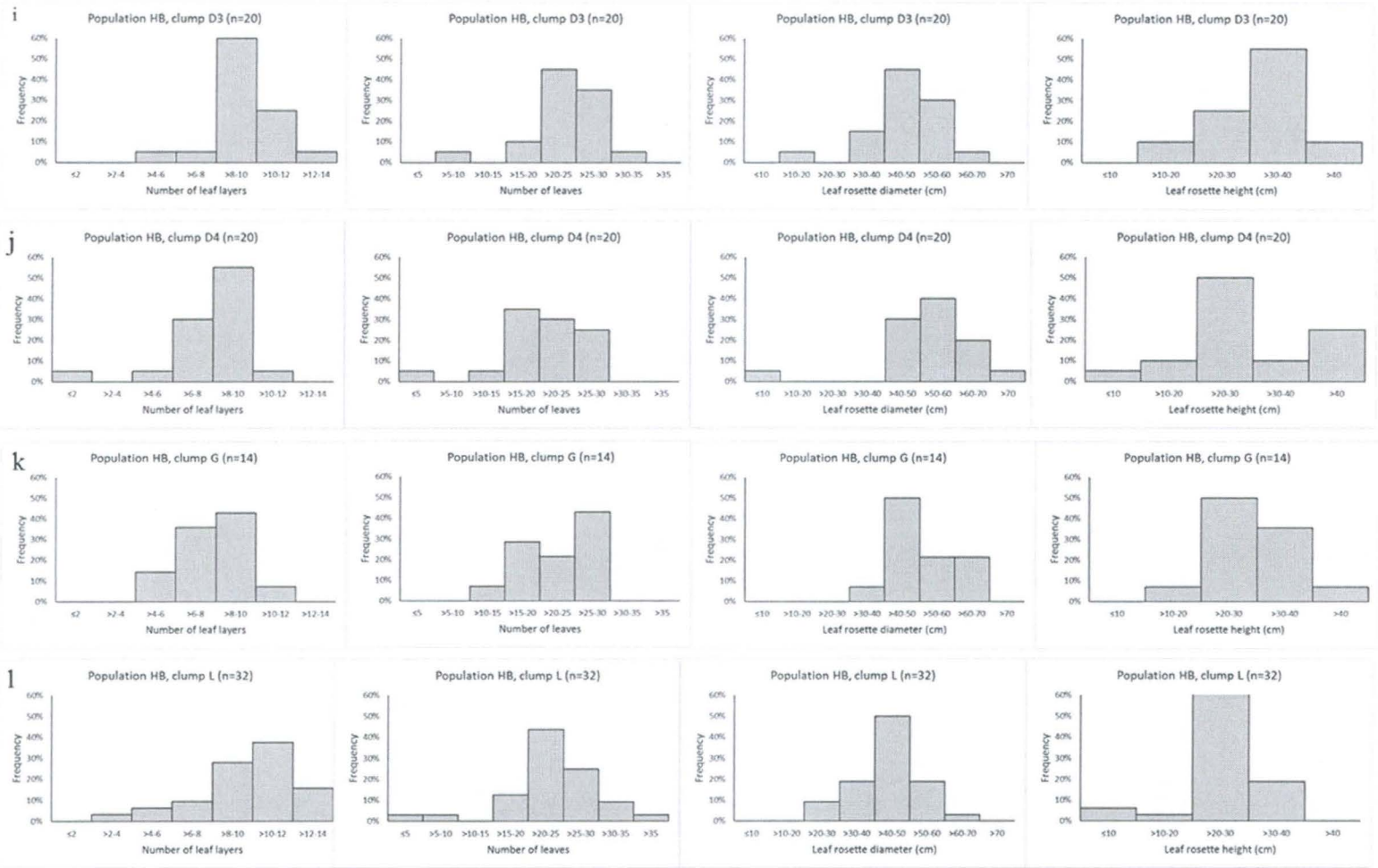
Appendix 2.3

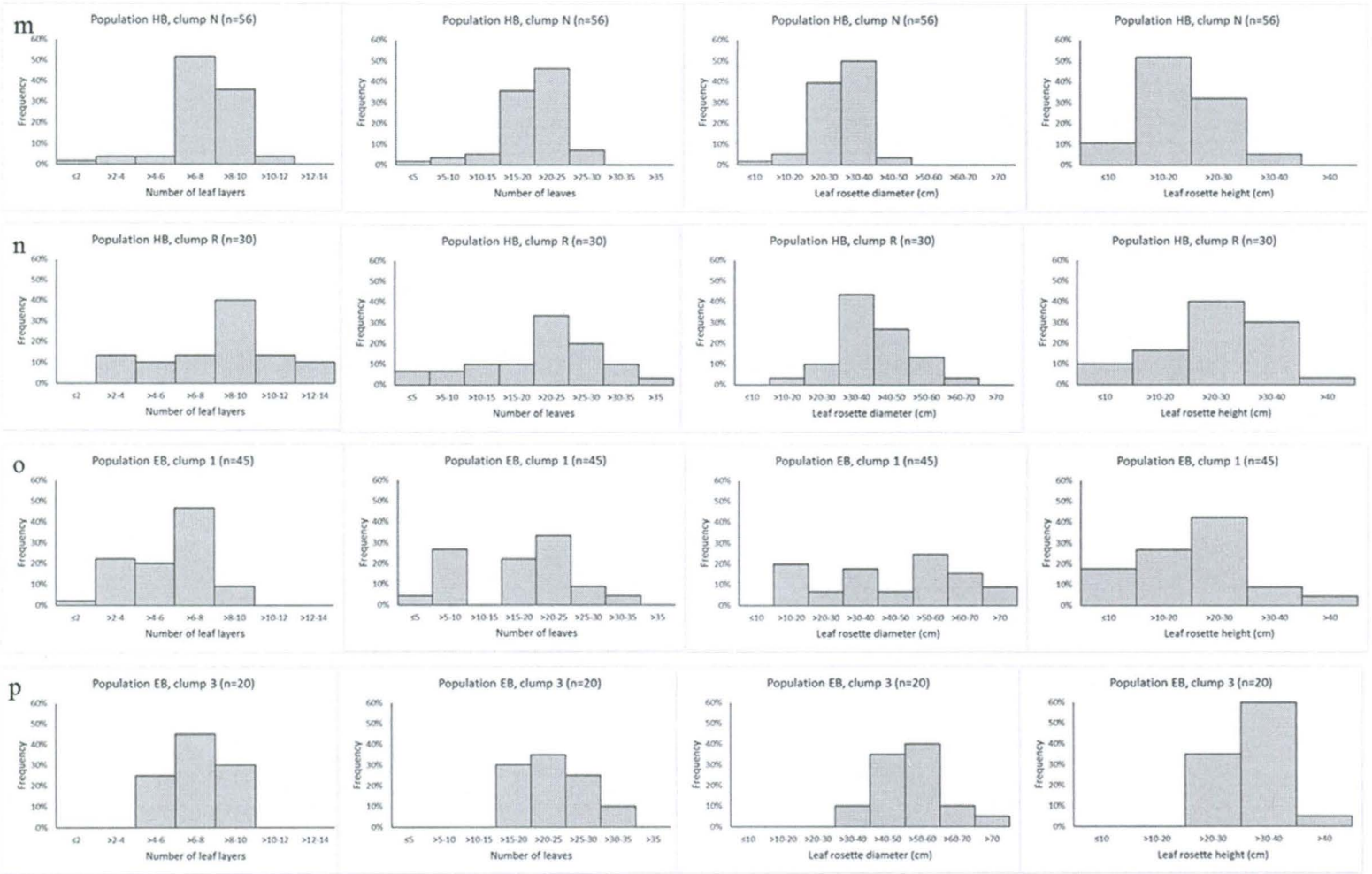
Size class distributions (SCDs) based on number of leaf layers and leaves, leaf rosette diameter and height for all *Aloe lettyae* clumps and populations surveyed, separated into groups (Bell-shaped, J-shaped, Inverse J-shaped and Irregular). Number of plants surveyed (n) on graphs in parentheses.

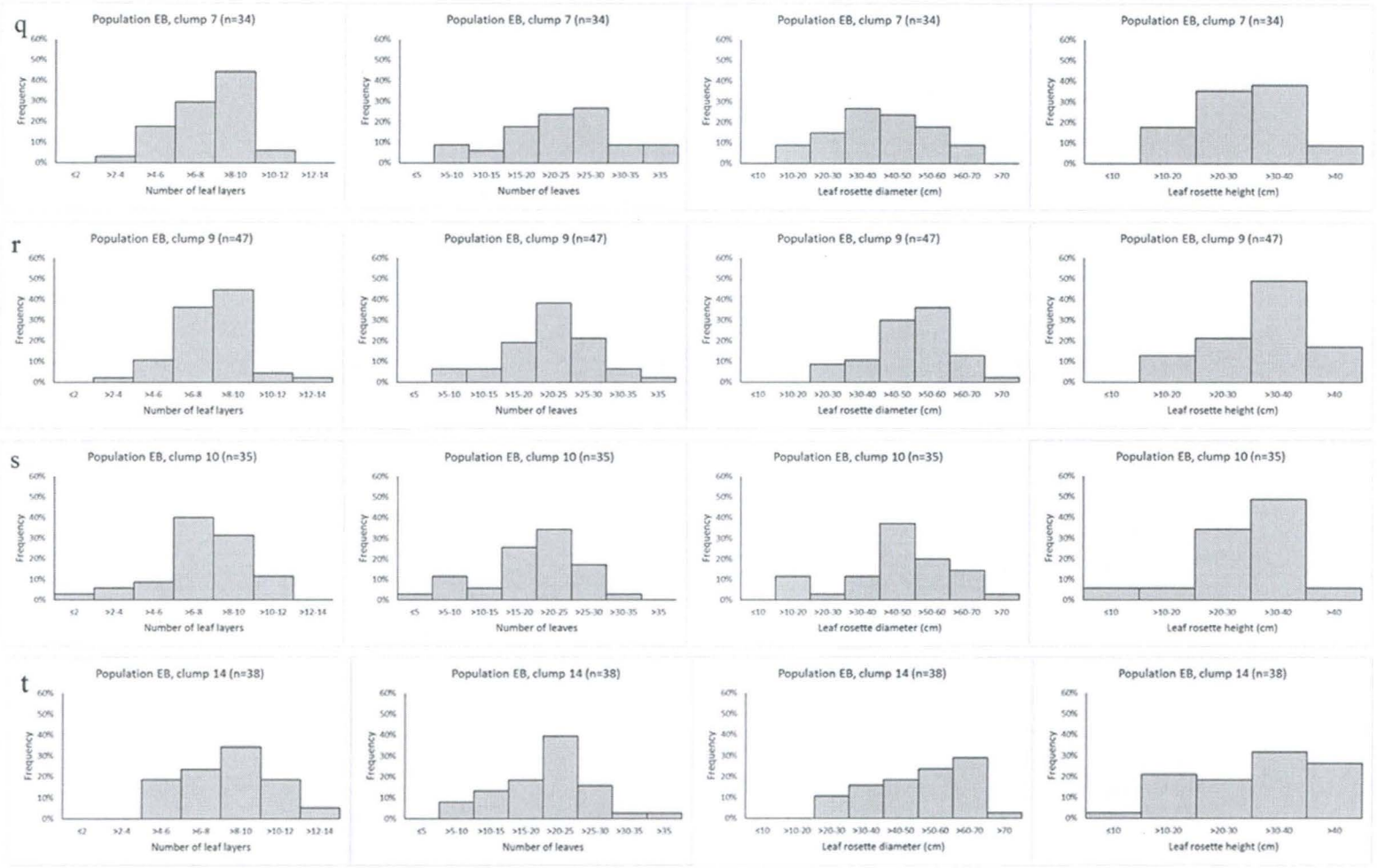
Group 1: Bell-shaped SCD

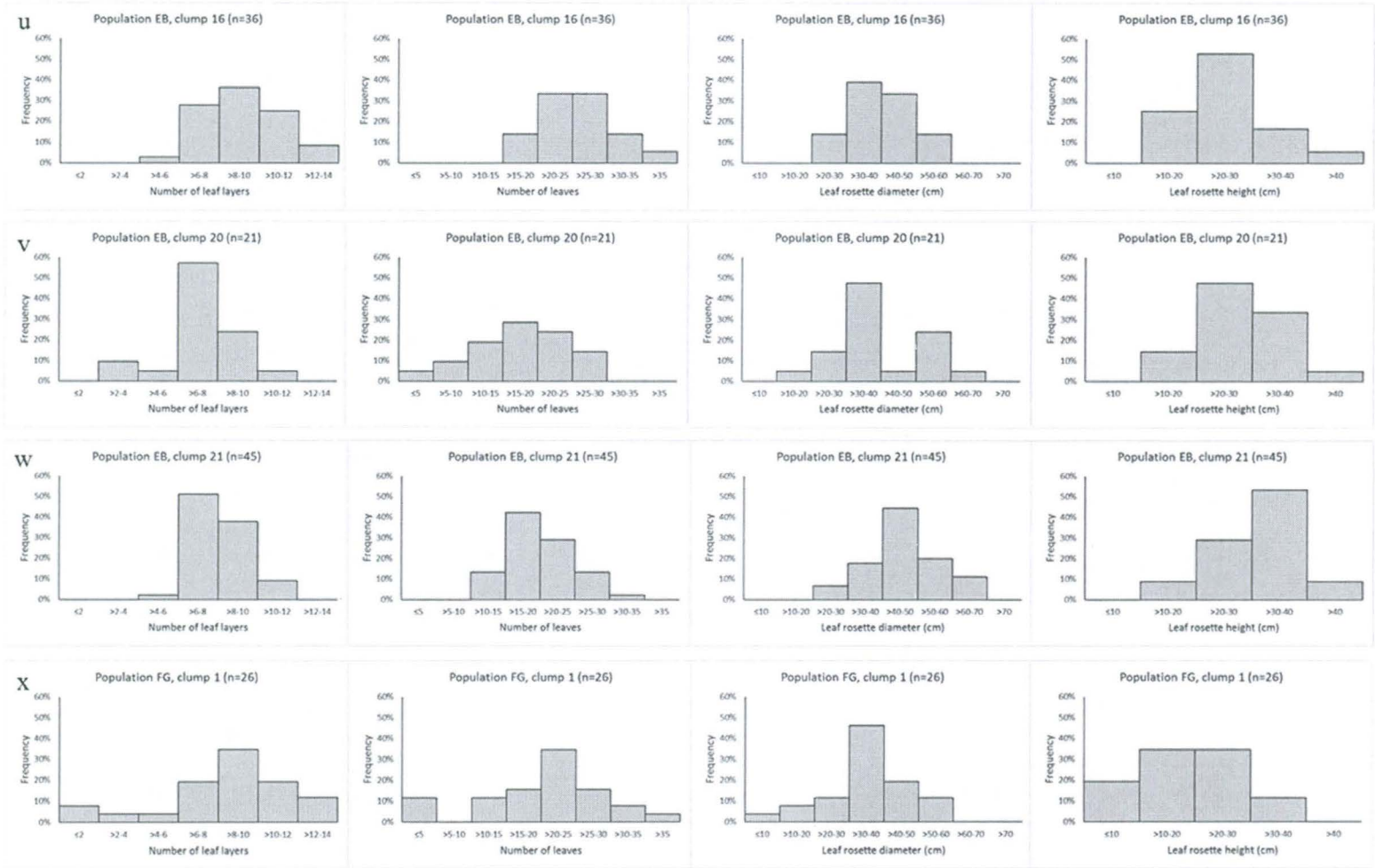


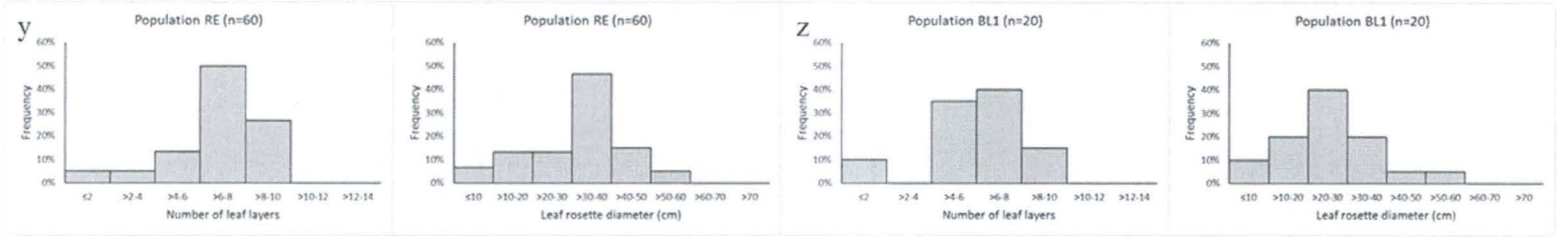

e
Population HB, clump A2 (n=53)
Frequency
Number of leaf layers
Number of leaves
Leaf rosette diameter (cm)
Leaf rosette height (cm)
f
Population HB, clump B (n=16)
g
Population HB, clump D1 (n=20)
h
Population HB, clump D2 (n=15)


i
Population HB, clump D3 (n=20)
Frequency
Number of leaf layers
Number of leaves
Leaf rosette diameter (cm)
Leaf rosette height (cm)
j
Population HB, clump D4 (n=20)
k
Population HB, clump G (n=14)
l
Population HB, clump L (n=32)

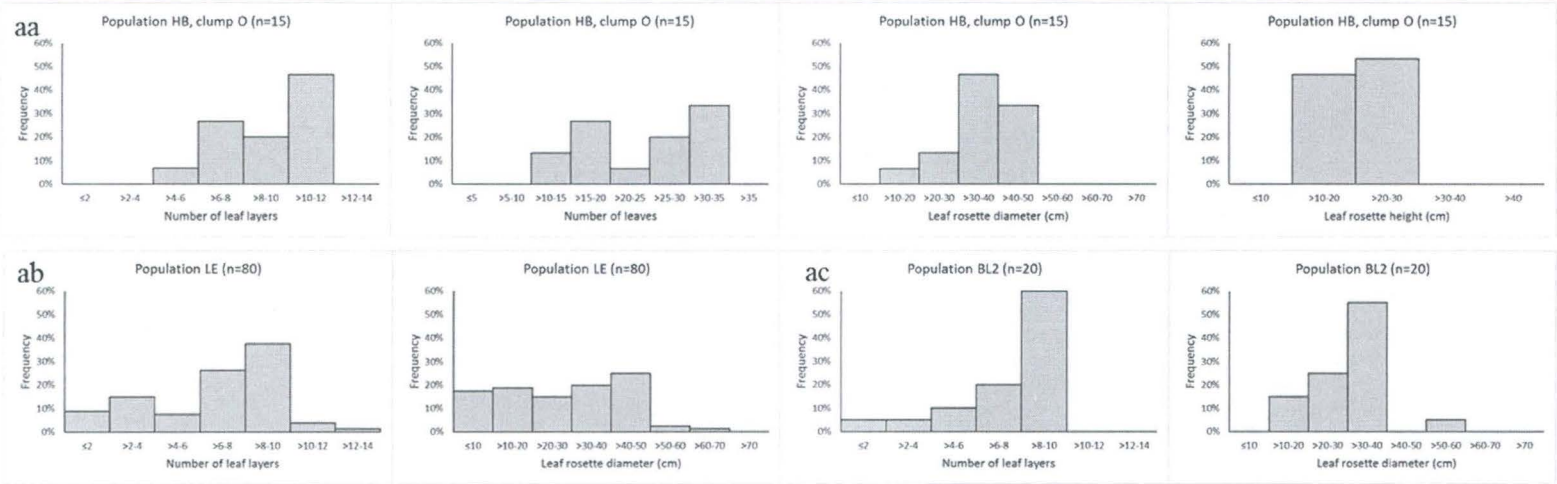

m
Population HB, clump N (n=56)
Frequency
Number of leaf layers
Number of leaves
Leaf rosette diameter (cm)
Leaf rosette height (cm)
n
Population HB, clump R (n=30)
o
Population EB, clump 1 (n=45)
p
Population EB, clump 3 (n=20)


q
Population EB, clump 7 (n=34)
Frequency
Number of leaf layers
Number of leaves
Leaf rosette diameter (cm)
Leaf rosette height (cm)
r
Population EB, clump 9 (n=47)
s
Population EB, clump 10 (n=35)
t
Population EB, clump 14 (n=38)

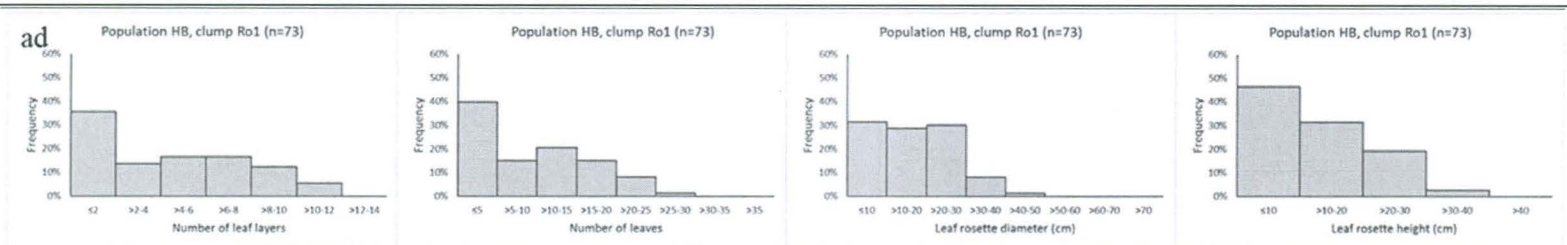

u
Population EB, clump 16 (n=36)
Frequency
Number of leaf layers
Number of leaves
Leaf rosette diameter (cm)
Leaf rosette height (cm)
v
Population EB, clump 20 (n=21)
w
Population EB, clump 21 (n=45)
x
Population FG, clump 1 (n=26)



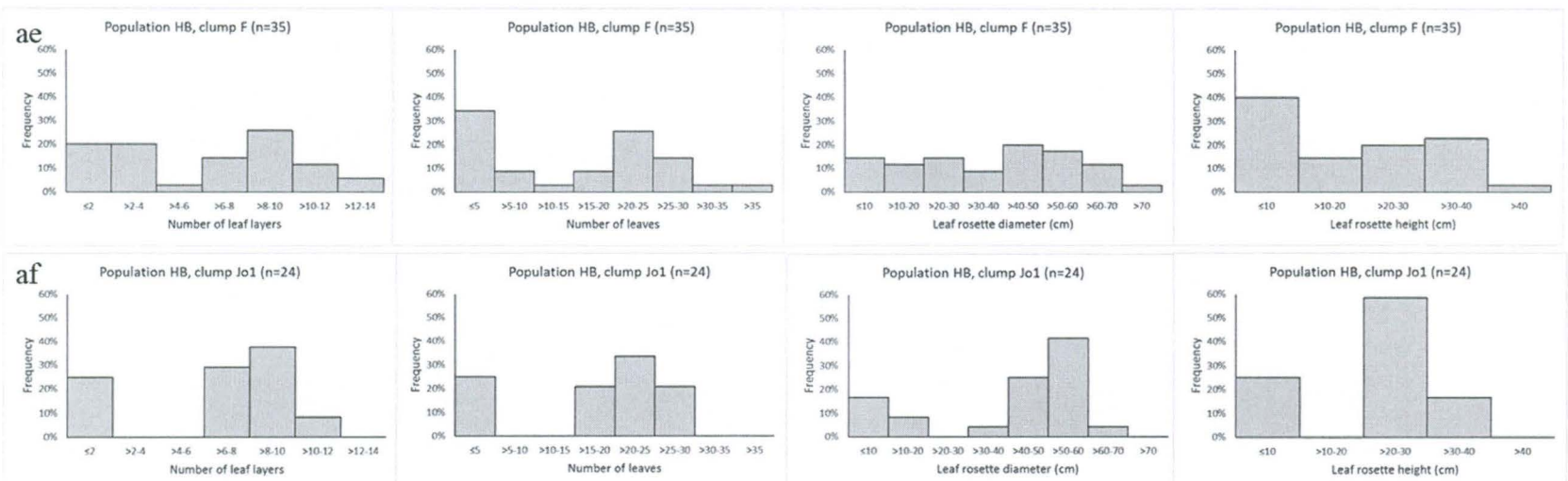
Group 2: J-shaped SCD



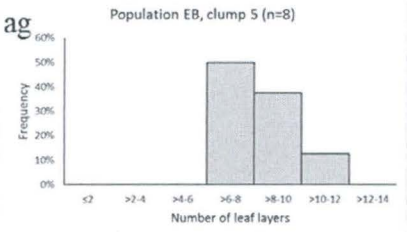
Group 3: Inverse J-shaped SCD

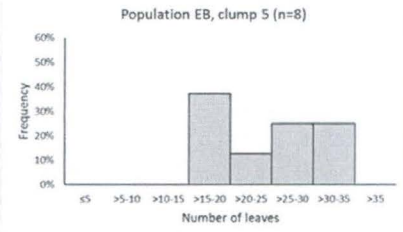


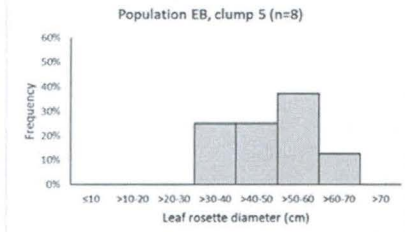
Group 4: Irregular SCD

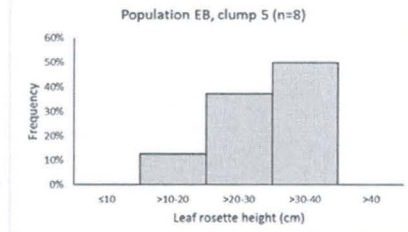


ag

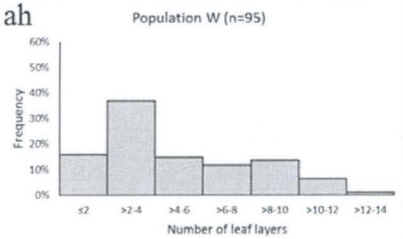

Population EB, clump 5 (n=8)
Frequency
60%
50%
40%
30%
20%
10%
0%
≤2
>2-4
>4-6
>6-8
>8-10
>10-12
>12-14
Number of leaf layers

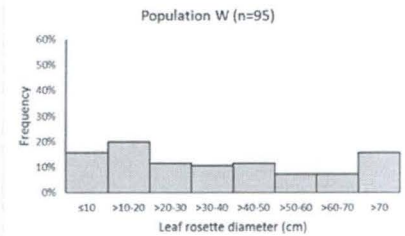

Population EB, clump 5 (n=8)
Frequency
60%
50%
40%
30%
20%
10%
0%
≤5
>5-10
>10-15
>15-20
>20-25
>25-30
>30-35
>35
Number of leaves


Population EB, clump 5 (n=8)
Frequency
60%
50%
40%
30%
20%
10%
0%
≤10
>10-20
>20-30
>30-40
>40-50
>50-60
>60-70
>70
Leaf rosette diameter (cm)


Population EB, clump 5 (n=8)
Frequency
60%
50%
40%
30%
20%
10%
0%
≤10
>10-20
>20-30
>30-40
>40
Leaf rosette height (cm)

ah


Population W (n=95)
Frequency
60%
50%
40%
30%
20%
10%
0%
≤2
>2-4
>4-6
>6-8
>8-10
>10-12
>12-14
Number of leaf layers


Population W (n=95)
Frequency
60%
50%
40%
30%
20%
10%
0%
≤10
>10-20
>20-30
>30-40
>40-50
>50-60
>60-70
>70
Leaf rosette diameter (cm)

Appendix 2.4

Identity (species, family) and abundance of the tallest plant species recorded in quadrats within 1 m of an *Aloe lettyae* individual (Near aloe) and ≥ 5 m away from *A. lettyae* plants in adjacent non-populated areas (Away from aloe) for all *A. lettyae* populations combined. Taxonomy follows Retief and Herman (1997) and SANBI (2017). Number of quadrats containing forb, woody and grass species respectively, in parenthesis. Abundance expressed as percentage of quadrats in which the respective species occurred, and species ranked according to their abundance in quadrats near the aloes. Alien invasive species marked with an asterisk (*).

Species	Family	Abundance (%)	
		Near aloe	Away from aloe
Forb			
	Number of quadrats	(364)	(426)
Berkheya setifera	ASTERACEAE	17.9	15.7
Helichrysum platypterum	ASTERACEAE	17.9	19.7
Afroaster comptonii (=*Aster comptonii*)	ASTERACEAE	6.3	6.6
Hilliardiella aristata (=*Vernonia natalensis*)	ASTERACEAE	5.8	5.6
Pteridium aquilinum	DENNSTAEDTIACEAE	4.7	3.5
Hilliardiella oligocephala (=*Vernonia oligocephala*)	ASTERACEAE	4.4	5.2
Acalypha sp.	EUPHORBIACEAE	3.0	2.3
Galopina aspera	RUBIACEAE	3.0	3.3
Aloe lettyae	ASPHODELACEAE	2.5	0
Gladiolus densiflorus	IRIDACEAE	2.2	1.4
Helichrysum umbraculigerum	ASTERACEAE	2.2	3.8
Afroaster sp. (=*Aster* sp.)	ASTERACEAE	1.6	0.7
Berkheya sp.	ASTERACEAE	1.6	1.2
Triumfetta welwitschii	MALVACEAE	1.6	0.2
Helichrysum nudifolium	ASTERACEAE	1.4	2.1
Pentanisia angustifolia	RUBIACEAE	1.4	0.2
Senecio inornatus	ASTERACEAE	1.4	2.1
Stomatanthes africanus	ASTERACEAE	1.4	1.9
Alepidea peduncularis	APIACEAE	1.1	1.2
Helichrysum chrysargyrum	ASTERACEAE	1.1	1.4
Helichrysum sp.	ASTERACEAE	1.1	1.2
Senecio venosus	ASTERACEAE	1.1	1.6
Berkheya insignis	ASTERACEAE	0.8	1.6
Cyphia elata	LOBELIACEAE	0.8	0.5
Eriosema nutans	FABACEAE	0.8	0.7
Helichrysum acutatum	ASTERACEAE	0.8	0.7
Helichrysum cephaloideum	ASTERACEAE	0.8	1.2
Helichrysum harveyanum	ASTERACEAE	0.8	0.2
Helichrysum odoratissimum	ASTERACEAE	0.8	1.2
Thunbergia atriplicifolia	ACANTHACEAE	0.8	0
Clutia monticola	EUPHORBIACEAE	0.5	0.5

Crabbea hirsuta	ACANTHACEAE	0.5	0.5
Hypoxis rigidula	HYPOXIDACEAE	0.5	0
Laggera crispata	ASTERACEAE	0.5	0
Schistostephium crataegifolium	ASTERACEAE	0.5	1.4
Acalypha peduncularis	EUPHORBIACEAE	0.3	0
Acalypha wilmsii	EUPHORBIACEAE	0.3	0.5
Lablab purpureus	FABACEAE	0.3	0
Ocimum obovatum	LAMIACEAE	0.3	0
Phyllanthus sp.	PHYLLANTHACEAE	0.3	0
Senecio gerrardii	ASTERACEAE	0.3	0.7
Senecio sp.	ASTERACEAE	0.3	0.2
Sonchus wilmsii	ASTERACEAE	0.3	0
Tephrosia sp.	FABACEAE	0.3	0
Thesium sp.	SANTALACEAE	0.3	0.2
Trichodesma physaloides	BORAGINACEAE	0.3	0.2
Watsonia transvaalensis	IRIDACEAE	0.3	0.2
Afroaster serrulatus (=*Aster harveyanus*)	ASTERACEAE	0	0.7
Basananthe sandersonii	PASSIFLORACEAE	0	0.2
Berkheya echinacea	ASTERACEAE	0	0.2
Chlorophytum krookianum	AGAVACEAE	0	0.7
Clutia sp.	EUPHORBIACEAE	0	0.5
Crassula cf. *capitella*	CRASSULACEAE	0	0.2
Crocosmia paniculata	IRIDACEAE	0	0.2
Gnidia microcephala	THYMELAEACEAE	0	0.2
Helichrysum herbaceum	ASTERACEAE	0	0.2
Helichrysum pallidum	ASTERACEAE	0	0.2
Kniphofia splendida	ASPHODELACEAE	0	0.5
Lasiosiphon kraussianus	THYMELAEACEAE	0	0.2
Macledium zeyheri	ASTERACEAE	0	0.2
Pearsonia sp.	FABACEAE	0	0.2
Plectranthus sp.	LAMIACEAE	0	0.2
Raphionacme hirsuta	APOCYNACEAE	0	0.2
No identification		2.7	3.3
Woody			
	Number of quadrats	(193)	(218)
Athrixia phylicoides	ASTERACEAE	20.7	19.3
Fadogia homblei	RUBIACEAE	14.0	17.9
Rabdosiella calycina	LAMIACEAE	11.9	6.9
Helichrysum setosum	ASTERACEAE	5.2	2.3
Helichrysum splendidum	ASTERACEAE	5.2	5.5
Syncolostemon rehmannii (=*Hemizygia rehmannii*)	LAMIACEAE	5.2	4.6
Pseudarthria hookeri	FABACEAE	5.2	6.0
Tenrhynea phylicifolia	ASTERACEAE	5.2	8.7
Indigofera sp.	FABACEAE	3.6	1.8
Helichrysum sp.	ASTERACEAE	3.1	1.8

Lippia javanica	VERBENACEAE	2.6	4.6
Gymnanthemum myrianthum (=*Vernonia myriantha*)	ASTERACEAE	2.6	1.4
Rhoicissus tridentata	VITACEAE	2.1	1.8
Otholobium wilmsii	FABACEAE	1.6	0.9
Tylosema fassoglense	FABACEAE	1.6	5.5
Gymnanthemum corymbosum (=*Vernonia tigna*)	ASTERACEAE	1.6	4.1
Searsia pondoensis	ANACARDIACEAE	1.0	0.9
Artemisia afra	ASTERACEAE	0.5	0.5
Diospyros lycioides	EBENACEAE	0.5	0.5
Lantana rugosa	VERBENACEAE	0.5	0
Nuxia floribunda	STILBACEAE	0.5	0
Pygmaeothamnus chamaedendrum	RUBIACEAE	0.5	0
**Rubus cuneifolius*	ROSACEAE	0.5	0.9
Searsia discolor	ANACARDIACEAE	0.5	0
Helichrysum lepidissimum	ASTERACEAE	0	0.5
Leonotis intermedia	LAMIACEAE	0	0.5
No identification		4.1	3.2
Grass			
	Number of quadrats	(377)	(442)
Cymbopogon nardus	POACEAE	46.7	47.7
Trachypogon spicatus	POACEAE	18.6	16.5
Loudetia simplex	POACEAE	11.4	14.0
Themeda triandra	POACEAE	4.5	6.6
Eulalia villosa	POACEAE	4.0	3.4
Cymbopogon caesius	POACEAE	2.9	0.9
Andropogon schirensis	POACEAE	2.1	1.1
Hyparrhenia sp.	POACEAE	1.9	0.9
Setaria cf. *sphacelata*	POACEAE	1.3	0.7
Digitaria diagonalis	POACEAE	0.8	0
Hyparrhenia tamba	POACEAE	0.8	0.7
Hyparrhenia filipendula	POACEAE	0.5	0.5
Eragrostis sp.	POACEAE	0.3	0
Hyparrhenia cf. *hirta*	POACEAE	0.3	0.9
Microchloa caffra	POACEAE	0.3	0
Monocymbium ceresiiforme	POACEAE	0.3	0.2
Cymbopogon sp.	POACEAE	0	0.2
No identification	POACEAE	3.4	5.7

CHAPTER THREE

REPRODUCTIVE ECOLOGY OF THE SUMMER-FLOWERING *ALOE LETTYAE*, A SOUTH AFRICAN GRASSLAND ENDEMIC

Abstract

I studied the reproductive ecology of the Woodbush Granite Grassland (WGG) endemic, summer-flowering, *Aloe lettyae* to inform conservation and management strategies for this species whose distribution has been severely reduced by habitat loss and fragmentation. In the *A. lettyae* populations surveyed in 2016/7, approximately 80% of the adult individuals were reproductive. *Aloe lettyae* flowered from early December to mid-February in the two largest populations, and from March to mid-April in the smaller populations located on the western and northern WGG periphery. Reproductive success in terms of fruit set and seed production was monitored in the two large *A. lettyae* populations with ~3000 plants each and four smaller populations with <350 plants each. Fruit set varied from 29% in the largest population to 3% in the smallest population. Total seed production per plant was high in the large populations (5200 and 2400), compared with the four smaller populations surveyed (130–920), which indicated that an Allee effect may be operating. Approximately 90% of the sampled intact fruit contained seed predators, a fly (*Apenthecia* cf. *crassiseta*, Drosophilidae) and/or a wasp (*Eurytoma* sp., Eurytomidae). During flowering, camera traps were used at four localities to identify the main floral visitors, and probe rates by bird visitors. Three bird species were observed feeding on *A. lettyae* nectar, Amethyst sunbirds (*Chalcomitra amethystina*) as well as Southern and Greater Double-collared sunbirds (*Cinnyris chalybeus* and *Cinnyris afer*). Amethyst sunbirds, the most frequent visitors (554 visits/1000 plants/h), were observed at all four localities monitored, while Double-collared sunbirds only occurred at two localities and had a much lower visitation rate (46 visits/1000 plants/h). The visitation rate of Amethyst and Double-collared sunbirds combined was 3.6 times higher in the large population than in the smallest population in which camera traps were used. *Aloe lettyae* nectar volume in unscreened flowers was very variable (range 5–85 µl; mean 15.5 ± 1.2 µl) and nectar sugar

concentration was high (range 11–25%; mean 18.6 ± 0.2%) confirming that *A. lettyae* flowers are adapted for specialist nectarivores. The contribution of different pollinator guilds (birds and insects) was determined through selective pollinator exclusion treatments which showed that birds contributed significantly more to *A. lettyae* fruit set (1.7 times), seed production per fruit (1.5 times) and seed production per plant (4.3 times) than insects. In a germination trial conducted under controlled conditions with seeds from the populations monitored for their reproductive success and seeds obtained from the pollinator exclusion trial, mean germination time was approximately 2.5 weeks and cumulative germination percentages were high (approximately 90%), and did not differ between populations/treatments. Overall, *in situ A. lettyae* seedling survival into the next growing season was 62%, being significantly lower in burned (26%) than in unburned areas (76%). Understanding *A. lettyae's* reproductive ecology is vital for managing remnant wild populations. In combination with baseline *A. lettyae* population data our understanding of *A. lettyae's* reproductive ecology will aid in designing an adaptive management strategy for WGG.

Key words:

Allee effect, *Aloe lettyae*, Amethyst sunbird, bees, bird pollination, grassland, habitat loss and fragmentation, seed germination, Woodbush Granite Grassland (WGG).

Introduction

Studies on the pollination biology of the genus *Aloe* have demonstrated that *Aloe* species are generally self-incompatible and therefore depend entirely on animal pollinators for reproductive output (e.g. Hoffman 1988; Botes *et al.* 2008; Botes *et al.* 2009a; Johnson *et al.* 2009; Wilson *et al.* 2009; Cousins and Witkowski 2012; Payne *et al.* 2016). *Aloe* benefits from different pollinator groups, mainly birds and insects. Knowledge of flower and nectar characteristics as well as observations of flower visitors are required to predict pollinator type (Hoffman 1988; Symes *et al.* 2009). The summer-flowering *Aloe lettyae* has long, tubular, brightly orange-red coloured flowers which suggest a bird pollination syndrome (Cronk and Ojeda 2008; Botes *et al.* 2009a; Cousins and Witkowski 2012). *Aloe*

species with long tubular flowers and included anthers are usually pollinated by long-billed sunbirds (Botes *et al.* 2009a). However, bees typically outnumber birds as floral visitors to aloes, and honey bees often crawl into the perianth tube to obtain nectar and, in so doing, contribute to seed production (Botes *et al.* 2009a). The relative contributions of bird and insect pollinators to reproductive output have been elucidated for several *Aloe* species, comprising winter-flowering species e.g. *A. ferox* (Hoffman 1988; Stokes and Yeaton 1995; Botes *et al.* 2009a; Kuiper *et al.* 2015), *A. marlothii* (Symes *et al.* 2008), *A. greatheadii* var. *davyana* (Symes *et al.* 2009) and *A. peglerae* (Arena *et al.* 2013), as well as summer-flowering *Aloe* species including *A. pruinosa* (Wilson *et al.* 2009), *A. ecklonis* (= *A. boylei*) (Johnson *et al.* 2009), *A. reitzii* var. *reitzii* (Symes 2017), *A. linearifolia* (Botes *et al.* 2009b), *A. minima* (Botes *et al.* 2009b) and *A. modesta* (Van der Riet 1977).

The demographic study of *A. lettyae* across its range revealed that population sizes of seven surveyed populations (of a total of 19 known localities) varied from 10 to 4000 plants, and that populations were typically dominated by adult plants (82%), the majority of which flowered in 2016/7 (80%). The low percentages of juveniles and non-reproductive subadults indicated an apparent current and historic prevailing lack of recruitment (Chapter Two). Small, sparse populations have been found to be less attractive to pollinators than larger, denser populations resulting in reduced reproductive output (Lamont *et al.* 1993; Kleizen *et al.* 2009) which may lead to the local extinction of smaller populations through the Allee effect (Courchamp *et al.* 1999). To inform conservation and management strategies for the Endangered *A. lettyae*, whose distribution has been severely reduced since 1910 by habitat loss and fragmentation, I studied the reproductive ecology of this species. The objectives of this study were (a) to document the flowering phenology of *A. lettyae* and to determine reproductive success in populations of different sizes; (b) to identify *A. lettyae* floral visitors and investigate nectar properties; (c) to test for self-pollination and determine the contributions of bird and insect pollinators to fruit set and seed production of *A. lettyae*, and (d) to quantify germination success of *A. lettyae* seeds.

Methods

Study site and species

This study was conducted in the remaining fragments of the Critically Endangered Woodbush Granite Grassland (WGG) (S23°56'55.2" E29°57'09.6", 1080–1800 m a.s.l.) occurring about 50 km east of Polokwane, Limpopo Province, South Africa. The WGG is situated on a mountainous plateau originally covered with grassland which has undergone major transformation during the last century, mainly due to silviculture (Mucina *et al.* 2006). The area immediately adjacent to the largest remaining WGG fragment, being Haenertsburg village, received 786 mm and 1192 mm of rain during the periods July 2015 to June 2016 and July 2016 to June 2017 respectively (SAEON 2017).

The maculate *Aloe lettyae* is endemic to the WGG and has a dense rosette of erectly spreading bluish green leaves with dull cream spots on both surfaces (Reynolds 1937, 1969). Adult *A. lettyae* plants generally produce one inflorescence which can be up to 2 m tall, branched from about the middle and has distinctly widely upwards curved side branches (Smith *et al.* 2012). The inflorescence has 8–12 branches, the lower ones of which are usually re-branched, and bears a total of 15–20 cylindric to slightly acuminate, laxly flowered racemes (Letty 1962; Reynolds 1969; Carter *et al.* 2011). The tubular flowers are orange-red, have pale margins and a remarkably large globose base (Reynolds 1969; Smith *et al.* 2012). Flowers are 38–42 mm long, 10–11 mm across the rounded ovary, constricted above to 6 mm and then widening towards the mouth. Flowers are protandrous (Kremer-Köhne, pers. obs.), and stamens and style are scarcely exserted (Reynolds 1937, 1969; Carter *et al.* 2011; Smith *et al.* 2012). The inflorescence is indeterminate, with flowers maturing from the bottom of each raceme towards the top, with only two or three flowers opening per raceme per day, resulting in an *A. lettyae* individual bearing flowers over a period of approximately four weeks (Kremer-Köhne, pers. obs.). *Aloe lettyae* fruits are oblong capsules (30 mm long, 15 mm in diameter at middle) which dry and dehisce from the apex when ripe (Reynolds 1969). Seeds are approximately 4 mm long, winged and charcoal grey (Kremer-Köhne, pers. obs.). Across its restricted range *A. lettyae* flowers in summer from early December to mid-April (Kremer-Köhne, pers. obs.). For the Duiwelskloof/Magoebaskloof area

flowering has been previously recorded from February to April (Jeppe 1969; Van Wyk and Smith 2014), February to March (Reynolds 1940) and March to April (Reynolds 1969).

Flowering and reproductive success

The relationship between plant size and inflorescence height was determined from all the reproductive adult *A. lettyae* plants surveyed in this study (Chapter Two). Correlations between inflorescence height and plant size in terms of the number of leaf layers and rosette diameter (n = 619) and the number of leaves and rosette height (n = 539) were calculated for all populations combined.

In summer 2017, fruit set and seed production were determined in six *A. lettyae* populations of different sizes in terms of number of plants and area of occupancy (AOO). This included the two large *A. lettyae* populations with ~3000 plants each and four smaller populations with <350 plants each (Table 3.1). The smaller populations selected for this study were likely remnant *A. lettyae* populations in small (<15 ha) remaining isolated WGG fragments surrounded mainly by forest and timber plantations (Appendix 2.1). Flowering *A. lettyae* plants with a fully expanded inflorescence and bearing at least one open flower were randomly selected within populations, marked with a wooden stake (Figure 3.1a), tagged and GPS co-ordinates recorded using a hand-held Garmin unit (Garmin, GPSmap 62sc, USA). On each selected plant, the height of the inflorescence was measured and the number of primary and secondary racemes borne on each inflorescence was counted. One of the three lowest primary racemes was randomly selected and tagged with a cable tie, and the raceme's length, degree of branching and number of flowers (or pedicels if flowers had fallen off) recorded. In populations HB (clump E), EB (clump 7), FG (clump 1) and BL (clump 2) (see Chapter Two), 20 *A. lettyae* plants each were selected in mid-January 2017. In the small populations BU and FH, flower development and consequently selecting plants were delayed by two and three weeks, respectively, and only 10 *A. lettyae* plants each could be identified for the trial (Table 3.1). Due to time constraints, size class distributions and life history stages were not determined in BU and FH.

Table 3.1. *Aloe lettyae* populations in which reproductive success was determined in 2017. For each population the following is listed: altitude (m a.s.l.), aspect, occurrence of fire in 2016, degree of isolation from main population HB (km), estimated total number of plants, percentage reproductive plants (n.d.=not determined), Area of Occupancy (AOO), population density (plants/ha, nearest-neighbour method), and the date on which plants were tagged.

Population	Altitude (m a.s.l.)	Aspect	Fire 2016	Isolation from main population (km)	*Aloe lettyae*				
					Number of plants	Reproductive (%)	AOO (ha)	Density (plants/ha)	Date tagged
HB	1470	W	Yes	0	3516	77	7.4	475	11.1.2017
EB	1400	SW	Yes	2	2683	87	2.5	1073	12.1.2017
BL	1480	NE	Yes	4	330	90	0.5	660	13.1.2017
FG	1710	E	No	4	145	71	0.4	363	12.1.2017
BU	1700	SE	No	5	85	n.d.	0.5	160	25.1.2017
FH	1670	NE	No	4	85	n.d.	0.1	850	7.2.2017

The period from flowering to fruit maturation (mid-January to mid-March) coincided with high summer rainfall (~450 mm in total). Approximately eight weeks after selecting and measuring the *A. lettyae* individuals, fruit set on the tagged racemes was recorded. Totals of flowers and fruit on a tagged primary raceme with one or several secondary racemes respectively were multiplied by the number of primary racemes to obtain the number of flowers and fruit per inflorescence (i.e. per plant). Fruit set was represented as the percentage of flowers that produced fruit. Where possible, up to three fully mature fruit that were neither mouldy, visibly predated (capsule eaten or entry/exit holes made by predators, Figure 3.2 g and h) nor dehisced were collected per tagged raceme (a total of 27, 12, 2, 1, 4 and 4 fruits were collected in populations HB, EB, BL, FG, BU and FH, respectively). Individual fruit were placed in labelled envelopes and air dried at room temperature (Arena *et al.* 2013; Payne *et al.* 2016). After fruit dehiscence the number of seeds in each fruit was counted, and seeds were categorized as intact/viable (>3 mm long, plump and black), undersized and aborted (small, flattened and brown) or predated (entry/exit holes made by predators clearly visible on seed coat or seed disintegrated). Seed counts were used to calculate the mean number of seeds produced per fruit. The mean total number of seeds and the mean number of intact/viable seeds per fruit were then multiplied by the number of fruit set per individual to calculate the estimated total seed production per replicate plant and the potential

number of viable seeds dispersed from the plant, respectively. Fruit and seed parasites (flies and wasps) found in the fruit capsule were collected, identified and voucher specimens (S. Kremer-Köhne 12, 13, 18, 19 and 20) were lodged at the Iziko South African Museum, Cape Town.

To assess *in situ* seedling survival into the next growing season, adult *A. lettyae* plants with a minimum of three seedlings growing within 30 cm from their leaf rosette were located in May 2017 (Figure 3.2i). The seedlings likely grew from seeds dispersed in 2016 and germinated during summer 2016–2017. In populations HB, EB and FG, thirty *A. lettyae* plants in total with a total of 237 seedlings (3–31 per mother plant) growing next to them were marked with metal stakes and their GPS co-ordinates recorded. Within 1 m of each marked mother plant, the percentage aerial cover of grass, forbs, woody plants, rock and bare soil was visually estimated, the height of grass, forbs and woody plants measured and the standing herbaceous biomass determined (Chapter Two). In July 2017, controlled burns (low fire intensity) were applied in some patches of HB and at FG (n = 21 aloes in unburned areas and n = 9 aloes in burned areas) and no unplanned fires occurred during winter. The marked plants were re-visited in December 2017, the surviving seedlings counted and percentage seedling survival calculated. Regression analyses were performed for relationships between the percentage seedling survival and the variables relating to the vegetation cover around the mother plant.

Figure 3.1. *Aloe lettyae* (white scale bars = 30 cm) (a) Plant with marked primary raceme selected to determine fruit set. (b) Selective pollinator exclusion treatment 'insects only' (15 mm bird netting). (c) Pollinator exclusion treatment 'no visitors' (1 mm mesh). (d) Motion-sensitive camera trap observing floral visitors. (e) Two male Amethyst sunbirds (*Chalcomitra amethystina*) probing flowers. Photographs: S. Kremer-Köhne.

Aloe lettyae visitors and nectar properties

Observations of *A. lettyae* visitors were conducted during flowering in January 2017 to identify the main floral visitors and probe rates by bird visitors. Six motion-sensitive camera traps (Bushnell, model 119740, China) with f 600 mm lenses were used, 24 hours/day. Three cameras each were

positioned in one locality for several days at a time (HB A2 and HB Jo1: 11–18 January 2017; EB 7: 20–25 January 2017; FG 1: 20 January – 1 February 2017). At each locality, two cameras were observing *A. lettyae* plants in open grassland (No bush treatment) while the third camera was monitoring an *A. lettyae* individual growing within 5 m of woody vegetation (Bush treatment). Total camera trap observation hours were calculated by multiplying the number of days by the hours per day and the number of cameras. The cameras were fastened to metal Y standards approximately 1.5 m above the ground, at least one metre from the *A. lettyae* plants, positioned to encompass the entire inflorescence, facing southwards to minimize sun glare, and downwards onto the aloe (Figure 3.1d). Memory cards (SanDisk 4GB) and batteries in the cameras were replaced as needed. Each camera was programmed at high sensitivity to take three (HB) or two (EB and FG) photographs when motion sensors were activated, with three-second intervals between activations. A visitor was defined as an individual which probed flowers (i.e. inserted the bill into the mouth of the flower or pierced either the large globose flower base or the floral tube) or perched on the inflorescence and therefore could potentially transfer pollen. The date and time of each visit were logged, and a visitor after an interval of 30 s between camera activations was considered as a new visitor. The visitors were identified from the images. Mean visitation rate per 1000 plants (visits/1000 plants/h) for probing and non-probing visitors combined was calculated for all localities (Arena *et al.* 2013; Payne *et al.* 2016). During field work, insects occurring on *A. lettyae* were collected to identify potential pollinators and to establish which insects are associated with the aloe. Insect specimens were kept in 95% ethanol until their identification. Voucher specimens (S. Kremer-Köhne 1, 3, 8 and 9) were lodged at the ARC Roodeplaat.

Aloe lettyae nectar volume (standing crop) and sugar concentration were measured at different flower stages (Symes and Nicolson 2008), i.e. immature phase (flower open, neither stamens nor style protruding), male phase (stamens protruding) and female phase (style protruding). As it proved very difficult to extract nectar from the *A. lettyae* floral tube, the globose flower base was pierced with a sharpened metal pin to extract the nectar with a disposable hematocrit tube (75 µl) (Figure 3.2a and b). The inflorescences were unscreened and may have been visited by feeding birds prior to sampling. The sugar concentration (% w/w) was determined with a handheld refractometer (Eclipse refractometer,

Bellingham + Stanley Ltd. UK, model 45-81, Ser. No. 019365). Three trials were undertaken during which only readings for flowers with nectar were recorded. Trial 1 male phase: 7.2.2017 13:00, 27°C, sunny after misty morning, population FH, nectar sampled from five male phase flowers from each of eight randomly selected individuals (n = 40). Trial 2 female phase: 8.2.2017, 9:30–12:30, 22–27°C, overcast, population HB A1 and A2, nectar sampled from two female phase flowers from each of 17 randomly selected individuals (n = 34). Trial 3: 13.2.2017, 14:30–16:30, 30–35°C, sunny and hot, population RE 3, nectar sampled from five open flowers (and their flower stage, male or female phase, recorded) from each of 10 randomly selected individuals (n = 50).

Pollinator guilds

Relative contributions of the different pollinator guilds (birds and insects) to reproductive success of *A. lettyae*, i.e. fruit set, seed production per fruit and per plant, were determined by conducting a selective pollinator exclusion experiment in population HB A2. *Aloe lettyae* plants with an inflorescence in bud (before first flowers opened) were randomly selected, marked with a wooden stake, and GPS co-ordinates recorded. Eleven *A. lettyae* plants were randomly assigned to each of three treatments in the first week of January 2017. A control (permitting all visitors) allowed unrestricted access by all floral visitors and was left uncovered; a bird exclusion treatment (permitting insects only; Figure 3.1b) allowed access by insect visitors and excluded birds using a bird net cage (Alnet bird netting black, 15 × 15 mm holes); and a total exclusion treatment (No visitors permitted; Figure 3.1c) excluded all floral visitors using a fine nylon mesh cage (1 × 1 mm holes). The sturdy cages (1 × 1 × 1.3 (height) m) covered the entire *A. lettyae* plant until the end of the flowering season and withstood thunderstorms. Approximately nine weeks after applying the treatments, the number of pedicels present after flowers had fallen off and the number of fruit set were counted on each replicate plant. Fruit set was represented as the percentage of flowers per inflorescence that produced fruit. Where possible, a sample of up to ten fully developed fruit that were neither mouldy, visibly predated (capsule eaten or entry/exit holes made by predators) nor dehisced was collected per replicate. Due to large differences in fruit production related to the treatment effect, the number of fruit sampled varied greatly (a total of

33 fruits from 6 plants, 15 fruits from 5 plants and 3 fruits from 1 plant were collected from treatments All visitors, Insects only and No visitors, respectively). Seeds per fruit and estimated total seed production per replicate plant were determined as described above for the assessment of reproductive success.

Seed germination

To determine the effect of reduced seed production on reproductive output germination success was established for 6-month-old *A. lettyae* seeds sourced from the populations monitored for their reproductive success and from each selective exclusion treatment. Intact seeds were randomly sampled from each fruit and pooled per replicate plant. The seed number varied (1–78 seeds/plant) depending on the number of seeds available from each plant. A total of 100 seeds each was used from populations HB, EB and BU as well as from the control and bird exclusion treatments, while a total of 48, 62, 64 and 11 seeds was used from populations FG, BL, FH and the total exclusion treatment respectively. Seeds were surface sterilised by dipping them in a 2% sodium hypochlorite solution for 5 min. Grouping seeds per replicate plant, seeds were placed on filter paper discs (Whatman, diameter 70 mm) and germinated on a Jacobsen germination table (Steinmetz Apparatebau, München, Germany) located on the veranda of the Merensky Timber nursery near Tzaneen (about 38 km away). The filter paper was kept moist by wicks which draw water containing a low concentration of sodium hypochlorite for prevention of fungal growth from a bath set at 30°C. The humidity around the seeds was kept high by means of glass domes with limited ventilation. During the day (05:00–17:59) ambient light and at night (18:00–4:59) light from fluorescent tube lamps was supplied. Temperatures were ambient with a mean daily maximum temperature of 27°C and a mean daily minimum temperature of 14°C. The *A. lettyae* seeds were sown on 29.9.2017. Taking germination as the emergence of the radicle by 2 mm from the seed testa, germinating seeds were counted every seven days for seven weeks, and germinated seeds were planted into seedling trays containing a germination mix (Culterra Professional Germination Mix).

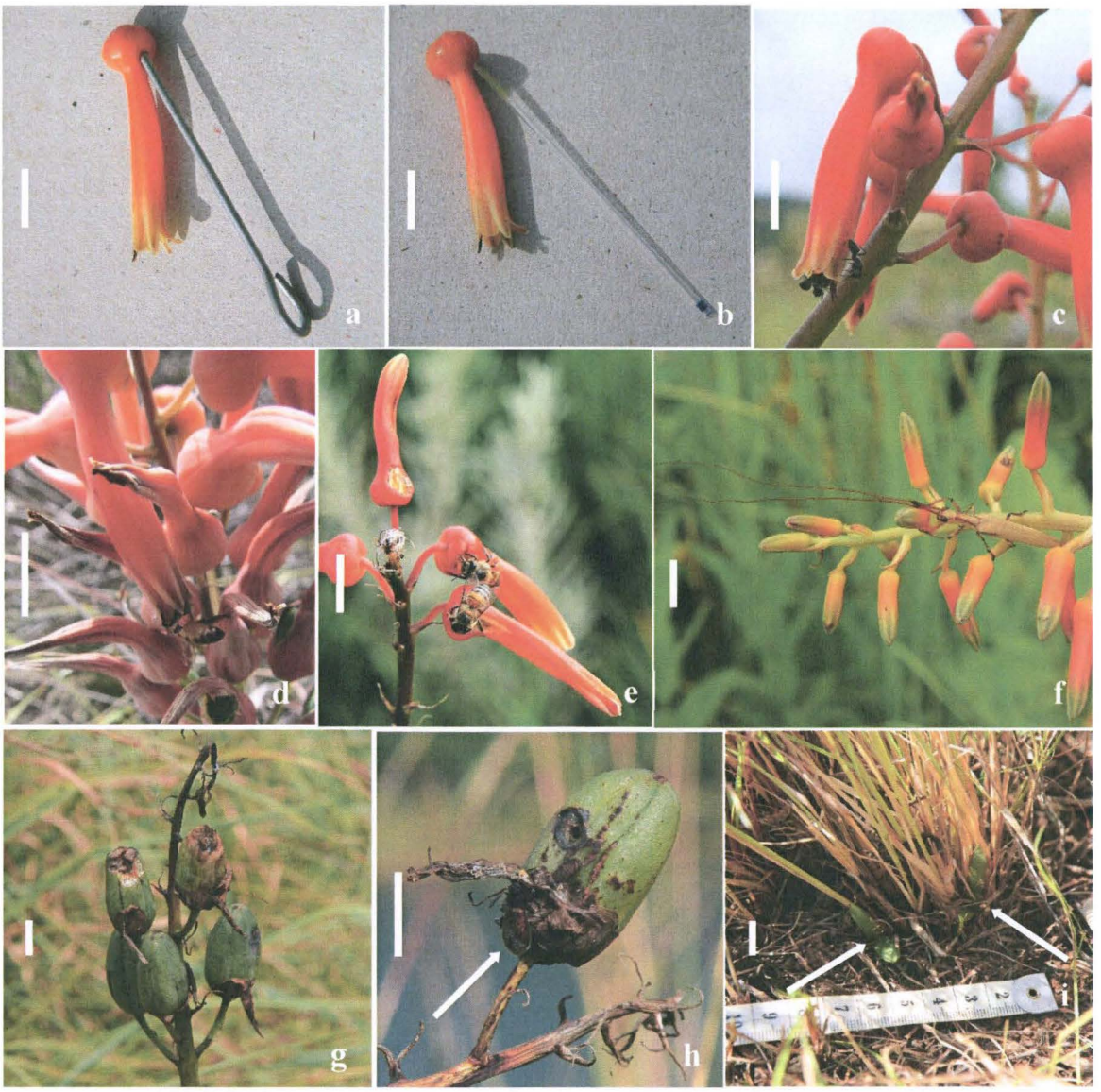


Figure 3.2. *Aloe lettyae* (white scale bars = 10 mm) (a) and (b) Nectar sampling by piercing globose flower base with metal pin to extract nectar with haematocrit tube. (c) Ants (*Camponotus* sp.) collecting nectar inside tubular flower. (d) Honey bee (*Apis mellifera* subsp. *scutellata*) at mouth of tubular flower. (e) Honey bees feeding on nectar from flower tubes damaged by sunbirds. (f) Herbivorous longhorn beetle (*Nemotragus helvolus*) feeding on flowers. (g) Predated fruit capsules. (h) Herbivorous beetle (*Lagrinii* sp.) feeding on fruit capsule with entry/exit hole. (i) Seedlings in grass tussock (20.4.2017). Photographs: S. Kremer-Köhne.

The germination trial was terminated after week seven on 16.11.2017. Results are presented as the cumulative percentage of germinated seeds. Mean Germination Time (MGT) in weeks was calculated for each population and treatment using the following equation:

$$\text{MGT} = \frac{\sum Wn}{\sum n}$$

where n is the number of seeds germinated in week W, and W is the number of weeks counted from the onset of germination (Zanjan and Asli 2012; Payne *et al.* 2016).

Statistical analyses

Data from the reproductive success trial (inflorescence structure, fruit set, seed production per fruit and per individual), from the pollinator exclusion trial (fruit set, seed production per fruit and per individual), the germination trial (percentage of germinated seeds, MGT) and nectar measurements (volume, sugar concentration) were tested for normality using the Kolmogorov-Smirnov normality distribution. Data were log transformed when not normal. One-way ANOVA or the non-parametric Kruskal-Wallis test was used to test for differences between populations/treatments. Tukey Post Hoc Tests or the Bonferroni inequality were applied to statistically significant results ($P < 0.05$). Analyses were performed using Statistica 13.2 (StatSoft, Tulsa, OK, USA, 2016). Values are presented as mean ± SE. Regression analyses were used to investigate the relationships between plant size (leaf rosette diameter, leaf rosette height, number of leaf layers, number of leaves) and inflorescence height; population size (number of plants per population, AOO) and reproductive output (fruit set, seed production); seedling survival and vegetation surrounding the mother plant (percentage aerial cover of grass, forbs, woody plants, and bare soil, standing herbaceous biomass). The best fit curve was selected. The regression analyses were done in Microsoft Excel 2016.

Results

Flowering and reproductive success

As shown in Chapter Two, *A. lettyae* populations were dominated by adult plants (82%), the majority of which flowered (80%) during this study. Preliminary observations indicated that conspicuous *A. lettyae* individuals along a hiking trail flowered in each of three consecutive years. Further, all 30 randomly selected plants marked for monitoring seedling survival aross three populations flowered in 2017, and again in 2018. During the summers 2015/6 and 2016/7, the big *A. lettyae* populations in the immediate vicinity of Haenertsburg (HB and EB) flowered from early December to mid-February; other conspicuously flowering plant species during the early part of this period were *Helichrysum platypterum* and *Berkheya setifera*. It is interesting to note that *A. lettyae* populations located further north and west on the WGG periphery (W, O, SK, VE, RE, DR and G; refer to Chapter Two Appendix 2.1) only started flowering in March. *Aloe lettyae* plants generally had one inflorescence. In many plants at locality O, however, a second inflorescence emerged when the first inflorescence started to set fruit. It is unknown whether the second inflorescence set fruit as locality O was not revisited. There was a moderate positive relationship between leaf rosette diameter and inflorescence height ($R^2 = 0.31$) (Figure 3.3a). However, plant size in terms of leaf rosette height ($R^2 = 0.12$), number of leaf layers ($R^2 = 0.01$) and leaves ($R^2 = 0.02$) was poorly correlated with inflorescence height (Figure 3.3b–d).

In the populations monitored for their reproductive success, inflorescence height ($F_{5,94} = 0.3656$, $P = 0.8709$) and the number of secondary racemes ($H_{5,100} = 9.2471$, $P = 0.0996$) did not differ between populations. Populations FG and BL had significantly shorter tagged primary racemes ($F_{5,94} = 3.5747$, $P = 0.0053$) and consequently fewer flowers per inflorescence (i.e. per plant) than the other populations, while populations BU and FH had long primary racemes and high numbers of flowers per plant ($F_{5,94} = 2.6962$, $P = 0.0254$) (Figure 3.4a). The proportion of flowers that set fruit differed significantly between populations ($H_{5,100} = 46.2644$, $P < 0.0001$) (Figure 3.4b). Fruit set was highest in the big populations HB (29%) and EB (22%). While fruit set in the small population FG (20%) did not differ from that in

the big populations, fruit set in three of the four small populations assessed for their reproductive success (BL, BU, FH) was significantly lower than in the big populations and ranged from 3% to 7%. A regression analysis between log AOO and log fruit set showed that population size had a significant positive effect on fruit set ($R^2 = 0.32$; $P < 0.0001$) (Figure 3.4c) and that excluding FG which had a high fruit set from the regression analysis would strengthen this relationship ($R^2 = 0.52$). In population BL, very poor fruit set was also observed in the preceding summer of 2016 (Kremer-Köhne, pers. obs.).

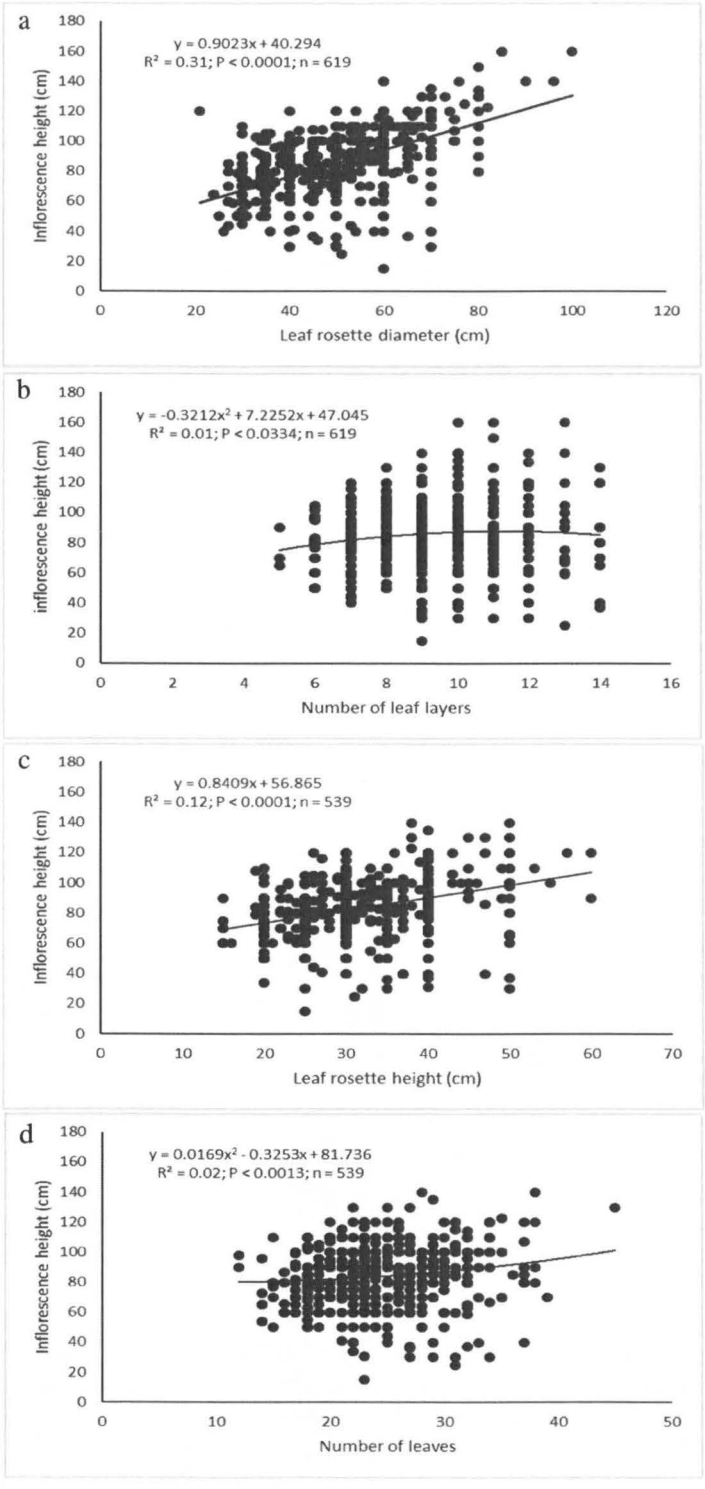


Figure 3.3. Relationship between plant size and inflorescence height for all reproductive adult *Aloe lettyae* plants surveyed in this study (Chapter Two). Correlations between (a) leaf rosette diameter and (b) number of leaf layers and inflorescence height (n = 619); (c) leaf rosette height and (d) number of leaves and inflorescence height (n = 539) were calculated. The best fit curve (linear or polynomial) was selected.

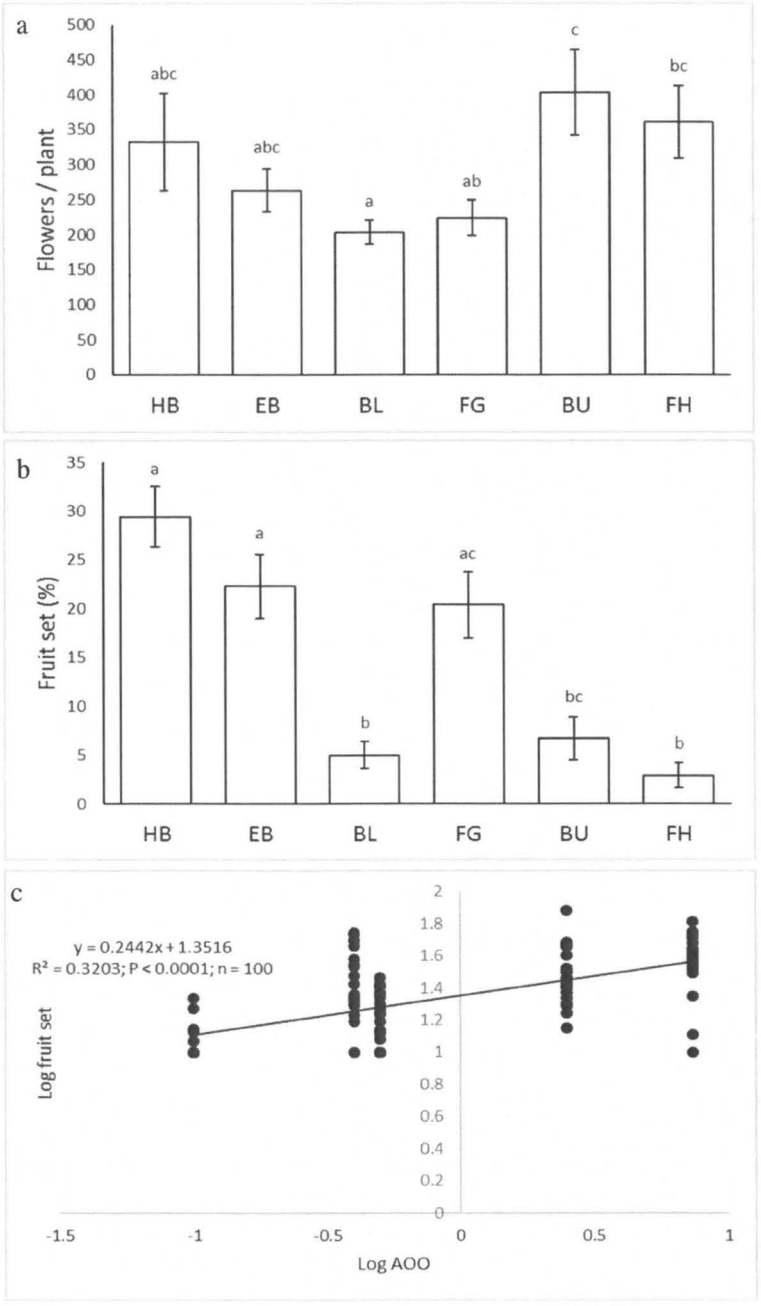


Figure 3.4. Reproduction in *Aloe lettyae* populations of different sizes (ranked from left to right according to decreasing population size, Table 3.1) in 2017. (a) Flowers per plant ($F_{5,94} = 2.6962$, $P = 0.0254$) and (b) Percentage fruit set (proportion of flowers that set fruit) ($H_{5,100} = 46.2644$, $P < 0.0001$). Mean ± SE. Significant differences are indicated by different letters. Populations HB, EB, FG, BL (n = 20); populations BU, FH (n = 10). (c) Relationship between population size (Log AOO) and fruit set (Log fruit set).

In situ, A. lettyae fruit capsules dehisced in mid-March, and seeds were gradually dispersed from the capsules over a period of about four months (Kremer-Köhne, pers. obs.). As very few intact fruit were available and weather conditions prevented access to the populations before fruit dehisced, insufficient fruit samples were collected in populations FG, BL, BU and FH (Table 3.2). No significant differences were found between populations in terms of percentages of seeds intact/viable, predated and undersized/aborted, and the total number of seeds/fruit (Kruskal-Wallis, $P > 0.05$). Across all populations, the percentage undersized/aborted seeds averaged 48% (range 31–72%). While seed production per fruit was similar in all populations, the estimated total seed production and the production of intact/viable seeds per plant was higher in the large populations HB and EB than in the four smaller populations surveyed (BL, FG, BU, FH) (Table 3.2). Estimates of the number of viable seeds dispersed from the plant varied greatly and ranged from 2106 in the largest population HB to 68 in the small population BL (Table 3.2).

Table 3.2. *Aloe lettyae* seed production per fruit and per plant in populations of different sizes (ranked from top to bottom according to decreasing population size) in 2017. Number of plants evaluated and number of intact fruit sampled per population; percentage of seeds intact/viable, predated and undersized/aborted; total number of seeds produced per fruit and per plant, and viable seed production per plant. Mean ± SE; means weighted according to the number of ^{P}plants and ^{F}fruit. Significant differences are indicated by different letters (Kruskal-Wallis, $P < 0.05$).

Population	Plants evaluated	Number of fruit sampled	Seeds (%)			Number of seeds		
			Intact/ viable	Predated	Undersized/ aborted	Total per fruit	Total per plant	Intact/viable per plant
HB	16	27	43 ± 4	11 ± 2	46 ± 4	57 ± 3	5144 ± 1049^{a}	2106 ± 497^{a}
EB	8	12	31 ± 7	18 ± 6	51 ± 8	49 ± 4	2390 ± 744^{a}	933 ± 419^{ac}
BL	11	2	53 ± 2	16 ± 9	31 ± 7	59 ± 3	128 ± 128^{b}	68 ± 68^{b}
FG	3	1	59	5	36	81	540 ± 540^{ab}	320 ± 320^{ab}
BU	6	4	42 ± 15	17 ± 4	41 ± 12	59 ± 14	919 ± 538^{ab}	468 ± 308^{ab}
FH	8	4	24 ± 6	4 ± 3	72 ± 5	67 ± 13	418 ± 217^{b}	107 ± 56^{bc}
Total/Sign.	52	50	NS	NS	NS	NS	$P < 0.05$	$P < 0.05$
Weighted means			39 ± 3^{F}	13 ± 2^{F}	48 ± 3^{F}	56 ± 3^{F}	2179 ± 451^{P}	895 ± 205^{P}

In populations HB and EB, 89% and 92%, respectively, of the apparently unpredated fruit (no visible entry/exit holes made by predators) contained a fly and/or a wasp (Table 3.6). Two wasp species were identified, the seed predating *Eurytoma* sp. (S. Kremer-Köhne 13) and the fly parasitoid *Afrostilba* sp. 1 (S. Kremer-Köhne 18, 19, 20) (Van Noort 2018). The fly was identified as the seed predating fruit fly *Apenthecia* cf. *crassiseta* (S. Kremer-Köhne 12) (McEvey *et al.* 1988) and confirmed as the *Afrostilba* sp. 1 host (Van Noort, pers comm.).

Overall, *in situ* seedling survival into the next growing season was 62% (Table 3.3). Relationships between seedling survival and percentages aerial cover (bare soil, forbs, woody plants, grass), height of woody plants and grass as well as calculated standing herbaceous biomass near mother plants were not significant ($P > 0.05$). However, forb height had a significant (but weak) positive effect on seedling survival (Table 3.4 and Figure 3.5). Rocks did not occur around the surveyed mother plants (Table 3.4). Seedling survival was significantly lower in burned areas (26%) than in unburned areas (76%) ($H_{1,30} = 11.53537$, $P = 0.0007$) (Table 3.3), but relationships between seedling survival in burned and unburned areas and the variables relating to the immediate habitat of the mother plants were not significant (regression analyses, $P > 0.05$). Some new seedlings ("recruits") appeared around 43% of the mother plants within the six months of them being marked, indicating ongoing germination in 2017 and possibly older seeds as well as seedling establishment, particularly in unburned areas (Table 3.3).

Table 3.3. *Aloe lettyae* seedling survival into the following growing season. Mother plants with 3–31 seedlings each marked in May 2017; surviving seedlings and new seedlings (recruits) counted in December 2017. Populations HB, EB and FG. Mean ± SE; values in parentheses are the ranges of the counts. Significant differences are indicated by different letters.

Fire			Seedlings			Recruits
July 2017	Mother plants		May 2017	19.12.2017	Survival (%)	19.12.2017
No	21	Total number	172	131	76.2[a]	21
		Per mother plant	8.2 ± 1.6 (4–31)	6.2 ± 1.1 (4–23)	81.2 ± 6.5 (0–100)	1.0 ± 0.3 (0–4)
Yes	9	Total number	65	17	26.2[b]	4
		Per mother plant	7.2 ± 1.8 (3–21)	1.9 ± 0.5 (0–4)	33.1 ± 10.5 (0–75)	0.4 ± 0.3 (0–3)
Overall	30	Total number	237	148	62.4	25
		Per mother plant	7.9 ± 1.2 (3–31)	4.9 ± 0.9 (0–23)	66.8 ± 6.8 (0–100)	0.9 ± 0.2 (0–4)

Table 3.4. R^2 values of regression analyses between percentage *Aloe lettyae* seedling survival and aerial cover (visually estimated percentage of rock, bare soil, forbs, woody plants and grass), vegetation height (cm) of forbs, woody plants and grass, and calculated standing plant biomass within 1 m of marked mother plants (n = 30). Populations HB, EB and FG; burned and unburned areas combined. Mean ± SE; bold value represents a significant relationship.

Variable	Mean ± SE	R^2	P
Bare soil (%)	8.1 ± 1.4	0.0005	0.9088
Rock (%)	0		
Grass cover (%)	50.5 ± 3.0	0.0284	0.3731
Forb cover (%)	29.7 ± 3.2	0.0154	0.5139
Woody cover (%)	11.7 ± 2.4	0.0039	0.7437
Grass height (cm)	140.7 ± 8.3	0.0809	0.1277
Forb height (cm)	78.8 ± 5.3	**0.1688**	**0.0299**
Woody height (cm)	92.6 ± 6.9	0.0163	0.6022
Standing biomass (kg/ha)	7426.0 ± 763.1	0.0246	0.4082

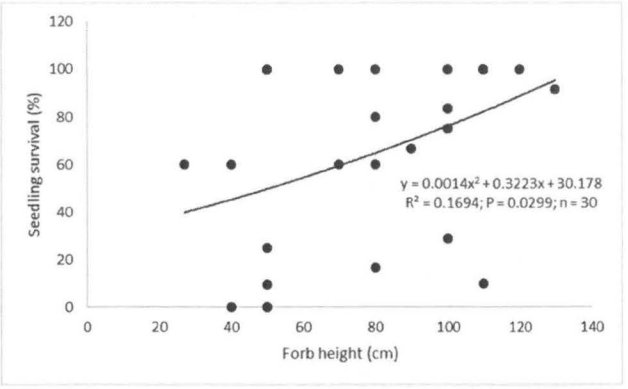


Figure 3.5. Relationship between percentage *Aloe lettyae* seedling survival (assessed in December 2017) and forb height (cm) measured in May 2017 around mother plants (n = 30). Populations HB, EB and FG; burned and unburned areas combined.

Aloe lettyae visitors and nectar properties

Although the camera traps were active for 24 hours, very few records were obtained during the night. Only at population FG, there was one record of a mouse climbing up the inflorescence rachis without coming near a flower, and ten incidents of a moth hovering near a flower were recorded. Therefore, only daylight hours (5:30–18:30) were considered, and the total camera trap observation hours came to 1209 hours with a total of 901 visitors observed. Some 3.6% of bird visitors could not be identified due to the bird moving or being obscured by poor light or the inflorescence itself. During data collection in the populations surveyed across *A. lettyae*'s range, Amethyst sunbirds (*Chalcomitra amethystina*), Southern and Greater Double-collared sunbirds (*Cinnyris chalybeus* and *C. afer*) as well as honey bees (Figure 3.2d), stinkbugs and longhorn beetles were seen visiting *A. lettyae* flowers. Camera traps confirmed the bird species observed, and nine additional species were recorded (Table 3.5). Both Southern Double-collared and Greater Double-collared sunbirds occur in the study area (Grosel 2016), and their females are indistinguishable (*Chittenden et al. 2016*). Further, some individuals within the Southern Double-collared sunbird population occurring in the Magoebaskloof/Haenertsburg area show uncharacteristically broad red chest bands (Grosel, pers. comm.). For these reasons, Southern Double-collared and Greater Double-collared sunbird observations were combined (Table 3.5 and Figure 3.6).

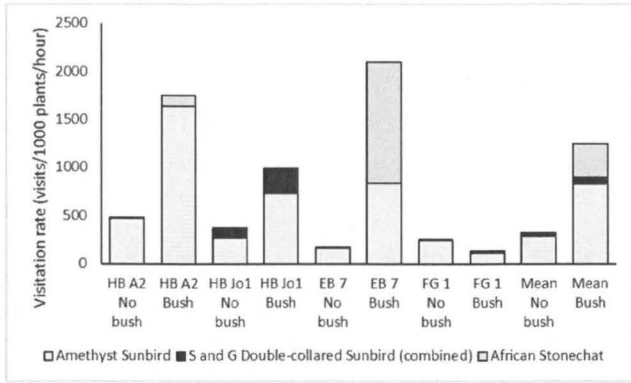


Figure 3.6. Visitation rates (number of visits/1000 plants/h) of Amethyst sunbirds, Southern and Greater Double-collared sunbirds (combined) and African Stonechat on *Aloe lettyae* inflorescences recorded by camera traps in open grassland (No bush) and within 5 m from a bush (Bush) at localities HB A2, HB Jo1, EB 7 and FG 1 in January 2017. Total camera trap observation hours (n = 1209); total visitors (n = 901).

Table 3.5. Bird species visiting *Aloe lettyae* inflorescences as observed by camera traps in January 2017, their main food (according to Hockey *et al.* 2005) and calculated mean visitation rates (number of visits/1000 plants/h ± SE) combined for localities HB A2, HB Jo1, EB 7 and FG 1. Total camera trap observation hours (n = 1209); total visitors (n = 901). Mean ± SE; percentage of total visitation in parenthesis for each species.

Bird species	Main food source	Visitation rate (number of visits/1000 plants/h)
Specialist nectarivores		
Amethyst Sunbird *Chalcomitra amethystina*	Nectar, insects	554.4 ± 180.3 (69.84)
Southern and Greater Double-collared Sunbird *Cinnyris chalybeus and C. afer*	Nectar, insects	46.2 ± 33.2. (5.82)
Total		600.6 ± 110.2 (75.66)
Occasional nectarivores		
Drakensberg Prinia *Prinia hypoxantha*	Insects, aloe nectar	2.7 ± 2.7 (0.34)
Cape White-eye *Zosterops virens*	Insects, fruit, nectar	0.3 ± 0.3 (0.04)
Total		3 ± 1.4 (0.38)
Generalists		
African Stonechat *Saxicola torquatus*	Insects	175.3 ± 155.7 (22.08)
Wailing Cisticola *Cisticola lais*	Insects	6.5 ± 3.6 (0.82)
Dark-capped Bulbul *Pycnonotus tricolor*	Fruit	4.6 ± 3.8 (0.58)
Red-collared Widowbird *Euplectes ardens*	Seeds	1.9 ± 1.9 (0.24)
African Firefinch *Lagonosticta rubricata*	Seeds, insects	1.4 ± 1.4 (0.18)
Dark-capped Yellow Warbler *Chloropeta natalensis*	Invertebrates	0.3 ± 0.3 (0.04)
African Dusky Flycatcher *Muscicapa adusta*	Insects	0.2 ± 0.2 (0.03)
Total		190.2 ± 22.5 (23.96)
Overall visitation rate		793.8 ± 276.4 (100)

Three specialist nectarivorous bird species accounted for 76% of the visits (Table 3.5) and were observed inserting their bills into *A. lettyae* flowers, Amethyst sunbirds (46% of visiting Amethyst sunbirds probing) as well as Southern and Greater Double-collared sunbirds (51% of visiting Double-collared sunbirds probing). Amethyst sunbirds (Figure 3.1e) had the highest visitation rate (Table 3.5) and were observed at all localities monitored by camera traps, while Double-collared sunbirds only occurred at localities HB Jo1 and FG 1 (Figure 3.6). The visitation rate of Amethyst sunbirds and Double-collared sunbirds combined differed between populations and was highest in the big population

HB, intermediate at EG and lowest at FG with 713, 385 and 199 visits/1000 plants/hour, respectively. Where sunbirds had pierced the corolla, honey bees were seen feeding on nectar (Figure 3.2e). Occasional nectarivores such as Drakensberg Prinia and Cape White-eye had very low visitation rates. Bill length would preclude probing of the perianth and these species were not observed feeding on *A. lettyae* nectar. The second highest visitation rate was recorded for the insectivorous African Stonechat which, like the other generalist bird species, only perched on *A. lettyae* inflorescences. Overall, four times as many birds appeared to be attracted to an *A. lettyae* inflorescence near a bush (Bush, total of 5065 visits/1000 plants/h) than to one in the open grassland (No bush, total of 1287 visits/1000 plants/h), except at FG 1 (Figure 3.6). This difference, however, was not significant ($P > 0.05$).

Table 3.6. Identity of insects collected from *Aloe lettyae* reproductive plant parts during January–March 2017. Plant parts the insects were found on or in, observations of herbivory and abundance (scored as low, moderate or high).

Order Family	Species	Reproductive plant part	Herbivore	Abundance
Coleoptera				
Chrysomelidae	*Bonesioides kirschi*	Flowers, fruit, inflorescence	Yes	High
Cerambycidae	*Nemotragus helvolus*	Flowers, inflorescence	Yes	High
Elateridae	Sp. indet.	Flowers	No	Low
Tenebrionidae	*Lagriini* sp.	Fruit	Yes	Moderate
Orthoptera				
Acrididae	*Heteracris* sp.	Inflorescence	No	Low
Hymenoptera				
Formicidae	*Camponotus* afrc-za53	Inflorescence	Yes	Moderate
Formicidae	*Camponotus* sp.	Flowers	No	Low
Eurytomidae	*Eurytoma* sp.	Fruit	Yes	High
Figitidae	*Afrostilba* sp. 1	Fruit	No	High
Apidae	*Apis mellifera*	Flowers	No	High
Diptera				
Drosophilidae	*Apenthecia* cf. *crassiseta*	Fruit	Yes	High
Lepidoptera				
Pieridae	*Catopsilia florella*	Flower	No	Low

Insects collected from *A. lettyae* inflorescences were identified to either species or genus level where possible (Table 3.6). A small blue metallic beetle *Bonesioides kirschi* (Kremer-Köhne 8) which has been collected from 'aloe' previously (Freund and Wagner 2003), and the longhorn beetle *Nemotragus helvolus* (Kremer-Köhne 1) were frequently observed feeding on reproductive plant parts. The beetle *Lagriini* sp. (Kremer-Köhne 9) was feeding on fruit (Figure 3.2h), while the African Migrant butterfly *Catopsilia florella* (Kremer-Köhne 11), an unidentified click beetle of the Elateridae family (Kremer-Köhne 3) and the grasshopper *Heteracris* sp. visited inflorescences without leaving feeding scars. The ants *Camponotus* afrc-za53 (Kremer-Köhne 14) were feeding on peduncles and *Camponotus* sp. (Kremer-Köhne 7) were seen collecting nectar inside the tubular flowers (Figure 3.2c). None of the insects listed in Table 3.6 appeared to be a potential pollinator of *A. lettyae*.

Camera trap observations showed that 60% of the nectar feeding occurred in the morning before 12 p.m., and as the inflorescences were unscreened, they may have been visited by sunbirds prior to sampling. Nectar volumes were highly variable, ranging from 5 µl to 85 µl, and did not differ between male and female flower stage ($H_{1,125} = 3.3502$, $P = 0.0672$), while nectar sugar concentrations were more consistent and ranged from 11% to 25% (Table 3.7). Nectar sugar concentration was significantly higher during the male flower stage than during the female stage ($F_{1,123} = 14.243$, $P = 0.00025$) (Table 3.7).

Table 3.7. *Aloe lettyae* nectar volumes (µl) and sugar concentrations (%) determined for male (stamens protruding) and female (style protruding) flower stages in three trials. Mean ± SE; values in parentheses are the ranges of the readings. Overall n = 125; no difference between flower stages was found in nectar volume (Kruskal-Wallis, $P > 0.05$); significant difference in sugar concentration indicated by different letters (One-way ANOVA, $P < 0.05$).

Trial	Flower stage	N	Nectar volume (µl)	Sugar concentration (%)
1	Male	40	20.1 ± 2.7 (5–83)	18.5 ± 0.3 (15–23)
2	Female	35	11.6 ± 1.1 (5–28)	17.5 ± 0.4 (11–23)
3	Male	34	13.6 ± 1.7 (5–51)	20.3 ± 0.4 (16–25)
	Female	16	16.7 ± 4.9 (5–85)	17.7 ± 0.7 (13–23)
Overall	Male	74	17.1 ± 1.7 (5–83)	19.3 ± 0.3[a] (15–25)
	Female	51	13.2 ± 1.7 (5–85)	17.6 ± 0.4[b] (11–23)

Pollinator guilds

In the Insects only treatment, honey bees were observed landing on the bird net, getting inside the cage and then flying on to an *A. lettyae* flower. In the No visitors treatment, two individually caged *A. lettyae* plants set fruit, however no damage was detected on the two cages. One plant produced three fully developed fruit; the other plant set but aborted eight fruit and was excluded from the statistical analysis. On one control plant (All visitors treatment) the inflorescence disappeared inexplicably during the fruit development period and was omitted from the statistical analysis. All plants (100%) in the All visitors treatment produced fruit compared with 82% and 18% of the plants in the Insects only and No visitors treatments, respectively. Fruit set differed highly significantly between treatments ($H_{2,32} = 21.2404$, $P < 0.0001$), being higher in the All visitors (15%) than in the Insects only (5%) and No visitors (0.2%) treatments (Figure 3.7a), respectively. In part due to the high late summer rainfall in 2017, no fruit samples could be collected from 40% and 44% of the plants that had set fruit in the All visitors and the Insects only treatments, respectively, as a very high proportion (84%) of the fruit in both treatments were mouldy, visibly predated or had already dehisced.

Seed production per fruit differed highly significantly between the All visitors and the Insect only treatments ($F_{2,47}$ = 11.822, P < 0.0001), and was 2.5 times higher in the All visitors (62.6 ± 6.2 seed/fruit) than the Insects only (25.2 ± 7.0) treatment (Figure 3.7b). The No visitors treatment was excluded from the statistical analysis as there were only three fruit (containing on average 43 seeds) produced by one plant. The proportion of undersized/aborted seeds (89%) was extremely high in the No visitors treatment. In this trial, fruit set in the All visitors treatment was lower (15%) than that obtained from the reproductive success trial in HB clump E (29%; Figure 3.4), while the number of seeds produced per fruit was similar (63 and 57; Table 3.2). For the All visitors treatment, the percentages of undersized/aborted (47%), predated (10%) and intact/viable (43%) seeds were consistent with those obtained from the reproductive success trial in HB clump E (Table 3.2). Total seed production per aloe differed between treatments ($H_{2,24}$ = 16.8583, P = 0.0002; No visitors treatment excluded from the statistical analysis), with five- and 124-fold greater seed production in the All visitors treatment (1484 ± 499) compared to the Insects only (280 ± 104) and no visitors (12 ± 12) treatments, respectively (Figure 3.7c). This is likely an overestimate of the actual number of seeds dispersed from a plant as a high percentage of fruit were mouldy or predated, as mentioned previously.

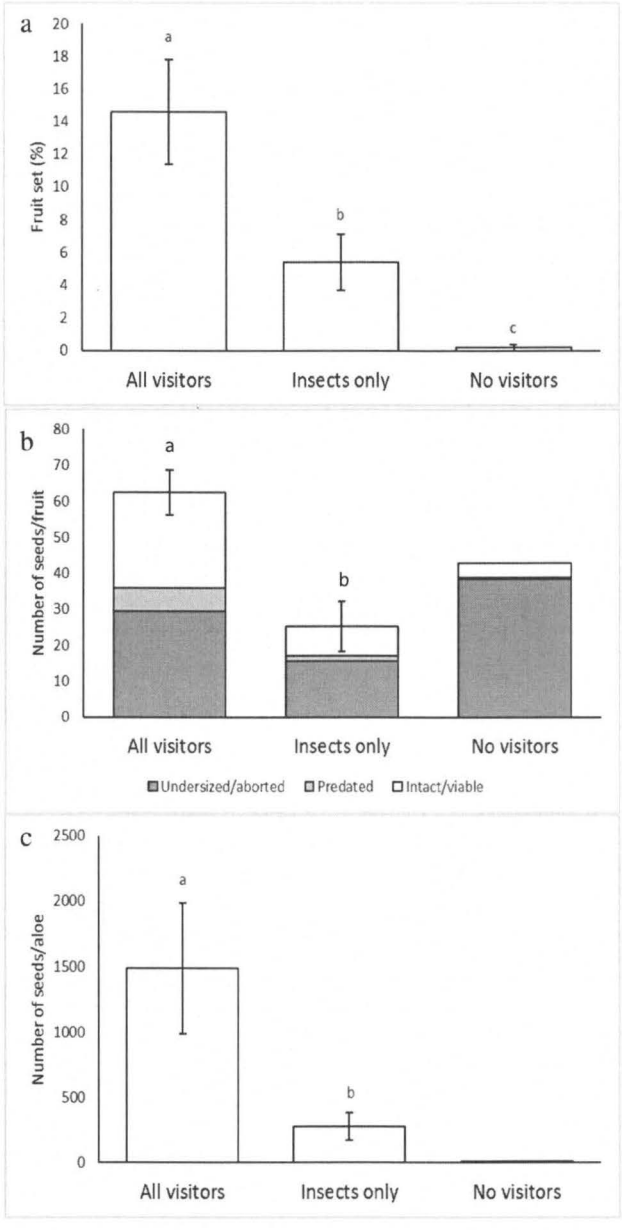


Figure 3.7. The contribution of different pollinator guilds (birds and insects) determined through pollinator exclusion treatments applied to *Aloe lettyae* plants (Insects only and No visitors n=11; All visitors n=10). (a) Percentage fruit set (proportion of flowers that set fruit) ($H_{2,32} = 21.2404$, $P < 0.0001$). (b) Seed production per fruit ($F_{2,47} = 11.822$, $P < 0.0001$; No visitors treatment excluded from the statistical analysis; error bars for cumulative number of undersized/aborted, predated and intact/viable seeds/fruit). (c) Seed production per aloe ($H_{2,24} = 16.8583$, $P = 0.0002$; No visitors treatment excluded from the statistical analysis). Mean ± SE; significant differences are indicated by different letters.

Seed germination

Six-month-old *Aloe lettyae* seeds from the populations monitored for their reproductive success and those from the pollinator exclusion trial started germinating within the first week after sowing and stopped germinating after week six. Cumulative germination percentages of seeds obtained from the populations of different sizes were high and ranged from 84% to 95% (Figure 3.8a). In the big populations HB and EB, MGT was 2.8 ± 0.1 weeks and 3.0 ± 0.2 weeks, respectively. Seeds from populations BU and FG (MGT both 2.1 weeks) germinated slightly faster than those from populations HB, EB, BL (MGT 2.5 weeks) and FH (MGT 2.7 ± 0.2 weeks) during the first three weeks of the trial. However, there were no significant differences between populations in terms of cumulative germination rates ($H_{5,30}$ = 6.0031, P = 0.3059) and MGT ($H_{5,30}$ = 6.995, P = 0.221). Cumulative germination percentages of seeds obtained from the pollinator exclusion trial were 81%, 91% and 93% for the Insect only, No visitors and the All visitors treatments, respectively (Figure 3.8b). MGT did not differ between treatments (All visitors 2.5 ± 0.1 weeks; Insects only 2.4 ± 0.1 weeks; No visitors 2.5 weeks) ($H_{2,12}$ = 0.0294, P = 0.9854), and neither did the cumulative germination rates HB ($H_{2,12}$ = 1.3158, P = 0.5179).

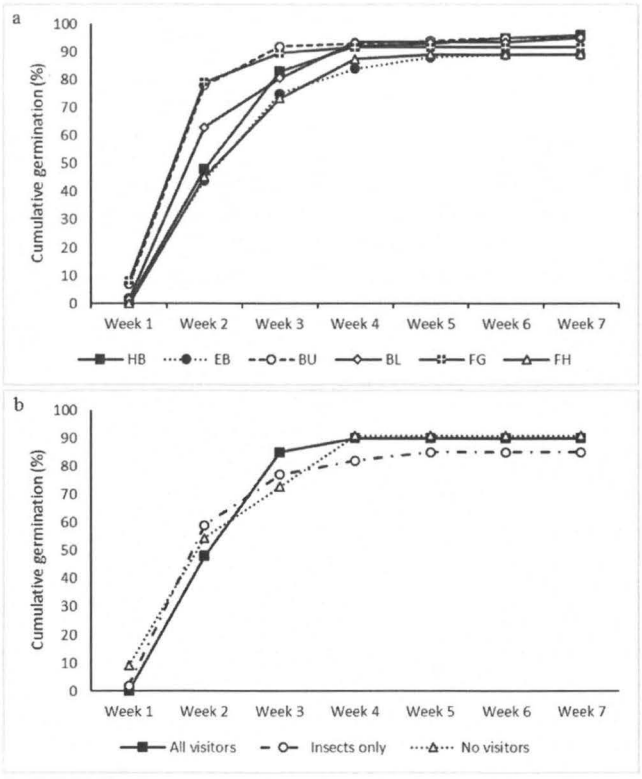


Figure 3.8. Cumulative germination of *Aloe lettyae* seeds from (a) populations HB (n=100), EB (n=100), BU (n=100), BL (n=62), FG (n=48), FH (n=64), ($H_{5,30}$ = 6.0031, P = 0.3059) and (b) the three pollinator exclusion treatments All visitors (n=100), Insects only (n=100), No visitors (n=11) applied in population HB ($H_{2,12}$ = 1.3158, P = 0.5179). The fruit were harvested in March 2017 and seeds sown at the end of September 2017. Germination under controlled conditions on a Jacobsen germination table.

Discussion

Flowering phenology and reproductive success

In the *A. lettyae* populations surveyed in this study, approximately 80% of the adult individuals were reproductive, and preliminary results indicate that *A. lettyae* plants flower in consecutive years. A similar high percentage (85%) of adult *A. plicatilis* were reproductive (Cousins *et al.* 2014b), while the flowering percentages for *A. peglerae* populations were lower, ranging from 23% to 67% (Arena *et al.* 2015; Phama *et al.* 2014) and varied highly between years (Arena *et al.* 2015; Scholes 1988). Bolus *et al.* (2004) observed that *A. pillansii* plants do not flower annually, but rather sporadically. The *A. lettyae* flowering season did not vary between years (2016–2018), an observation consistent with that made by

Botes *et al.* (2008) who found that peak flowering times of *A. pluridens*, *A. speciosa*, *A. africana*, *A. ferox* and *A. lineata* var. *muirii* did not differ between two years. *Aloe lettyae* reproductive output declined severely as population size declined, which indicated that the Allee effect may be operating (Lamont *et al.* 1993; Kleizen *et al.* 2009). However, more extensive sampling of small (defined here as less than 350 individuals) *A. lettyae* populations is required to confirm this. Reduced reproductive fitness has also been found in isolated *A. ferox* plants that flowered later in the season than most of the clumped, high density population, attracted fewer birds and had a lower fruit set (Stokes and Yeaton 1995). Further, the Allee effect has been shown for the related bird-pollinated Asphodelaceae taxa *Kniphofia umbrina* (Witkowski *et al.* 2001), *Kniphofia linearifolia* (Duffy et al. 2013) and *Kumara plicatilis* (=*Aloe plicatilis*) (Cousins *et al.* 2013, 2014b). In contrast, Wilson *et al.* (2009) showed that the insect-pollinated *A. pruinosa* is not experiencing Allee effects. The findings of the present study suggest that small *A. lettyae* populations are potentially vulnerable to long-term decline associated with Allee effects, which are exacerbated by the ongoing reduction of the remaining small extent of WGG and may lead to the local extinction of small and /or sparse populations (Courchamp *et al.* 1999). Delays between the start of habitat change and local extinction of populations may be substantial and have been estimated at 50–100 years (Lindborg and Eriksson 2004, Krauss 2010).

The quantity of intact seeds dispersed from *A. lettyae* plants is considerably reduced by insect-related fruit and seed predation. Seed predation by *Eurytoma* sp. wasps has also been observed in the summer-flowering *A. vossii* (Willis and Willis 1995) and in grass aloes (Craib 2005), as well as in the winter flowering *A. ferox* (Van Noort 2018) and *A. pretoriensis* (Van den Bosch and Cron 2018). *Aloe lettyae* fruit capsules dehisced in mid-March, and seeds were gradually dispersed from the capsules over a period of about four months. *Aloe* seeds are wind dispersed (Cousins and Witkowski 2012), and seed dispersal distances are estimated to be three times the height of seed release (Stokes and Yeaton 1995). In *A. lettyae*, with an inflorescence height of approximately 1 m as measured in this study, the seeds do not disperse far from the mother plant when compared to taller aloes such as e.g. *A. ferox* (=*A. candelabrum*) (Stokes and Yeaton 1995) and *Aloidendron pillansii* (=*Aloe pillansii*) (Bolus *et al.* 2004) which disperse their seeds over greater distances. *Aloe lettyae* seedlings were found growing within 30

cm from the leaf rosette of the mother plant. It is, however, possible that seedlings further away from the mother plant (within a 3 m radius of the mother plant) remained undetected in the dense herbaceous layer. As observed in this study, *A. lettyae* seed germination and seedling establishment appear to be occurring in small sporadic numbers over a long period of time, require the protection of nurse plants such as grass tussocks (Smith and van Wyk 2008) and are likely dependent on rainfall (Witkowski *et al.* 2001; Cousins *et al.* 2013). The slow release of seeds from the capsules combined with continuous seed germination and seedling establishment increase *A. lettyae's* chance to survive disturbances such as drought, aseasonal rainfall and fire. *A. lettyae* seedling survival rates in unburned and burned, generally non-rocky grassland areas, increased with increasing forb height around the mother plant. This differs from the results obtained in an *A. peglerae* study, which found that species persists in rocky sites with low surrounding vegetation (Arena *et al.* 2015).

Aloe lettyae visitors and nectar characteristics

Specialist nectarivorous Amethyst sunbirds account for 70% of the bird visits to *A. lettyae* in this study, with a very high visitation rate of 554 visits/1000 plants/h. This is four times the visitation rate that has been recorded for the generalist nectarivore Cape rock-thrush, the most frequent visitor to the winter-flowering *A. peglerae* (Payne *et al.* 2016) which provides abundant nectar during a time when other food sources are limited. Amethyst sunbirds have been recorded visiting several winter-flowering aloes (Botes *et al.* 2008; Hargreaves *et al.* 2010; Arena *et al.* 2013) as well as the summer flowering, insect-pollinated *A. pruinosa* (Wilson *et al.* 2009) and several grass aloes (Craib 2005). Southern and Greater Double-collared sunbirds contributed far less to the bird visits (6%) to *A. lettyae.* Nectarivorous sunbirds probe flowers rather indiscriminately, not only those that contain nectar (Oatley and Skead 1972). Amethyst sunbirds as well as Southern and Greater Double-collared sunbirds are known to feed on, *inter alia*, *Aloe* spp. nectar, either by inserting the bill into the mouth of the flower or piercing the base or side of the flower if the corolla tube is longer than the bill (Reynolds 1969; Hockey *et al.* 2005), robbing nectar when using the latter method, without rendering a pollination service (Geerts and Pauw 2009). *Aloe lettyae* has a limited number of open flowers on each raceme at any one time, resulting in

pollinators finding nectar in a few flowers only and being forced to move to another inflorescence on a different plant (Hoffman 1988). In this study, the visitation rate of Amethyst and Double-collared sunbirds combined was 3.6 times higher in the large main population than in the smallest population in which camera traps were used. Bird activity has been observed to be greatest where flowering aloes are most dense (Stokes and Yeaton 1995). Conversely, bird activity is low in small, sparse populations such as the isolated *A. lettyae* population FG (Figure 3.6) that may not be able to support birds. Despite that, birds have been recorded foraging regularly over distances of one km or more visiting several widely scattered nectar sources and are probably more mobile than insects (Stiles 1978).

Bees are frequently visiting aloe flowers (Hoffman 1988; Botes *et al.* 2008; Cousins and Witkowski 2012). In aloes with long tubular flowers and included filaments, e.g. *A. lettyae*, bees often crawl into the floral tube to obtain nectar and contribute to seed production (Botes *et al.* 2009a). However, bees are unable to access nectar in the bulb of maculate aloe flowers (Human and Nicolson 2008). As in *A. lettyae*, ants (Formicidae) have been observed as rare visitors in *A. ferox* (Hoffman 1988) and *A. divaricata* (Ratsirarson 1995), but they do not seem to be effective pollinators.

Flowers of maculate *Aloe* species have a swelling at the base of the flower which is usually full of nectar (Jordan 1996), and nectar concentration does not differ between the basal swelling (bulb) and the floral tube (Human and Nicolson 2008). Nectar is continuously available and its concentration remains relatively constant throughout the day (Nicolson and Nepi 2005; Human and Nicolson 2008; Symes and Nicolson 2008; Symes 2017). Nectar properties are associated with a specificity in bird pollination systems where flowers adapted for specialist nectarivores (e.g. sunbirds) have relatively low volumes of concentrated nectar (approximately 10–30 µl, 15–25% w/w per flower) and those adapted for generalist bird pollinators have high volumes of dilute nectar (approximately 40–100 µl, 8–12% w/w per flower) (Johnson and Nicolson 2008). *Aloe lettyae* nectar volumes from unscreened flowers were 15.5 ± 1.2 µl, and the nectar concentration was 18.6 ±0.2% w/w, confirming that *A. lettyae* flowers are adapted for specialist nectarivores. Nectar concentration has been shown to differ between flower stages (Human and Nicolson 2008; Symes and Nicolson 2008), and in male stage *A. lettyae* flowers sugar

concentration is significantly higher than in female stage flowers. Further, nectar properties vary during the flowering season with nectar volume being lowest and nectar concentration highest late in the flowering season of the maculate winter-flowering *A. greatheadii* var. *davyana* (Human and Nicolson 2008).

Pollinator guilds

Plant species producing significantly fewer fruits and seeds from self-pollination compared to cross-pollination are considered as self-incompatible (Johnson *et al.* 2009). In the absence of pollinators *A. lettyae* fruit set was very low (0.2%), and the few fruit that developed contained a very high proportion of undersized/aborted seeds (89%), suggesting self-incompatibility which has also been observed in various other *Aloe* species (e.g. Hoffman 1988; Botes *et al.* 2008; Botes *et al.* 2009a; Johnson *et al.* 2009; Wilson *et al.* 2009; Cousins and Witkowski 2012; Payne *et al.* 2016). *Aloe lettyae* is therefore entirely dependent on animal pollinators for reproductive output. Our study shows that birds contributed significantly more to *A. lettyae* fruit set (1.7 times), seed production per fruit (1.5 times) and seed production per plant (4.3 times) than insects. These results, and the camera trap observations, indicate that *A. lettyae* is primarily reliant on Amethyst sunbirds (*Chalcomitra amethystina*) for pollination and that insects, probably bees, are co-pollinators. The relative contributions of bird and insect pollinators to reproductive output vary between summer-flowering *Aloe* species. *Aloe ecklonis* (= *A. boylei*) and *A. reitzii* var. *reitzii* are predominantly sunbird-pollinated (Johnson *et al.* 2009; Symes 2017), *A. pruinosa* is exclusively bee-pollinated but is also visited by Amethyst sunbirds (Wilson *et al.* 2009), while. *A. linearifolia* (Botes *et al.* 2009b), *A. minima* (Botes *et al.* 2009b) and *A. modesta* (Van der Riet 1977) have been shown to be mainly bee-pollinated despite sunbirds being present in the study areas.

Seed germination

Under controlled conditions, *A. lettyae* seeds from the populations monitored for their reproductive output and those from the pollinator exclusion trial started germinating within the first

week after sowing which is consistent with results obtained with seeds of other *Aloe* species (Beverly 1979; Cousins *et al.* 2013; Payne *et al.* 2016). *Aloe lettyae* mean germination time and cumulative germination percentage varied very little, indicating that reduced seed production linked to either pollinator exclusion treatment or population size had no effect on seed germination. The lack of differences in germination results of *A. lettyae* seeds from the selective pollinator exclusion treatments is consistent with the findings in *A. peglerae* (Arena *et al.* 2013; Payne *et al.* 2016). Seeds of most *Aloe* species are known to have limited longevities of up to two years (Giddy 1973; Smith and Correia 1992; Symes 2012) and therefore Asphodelaceae are unlikely to form persistent seed banks (Witkowski *et al.* 2001; Cousins and Witkowski 2012). However, storing *A. lettyae* seeds under suitable conditions would be a low-cost option to maintain *A. lettyae* genetic material for *in situ* and *ex situ* conservation (Cousins *et al.* 2014a).

Conclusion

This study showed that *A. lettyae* is primarily reliant on specialist nectarivorous Amethyst sunbirds (*Chalcomitra amethystina*) for pollination, while bees are probably co-pollinators. The visitation rate of Amethyst sunbirds was lower in a small *A. lettyae* population than in a large one, resulting in a significantly lower reproductive output in terms of fruit set and seed production in small *A. lettyae* populations which indicates that an Allee effect may be operating. Seed germination, however, was not affected by population size. In large *A. lettyae* populations, recruitment is likely limited at the seedling establishment and juvenile maturation stages by a disturbance such as fire, and possibly by fruit and seed predation. Long-term monitoring of individual plants in selected populations is recommended to assess the short- and long-term effects of disturbances such as fire of different intensities and frequencies on this long-lived and slow-recruiting species. In combination with baseline *A. lettyae* population data provided by this study (Chapter Two), the understanding of *A. lettyae's* reproductive ecology will aid in designing an adaptive WGG management strategy.

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CHAPTER FOUR

GENERAL CONCLUSION

This is the first scientific study on the Endangered *Aloe lettyae* endemic to the highly threatened Woodbush Granite Grassland (WGG) in Limpopo Province, South Africa. I established *A. lettyae*'s range and showed how population size and structure are interlinked with the species' reproductive ecology (Figure 4.1).

Aloe lettyae's distribution has been severely reduced by habitat loss and fragmentation and most populations were clustered on the south western side of the WGG polygon, with all known localities less than 40 km apart within this fragmented vegetation type, confirming *A. lettyae* as a WGG endemic. Nineteen *A. lettyae* populations, including two large and 17 remnant populations, with a total area of occupancy of 17.5 ha and an estimated total of 8500 individuals were documented. *Aloe lettyae* populations are aggregated and occur in tall, dense grassland vegetation in which plants are difficult to detect, impeding locating populations and slowing down sampling procedures. Therefore, it is important to sample early in the flowering season when the grass is still relatively short and individual plants are less likely to be overlooked. Based on number of leaf layers and leaf rosette diameter, across all surveyed populations 15% of the plants were juveniles, 9% subadults, 15% non-reproductive adults and 61% reproductive adults. There was a clear difference in population structure between the main population and the second largest population, with the main population having a higher proportion of juveniles (14%) than the second largest population (3%). Most *A. lettyae* size class distributions were bell-shaped as Cousins *et al.* (2014) also found for *Kumara plicatilis* and suggested that populations comprising medium to large adult individuals with low recruitment rates are considered stable. Nevertheless, the low percentages of *A. lettyae* juveniles and non-reproductive subadults indicating an apparent lack of recruitment are a cause for concern. To observe changes in population structures by means of routine monitoring, counting leaf layers and measuring the leaf rosette diameter is recommended as these are very efficient and a fair proportion of juveniles are detected, i.e. these metrics

most appropriately reflect life history stages. For all surveyed populations combined, habitat profile variables only differed significantly with regard to the maximum height of woody plants which were slightly taller near an *A. lettyae* individual than further away, suggesting that *A. lettyae* is mostly unaffected by these variables. Habitat variables differed significantly between populations, possibly due to differences in disturbance history for which no records exist. Percentage aerial cover for grass, forbs and woody species were 50%, 37% and 5% for the main population and 49%, 26% and 15% for the second largest population, respectively, with the small remaining percentages being made up of bare soil and rock cover. The higher percentage forb and lower percentage woody species cover in the main population was associated with a higher proportion of juveniles (14%) when compared to the second largest population (3%). The main threats to *A. lettyae* were identified as misapplication of fire (too frequent or infrequent), cattle grazing and invasive alien plants, the level of which varied between WGG fragments. Therefore, it is recommended to draw up a management plan compliant with the National Environment Management Protected Areas Act for each individual, Critically Endangered WGG remnant to address these threats and manage their mitigation. Since both fire and grazing impact on grassland forbs (Witkowski *et al.* 2001; Fynn *et al.* 2004; Uys *et al.* 2004; Scott-Shaw and Morris 2015; Drury *et al.* 2016) thorough assessments of WGG fragments, including a quantification of the species richness, are needed to evaluate the effects of disturbances such as fires of different intensities and frequencies and adjust grassland management accordingly.

A selective pollinator exclusion experiment and camera trap evidence showed that *A. lettyae* is primarily reliant on specialist nectarivorous Amethyst sunbirds (*Chalcomitra amethystina*) for pollination, while bees are probably co-pollinators. The visitation rate of Amethyst sunbirds was lower in a small *A. lettyae* population than in a large one as pollinators are less attracted to small, sparse populations than to larger, denser populations resulting in increased levels of inbreeding and reduced fitness, i.e. reduced reproductive output (Lamont *et al.* 1993). In small *A. lettyae* populations, reproductive output in terms of fruit set and seed production was significantly lower than in large populations, indicating that an Allee effect may be operating. Further, low reproductive success has been shown to precede the extinction of small and isolated plant patches (Groom 1998). In large *A.*

lettyae populations, however, recruitment is likely limited at the seedling establishment and juvenile maturation stages by a disturbance such as fire, and possibly by fruit and seed predation.

This study suggests that small *A. lettyae* populations are potentially vulnerable to long-term decline associated with Allee effects, which are exacerbated by the ongoing reduction of the remaining small extent of WGG and may lead to the local extinction of small and/or sparse populations (Courchamp *et al.* 1999). Delays between the start of habitat change and local extinction of populations may be substantial and have been estimated at 50–100 years (Lindborg and Eriksson 2004; Krauss *et al.* 2010). The history of habitat loss and fragmentation, coupled with the severe reproductive decline as *A. lettyae* population size declines (Allee effect), points to the necessity to conserve the remaining small extent of WGG.

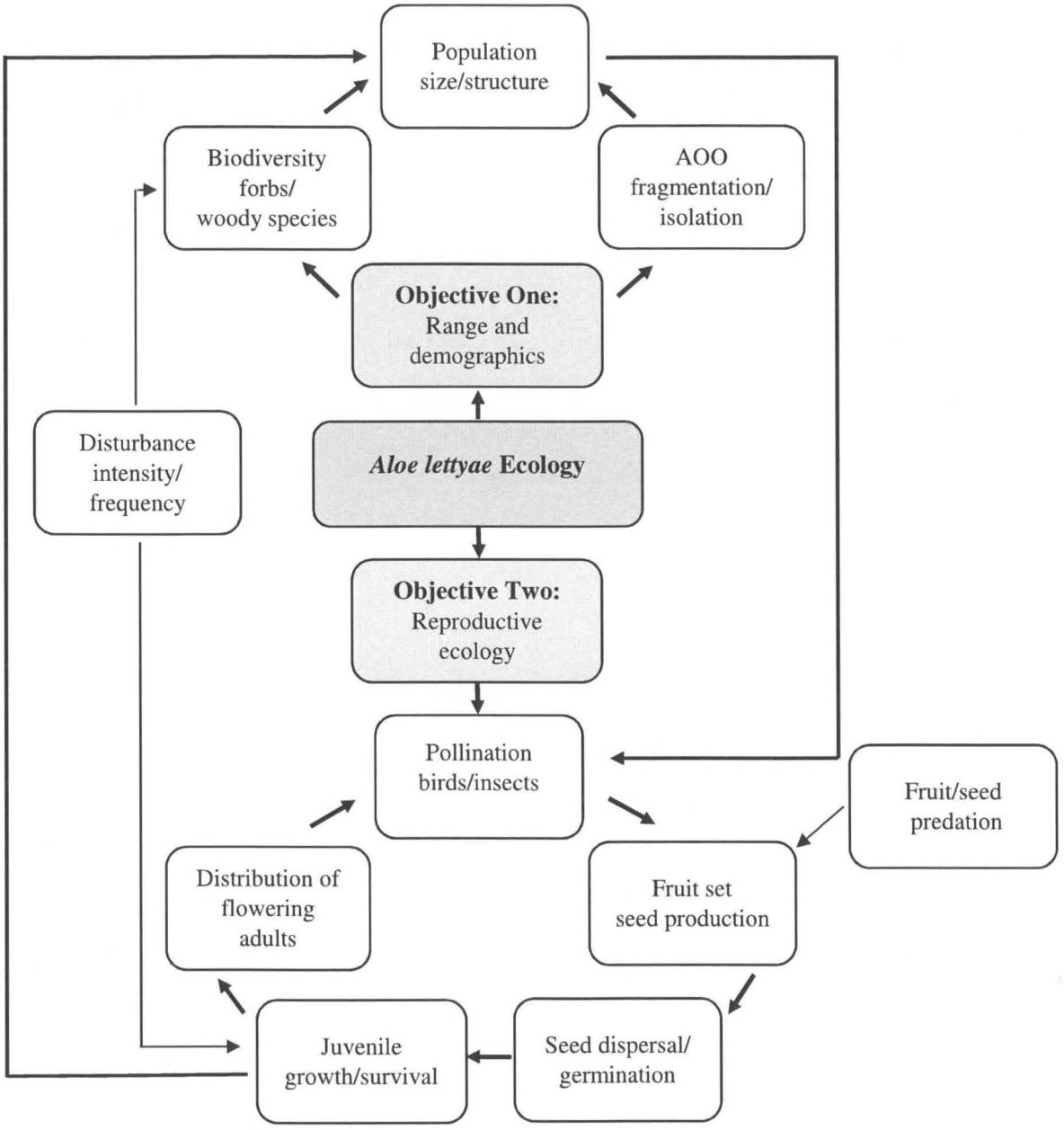

Figure 4.1. Synthesis of the results of the *Aloe lettyae* study, illustrating how population size and structure are interlinked with the species' reproductive ecology.

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