Aloe Pollination: Floral visitors, reproductive output and gender expression

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ABSTRACT

Suites of plant floral traits, such as floral colour and shape, nectar properties, scent, and timing of anthesis are important attractants and/or rewards for visitors, to ensure successful pollination events. This has resulted in a wide range of floral diversity within the angiosperms, and floral traits are often used to predict the most effective pollinators of a plant.

The genus *Aloe* comprises of > 500 species, with the greatest concentration and diversity of species found in southern Africa. Twelve different growth forms across ~140 taxa are recognised in South Africa alone, with a wide variety of floral traits. These traits suggest specialist nectarivorous bird (e.g. sunbirds (Nectariniidae) and sugarbirds (Promeropidae), generalist (opportunistic) nectar-feeding birds (e.g. weavers (Ploceidae), thrushes (Muscicapidae), white-eyes (Zosteropidae), etc.), or insect (e.g. halictid, allodapine and honeybees) pollination. Floral colour, nectar, and pollen rewards are the most important attractants for these visitors (both predicted and unexpected), and nearly all aloes are self-incompatible and reliant on pollen vectors for successful pollen transfer. This thesis investigated the role of *Aloe* floral traits in the attraction of multiple visitor/pollinator guilds, and the consequent effect on plant reproductive output, using three South African endemic *Aloe* species as model species.

Aloe peglerae is a Critically Endangered, stemless aloe, which flowers during winter (June – July). Floral characteristics suggest bird pollination (red flowers that produce large amounts of nectar), although small mammals were reported as occasional nocturnal visitors to the aloes. To compare the role of diurnal bird visitors and nocturnal small mammal visitors

in the pollination, and subsequent reproductive output of *A. peglerae*, day/night exclusion experiments were conducted during the 2013 flowering season. These experiments confirm that generalist nectar-feeding birds, especially the Cape Rock-Thrush (*Monticola rupestris;* 80% of all diurnal visits), are the primary pollinators of *A. peglerae*. Small, non-flying mammals, primarily the Namaqua Rock Mouse (*Micaelamys namaquensis*), successfully pollinate the aloe at night (70% of nocturnal visits), despite not being predicted by floral traits. This is the first quantification of small mammal pollination in *Aloe.*

Over two flowering seasons with different flowering success rates (2013: a "good" flowering year, and 2014: a "poor" flowering year), day/night exclusion experiments were conducted to assess the effect of annual fluctuations in flowering success on the pollinator visitation and subsequent plant reproductive output. Small mammals are suitable, alternative pollinators, especially in years when flowering success is low. Mobile birds are able to move to more energetically viable food resources, while mice are forced to rely on their immediate surroundings. In a poorer flowering year, *Aloe peglerae* reproductive output was lower in bird-pollinated plants (fruit set: 2013: 23.3 ± 4.9%; 2014: 0%; mean ± S.E.), while reproductive output by small mammal pollinated plants was similar between years (2013: 8.9 ± 2.7%; 2014:4.9 ± 4.0%). This emphasizes the effectiveness of small mammals as pollinators in flowering years with low primary pollinator (bird) abundance.

The production of *A. peglerae* nectar over a 24-hour period was measured, to assess the effect of constant nectar production on the temporal partitioning of pollinator visitation and resource use. *Aloe peglerae* nectar is produced continuously over 24-hours, with peaks in bird and small mammal visitation tracking periods of peak nectar production. Diurnal birds and nocturnal small mammals partition this nectar resource temporally, with overlaps in visitation at dusk and dawn. The increased nectar production during the day attracts birds, but

residual and freshly secreted (albeit reduced) nectar attracts small mammals at night, ensuring pollination events throughout the flowering period of the aloes.

Aloe reitzii var. *reitzii* flowers in summer (February – March), and flowers produce nectar throughout the day, with properties (volume and concentration) that range across both specialist- and generalist nectarivorous bird preferences. The contributions, and temporal partitioning, of both specialist- and generalist nectar feeding birds to the visitation to this aloe were investigated. Both these bird guilds regularly visit the aloes, with specialist visitation dominated by the Malachite Sunbird (*Nectarinia famosa*; 49% of all visits), and generalist visitation dominated by the Cape Weaver (*Ploceus capensis*; 41% of all visits). This may be indicative of pollinator specialisation by one generalist and one specialist nectarivore in *A. reitzii* var. *reitzii*. Visitation by these birds is temporally separated, likely due to territoriality and competition for nectar resources. Cape Weaver visitation peaks early in the morning, while Malachite Sunbirds are dominant from the late morning, and are more consistent for the rest of the day. Hawkmoths visit the aloes at night, and honeybees are regular visitors during day. Insects likely play an important role in the pollination of these aloes, as with many summer-flowering aloes.

Aloe petricola is a winter flowering (July – August) aloe, with three different floral colour morphs co-existing in different proportions within the same populations (orange buds, yellow flowers (55% of sampled population); red buds, white flowers (22%); and red buds, yellow flowers (22%)). The potential influence of pollinator-mediated selection for floral colour (flower constancy) by birds, on the proportions of different floral morphs within a sub-population of *A. petricola*, along with the resulting reproductive output of each color morph was assessed. Plant morphometrics (leaf rosette diameter and height, number of racemes, and raceme length) and nectar volume and concentration do not differ among morphs. Generalist nectar-feeding birds (six species recorded), especially the Dark-capped Bulbul (*Pycnonotus*

tricolor; 43.2% of visits), regularly visited the aloes, with the White-bellied Sunbird (*Cinnyris talatala*; 10.2% of visits) making up most of the specialist nectarivore visits (four species recorded). Honeybees and butterflies were observed visiting the plants. Flower colour does not appear to confer a reproductive advantage, as bird visitors do not preferentially select among morphs and reproductive output was similar among the three morphs (fruit set/plant: orange-yellow: 21.9 ± 4.1%; red-white: 16.9 ± 2.3%, and red-yellow: 21.4 ± 5.4%) Visitors are likely foraging at any available food resources in the resource-limited winter. Interactions and functional trade-offs between biotic (pollinators, floral pigments) and abiotic factors (spatial and temporal environmental variation, resource availability and use), rather than pollinator-mediated selection and flower constancy, may be driving and maintaining polymorphism, and optimising plant fitness in this *Aloe* species.

Despite the roles these floral characteristics play in attracting visitors and pollinators, 60% of published *Aloe* studies report fruit set (proportion of flowers that set fruit) of < 30%, with some plants producing no fruit at all. A review of published *Aloe* studies was conducted, where reports of pollinator visitation, surplus flower production and low fruit set were reviewed, and the theory of gender expression discussed in the context of *Aloe*. Many aloe species appear to produce surplus flowers that do not develop into fruit, likely as a result of the limited resources available, as these plants often grow in dry, resource-limited soils/environments. Fruit and seed maturation (female function) is resource-intensive, and so plants may allocate the limited resources to the "cheaper" male function of producing pollen. Therefore, these plants may be "functionally male", with surplus flowers performing multiple functions, apart from fruit production. They may serve as additional pollinator attractants, by making inflorescences larger and more conspicuous (which is important during pollinator limitation), or providing additional nectar and pollen rewards. Surplus flowers may also compensate for flowers that are lost due to transfer of self-pollen (self-incompatibility

reaction), and florivory. Structural limitations may cause low fruit set, as the infructescence cannot physically accommodate each flower as a fruit capsule; reported "low" fruit set may be the physical maximum that a plant can achieve. It appears that a trade-off between male and female function is made to optimise overall plant fitness. A broad scope for experimentation exists, as specific studies are required to define the role of surplus flowers in *Aloe*.

Plant-pollinator interactions in *Aloe* are complex, and are further confounded by other factors, such as resource limitation, and climate variability. Long term studies on the variability in flowering and fruiting, as a result of resource availability, use, and storage, may prove invaluable in understanding these interactions. Further studies should avoid treating extrinsic (pollinators and resource availability) and intrinsic factors (plant size, gender expression) as mutually exclusive, but rather take the interactions into consideration. Resources are allocated to the production of pollinator attractants, and visitors of multiple guilds (specialist and generalist birds, small mammals, and insects) play a vital role in the pollination of aloes. Aloes are also important food resources for these visitors, especially in resource-limited winter months. Visitors do not appear to preferentially select plants based on specific colours or nectar properties, but rather feed at any available resource. Within each guild, there appears to be a dominant species which may be acting as the "specialist" pollinator for specific *Aloe* species. However, the efforts of these pollinators may be futile if remaining resources are not sufficient for fruit and seed maturation. If resources for female function are limiting, then the production of surplus flowers still ensures gene transfer. Inadequate resource supply, storage, and allocation may jeopardise the overall fitness of the plant and, ultimately, the species, many of which are sensitive to habitat transformation and land degradation.

DECLARATION

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



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29 May 2019

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PREFACE

This thesis is comprised of chapters prepared for publication as Original Research Articles. Therefore, there is some repetition of information among chapters, especially in the introductions, study species and site descriptions, and methods. Although every effort was made to minimise the repetition, omission of some information would render the chapters incomplete, and so some of the repeated information was retained.

Chapter Two is published in *Austral Ecology* as Payne *et al*. (2016), and Chapter Three has been published in the *Australian Journal of Botany* as Payne *et al*. (2019). The formatting for these two chapters is according to journal requirements, and differ from the rest of the thesis. A popular article was published in *Veld & Flora* (Payne *et al*. 2017), and has been attached as Appendix 8.2 in the thesis. This article does not match the formatting of the rest of the thesis.

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GLOSSARY OF ABBREVIATIONS

ALL$_{TRT}$: All Visitors Treatment, acting as a control. A selective exclusion treatment, where *Aloe peglerae* plants were left exposed i.e. not covered with mesh cages, for the entire duration of the flowering period, allowing access to the plant and/or flowers by visitors of all types, both diurnal and nocturnal.

CV: Coefficient of Variation. The quotient of standard deviation and mean, multiplied by 100.

DIUR$_{TRT}$: Diurnal Visitors Treatment. A selective exclusion treatment, where *Aloe peglerae* plants were covered with mesh cages at night, but cages were removed during the day, allowing access to plants and/or flowers by diurnal visitors only.

GLM: Generalised Linear Model.

MGT: Mean Germination Time. Measured in days, Mean Germination Time is the approximate time take for seeds to germinate.

MMR: Magaliesberg Mountain Range, between the Gauteng, North-West and Limpopo provinces, South Africa.

NOCT$_{TRT}$: Nocturnal Visitors Treatment. A selective exclusion treatment, where *Aloe peglerae* plants were covered with mesh cages during the day, but cages were removed at night, allowing access to plants and/or flowers by nocturnal visitors only.

NOVIS$_{TRT}$: No Visitors Treatment. A selective exclusion treatment, where *Aloe peglerae* plants were covered with mesh cages for the entire duration of the flowering period, preventing visitors (both diurnal and nocturnal), of all types from accessing the plants and/or flowers.

S-C: Self-Compatible; plant species that are able to produce viable seed after flowers are pollinated from pollen from the same flower and/or plant.

S-I: Self-Incompatible; plant species that require conspecific pollen from a different plant of the same species to produce viable seed.

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CHAPTER ONE

General Introduction



Two flowering *Aloe peglerae* plants in The Peglerae Conservancy, Gauteng (Craig Symes)

1.1 Introduction and background information

Phenotypic floral traits, such as corolla colour, floral shape and size, nectar volume and concentration, scent, and timing of anthesis are often directed to attract potential pollinators (Faegri and van der Pijl 1979; Ollerton *et al*. 2009). However, because floral visitors differ in their effectiveness, these traits also inhibit inefficient visitors from accessing pollen and nectar rewards (Thomson and Thomson 1992; Castellanos *et al.* 2004; Johnson *et al.* 2006; Johnson 2010). These traits have given rise to pollination syndromes, where suites of floral characteristics can be used to predict the most efficient pollinating guild of a particular plant species (Faegri and van der Pijl 1979). These characteristics likely co-evolved to ensure "blossom - visitor harmony" (Faegri and van der Pijl 1979), through selective pressures for efficient pollination (Stebbins 1970; Hargreaves *et al*. 2004; Johnson 2010). These syndromes, however, are often highly generalised (e.g. Hingston and McQuillan 2000), and are more suited to formulating testable hypotheses for field experiments (Hargreaves *et al*. 2004; Johnson 2010).

In spite of floral characteristics being suited to a specific pollinator guild, many other visitors that are not predicted by the same floral traits are also attracted to these flowers, introducing the "Plant - Pollinator Paradox" (Ollerton 1996). Therefore, flower colour and shape (e.g. Raven 1972; Castellanos *et al*. 2004), nectar secondary compounds (e.g. Johnson *et al.* 2006; Nicolson *et al*. 2015), and timing of anthesis and nectar provision (e.g. Percival 1974; Kaczorowski *et al*. 2005), may also serve to prevent visitation by inefficient visitors, while attracting suitable pollinators.

Floral colour

Pollinators often select flowers visually, based on colour (e.g. Mogford 1974; Waser and Price 1981; Stanton *et al*. 1989; Comba *et al*. 2000; Jones and Reithel 2001). Birds are often

attracted to scentless, bright red, yellow, or orange tubular flowers that produce substantial (10 - 100 µL; Johnson and Nicolson 2008) amounts of nectar, while bees are attracted to white and yellow, heavily scented flowers with less nectar ("ornithophilous" and "melittophilous" pollination syndromes respectively; Faegri and van der Pijl 1979). Red flowers are particularly attractive to birds, since the bright colours are conspicuous against the surrounding vegetation, and signal a high caloric reward (Raven 1972). The most recently-defined pollination syndrome is the small, non-flying mammal syndrome, with geoflorous (close to the ground), bowl-shaped, white and cream flowers, which emit a yeasty smell ("therophilous" pollination syndrome; Rourke and Wiens 1977; Faegri and van der Pijl 1979; Wiens *et al*. 1983; Rebelo and Breytenbach 1987).

In angiosperms, different corolla/perianth colour phenotypes can occur within the same species, known as flower colour polymorphism (e.g. Asteraceae: de Jager and Ellis 2014; Ericaceae: Heystek *et al*. 2014; Solanaceae: Smith *et al*. 2008; Ng and Smith 2016a, b). These colours are often the result of shifts in biochemical pathways that drive pigment compositions and concentrations within the floral tissue (Tanaka *et al*. 2008; Ng and Smith 2016a, b). Such plants may co-exist in one population, or occur in geographically isolated populations (e.g. de Jager and Ellis 2014). Pollinator-mediated selection may be affecting the proportions and persistence of each morph within a specific population, as pollinators may preferentially visit one colour morph over another, increasing reproductive output of the selected colour morph (Mogford 1974; Stanton 1987; Stanton *et al*. 1989; Jones and Reithel 2001). Flower constancy – strict flower colour preference – is either innate or learned through previous visits and experiences (Chittka *et al*. 1999; Jones and Reithel 2001; Heystek *et al*. 2014), and can result in

geographically isolated populations of each colour, as it drives floral divergence (Waser 1986; Grant 1994).

Nectar properties and availability

Nectar is an important attractant and food resource for birds, small mammals and insects (e.g. Fleming and Nicolson 2002; Kaczorowski *et al*. 2005; Symes *et al*. 2008, 2009, 2011; Schmid *et al*. 2015; Steenhuisen *et al*. 2015). Nectar offers a high caloric reward and may be an essential food source in resource-limited winter months (Malizia 2001; Fleming and Nicolson 2002; Symes and Nicolson 2008). Nectar properties and production rates are often influenced by selection pressure and competition to attract pollinators (Waser 1983; Aizen and Basilio 1998; Varassin *et al*. 2001; Castellanos *et al*. 2002; Kaczorowski *et al*. 2005), and so nectar properties often reflect the preferences of the most efficient pollinators (Faegri and van der Pijl 1979; Johnson and Nicolson 2008). Specialist, nectarivorous birds (e.g. sunbirds (Nectariniidae) and sugarbirds (Promeropidae)) prefer concentrated nectar that is produced in small quantities (15 - 25% w/w, 10 - 30 µL), while generalist (opportunistic) nectarivores (e.g. weavers, thrushes, etc.) are attracted to large amounts of dilute nectar (8 - 12% w/w, 40 - 100 µL; Johnson and Nicolson 2008). Secondary compounds, such as phenolic compounds, are present in some plant nectars (e.g. *Aloe vryheidensis* and *Erythrina* spp.), and may be deterring inefficient pollinators, who find the nectar unpalatable, from visiting these plants (Johnson *et al*. 2006; Nicolson *et al*. 2015).

Plants may also alter their nectar secretion/production rates to manage visitation by different pollinator guilds (Cruden *et al*. 1983). Consequently, visitors may alter their foraging behaviour to coincide with varying nectar secretion patterns, by altering the timing of their visits, or increasing the amount of time spent visiting at a flower or plant (Rathcke 1992). Some pollinating guilds and species may be competitively superior in different times and spaces (e.g.

Perrin and Kotler 2005; Abu Baker and Brown 2014), and visitation by multiple guilds may become temporally and spatially partitioned, if food resources are available at their preferred foraging times (Crome and Irvine 1986; Abu Baker and Brown 2014; Zoeller *et al*. 2016). Foraging tactic, scale and abilities of visitors may also allow for co-existence, if one species is more efficient at high resource abundance, while the other is more efficient during food resource limitation (Brown *et al.* 1997; Perrin and Kotler 2005).

Changes in nectar resource availability can impact visitor abundance and frequency, especially of more mobile visitors (e.g. birds), that can move to areas with more abundant resources (e.g. Brown and Hopkins 1996; MacNally 1996; Symes *et al*. 2001, 2008; Cotton 2007; Forbes *et al.* 2009; Las-Casas *et al*. 2012; Kuiper *et al*. 2015). Such species are able to track resources, and select areas with more energetically viable resources (Johnson and Sherry 2001; Malizia 2001; Cotton 2007). Some bird species, such as the Malachite Sunbird (*Nectarinia famosa*), are known to actively move between flowering plant populations, often in a predictable route, following the seasonal production of abundant nectar resources (Craig and Hulley 1994; Symes *et al*. 2001). Conversely, small, non-flying mammals have smaller home ranges (e.g. Ribble and Perrin 2005), and are more dependent on resources present in their immediate surroundings, regardless of the amount available. However, some Four-striped Grass-mouse (*Rhabdomys pumilio*) females expand their home-ranges in periods of low resources availability, rather than migrating to new habitats to avoid the increased energy requirements and predation risks that are coupled with long-distance migrations (Schradin and Pillay 2006). These fluctuations in pollinator abundance may negatively affect plant reproductive output, especially during poor flowering seasons with fewer nectar resources.

Resource limitation and gender expression

In plants, flower and fruit production is limited by resources that are available (e.g. nitrogen, phosphorous and potassium: Witkowski 1990; Lau and Stephenson 1994; Erel *et al.* 2008; sunlight: Witkowski and Wilson 2001; water: Ogaya and Peñuelas 2007). Plant size also influences the number of flowers and fruit produced, as well as the annual flowering and fruiting patterns of an individual plant (Schmitt 1983; Melville 1988; Scholes 1988; Ollerton and Lack 1998; Wilson and Witkowski 2003). In hermaphroditic plant species, fruit set (proportion of flowers that set fruit and seed) is often low (~22%, Sutherland and Delph 1984). It appears that more flowers are produced than the plant can sustain as fruit, usually a result of limited resources available prior to, or during, flowering and fruiting (e.g. Willson and Rathcke 1974; Stephenson 1980, 1981). Fruit and seed production is resource intensive – in nutrient poor-environments, 48% of P and 24% of N is allocated to seed production, with seeds only representing 0.5% of overall plant biomass (Witkowski and Lamont 1996). Therefore, to optimise plant fitness, the plant may need to make a trade-off between the "cheaper" male function of producing pollen, and the female function of producing and maturing fruit and seed (Charnov 1979; Charlesworth and Charlesworth 1981; Sutherland and Delph 1984; Sutherland 1986a; Ashman 1994).

These "surplus" flowers produced may be performing other functions within the plant, rather than being produced for the purpose of setting seed. Extra flowers may be increasing the size and attractiveness of the floral displays for pollinators, thereby increasing the probability of visitation and successful pollination (Willson and Rathcke 1974; Sutherland and Delph 1984; Sutherland 1986b; Broyles and Wyatt 1990). Larger floral displays may be important to increase visitation to small, geographically isolated populations (Kunonga *et al*. 2019). Surplus flowers may be produced as a form of "bet-hedging", where the plant produces excess flowers to

compensate for variation in pollination success and resources available for fruit production and seed maturation (Stephenson 1980, Sutherland and Delph 1984; Sutherland 1986b). In some cases, more fruit are initiated than can be matured, and the plant will selectively abort fruit of a poorer quality, to redirect resources to maturation of fruit and seed of a higher genetic quality (selective abortion; Stephenson 1980, 1981; Sutherland and Delph 1984; Sutherland 1986b). The pollen donation hypothesis suggests that some flowers are "functionally female", and can produce fruit and seed, while the remaining individual flowers act as "functional males", producing pollen (Willson and Rathcke 1974; Sutherland and Delph 1984; Collins and Rebelo 1987; Sutherland 1987; Broyles and Wyatt 1990). Therefore, the gender of the plant as a whole may be determined by the number of functionally male flowers on the inflorescence (Lloyd 1979, 1980; Sutherland and Delph 1984). One, or a combination, of these theories may be contributing to the seemingly low fruit set in hermaphroditic angiosperms.

The genus Aloe

Aloe (Asphodelaceae) is a mostly succulent genus, endemic to sub-Saharan Africa, Madagascar and surrounding oceanic islands (Reynolds 1950; Cousins and Witkowski 2012). Over 550 species are recognised (Newton and Rowley 2001; Lüthy 2007), with the greatest diversity of species concentrated in southern and eastern Africa, and Madagascar (± 350 species; Klopper and Smith 2007; Carter *et al*. 2011; Cousins and Witkowski 2012). Recently, the genus *Aloe* has undergone reclassification, to better reflect phylogenetic relationships between species and reduce heterogeneity in the genus (Grace *et al*. 2013). This has resulted in the reinstating of the genus *Kumara* (i.e. *Aloe plicatilis* = *Kumara plicatilis*), and the establishment of two new genera, *Aloidendron* (tree aloes) and *Aloiampelos* (scrambling aloes; Grace *et al*. 2013).

However, these genera still form part of the Asphodelaceae family, and are included in many *Aloe* field guides (e.g. Van Wyk and Smith 2014).

Approximately 140 taxa are found inhabiting a variety of environments in South Africa alone (Holland 1978; Van Jaarsveld 1989; Klopper and Smith 2007; Klopper *et al*. 2009; Cousins and Witkowski 2012). Aloes are of ecological importance within the ecosystem, as flowers are an important nectar and pollen sources for birds, insects and small mammals, while the fleshy leaves and stems are food sources for larger mammals, such as African Elephant (*Loxodonta africana*), Black Rhinoceros (*Diceros bicornis*) and Greater Kudu (*Tragelaphus strepsiceros*; Steyn and Stalmans 2001; Shackleton and Gambiza 2007; Parker and Bernard 2009). Aloes are also of horticultural and medical (traditional and western) importance (e.g. Holland 1978; Grace *et al*. 2009), and are threatened by habitat loss, unsustainable (and sometimes illegal) harvesting, and poor farming and mining practices (e.g. Pfab and Scholes 2004; Grace *et al*. 2009; Phama *et al*. 2014; Kunonga *et al*. 2019). These threats are further exacerbated by the aloes' very restricted natural ranges and/or distributions (Holland 1978; Cousins and Witkowski 2012).

Twelve different *Aloe* growth forms are recognised in southern Africa (Van Wyk and Smith 2014). These range from small grass (e.g. *A. minima*, ~35 cm) and bulbous (e.g. *A. bowiea*, ~14 cm) aloes to large single-stemmed (e.g. *A. ferox*, ~2 - 5 m) and tree aloes (e.g. *Aloidendron* (*Aloe*) *barberae*, ~18 m; Van Wyk and Smith 2014; Figure 1.1). A wide variety of floral morphologies are displayed within these growth forms. Larger species usually produce multiple, large inflorescences, tightly packed with brightly coloured flowers (e.g. *A. reitzii* var. *reitzii*, *A. spicata*), while smaller species will have fewer, dull flowers that are loosely arranged on the inflorescence (e.g. *A. albida*; Jeppe 1969; Van Wyk and Smith 2014; Figures 1.1 and 1.2).

Nearly all tested *Aloe* species are self-incompatible (*n* = 17 species; Hoffman 1988; Johnson *et al*. 2006; Botes *et al*. 2009a, b; Hargreaves *et al*. 2008, 2010, 2012; Symes *et al*. 2009; Wilson *et al*. 2009; Duffy *et al*. 2014), the exception being *A. thraskii*, which is able to autogamously self-pollinate, although fruit production is not as successful as when xenogamously pollinated (Patrick *et al*. 2018). Therefore, *Aloe* species are heavily reliant on external pollen vectors for successful pollen transfer. The floral displays of aloes, along with nectar and pollen rewards produced by the flowers, play an important role in pollinator attraction.

Aloe *floral traits*

Many *Aloe* species display ornithophilous, or bird, pollination syndromes, with tubular red, yellow or orange flowers (Reynolds 1950; Faegri and van der Pijl 1979; Cousins and Witkowski 2012; Van Wyk and Smith 2014; Figure 1.2). Nectar properties vary among species, and help refine predictions of visiting and pollinating birds (i.e. specialist nectarivores *versus* generalist, opportunistic nectar feeders; Johnson and Nicolson 2008).

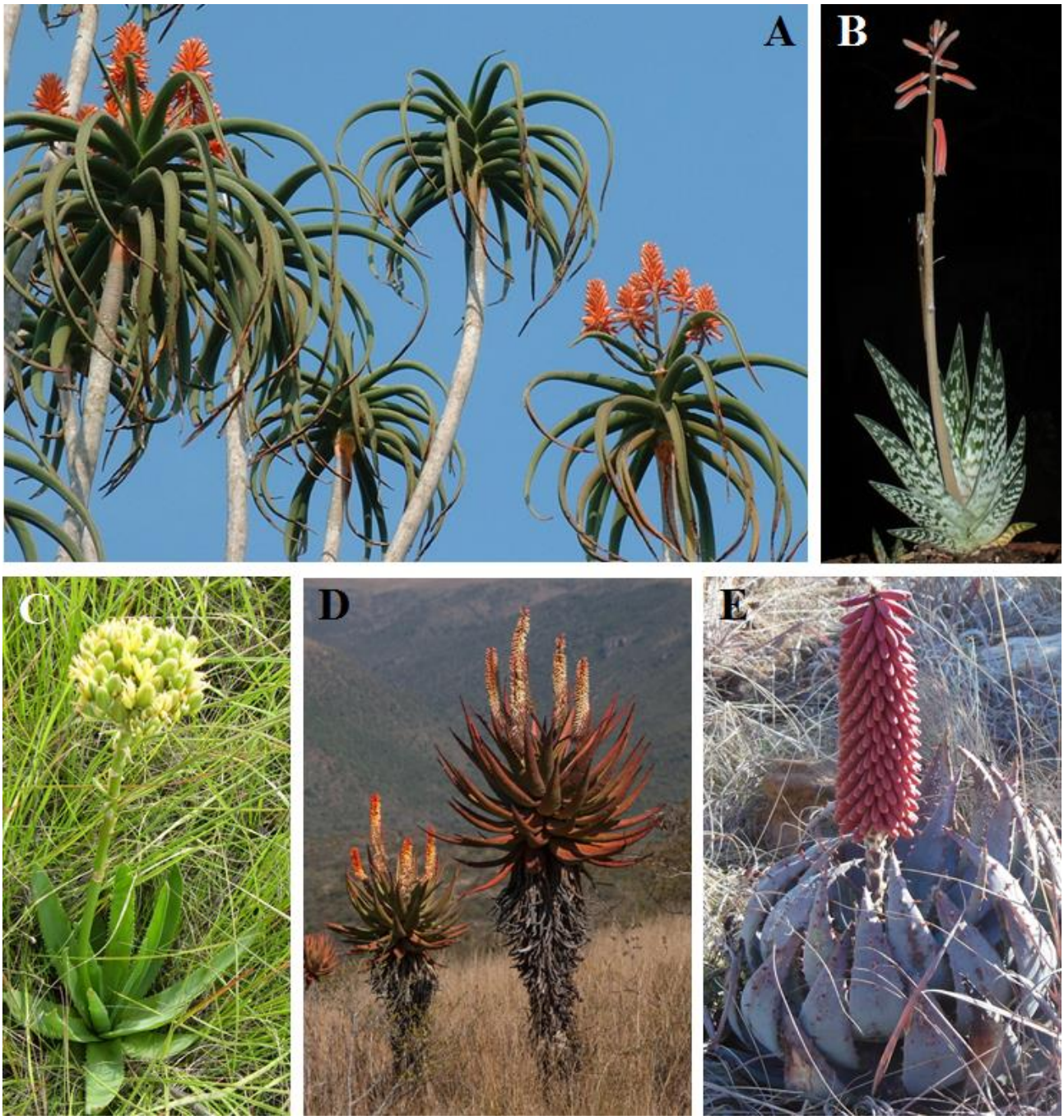


Figure 1.1. Representatives of five of the 12 different *Aloe* growth forms recognised in southern Africa. A) *Aloidendron (Aloe) barberae*, a tree aloe; B) *Gonialoe (Aloe) variegata*, a dwarf aloe; C) *Aloe ecklonis*, a slender aloe; D) *Aloe ferox*, a single-stemmed aloe; and E) *Aloe peglerae*, a stemless aloe. Photographs A, B, and D: Duncan McKenzie, photograph C: Craig Symes, and photograph E: Stephanie Payne.

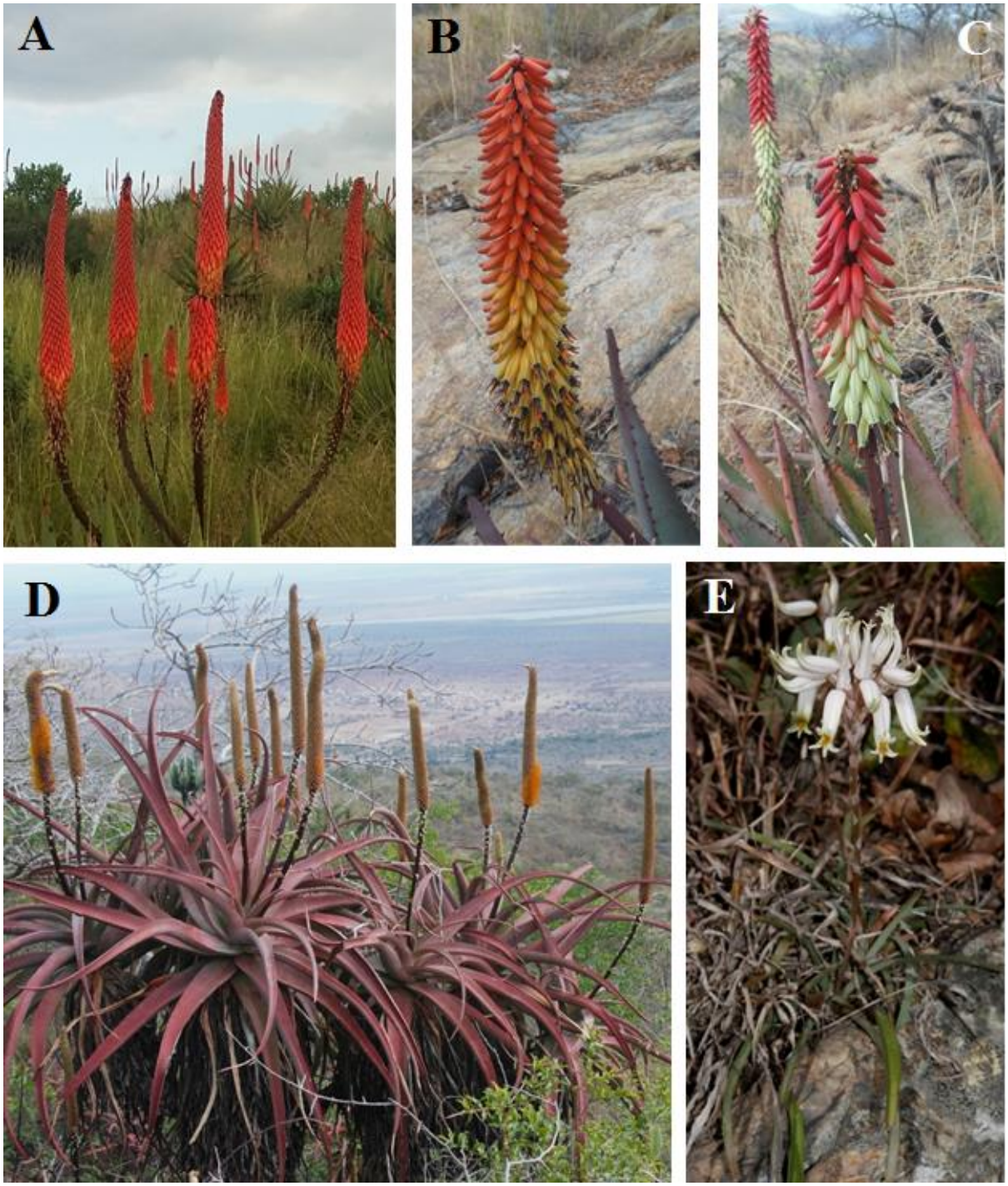


Figure 1.2 Different, floral colours and/or growth forms of species within the genus *Aloe*. A) *Aloe reitzii* var. *reitzii*, a stemless aloe with multiple racemes, and bright red, tubular flowers; B) A raceme of an orange-yellow morph of the polymorphic, stemless *Aloe petricola*; C) A raceme of a red-white morph of *Aloe petricola*; D) The multi-stemmed *Aloe spicata*, with brown flowers and yellow exserted anthers; and E) *Aloe albida*, a grass aloe with white flowers. Photographs A – C: Stephanie Payne, photographs D and E: Duncan McKenzie.

Aloes produce nectar consistently throughout the day (e.g. *A. castanea*: Nicolson and Nepi 2005; *A. greatheadii* var. *davyana*: Human and Nicolson 2008), with *A. marlothii* producing nectar over a 24-hour period (Symes and Nicolson 2008).

Indeed, many aloes are pollinated by specialist and/or generalist nectar-feeding birds, and these nectar properties have proven accurate in predicting the primary pollinators of some species (e.g. Hoffman 1988; Ratsirarson 1995; Botes *et al*. 2008, 2009a; Symes and Nicolson 2008; Symes *et al*. 2008, 2009; Wilson *et al.* 2009; Hargreaves *et al*. 2012; Arena *et al*. 2013; Cousins *et al*. 2013). All of these species are regularly visited by insects in search of nectar and pollen, usually honeybees, although they play an insignificant role in the pollination of the aloes (e.g. Hoffman 1988; Stokes and Yeaton 1995; Symes *et al*. 2008; Arena *et al*. 2013). However, some *Aloe* species are effectively pollinated by bees (Botes *et al*. 2009b, Symes *et al*. 2009; Wilson *et al*. 2009; Hargreaves *et al*. 2010; Patrick *et al*. 2018*),* with *A. inconspicua* exclusively pollinated by *Amegilla fallax* (Hymenoptera: Apidae; Hargreaves *et al*. 2008). Small mammals (Namaqua Rock Mouse *Micaelamys namaquensis* and Sengi *Elephantulus* sp.) were recorded visiting the flowers of *A. peglerae* at night, despite floral traits not matching small mammal preferences; their role in the pollination of this aloe has yet to be quantified (Arena 2013). Other small and medium-sized mammals, such as Chacma Baboon (*Papio hamadryas ursinus*), Four-striped Grass-mouse, Namaqua Rock Mouse and Slender Mongoose (*Galerella sanguinea*), are known to feed on *A. marlothii* nectar during the day, with the baboons damaging inflorescences to suck the nectar out of the flowers (Symes and Nicolson 2008). Fifty-five percent of southern African aloes flower in winter (N =158 species in southern Africa; Van Wyk and Smith 2014), and these aloes may be providing important food resources for these small mammals while other food sources are limited (Symes *et al*. 2011).

Since multiple guilds (specialist and generalist nectar feeding birds, small mammals) appear to be attracted to the aloes, it is possible that they are separating visitation to these plants temporally and spatially (e.g. Zoeller *et al*. 2016). Small mammal visitation suggests that there is likely some reward available, and that the aloes are producing nectar throughout the night (Symes and Nicolson 2008). Fluctuations in the proportions of flowering plants among flowering seasons may also be affecting pollinator visitation and the separation thereof. The abundance of specialist and generalist nectar-feeding birds (individuals and species) increases significantly during the peak of the flowering season of *A. marlothii* (Oatley 1964; Symes *et al*. 2008), although this influx is not as marked in poorer flowering years as compared to good flowering years (Oatley 1964). A similar trend has been recorded for *A. ferox*, where Malachite Sunbirds and *Ploceus* weaver species flock to stands of flowering aloes on an annual basis (Forbes *et al*. 2009; Kuiper *et al*. 2015). This suggests that these nectar-feeding birds are tracking the availability of nectar resources (and the relative abundance thereof), and are moving between sites as nectar becomes available, often in a predictable route (Craig and Hulley 1994; Symes *et al*. 2001; Kuiper *et al*. 2015).

Aloe resource limitation

Many *Aloe* species are slow-growing, but long-lived, and once they reach reproductive size, flowering and fruiting is erratic and can vary considerably between years (Scholes 1988; Weisser and Deall 1989; Kuiper *et al*. 2015). This affects pollinator visitation, and might be a consequence of environmental resource limitation. Aloes grow in dry habitats with shallow soils, such as the arid, water-limited savanna system, which are often in water deficit, even during rainy seasons (Van Jaarsveld 1989; Scholes and Walker 1993; Cousins and Witkowski 2012). When available, water is stored in the fleshy leaves and stems of the plants, and the shallow

roots, that lie close to the soil surface allow for the quick absorption of water during irregular rainfall events (Van Jaarsveld 1989; Cousins and Witkowski 2012). However, most species do not grow well in water-logged soil, as their roots begin to rot (Cousins and Witkowski 2012). Instead, they prefer well-drained, sandy soils and rocky sites, which are often nutrient poor (e.g. calcium, magnesium, potassium, phosphorous, nitrogen; Scholes 1988; Weisser and Deall 1989; Cousins and Witkowski 2012). In general, there has been little study on the ecophysiology of aloes, particularly relating to nutrient/energy resource requirements, uptake, storage, and use.

Pollination studies have been conducted on 28 South African *Aloe* species, and 60% of studies where reproductive output was assessed (n = 15 studies), reported low fruit set despite regular pollinator visitation (≤ 30%; e.g. Hoffman 1988; Botes *et al*. 2008, 2009a, b; Symes *et al*. 2009; Arena *et al*. 2013; amongst others). A similar phenomenon has recently been reported in the Zimbabwean *A. ortholopha*, where flowering plants produced many flowers but a low percentage of fruit and seed (Kunonga *et al*. 2019). In *A. peglerae*, many plants produced little to no fruit, despite receiving multiple pollinator visits while flowers were receptive (Figure 1.3; Arena 2013). Arena (2013) also found that bird and small mammal visitation rates did not correlate to reproductive output (fruit set, mean seed per fruit, total seed production per plant), suggesting that factors other than pollinator visitation were affecting fruit and seed production.

1.2 Rationale

Floral traits appear to play an important role in pollinator attraction in the genus *Aloe*, as multiple guilds, both predicted and unexpected, have been recorded visiting aloes. However, particularly in the case of small, non-flying mammals, their potential role in the pollination of *Aloe* has not been quantified. Nectar is often a reliable predictor of primary pollinator(s) of an *Aloe* species, and the partitioning of these resources by the primary pollinators, and other visitors

has not been explored. The effect of temporal fluctuations in the availability of this resource, both over a day, and among flowering seasons, on multiple visiting guilds, and consequent reproductive output is unknown.

Flower colour is an important attractant, especially since these colours create a stark contrast with the surrounding environments (Raven 1972). Pollinator-mediated selection and flower-constancy may be influencing pollinator visitation and reproductive success of floral morphs, which may be driving flower colour polymorphism within certain *Aloe* species. Flower colour polymorphism is very common in 20 out of 37 polymorphic South African *Aloe* species (Van Wyk and Smith 2014). Pollinator preferences for each morph and the role these preferences may have in reproductive output and consequent proportions of each morph, has not been studied in the genus.

Understanding flowering, fruiting, and resource availability in this genus is complex. There is no clear pattern in annual plant flowering, even with the best dataset available (10 years of *Aloe peglerae*; Scholes 1988). It is possible to predict the size and/or the age of the plants that are able to flower (e.g. adult *A. peglerae* plants > 20cm in diameter will start flowering; Scholes 1988), but it is difficult to predict the annual frequency of flowering and fruiting thereafter. Plant height, number of racemes and raceme size are also not a reliable predictors of fruit set (*A. ferox*; Hoffman 1988). Very little information is available on the soil requirements and energy and/or nutrient storage or nutrients in the genus (Scholes 1988; Weisser and Deall 1989). These resource requirements and limitations might be affecting the production of floral attractants (flowers, nectar and pollen), and ultimately, the production of fruit and seed, which appears to be highly erratic (e.g. Scholes 1988). Most South African aloes produce flowers and fruit during the resource-limited (especially water) winter months (~ 55% of species, June-August; Van Wyk

and Smith 2014), and so plants likely rely on stored resources during this time. Due to limited resources, *Aloe* species may also be producing more flowers than they can mature as fruit, rendering the plants "functionally male", to optimise overall plant and population fitness (Charnov 1979; Sutherland and Delph 1984).

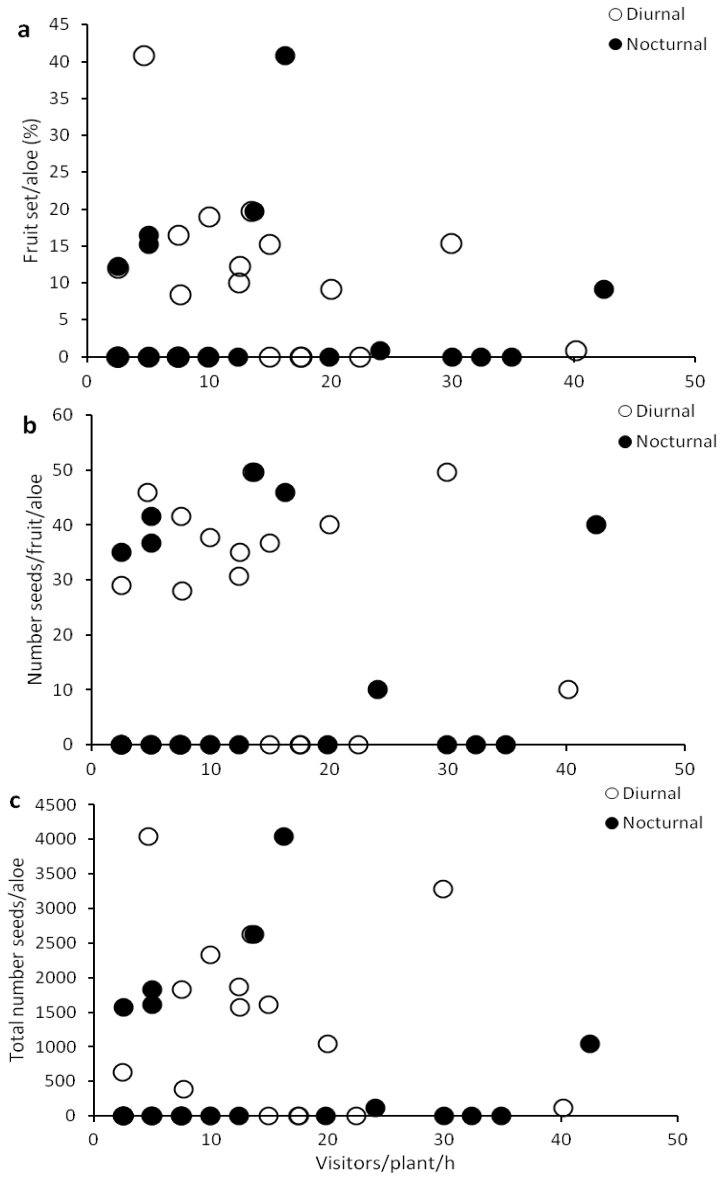


Figure 1.3. The effect of diurnal (bird) and nocturnal (small mammal) visitation rate per *Aloe peglerae* plant per hour in 2012 (n = 30) on, a) fruit set (%), b) seed set and, c) total seed production per plant. Many plants received high visitation rates, yet produced zero fruit and seed. Linear relationships are not significant (Spearman Rank Order Correlation; $P > 0.05$). Extracted, with permission, from Arena (2013).

1.3 Research aim and Objectives

This study aimed to assess the role of floral traits, particularly floral colour and nectar properties, in floral visitation by multiple guilds and reproductive output in *Aloe* pollination (Figure 1.4). Reasons behind the apparent overproduction of flowers within the genus *Aloe*, and the implications this may have on visitor/pollinator visitation, and fruit production were reviewed and discussed (Figure 1.4). This was achieved through the following objectives:

1.3.1. Objective 1: To measure and compare the contributions of diurnal (birds) and nocturnal (small, non-flying mammals) visitors to the pollination and reproductive output of *Aloe peglerae* (Chapter Two). The specific questions addressed are:

1) Are nocturnal small mammals effective pollinators of *A. peglerae*?
2) How do the contributions of nocturnal small mammals to visitation and reproductive output of *A. peglerae* compare to that of the diurnal birds?

1.3.2. Objective 2: To assess the effect of annual fluctuations in the proportions of plants flowering in an *Aloe peglerae* sub-population on diurnal and nocturnal pollinator visitation (resource tracking), and the consequent effect on plant reproductive output (Chapter Three). The following specific questions were addressed:

1) How does bird and small mammal visitation to *A. peglerae* differ seasonally?
2) How do fluctuations in bird and small mammal visitation rates affect *A. peglerae* reproductive output?

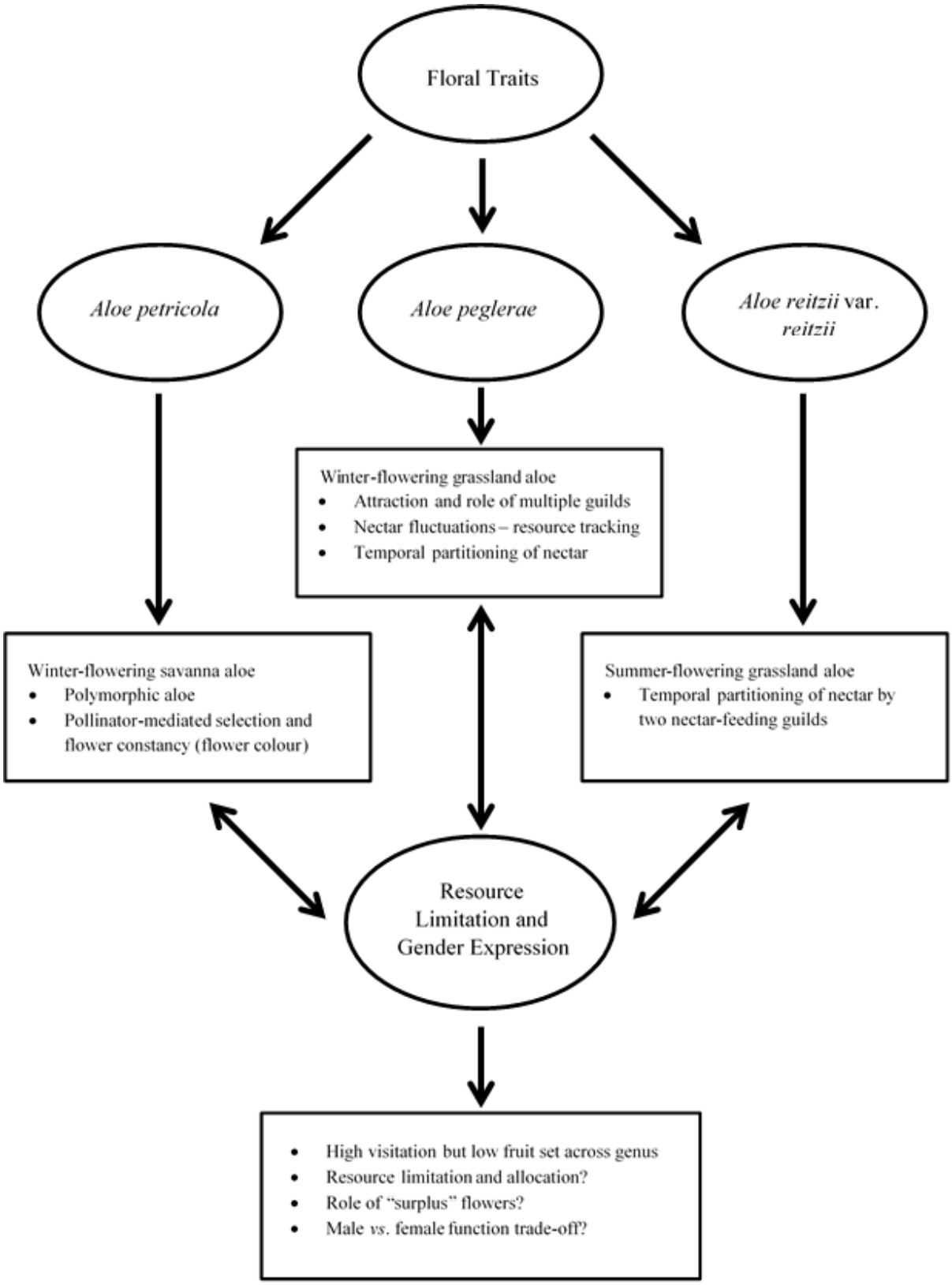


Figure 1.4. An overview of the aims and objectives of this study. Model species studied are in solid oval boxes, with objectives in rectangular boxes below.

1.3.3. Objective 3: To assess the production of nectar over 24-hours, and the effect on temporal partitioning of pollinator visitation and resource use, using *Aloe peglerae* as a model species (Chapter Four).

1) How does *A. peglerae* nectar production, and the properties thereof, change over a 24-hour period?
2) Are diurnal bird and nocturnal bird visitation patterns coordinated with *A. peglerae* nectar production patterns?
3) Do diurnal birds and nocturnal small mammals partition visitation to, and nectar resources of, *A. peglerae*?

1.3.4. Objective 4: Investigate the contributions, and temporal partitioning, of generalist and specialist nectar-feeding birds to visitation events, along with changes in nectar properties (a distinguishing feature used to separate generalist and specialist pollinated plants), using *Aloe reitzii* var. *reitzii* as a model species (Chapter Five).

1) What is the population structure of the assessed *A. reitzii* var. *reitzii* sub-population?
2) Which species visit, and subsequently dominate visitation to, the aloe during the peak of the *A. reitzii* var. *reitzii* flowering period?
3) Do *A. reitzii* var. *reitzii* nectar resources change throughout the day?
4) Do the specialist and generalist nectar-feeding birds partition their visits to *A. reitzii* var. *reitzii*, and the nectar resource, temporally?

1.3.5. Objective 5: To assess if pollinator-mediated selection for floral colour (flower constancy) may have influenced the proportions of different floral colours, and resulting

reproductive output, in a sub-population of the polymorphic *Aloe petricola* (Chapter Six). Specific questions addressed were as follows:

1) Are there differences in morphometrics (apart from flower colour) and nectar properties among the three *A. petricola* morphs?
2) What visits the *A. petricola* flowers, and do visiting species or guilds preferentially visit one colour morph over the others?
3) Does floral colour selection by visitors confer a reproductive advantage for *A. petricola*?

1.3.6. Objective 6: Review literature and supplied data for pollinator visitation, surplus flower production and low fruit set in the genus *Aloe*, and discuss the theory of gender expression in the context of the genus (Chapter Seven). The following questions were addressed:

1) Given that fruit production is resource intensive, and most of the flowers produced do not set fruit, why do *Aloe* plants allocate resources to the production of "excess flowers", rather than to fruit production?
2) What function do these excess *Aloe* flowers perform, if any?

1.4 Structure of the thesis

The thesis is comprised of six research chapters (Chapters Two – Seven). An introductory chapter (Chapter One) includes a literature review, describes the rationale for the study and outlines thesis aims and objectives. A concluding chapter (Chapter Eight) provides an overall summary of the thesis, places the study in the context of other *Aloe* studies, and suggests areas of further research. Appendices include a table of available information for South African *Aloe*

species (Appendix 8.1), and Payne *et al*. (2017), a popular article on *Aloe peglerae*, published in *Veld & Flora* (Appendix 8.2).

Details of publication and conference proceedings of the content chapters are as follows:

a) Chapter Two is published in *Austral Ecology* as Payne *et al*. (2016) and is formatted accordingly. This chapter began as an honours project in 2013, and data have been re-analysed and the chapter restructured for the thesis. It has been presented at the South African Association of Botanists Conference in Tshipise, Limpopo Province, during 11 - 15 January 2015. This chapter compares contributions of diurnal and nocturnal visitors to *Aloe* pollination.
b) Chapter Three has been accepted for publication in the *Australian Journal of Botany*, as of 07 March 2019. This chapter was presented at the BirdLife Flock/LAB (Learn About Birds) Conference at Skukuza (Kruger National Park), Mpumalanga, during 10 - 11 March 2016, and investigates the effect of fluctuations in *Aloe* flowering success on pollinator visitation and plant reproductive output.
c) Chapter Four investigates 24-hour nectar production, and temporal partitioning of pollinators in *Aloe*.
d) Chapter Five was presented at the 45th South African Association of Botanists Conference at the University of Johannesburg, during 8 - 11 January 2019. This chapter assessed the temporal partitioning of visitation to *Aloe* by multiple bird guilds.
e) Chapter Six was presented at the 44th South African Association of Botanists Conference at the University of Pretoria, during 9 - 12 January 2018. This chapter investigates pollinator-mediated selection based on flower colour in a polymorphic *Aloe*.

f) Chapter Seven was presented at the 45th South African Association of Botanists Conference at the University of Johannesburg, during 8 - 11 January 2019. This chapter reviews *Aloe* reproductive output and gender expression.

Each chapter is written with the intent to publish as an original research article. Therefore, there is some repetition between chapters, especially in the introductions, species and site descriptions, and methods. Although an attempt was made to minimise repetition, omitting some information would render the chapters incomplete and, therefore, some duplicate information was retained. Each chapter has its own reference section. Chapters Two and Three are formatted for their respective journals, and therefore formatting for these chapters differs from the rest of the thesis.

1.5. Author contributions

The published and/or accepted Chapters Two and Three have multiple co-authors. The contributions of each co-author are as follows:

a) S.L. Payne: Primary author, conducted all field work, data collation, statistical analyses, and write-ups;
b) C.T. Symes: PhD supervisor, provided guidance regarding project design, data analysis, chapter structures, and reviewed/commented on multiple drafts of all chapters and/or papers;
c) E.T.F. Witkowski: PhD supervisor, provided guidance regarding project design, data analysis, chapter structures, and reviewed/commented on multiple drafts of all chapters and/or papers;

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CHAPTER TWO

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A flowering *Aloe peglerae* plant with bright red buds, with exclusion cage in the background, in The Peglerae Conservancy, Gauteng (Maxine Semple)

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Of feathers and fur: Differential pollinator roles of birds and small mammals in the grassland succulent *Aloe peglerae*

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Abstract The pollination syndrome of the genus *Aloe*, with gaudy inflorescences and orange–red flowers, suggests bird pollination. However, the diversity of flowering phenologies and structures suggests that generalizations within the group are currently uninformative because few studies have addressed the role of multiple pollinator guilds, especially mammals. *Aloe peglerae*, endemic to montane grassland of the Magaliesberg Mountains, South Africa, is primarily bird pollinated, although small mammals are recorded nocturnal visitors. We compared the independent contributions of diurnal and nocturnal visitors, i.e. birds and small mammals respectively, to reproductive success by assigning 12 flowering *A. peglerae* plants to each of four pollinator selective exclusion treatments: (i) no-visitors; (ii) nocturnal-visitors; (iii) diurnal-visitors; and (iv) all-visitors (control). Birds alone contributed more to fruit set (1.2–2.6 times), average seed/fruit (1.0–1.8 times) and total seed production (1.1–2.8 times) than both the all visitors (birds and small mammals combined) and the small mammal visitors respectively. The exclusion of all visitors resulted in no fruit set, suggesting that *A. peglerae* requires pollinators to set seed. Germination trials over six weeks and viability testing with tetrazolium staining identified no significant differences among the three treatments that produced fruit and seed. Germination success was 90–97%, indicating that seed quality of small-mammal and bird-pollinated plants are similar. *Aloe peglerae* inflorescences were visited continuously throughout the day and night, with the Cape rock-thrush (*Monticola rupestris*) and dark-capped bulbul (*Pycnonotus tricolor*) being the most abundant pollinators, accounting for 68.2% and 13.7% of all visits respectively in the control treatment. Small mammals, i.e. Namaqua rock mouse (*Micaelamys namaquensis*) and eastern rock sengi (*Elephantulus myurus*), while not increasing reproductive success in the absence of birds, are suitable alternative pollinators. The presence of diurnal bird and nocturnal small mammal visitors provides a diversity of pollinators in this resilient pollination syndrome.

Key words: *Aloe peglerae*, bird pollination, Cape rock-thrush, *Elephantulus*, mammal pollination, *Micaelamys*.

INTRODUCTION

Phenotypic traits of a flower, including shape, colour, corolla length, nectar concentration, timing of anthesis and fragrance, are directed strongly to attract specific pollinators (e.g. insects, birds and bats; Faegri & van der Pijl 1979; Ollerton *et al.* 2009). The variation in these floral traits is used to create pollination syndromes which, although highly generalized (e.g. Hingston & McQuillan 2000), can be used to test hypotheses regarding potential pollinators in the field (Hargreaves *et al.* 2004; Johnson 2010). The floral characteristics for these syndromes are thought to be the result of co-evolution for blossom-visitor harmony (Faegri & van der Pijl 1979), resulting from selective pressures by efficient pollinators and successful pollination events (Stebbins 1970; Castellanos *et al.* 2004; Hargreaves *et al.* 2004; Johnson 2010). However, most angiosperms are visited by a variety of different animals (Faegri & van der Pijl 1979; Carthew 1993; Botes *et al.* 2009a), introducing an apparent paradox, the 'Plant-Pollinator Paradox' (Ollerton 1996), where a plant with evolved floral traits to attract a specific pollinator may be visited by a wide variety of animals (Ollerton 1996). It has been suggested that because different pollinators tend to vary in their effectiveness (Schemske & Horvitz 1984; Ollerton 1996), floral traits act as filters to attract efficient pollinators and inhibit inefficient pollinators from accessing pollen and nectar rewards (Thomson & Thomson 1992; Castellanos *et al.* 2004; Johnson *et al.* 2006; Johnson 2010).

Von Marilaun (1904) highlighted the concept of mammal pollination by suggesting that the Australian genus *Banksia* (Proteaceae) was pollinated by kangaroos, which was later discovered to be pollinated by small marsupials (e.g. possums) and rodents (Carpenter 1978; Cunningham 1991; Goldingay *et al.* 1991; Carthew 1993). Until recently, this pollination guild was given little recognition (Faegri & van der Pijl 1979), and most evidence for mammal pollination was inferred through circumstantial observations (Rourke & Wiens 1977; Carthew & Goldingay 1997). Indeed, many studies have highlighted the greater importance of this guild as pollinators (e.g. Rourke & Wiens 1977; Wiens *et al.* 1983; Johnson *et al.* 2001; Balmford *et al.* 2006; Kleizen *et al.* 2008; Biccard & Midgley

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2009; Wester *et al.* 2009; Wester 2010; Johnson & Pauw 2014; Melidonis & Peter 2015). The mammalian, or therophilous, pollination syndrome is thought to be derived from the bird (ornithophilous) pollination syndrome, and has only recently been understood in more detail (Rourke & Wiens 1977; Johnson 2004). However, because of the wide variation in mammal morphology and behaviour, this syndrome is not as clearly defined as other syndromes and a wide variety of floral traits may therefore reflect mammal pollination (Carthew & Goldingay 1997; Wester *et al.* 2009).

Nectar is suggested as the primary attractant for mammals (Rourke & Wiens 1977; Johnson 2004; Steenhuisen *et al.* 2015). Plants exhibiting this syndrome often flower during winter when mammals may have to supplement their diet with nectar when traditional food resources are low (Rourke & Wiens 1977; Johnson 2004; Kleizen *et al.* 2008; Wester *et al.* 2009). When these animals visit plants in search of nectar, the transfer of pollen on the fur between different flowers is ensured (Carthew 1993; Carthew & Goldingay 1997). Verifying the contribution of mammals to plant reproduction is often difficult because of their nocturnal nature (Kleizen *et al.* 2008). Therefore, alternative methods to actual observations are needed to quantify the contributions by visitors. Such methods include trapping the animal and measuring pollen loads on fur and in faeces (Carthew & Goldingay 1997; Kleizen *et al.* 2008) and conducting selective pollinator exclusion experiments to assess the variable role of specific pollinator guilds to reproductive output (Kleizen *et al.* 2008). Recently, camera traps have been used to record and identify pollinating visitors to plants, especially in cases where potential pollinators, such as small mammals, may be shy to human presence (e.g. Letten & Midgley 2009; Melidonis & Peter 2015).

Aloes (Xanthorrhoeaceae, formerly Asphodelaceae) are succulent plants endemic to arid and semi-arid sub-Saharan Africa, with the highest diversity in southern Africa (Cousins & Witkowski 2012). Most *Aloe* species, with long, tubular flowers that range from yellow to red, suggest pollination by birds (Reynolds 1950; Cousins & Witkowski 2012). This has been confirmed in a number of studies identifying both generalist and specialist nectar feeding bird species as primary pollinators (e.g. *Aloe divaricata*, Ratsirarson 1995; *A. vryheidensis*, Johnson *et al.* 2006; *A. africana*, *A. speciosa* and *A. ferox*, Botes *et al.* 2009a; *A. marlothii*, Symes *et al.* 2009). However, *A. inconspicua* (Hargreaves *et al.* 2008), *A. minima*, *A. linearifolia* (Botes *et al.* 2009b) and *A. greatheadii* var. *davyana* (Symes *et al.* 2009) are successfully, and sometimes exclusively, pollinated by insects (e.g. honeybees [*Apis mellifera capensis* and *A. m. scutellata*] and *Amegilla fallax*; Hargreaves *et al.* 2008; Botes *et al.* 2009b; Symes *et al.* 2009) where certain floral traits (e.g. UV reflectance, floral scent, small quantities of concentrated nectar), re-inforce the argument for this alternate pollinator guild.

Aloe peglerae Schönland produces a single raceme during July–August (Scholes 1988), with flowers typical of an ornithophilous pollination syndrome (Arena *et al.* 2013; Fig. 1a). Exclusion experiments have demonstrated that generalist avian nectarivores, primarily the Cape rock-thrush (*Monticola rupestris*), are the major contributors to reproductive success (about 60% of all probes by birds), with insects making a minor contribution (Arena *et al.* 2013). *A. peglerae* inflorescences seldom reach >40 cm above ground and, in the grassland environment in which they flower, suggest that visitations from terrestrial small mammals are likely. Indeed, subsequent camera trap footage has revealed small mammals, i.e. eastern rock sengi (*Elephantulus myurus*) and Namaqua rock mouse (*Micaelamys namaquensis*) visiting aloes at night (G. Arena, unpubl. data 2011), thereby prompting an investigation into the role that they may play in pollination. Given that a number of *Aloe* species flower in winter (55% of 158 species during June–August in South Africa; van Wyk & Smith 2014), it is not surprising that there are records of mammal visitors, including chacma baboon (*Papio hamadryas ursinus*), slender mongoose (*Galerella sanguinea*), four-striped grass mouse (*Rhabdomys pumilio*) and Namaqua rock mouse to *A. marlothii* (Symes & Nicolson 2008; Symes 2010), and vervet monkey (*Cercopithecus aethiops*) and *Rattus* sp. to *A. ferox* (CTS pers. obs.). However, no studies have quantified the role of mammals as pollinators of *Aloe*. This study used reproductive success in different selective exclusion treatments to compare the effectiveness and contributions of diurnal (birds) and nocturnal (small-mammals) visitors to the pollination of *A. peglerae*.

METHODS

Study site and species

The study was conducted in the Peglerae Conservancy (25°41′ S 27°59′E, 1600–1650 m a.s.l., about 56 ha), situated in the Magaliesberg Mountain Range, approximately 25 km west of Pretoria, South Africa. The area received 530 mm of precipitation in 2013 (South African Weather Services 2016) and is classified as Gold Reef Mountain Bushveld, characterized by rocky slopes and ridges, with vegetation consisting of grasses, scattered trees and shrubs (Mucina & Rutherford 2006; Fig. 1a and b in Arena *et al.* 2015). Where aloes grow (about 5 ha; Arena *et al.* 2015) in the Conservancy, the estimated mean density of plants is 142 plants.ha^{-1}.

Aloe peglerae has a limited distribution in South Africa, consisting of only three known populations, the largest being along the Magaliesberg Mountain Range (Pfab & Victor 2003). It is a stemless aloe with a single rosette of green leaves edged with red spines (Scholes 1988; Fig. 1 in Arena *et al.*

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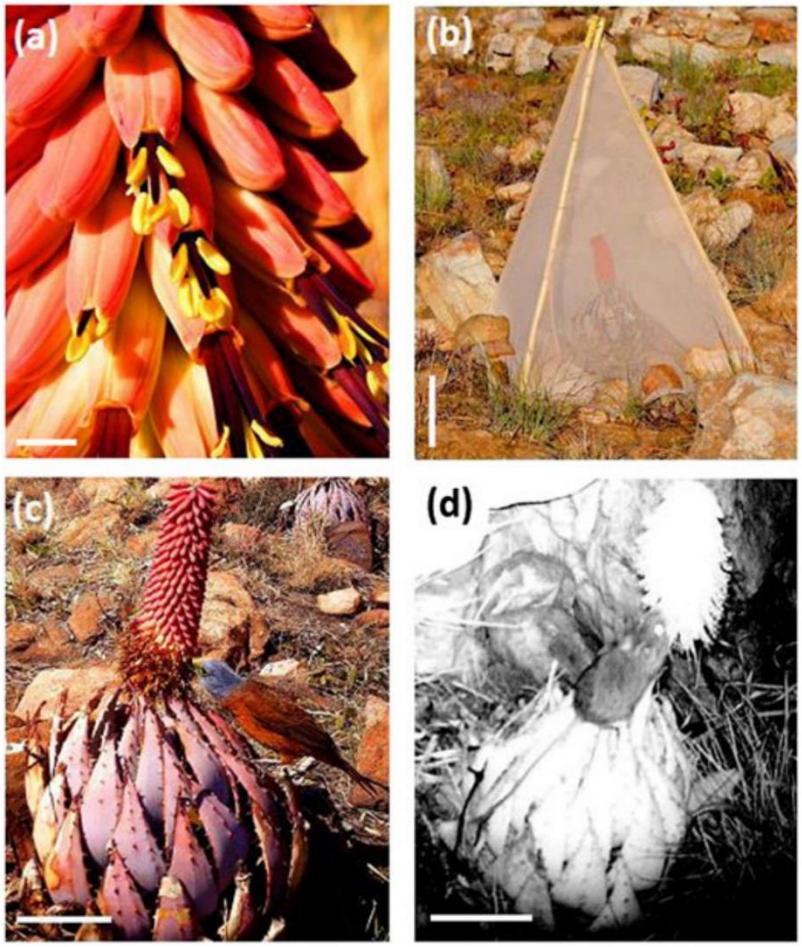


Fig. 1. (a) The tubular, dull red flowers of *Aloe peglerae*, that become a pale greenish-yellow as flowers mature, and contrast with exserted deep purple stamens and yellow anthers, suggest bird pollination (scale bar = 1 cm); (b) Exclusion cage constructed from a bamboo tripod frame and covered with nylon mesh (1 mm × 1 mm pores), to prevent visitors from accessing aloes (scale bar = 15 cm); (c) Cape rock-thrush (*Monticola rupestris*) probing *A. peglerae* flowers, with pollen deposited on the feathers around the beak (scale bar = 10 cm); (d) Namaqua rock mouse (*Micaelamys namaquensis*) feeding on *A. peglerae* nectar and/or flowers at night, likely collecting pollen in the fur on its face (scale bar = 10 cm). The figure is available in full colour in the online version. Photographs: A, Craig Symes; B, Maxine Hullett; C and D, Camera traps.

2013; Fig. 1c). Adult plants (≥20 cm in diameter) produce a single, densely packed raceme during winter (July–August) and have been known to produce more than one raceme on rare occasions (Scholes 1988). Although flowering is annual at the population level, it is variable between individual plants each year, while fruit and seed set can be even more variable between years (Scholes 1988). Flowers are tubular and approximately 30 mm in length; buds are a dull red, becoming a greenish-yellow as the flowers mature, with deep purple stamens and anthers that become exserted (about 25 mm) at maturity (Scholes 1988; van Wyk & Smith 2014; Fig. 1a). Flowers open sequentially from the base of the inflorescence, over a period of approximately 15 days (SLP unpubl. data 2013). Each flower produces copious amounts of dilute nectar (standing crop mean volume = 47 ± 7 (SE) μL, range = 1–150 μL; mean concentration = 10.2 ± 0.5 (SE) % w/w, range = 7.5–23.0% w/w, n = 36 flowers; measured with hand-held refractometer, Bellingham & Stanley, Tunbridge Wells, UK; CTS unpubl. data 2010). *A. peglerae* is slow-growing (Scholes 1988), and listed as Endangered because of habitat transformation and the illegal collection and trade of seeds and whole plants (Pfab & Victor 2003; Pfab & Scholes 2004; Phama *et al.* 2014).

Diurnal and nocturnal pollinator visitation

Exclusion experiments were conducted to measure the contributions of diurnal and nocturnal visitors. In a previous study of the same Peglerae Conservancy sub-population, each plant

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($n = 550$) was numbered and labelled with an engraved metal tag, and the GPS co-ordinates recorded using a hand-held GPS (Garmin, GPSMAP 60Cx. USA; Arena *et al.* 2013). Shortly after the flowering season started, 56 flowering aloes, whose flowers had not yet opened, were randomly selected and assigned to one of four treatments, with 14 plants per treatment, namely:

i No-visitors ($NOVIS_{TRT}$) – Total exclusion, plants covered with mesh cages for the entire flowering period, all diurnal and nocturnal visitors excluded;
ii Nocturnal-visitors ($NOCT_{TRT}$) – Diurnal-visitor exclusion, plants covered with a mesh cage during the day to exclude diurnal visitors, cages removed at night allowed access by nocturnal visitors;
iii Diurnal-visitors ($DIUR_{TRT}$) – Nocturnal-visitor exclusion, plants covered with a mesh cage during the night to exclude nocturnal visitors, cages removed during the day allowed access by all diurnal visitors;
iv All-visitors 'control' (ALL_{TRT}) – plants exposed for the duration of the flowering period, allowing access by diurnal and nocturnal visitors.

Exclusion cages (Fig. 1b) were constructed of a bamboo tripod frame (height = about 0.9 m, basal area covered = about 0.3 m^2), covered with a fine nylon mesh (1 × 1 mm pores), which restricted visitors of all sizes, including insects. Cages were shared between aloes in close proximity, i.e. a $DIUR_{TRT}$ aloe was 'paired' with a $NOCT_{TRT}$ aloe, because these treatments required cages at different times, and to facilitate the efficient changeover of cages between the two exclusion treatment groups at dusk and dawn. Care was taken when removing the cages from the aloes to avoid transfer of pollen from the flowers onto the cage and prevent the accidental transfer of pollen between plants during the changeovers. Cages were also large enough to prevent any contact of the mesh with the inflorescence and inadvertent pollen transfer between paired plants, even in windy conditions that are common during the flowering period. Eventually, as the flowering season drew to an end and more cages became available, remaining plants in the trials had their own cages. We also ensured that exclusion cages covered individual plants for the entire sampling period (25 June-27 July 2013) when their flowers were open.

Temperature data loggers (accurate to 0.5 °C, Thermocron iButtons, Australia), programmed to record temperature every hour for 25 days of the sampling period were placed inside the rosettes of five ALL_{TRT} and five $NOVIS_{TRT}$ plants to compare the temperatures of the micro-habitat environment of the exclusion cage space with that of un-caged plants.

Camera traps (Bushnell, models 119456 and 119477, China) were used to confirm the presence of the expected visitors for the treatment to which the aloe was assigned, i.e. bird visitors to $DIUR_{TRT}$ plants ($n = 12$ cameras during day, one not paired), small mammal visitors to $NOCT_{TRT}$ plants ($n = 11$ cameras during night) and both groups to the ALL_{TRT} (n = nine cameras during day and night). To verify the effectiveness of the exclusion cages in excluding all visitors, cameras were set at two $NOVIS_{TRT}$ plants. Each camera was programmed at high sensitivity to take two photographs when motion sensors were activated, with three-second intervals between activations. Cameras were placed on tripods at least one metre from the plants, facing southwards to minimize sun glare, and downwards onto the aloe to minimize vegetation moving in the wind activating the camera.

Changeovers of camera traps and cages took place in the hour after sunrise (06:45–07:45) and the hour before sunset (16:30–17:30). This strategy attempted to select for the visitors relevant to each exclusion treatment but because of the gradual transition of day to night, and night to day, 'inappropriate' visitors, (e.g. birds feeding very early in the morning or very late in the evening on $NOCT_{TRT}$ plants), could not be controlled for (see later). The 'diurnal' period was defined as 07:00–17:59, and the 'nocturnal' period as 18:00–06:59; small-mammals and birds visiting during each of these periods respectively were defined as 'inappropriate' visitors. Memory cards (SanDisk 2GB and 4GB) and batteries in the cameras were replaced as needed. Three cages were torn and broken by chacma baboons and strong winds, but were replaced with undamaged cages during a changeover period, i.e. within 12 h of damage.

A visitor was considered a legitimate visitor if it touched the plant or inflorescence (e.g. probing flowers, standing on leaves and/or inflorescence), because the visitor had the potential to transfer pollen. All visitors were identified to species. The time and date of each visit were recorded, and a visitor was recorded as the 'same visitor' if it was recorded in a similar position or a specific feature had remained the same (e.g. aberrant feather positioning on bird, pollen deposition patterns, etc.) after successive three-second intervals between activations. A visitor after a period of 30 s between camera activations was recorded as a new visitor, unless it was clear that it was the same individual as the previous visitor, as noted above. Mean visitation rate per 1000 plants (visits/1000 plants/h) for each treatment was calculated for all visitors (Table 1).

Reproductive success

Reproductive success, used as a measure of pollinator contributions, could only be measured for 12 aloes in each treatment, as two inflorescences per treatment were either broken or missing between flowering and fruiting (a period when no camera trap footage was available). Approximately five weeks after the end of the flowering season (9 September 2013), fruit produced by each aloe were removed and counted. A maximum of five fruit per plant were randomly selected and the remaining fruit scattered around the aloe (Arena *et al.* 2013). The number of flowers on each raceme (represented by the number of peduncles; Symes *et al.* 2009; Arena *et al.* 2013) was counted to calculate the mean number of flowers/plant. Fruit set (proportion) was calculated by dividing the number of fruit by the number of flowers (peduncles). The harvested fruit were left in open bags to dry at ambient laboratory temperatures at the University

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Table 1. Percentage contribution to visitation by each species in each of the exclusion treatments. ($NOCT_{TRT}$, $DIUR_{TRT}$ and ALL_{TRT} = Nocturnal-Visitors, Diurnal-Visitors and All-Visitors treatments respectively). Bold values indicate visitors on the 'correct' treatment (i.e. appropriate visitors); values in parentheses are mean visitation rates (number of visits/1000 plants/h ± SE). Significant differences in overall visitation rates per treatments are indicated by different letters (Kruskal–Wallis multiple comparison test; $P < 0.05$). An animal was recorded as a visitor if it touched the plant (e.g. perched) or inflorescence in any way. No visitors were recorded inside the exclusion cages of the total exclusion treatment. Taxonomy for birds follows Hockey *et al.* (2005) and Chittenden (2014).

Visiting species	$NOCT_{TRT}$ (2274 h; 11 plants)	$DIUR_{TRT}$ (1599 h; 12 plants)	ALL_{TRT} (3316 h; 9 plants)
Birds:			
Cisticolidae			
Wailing cisticola	0.8	**0**	**0**
Cisticola lais†#	(0.52 ± 0.52)	**(0)**	**(0)**
Dicruridae			
Fork-tailed drongo	1.5	**0.4**	**0.7**
Dicrurus adsimilis†#	(1.12 ± 1.12)	**(0.56 ± 0.56)**	**(0.56 ± 0.56)**
Fringillidae			
Streaky-headed seed-eater	3.8	**7.9**	**4.1**
Crithagra gularis†§	(1.79 ± 0.96)	**(12.59 ± 2.95)**	**(4.29 ± 1.29)**
Lybiidae			
Crested barbet	0	**0.4**	**1.7**
Trachyphonus vaillantii†#	(0)	**(0.56 ± 0.56)**	**(1.76 ± 1.18)**
Motacillidae			
Long-billed pipit	0	**0.4**	**0**
Anthus similis‡#	(0)	**(0.54 ± 0.54)**	**(0)**
Muscicapidae			
Cape rock-thrush	16.0	**80.5**	**68.2**
Monticola rupestris†	(9.30 ± 3.32)	**(134.02 ± 22.40)**	**(60.19 ± 7.58)**
Familiar chat	0	**1.1**	**1.0**
Cercomela familiaris‡	(0)	**(1.87 ± 1.29)**	**(0.66 ± 0.66)**
Mountain wheatear	0	**0.4**	**0**
Oenanthe monticola‡	(0)	**(0.69 ± 0.69)**	**(0)**
Pycnonotidae			
Dark-capped bulbul	4.6	**7.1**	**13.7**
Pycnonotus tricolor†	(2.43 ± 1.06)	**(12.40 ± 4.20)**	**(12.92 ± 5.12)**
Sturnidae			
Red-winged starling	0.8	**0**	**0**
Onychognathus morio†#	(0.52 ± 0.52)	**(0)**	**(0)**
Zosteropidae			
Cape white-eye	1.5	**0**	**0**
Zosterops virens†	(0.67 ± 0.67)	**(0)**	**(0)**
Mammals:			
Cercopithecidae			
Chacma baboon	0.7	**0**	**1.0**
Papio hamadryas ursinus‡§#	(0.38 ± 0.38)	**(0)**	**(1.18 ± 0.65)**
Macroscelididae			
Eastern rock sengi	**3.8**	0	**0**
Elephantulus myurus†#	**(2.28 ± 2.28)**	(0)	**(0)**
Muridae			
Namaqua rock mouse	**66.4**	0	**9.6**
Micaelamys namaquensis†§#	**(40.17 ± 18.75)**	(0)	**(7.83 ± 2.45)**
Reptiles:			
Agamidae			
Ground agama	0	**1.9**	**0**
Agama aculeata‡#	(0)	**(3.95 ± 2.21)**	**(0)**
Total number of visitors	**92,** 39 (59.17 ± 20.33)[b]	**266,** 0 (167.18 ± 23.75)[a]	**292,** 0 (89.39 ± 13.28)[ab]

†Visitors that touched the plant and/or inflorescence and probed flowers. ‡Visitors that touched the plant and/or inflorescence only. §Visitors that fed on flowers. #Visitors not previously recorded by Arena *et al.* (2013).

of the Witwatersrand, Johannesburg. When the fruit opened, the number of seeds from each fruit was counted and the average number of seeds/fruit for each plant was calculated (seed set). The estimated total seed production for each plant was calculated by multiplying the average seed set by the total number of fruit produced by that specific plant.

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Germination trials and seed viability

A maximum of 10 seeds randomly selected from each fruit were pooled, resulting in a total of up to 50 seeds/plant. Seeds were placed into sterilized petri dishes on top of two filter papers (Boeco Germany, 3 hw, 65 $g.m^{-2}$, 90 mm diameter), covered with one filter paper and saturated with distilled water. The petri dishes were kept in an environmental control chamber, with daytime temperatures of 25 °C and night-time of 15 °C (12 h day–night cycle) and a relative humidity of 50%. Germinating seeds, identified by a 2 mm radicle protruding from the seed, were counted every day for three weeks and, thereafter, three times a week for an additional three weeks.

Seeds that had not germinated after the six week period were removed from the control chamber. Empty seed coats were removed and the seed recorded as 'dead'. Any intact seeds were sliced in half to expose the embryo and covered in a 1.0% 2,3,5-triphenyltetrazolium chloride (tetrazolium) solution overnight. The following day, seeds with embryos that had turned a pale pink were considered to be 'viable', while seeds with embryos that remained white were recorded as 'dead' (ISTA 2003). Total seed viability was calculated as the total sum of all germinated and viable seeds after tetrazolium testing.

Mean Germination Time (MGT) was calculated using the following equation:

$$\mathrm{MGT} = \frac{\Sigma Dn}{\Sigma n}$$

where n is the number of seeds germinated on day D, and D is the number of days counted from the beginning of germination (Zanjan & Asli 2012).

Data analysis

A non-parametric Kruskal–Wallis rank sum test was used to test for differences among treatments in: (i) visitation rates; (ii) mean number of flowers; (iii) fruit set; (iv) seed set; (v) total seed produced/plant; (vi) percentage germination; (vii) MGT, and (viii) total seed viability (Shapiro–Wilk normality distribution test, $P < 0.05$). Kruskal–Wallis multiple comparison *post-hoc* tests were performed on datasets with significant differences ($P < 0.05$). The microhabitat temperatures inside and outside the cages, during the day (07:00–17:59) and night (18:00–06:59), were compared using an Independent Student's *t*-test (these data were normally distributed; Shapiro–Wilk, $P > 0.05$). All statistical analyses were performed in R (Version 3.1.2. R Core Team 2014), and are presented as mean ± standard error (SE).

RESULTS

Diurnal and nocturnal pollinator visitation

Although a Kruskal–Wallis test indicated that there was a significant difference in the visitation rates among treatments ($H_{2,28} = 12.72$, $P = 0.0017$), the *post-hoc* test indicated that only the $DIUR_{TRT}$ and $NOCT_{TRT}$ were significantly different from each other. The $DIUR_{TRT}$ had a significantly higher visitation rate (167.18 ± 23.75 visits/1000 plants/h), with 98.1% ($n = 266$ visits) of these visits by birds, compared to the $NOCT_{TRT}$ (59.17 ± 20.33 visits/1000 plants/h), with 70.2% ($n = 131$ visits) of all night visits made by small-mammals. The ALL_{TRT} had a mean visitation rate of 89.39 ± 13.28 visits/1000 plants/h which did not differ significantly from the other two treatments, with birds and small mammals making up 89.4% and 9.6% of all visits ($n = 292$ visits) respectively. The Cape rock-thrush (Fig. 1c) was the most common diurnal visitor, in the $DIUR_{TRT}$ and ALL_{TRT} (80.5% and 68.2% of all visits respectively) and the Namaqua rock mouse (Fig. 1d) the most common nocturnal visitor, in the $NOCT_{TRT}$ and ALL_{TRT} (66.4% and 9.6% of all visits respectively). The balance of visits in the $DIUR_{TRT}$ and ALL_{TRT} includes a combination of eight (19.5%; seven bird and one reptile species) and six species (22.3%; five bird and one mammal species) respectively. Table 1 lists visitation rates and activities for individual visiting species for each treatment. Rates differ from those presented by Arena *et al.* (2013), a similar study conducted a few years prior in the same *A. peglerae* sub-population. This is likely the result of different sampling techniques: Arena *et al.* (2013) conducted 20 min observations during daylight hours, three times/day (06 h00–10 h00, 10 h00–14 h00, 14 h00–18 h00) over a two week period, and used only six camera traps, while our study used 32 camera traps continuously throughout the day and night for the five week flowering period.

Insects were observed visiting all plants exposed during the day, but because their contribution to the reproductive success of the aloe has been shown to be insignificant, and they occurred at very low abundance, their contribution is not considered further (Arena *et al.* 2013). All $DIUR_{TRT}$ plants received only bird visitors, except two plants visited once each by a ground agama (*Agama aculeata*). Each ALL_{TRT} plant received both diurnal and nocturnal visitors, with the exception of one plant which received only diurnal visitors. Nine $NOCT_{TRT}$ plants received visits from the Namaqua rock mouse and/or the eastern rock sengi, while two did not receive any small mammal visits. Nine $NOCT_{TRT}$ aloes also received at least one avian visit (a total of seven bird species), accounting for 29% of all the visits to $NOCT_{TRT}$ plants (Table 1). These visits occurred just before sunset (21%) or just after sunrise (79%) when the cages and cameras were being changed between $DIUR_{TRT}$ and $NOCT_{TRT}$ plants. Therefore, to account for the avian visits to $NOCT_{TRT}$ aloes, an 'Inappropriate Visitors Correction' was applied to each of these aloes by subtracting the proportion of bird visits from each of the different measures of reproductive success. This correction did not make a significant difference to the statistical outcomes so the original, uncorrected dataset was used.

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Flower herbivory occurred on multiple occasions at five plants, where both small mammals and birds were photographed tearing perianths and/or removing whole flowers. One plant experienced severe herbivory where every flower was removed by mice over a period of 10 days and this plant produced no fruit at all. Chacma baboons were twice recorded pulling the racemes and leaves of the plants, as well as feeding on some flowers. The mocking cliff-chat (*Thamnolaea cinnamomeiventris*) was as abundant as the Cape rock-thrush in the Conservancy (SLP & CTS pers. obs. 2013), but not recorded on flowering plants, despite being known to occasionally feed on *Aloe* nectar (Symes 2010). Wailing cisticola (*Cisticola lais*), long-billed pipit (*Anthus similis*), red-winged starling (*Onychognathus morio*), Cape white-eye (*Zosterops virens*) and eastern rock sengi were only recorded visiting a single aloe, either on one or multiple occasions. Camera traps also recorded other mammal species: one slender mongoose, two large-spotted genet (*Genetta tigrina*), 12 Smith's red rock rabbit (*Pronolagus rupestris*), one Cape porcupine (*Hystrix africaeaustralis*) and one black-backed jackal (*Canis mesomelas*) near, but not in any way associating with, the flowering aloes. Species recorded visiting aloes by Arena *et al.* (2013) but not recorded in this study include amethyst sunbird (*Chalcomitra amethystina*), Cape bunting (*Emberiza capensis*), and lazy cisticola (*Cisticola aberrans*).

While the high sensitivity setting of the cameras resulted in a substantial number of photographs (5749, 15 331 and 39 127 in the $NOCT_{TRT}$, ALL_{TRT} and $DIUR_{TRT}$ respectively), largely because of vegetation moving in the wind, it also ensured that all visitors were detected and, therefore, we are confident that the 689 visitors recorded across the three treatments comprehensively surveyed all visitation events. Usually plant visits were short (<3 s, but still allowing time to touch the plant or probe at least once) although a single female Cape rock-thrush was recorded on an aloe for three minutes and 35 s.

The nylon mesh cages did not significantly alter the micro-habitat temperature for the plants in the cages, during the day or night, as there were no differences between the mean hourly temperatures for the ALL_{TRT} plants and $NOVIS_{TRT}$ plants (Daytime: $ALL_{TRT} = 20.8 \pm 1.5\,°C$, $NOVIS_{TRT} = 20.4 \pm 1.4\,°C$, $t_{20} = -0.22$, $P = 0.83$; Night-time: $ALL_{TRT} = 11.1 \pm 0.5\,°C$, $NOVIS_{TRT} = 12.1 \pm 0.5\,°C$, $t_{24} = 1.25$, $P = 0.22$).

Reproductive success

The mean number of flowers/plant did not differ significantly among treatments; $NOVIS_{TRT}$ (224 ± 35 flowers), $NOCT_{TRT}$ (242 ± 21), $DIUR_{TRT}$ (227 ± 24), ALL_{TRT} (227 ± 19) ($F_{3,44} = 0.372$, $P = 0.77$).

Mean fruit set differed among treatments ($H_{3,44} = 23.31$, $P < 0.0001$; Fig. 2a). The $NOVIS_{TRT}$ plants produced no fruit, which differed significantly from the $DIUR_{TRT}$ and ALL_{TRT}. Of the 12 plants/treatment, four of the $NOCT_{TRT}$, one of the $DIUR_{TRT}$ and three of the ALL_{TRT} plants did not produce any fruit. Fruit set did not differ significantly between the $DIUR_{TRT}$ (23.3%) and $NOCT_{TRT}$ (9.0%), or between the $NOCT_{TRT}$ and $NOVIS_{TRT}$ (0.0%; Fig. 2a). Coefficients of variation (CV) were high; 104%, 72% and 71% for the $NOCT_{TRT}$, $DIUR_{TRT}$ and ALL_{TRT} respectively.

A total of 144 fruit were collected (39 from $NOCT_{TRT}$, 55 from $DIUR_{TRT}$, 50 from ALL_{TRT} and zero from $NOVIS_{TRT}$). Although there was an overall significant difference among the four treatments for average seed/fruit ($H_{3,44} = 25.64$, $P < 0.0001$), there was no significant difference between the ALL_{TRT} and $DIUR_{TRT}$ (43 ± 6 and 43 ± 5 seeds/fruit respectively; Fig. 2b), nor between these two treatments and the $NOCT_{TRT}$, which produced 24 ± 19 seeds/fruit. Variation among plants was higher in the $NOCT_{TRT}$, with a CV of 80%, compared to the ALL_{TRT} and $DIUR_{TRT}$ (48% and 35% respectively).

The estimated total seed produced/plant varied significantly ($H_{3,44} = 23.52$, $P < 0.0001$; Fig. 2c), specifically between the $NOVIS_{TRT}$ and both the $DIUR_{TRT}$ and ALL_{TRT}. The $DIUR_{TRT}$ plants produced the most seed in total, with a mean of 2433 ± 456 seeds/plant, followed by the ALL_{TRT} (2129 ± 424 seeds/plant) and the $NOCT_{TRT}$ (861 ± 20 seeds/plant). Variation was high within each treatment (CVs of 129%, 65% and 68% for the $NOCT_{TRT}$, $DIUR_{TRT}$ and ALL_{TRT} respectively).

Germination trials and seed viability

Seeds in all treatments began germinating within four days, with the $DIUR_{TRT}$ and ALL_{TRT} having a MGT of 6.44 ± 0.24 and 6.46 ± 0.30 days respectively (Fig. 3). The ALL_{TRT} had the highest cumulative percentage germination (97%), then the $DIUR_{TRT}$ (92%), and $NOCT_{TRT}$ (90%), which also took longer to germinate (MGT = 7.30 ± 0.48 days). There was no significant difference in percentage germination ($H_{2,26} = 4.57$, $P = 0.10$; Fig. 3), or MGT among treatments ($H_{2,26} = 3.02$, $P = 0.22$). Germination slowed after approximately 10 days, and the final seed germinated 24 days into the trial. Total seed viability (germination and tetrazolium testing combined) ranged from 90 to 98% and did not differ significantly among treatments ($H_{2,29} = 5.80$, $P = 0.06$).

DISCUSSION

Birds were identified as the most abundant pollinators of *A. peglerae*, contributing more to all measures of reproduction, when compared to the $NOVIS_{TRT}$. Birds alone appeared to make more of a contribution to

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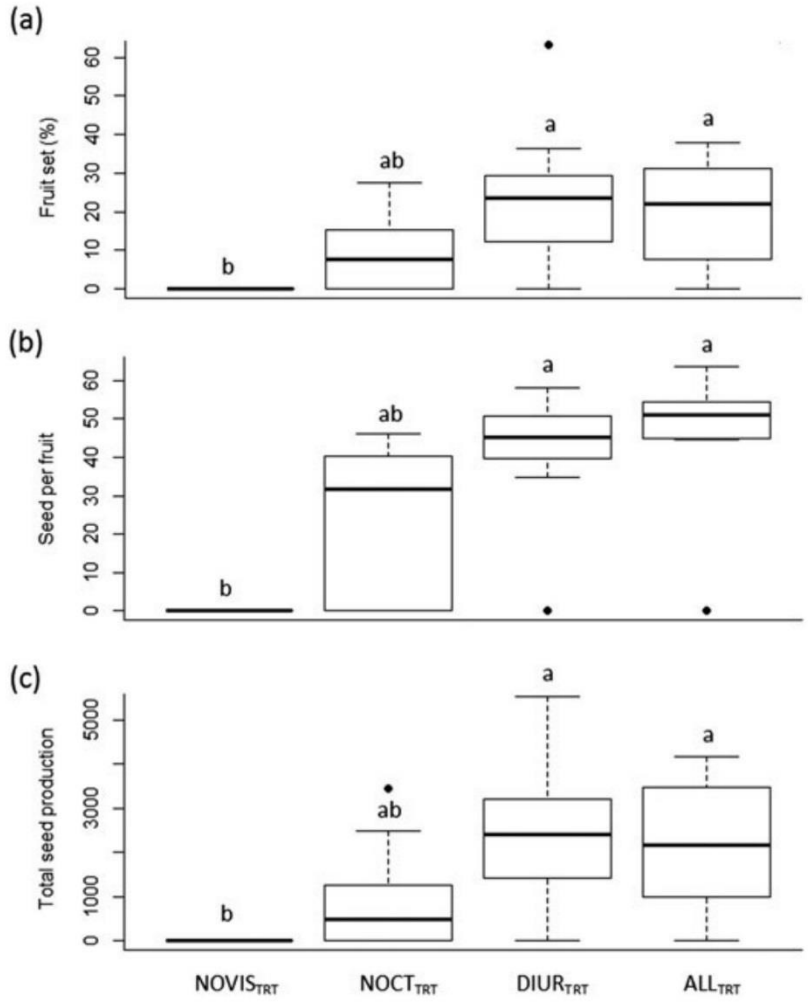


Fig. 2. Reproductive success of *Aloe peglerae* for pollinator selective exclusion experiments in 2013 (NOVIS$_{TRT}$, NOCT$_{TRT}$, DIUR$_{TRT}$ and ALL$_{TRT}$ = No-Visitors, Nocturnal-Visitors, Diurnal-Visitors and All-Visitors treatments respectively), as measured by, (a) fruit set (proportion of flowers that set fruit), (b) number of seed produced/fruit, (c) total seed produced/plant ($n = 12$ plants/treatment). Boxes represent median and inter-quartile range, bars represent minimum and maximum and solid circles represent outliers. Treatments with different letters are significantly different (Kruskal–Wallis multiple comparison test; $P < 0.05$).

reproduction when compared to the ALL$_{TRT}$ (birds and small-mammals combined, where the small mammals may be reducing plant reproductive success through herbivory), and the NOCT$_{TRT}$ (small-mammals only), i.e. fruit set (1.2–2.6 times greater), average seed/fruit (1.0–1.8 times greater) and total seed production (1.1–2.8 times greater), than the ALL$_{TRT}$ and NOCT$_{TRT}$ respectively. DIUR$_{TRT}$ and ALL$_{TRT}$ aloes received multiple bird visitors; in the ALL$_{TRT}$ the Cape rock-thrush (68.2%) and dark-capped bulbul (13.7%) were the most common visitors. These results are consistent with those of Arena *et al.* (2013), who showed that the Cape rock-thrush and dark-capped bulbul were the most important pollinators, with the Cape rock-thrush accounting for about 60% of all probing visits that contribute to pollination and reproductive success. In addition, 12 other bird species have been recorded visiting *A. peglerae* (Arena *et al.* 2013; this study) but less frequently, and all of these were recorded as visitors to other aloe species, such as *A. vryheidensis* (five generalist bird species), *A. marlothii* (39 bird species) and *A. ferox* (16 bird species) (Johnson *et al.* 2006; Symes *et al.* 2008; Kuiper *et al.* 2015). This finding therefore specifically emphasizes the important role that a single generalist avian nectarivore, i.e. the Cape rock-thrush, plays in the pollination of *A. peglerae*.

The relatively high abundance of Cape rock-thrushes in the area may be a response to nectar availability at high altitudes in resource-limited winters, as they appear to be transient at the site; only one Cape rock-thrush was

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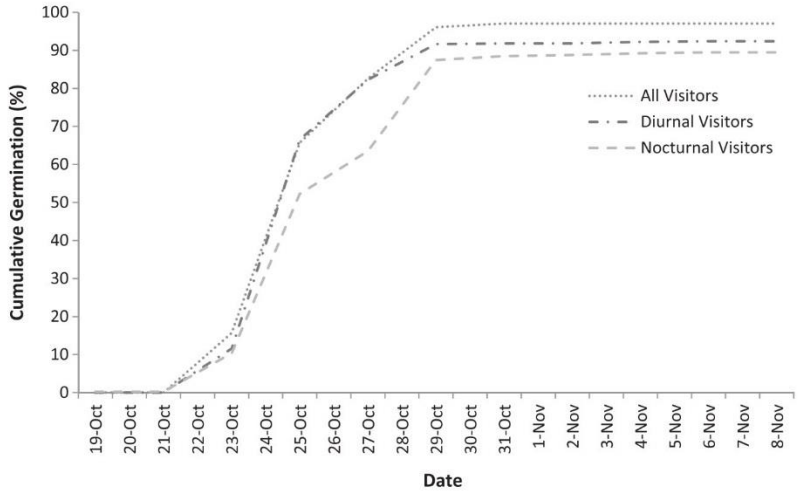


Fig. 3. Cumulative percentage seed germination over the six week germination trial between the three pollinator exclusion treatments on *Aloe peglerae*. Seeds stopped germinating after 29 October and the trial was terminated on 30 November 2013.

re-sighted 2 h after ringing six individuals in two flowering seasons (SLP and CTS unpubl. data 2012, 2014). The Cape rock-thrush is a resident species, but during resource-limited winter months, migrates to lower altitudes (<900 m; Johnson & Maclean 1994), yet *A. peglerae* is found at altitudes greater than 1500 m (Arena *et al.* 2013; SLP unpubl. data 2013), suggesting that the birds are temporarily moving to higher altitudes in search of resources. This seasonal movement of pollinators in response to nectar availability has been recorded in *A. marlothii* and *A. ferox*, where the peak abundance of nectarivores coincided with peak flowering and nectar availability in aloes (Symes *et al.* 2008; Kuiper *et al.* 2015). However, the lack of re-sightings of marked individual birds, given the patchy availability of nectar is intriguing, and requires further research; the temporary presence and continuous movement of birds through the area may suggest that, (i) alternative food resources elsewhere are available to sustain birds and/or, (ii) the nectar resource is not that critical for these birds.

The role that small-mammals appear to play in pollination cannot be overlooked in *A. peglerae*. Small mammals, (e.g. rodents), in South Africa, have been identified as the primary pollinators of *Protea nana* (Proteaceae; Biccard & Midgley 2009), *Leucospermum arenarium* (Proteaceae; Johnson & Pauw 2014), *Liparia parva* (Fabaceae; Letten & Midgley 2009), *Whiteheadia bifolia* (Hyacinthaceae; Wester *et al.* 2009; Wester 2010), *Massonia depressa* (Hyacinthaceae; Johnson *et al.* 2001), and Cape grey mongoose (*Galerella pulverulenta*) and large-spotted genet have been suggested to occasionally pollinate four *Protea* spp. (Steenhuisen *et al.* 2015). Although there are reports of mammals feeding on *Aloe* nectar (Symes & Nicolson 2008; Symes 2010) this, to our knowledge, is the first study to quantify the contribution of small mammals to pollination in an *Aloe* species. Arena *et al.* (2013) noted small mammals visiting *A. peglerae* plants at night, and suggested that they may contribute to pollination. Indeed, the results of this study suggest that small mammal, i.e. Namaqua rock mouse and eastern rock sengi, contributions to reproduction, as measured by fruit set, seed set and total seed production, were important. It is likely that these mammals are visiting the plants in search of nectar during the resource-limited winter, as they were also recorded removing flowers from the inflorescence (this study), and collecting and/or transferring pollen between flowers in the process. Despite this herbivory it may still be possible that seeds are produced so long as flower destruction is not too severe and the pistil remains intact (Faegri & van der Pijl 1979).

The effectiveness of small mammals as pollinators of *A. peglerae* is furthermore questioned. A visitor was considered an effective pollinator, or effective at transferring conspecific pollen, if plant visits were regular and non-destructive, and viable seed was produced (Carthew & Goldingay 1997). The small mammals, particularly the Namaqua rock mouse, were sometimes regular visitors, removing all flowers such that no fruit was produced. However, when the mammal-pollinated aloes produced seed, the seeds were of a similar quality to that of the bird-pollinated plants, as demonstrated by the germination trial. This suggests that small mammals can be effective pollinators of *A. peglerae*, provided that herbivory is not too great. In a poor flowering year, the effects of herbivory may have counter-productive effects on overall reproductive success because of the high proportion of herbivores to flowering plants. Following the 'predator-satiation' hypothesis (Silvertown 1980),

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rodent populations in various mast-fruiting North American oak species have been shown to be affected by variations in fruit availability (Clotfelter *et al.* 2007), although it is unclear how important *A. peglerae* is for small mammals during cold, dry winters. Birds can also be destructive visitors to the aloe. The streaky-headed seedeater (*Crithagra gularis*) tears the perianths of the flowers to access the nectar, feeds on the flowers and damages fruit to feed on the seeds (Symes *et al.* 2009; Arena *et al.* 2013; this study). However, this destruction may have a limited effect on overall reproductive success, because the pistils of the flowers do not appear to be damaged (Faegri & van der Pijl 1979; SLP pers. obs. 2013). Similarly for small mammal herbivory, the overall flowering success between years may play a role in overall reproductive success within the *A. peglerae* sub-population; if the proportion of flowering plants is low then the relative impact of herbivory may be greater.

It is unclear how effective feathers (birds) and fur (mammals) are at transporting pollen (Rourke & Wiens 1977; Cunningham 1991). However, more important may be the distances that pollen is transferred. Less mobile mammals have smaller home ranges (e.g. *E. myurus* males have a home range of approximately 3958 m^2; Ribble & Perrin 2005), and limited vagility compared to birds, restricting movement between individual plants (Faegri & van der Pijl 1979; Cunningham 1991). They may compensate for this by spending more time foraging at aloes, thereby picking up greater amounts of pollen on their fur than birds do on their feathers (Rourke & Wiens 1977; Cunningham 1991). Therefore, even though small mammals may be visiting fewer aloes over a smaller area, they may be transferring more pollen because of their potentially higher pollen load. The germination trial indicated that seed produced on mammal-pollinated plants was equally viable compared to seed produced by bird-pollinated plants, suggesting that the limited mobility of mammals compared to birds in visiting a greater number of inflorescences over a wider area may not significantly affect plant reproductive fitness. Therefore, the contributions of diurnal and nocturnal visitors on an individual plant may depend on the circumstances of that plant, whether it falls within the home range of a small mammal, and the degree of damage by visitors (Carthew & Goldingay 1997). This may also explain the high variation in each treatment and the variable effectiveness of different visiting species.

It appears that *A. peglerae* is visited throughout the course of the day and night, and is reliant on these visitors for the transfer of pollen. The lack of fruit and seed production in the total-exclusion treatment suggests that *A. peglerae* is self-incompatible but this remains to be tested. However, it is clear that *A. peglerae* needs to rely on an external vector for pollen transfer, as has been found in other *Aloe* species (e.g. Hoffman 1988; Botes *et al.* 2009a; Symes *et al.* 2009; Wilson *et al.* 2009). Although birds are the primary pollinators, visiting during the day, this is the first quantified evidence of small mammals visiting and feeding on aloe nectar at night. Although not measured, it is possible that *A. peglerae* produces nectar constantly, to replace that consumed by visitors, as with *A. castanea*, *A. vryheidensis* and *A. marlothii* (Nicolson & Nepi 2005; Johnson *et al.* 2006; Symes & Nicolson 2008). Should nectar production be constant, it could serve to attract both diurnal and nocturnal pollinators, increasing the probability of successful pollination. The height of the plant and the inflorescence (approximately 25 cm and 17 cm high respectively, resulting in a total height of about 42 cm; Arena *et al.* 2013; SLP unpubl. data 2014) may also facilitate small mammal visitation, as the flowers are more accessible to the mammals, compared to the flowers of taller *Aloe* species. At the same time, the inflorescences are conspicuous enough above the low grass layer (about 33 cm; Arena *et al.* 2015) to attract birds. The difference in percentage of bird visits in the ALL_{TRT} (89.4%) and the $DIUR_{TRT}$ (98.1%) suggests that the potential depletion of nectar by foraging small mammals at night could decrease the nectar available to the birds early in the morning, thereby decreasing the visitation by birds and the effect they may have on pollination.

CONCLUSION

Succulent aloes flower predominantly in winter (Cousins & Witkowski 2012; Symes 2012), and can provide important food resources for many animals during cold, dry and resource-limited winter periods (Johnson 2004). For example, Symes *et al.* (2011) found that *A. marlothii* nectar is an important source of nutrition for opportunistic avian nectarivores during the dry winter months. There are reports of sengis visiting the flowers of the summer-flowering *A. reitzii*, an endemic grassland aloe to the eastern grasslands in South Africa (CTS unpubl. data 2014). The evolutionary relationship between diurnal avian and nocturnal small-mammal pollinators in winter-flowering aloes may be more common than originally thought, and we predict it to be evident in other *Aloe* spp. that have similar growth forms and morphology (e.g. *Aloe aculeata*, *A. melanacantha*, *A. petricola*, *A. pratensis*).

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CHAPTER THREE

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A flowering *Aloe peglerae* plant with red buds, in The Peglerae Conservancy, Gauteng (Maxine Semple)

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Good times, bad times: inter-annual reproductive output in a montane endemic succulent (*Aloe peglerae*; Asphodelaceae) driven by contrasting visitor responses of small mammals and birds

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Abstract. The movements of birds and small mammals may be affected by spatial and temporal variation in resource availability. For the genus *Aloe*, bird abundance and diversity increase in response to increases in nectar availability during flowering. *Aloe peglerae* Schönland, endemic to the Magaliesberg Mountain Range, South Africa, is primarily pollinated by the Cape rock-thrush (*Monticola rupestris*), but the nocturnal Namaqua rock mouse (*Micaelamys namaquensis*) contributes to pollination. To test the independent contributions of different pollinator guilds, i.e. birds and small mammals, we conducted selective pollinator exclusion experiments during two consecutive flowering seasons – one 'good' year and one 'poor' year (14 and 7% of plants flowering respectively). Reproductive output for both years was measured for *A. peglerae* plants assigned to selective exclusion treatments: (i) no visitors; (ii) nocturnal visitors; (iii) diurnal visitors; and (iv) all visitors, with camera traps recording visitors to each treatment. Bird visitation rates to the 'diurnal visitors' treatment were higher during the good year; subsequently, reproductive output in the poor year was lower, with plants in this treatment producing no fruit in the poor flowering year. In the 'nocturnal visitors' treatment, nocturnal visitation rates were similar with no significant difference in reproductive output between years. Seed viability was maintained between years, although less seed was produced during the poor flowering season. Mobile birds are less abundant in the *A. peglerae* population during poor flowering years – years when nectar resources are less abundant than in good flowering years, resulting in reduced plant reproductive output. However, the presence of non-flying, small mammals that cannot relocate to better feeding grounds compensates for the absence of birds in poor flowering years. This aloe may be resilient against Allee effects in the absence of primary pollinators. Nonetheless, conservation of *A. peglerae* should still be prioritised since low flowering densities and ongoing anthropogenic disturbance could be detrimental to the species' survival.

Additional keywords: Cape rock-thrush, inter-annual variation, Namaqua rock mouse, resource tracking.

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Introduction

Animals move across landscapes in search of suitable breeding areas, mates and food resources (Wilmshurst *et al*. 1999; Carter 2004; Tellería and Pérez-Tris 2007; Dzerefos *et al*. 2015). The ability of birds to move effectively between patches in space and time has been well documented, and is facilitated by their ability to fly (e.g. Brown and Hopkins 1996; Franklin 1999; Malizia 2001; Cotton 2007; Symes *et al*. 2008; Las-Casas *et al*. 2012). This movement can occur at multiple spatial scales as defined by a species' diet, e.g. frugivore, insectivore or nectarivore (Loiselle and Blake 1991; Craig and Hulley 1994; MacNally 1996; Johnson and Sherry 2001; García and Ortiz-Pulido 2004). Nectar is an important food source for birds during periods when other resources may become limited, and changes in the abundance of nectar feeders in response to fluctuations in nectar availability have been recorded (e.g. Brown and Hopkins 1996; MacNally 1996; Symes *et al*. 2001, 2008; Cotton 2007; Forbes *et al*. 2009; Las-Casas *et al*. 2012; Kuiper *et al*. 2015). This is true of opportunistic and generalist nectarivores that make use of abundant nectar resources that specialists may be unable to completely exploit (Oatley and Skead 1972; Franklin and Noske 1999; Franklin 1999).

Small, non-flying mammals are less mobile and restricted to smaller home- and foraging ranges, compared with birds. Migrations over large distances in search of food are often energetically impractical and may also have increased predation risks (Schradin and Pillay 2006), which results in a higher dependence on resources in the immediate surroundings. This may also explain why some small flying mammals such as gliders and bats have evolved to include nectar as an important

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part of their diet (e.g. Goldingay 1990). Recently, more studies have begun to highlight small mammal nectar feeding associations, and the contributions these non-flying species make towards plant pollination, despite their limited mobility (e.g. Fabaceae: Letten and Midgley 2009; Hyacinthaceae: Wester *et al*. 2009; Proteaceae: Johnson and Pauw 2014; Melidonis and Peter 2015; Schmid *et al*. 2015; Steenhuisen *et al*. 2015; Zoeller *et al*. 2016).

The genus *Aloe* (Asphodelaceae) is widespread across subSaharan Africa, with the highest density and diversity found in southern Africa (Carter *et al*. 2011; Cousins and Witkowski 2012). Many aloes display a bird pollination syndrome, with tubular flowers, that range from yellow to red, and no discernible scent (Reynolds 1950; Van Wyk and Smith 2014). Numerous studies have highlighted the importance of birds as pollinators of aloes (e.g. Hoffman 1988; Botes *et al*. 2008; Botes *et al*. 2009*b*; Symes *et al*. 2009; Hargreaves *et al*. 2012) with a few species pollinated by insects (e.g. Hargreaves *et al*. 2008; Botes *et al*. 2009*a*; Symes *et al*. 2009; Hargreaves *et al*. 2012; Duffy *et al*. 2014). Aloe nectar is an important food resource for birds, especially for generalist (opportunistic) species in dry, resource limited winters (Symes *et al*. 2008, 2011; Kuiper *et al*. 2015), with different pollinators showing preferences for nectar with different properties (Johnson *et al*. 2006; Johnson and Nicolson 2008). Specialist (true) nectarivores (e.g. sunbirds with long, narrow bills) are attracted to low volumes of concentrated nectar (10–30 µL, 15–25% w/w), whereas generalist or opportunistic nectarivores (e.g. short-billed birds) are attracted to dilute nectar which is produced in large volumes (40–100 µL, 8–12%; Johnson and Nicolson 2008).

Consequently, birds track the flowering periods of aloes, when nectar is most abundant (Symes *et al*. 2008; Forbes *et al*. 2009; Kuiper *et al*. 2015). Oatley (1964) noted that the number of bird species present and feeding in a stand of flowering *Aloe marlothii* plants increased in a year where the plants flowered well, as opposed to the previous year of lower flowering density. Symes *et al*. (2008) found that there was an increase in the abundance and diversity of nectar feeding bird species during the flowering season of *A. marlothii*, with some nectar feeding species only present during the flowering season. Most bird species that arrived during peak flowering were generalist nectarivores, whereas specialist nectarivores showed no significant change in abundance in response to changes in nectar availability (Symes *et al*. 2008). In contrast, Forbes *et al*. (2009) recorded an influx of both specialist and generalist nectar feeding birds at the onset of flowering in *Aloe ferox*. This influx was annual, consisting predominantly of Malachite Sunbirds (*Nectarinia famosa*) and *Ploceus* weaver species (Kuiper *et al*. 2015). During the peak flowering period, the number of birds increased between three and five times compared with the rest of the year (Kuiper *et al*. 2015). However, the peak flowering period of *A. ferox* did not influence the population and/or number of birds from other feeding guilds, i.e. frugivores and insectivores, suggesting that nectarivores are responding to the peak in nectar availability by tracking resources (Kuiper *et al*. 2015).

Aloe peglerae Schönland is a critically endangered stemless aloe, endemic to the Magaliesberg Mountain Range, South Africa (Reynolds 1950; Van Wyk and Smith 2014; Pfab *et al*. 2016). The aloe produces a single raceme with red tubular flowers producing copious amounts (47 ± 7 µL; mean ± s.e.) of dilute (10.2 ± 0.5% w/w; mean ± s.e.) nectar (Payne *et al*. 2016). Although not explicitly tested, exclusion of all biotic visitors suggests that *A. peglerae* is self-incompatible (Arena *et al*. 2013; Payne *et al*. 2016), and like many *Aloe* species (e.g. Hoffman 1988; Botes *et al*. 2009*b*; Symes *et al*. 2009; Wilson *et al*. 2009), is reliant on external pollen vectors. The flowers are typical of a bird pollination syndrome (Faegri and van der Pijl 1979), and exclusion experiments, with low seed set in bird exclusions, have shown birds to be the primary pollinators (Arena *et al*. 2013; Payne *et al*. 2016). The Cape rock-thrush (*Monticola rupestris*) accounts for ~68% of visits (Payne *et al*. 2016) and up to 60% of probing visits (Arena *et al*. 2013). Recently, small mammals, although less effective than avian pollinators, have been shown to successfully pollinate *A. peglerae* at night, with the Namaqua rock mouse (*Micaelamys namaquensis*) an important nocturnal visitor (9.6% of visits; Payne *et al*. 2016).

Aloe peglerae flowering is annual at a population level, but variable at an individual level, with fruit and seed production more irregular than flowering (Scholes 1988; Arena *et al*. 2013). It is unknown to what extent the variability of flowering frequency affects pollinator abundance, and what subsequent effect this may have on visitation by these two different pollinator guilds, i.e. diurnal birds and nocturnal small mammals, and on aloe reproductive output. Thus, the aim of this study was to investigate the effect of annual variation in the proportion of flowering *A. peglerae* plants on reproductive output (fruit set, seed set, total seed production, and seed viability) by birds and small mammals, as determined from selective pollinator exclusion experiments. Since birds are known to track resources and aggregate in areas with increased nectar availability, it was predicted that fewer birds would be present in poor flowering years when nectar rewards are low. It was expected that this would consequently result in decreased reproductive output of exclusively bird-pollinated plants. In contrast, small mammals that are less mobile, would be expected to remain restricted to their home ranges (e.g. Ribble and Perrin 2005), and reliant on resources in their immediate environments. We expected this to result in an increase in overall visitation rates by small mammals in poor flowering years, since fewer plants were flowering and more small mammals may be visiting (more frequently) at the same plants. A consequent increase in reproductive output for plants visited exclusively by small mammals in poor flowering years was expected. Although interactive effects of diurnal birds and nocturnal small mammals were more difficult to predict, our experimental design addressed this.

Materials and methods

Study site and species

This study was conducted in the Peglerae Conservancy (1650 m above sea level), in the Magaliesberg Mountain Range, ~25 km west of Pretoria, South Africa. The Conservancy was established to protect *Aloe peglerae* Schönland from anthropogenic disturbances, such as habitat transformation and the unregulated and illegal harvesting of seed and whole plants, as well as to facilitate monitoring for conservation (Pfab and

Scholes 2004; Wesson 2007). The site occurs in Gold Reef Mountain Bushveld (Mucina and Rutherford 2006), with shallow soils dominated by grasses, shrubs and scattered trees (Mucina and Rutherford 2006; Arena *et al.* 2013, 2015). Rainfall is seasonal, falling mostly in summer, but can vary significantly between years (530 mm in 2013, and 289 mm in 2014; South African Weather Service 2016). The Conservancy (~56 ha) has a mean density of 142 *A. peglerae* plants ha^{-1} in the areas where *A. peglerae* is found (~5 ha; Arena *et al.* 2015).

Adult *A. peglerae* plants (>20 cm in diameter) produce a single raceme of densely packed, tubular flowers during winter (June–July; Scholes 1988; Fig. 1*a*). Flowers (~30 mm in length) are dull red in bud, and become a yellowish-green with deep purple stamens and exserted anthers (~25 mm past the mouth) when mature (Scholes 1988; Van Wyk and Smith 2014; Fig. 1*a*). These exserted anthers, along with the long, tubular corollas of the flowers suggest bird pollination, as was confirmed by exclusion experiments (Botes *et al.* 2009*b*; Hargreaves *et al.* 2012; Arena *et al.* 2013). Flowers open sequentially from the base of the raceme, over ~15 days (Payne *et al.* 2016).

Visitation rates

Vertebrate visitation rates were measured through selective exclusion experiments conducted during the flowering seasons of 2013 (14% of *A. peglerae* plants flowering, representing a 'good' flowering year) and 2014 (7% flowering, representing a 'poor' flowering year). These flowering proportions in the population compare to the average of 29% of plants that have flowered per year over five flowering seasons, with the proportion of flowering plants declining in each year (C. T. Symes, unpubl. data; Arena 2013). In previous studies of the same population, individual plants were labelled with an engraved metal identification tag and their locations recorded with a handheld GPS (Garmin, GPSMAP 60Cx. USA; Arena *et al.* 2013; Payne *et al.* 2016), resulting in a total of 609 plants. Before flower opening, 14 and seven *A. peglerae* plants in 2013 and 2014, respectively, were assigned to each of the following selective exclusions:

(i) no visitors treatment ($NOVIS_{TRT}$) – plants covered with mesh cages (that exclude visitors of all types, including insects) for the entire duration of the flowering season, excluding all visitors (diurnal and/or nocturnal) from visiting flowers;
(ii) nocturnal visitors treatment ($NOCT_{TRT}$) – plants covered with mesh cages during the day and uncovered at night to allow visitation by nocturnal visitors;
(iii) diurnal visitors treatment ($DIUR_{TRT}$) – plants covered with mesh cages at night, and uncovered during the day to allow for visitation by diurnal visitors; and
(iv) all visitors (control) treatment (ALL_{TRT}) – plants uncovered for the entire flowering season, allowing diurnal and nocturnal visitor access to flowers.

Exclusion cages, large enough to not touch the flowers, were constructed from a bamboo tripod frame (height = ~0.9 m, basal area = ~0.3 m^2, fig. 1*b* in Payne *et al.* 2016), and covered with a pale brown, fine nylon mesh (1 mm^2 pores), which excludes all visitor types, including insects. Cages were secured along all edges with large rocks, to prevent visitors accessing the plants. Effectiveness of cages was tested with camera traps focusing on $NOVIS_{TRT}$ aloes, and a mouse was identified trying to access the plant but could not get through the cage. In 2013, aloes in close proximity shared cages, i.e. a $NOCT_{TRT}$ and a $DIUR_{TRT}$ aloe shared a cage, since treatments required cages at different times, facilitating the efficient moving of cages at dusk and dawn. In 2014, lower numbers of flowering aloes allowed for a cage to be assigned to each plant. Care was taken when placing or removing cages, to avoid pollen transfer between aloes via the mesh. Cages do not significantly alter microhabitat temperature within the cage (Payne *et al.* 2016).

Sampling lasted for four-five weeks in both seasons (25 June–27 July 2013; 02 July–30 July 2014). The same plants could not be sampled in both years due to the annual flowering variability. Although 42 plants were recorded as flowering in 2014, only seven plants were sampled per treatment, due to the unsuitability of some individuals for the experiment (e.g. damaged inflorescences).

An animal was considered a visitor if it touched the aloe in any way (the inflorescence and/or the leaves), since it had the potential to transfer pollen, due to the close proximity of the raceme and flowers to the leaves. The date and time of each visit was recorded, and each individual was identified to species, where possible. An individual was recorded as a 'new visitor' if the period between photographs was >30 s, or if the visitor was identified as a different individual (e.g. aberrant feather positioning or unique feather markings on birds). Often a visitor was acknowledged as the same individual when it remained in the same position in successive (3 s intervals) photographs. Mean visitation rates were calculated as the number of visitors per 1000 plants h^{-1} (Payne *et al.* 2016).

Camera traps and exclusion cages were changed daily within an hour at dawn (06:45– 07:45 hours) and within an hour at dusk (16:30–17:30 hours). These timings were chosen to select for the visitors relevant to each treatment, but in the transition from day to night and night to day, some visitors (e.g. birds feeding on $NOCT_{TRT}$ aloes during the early morning or late evening) could not be controlled for (see later; Payne *et al.* 2016). The diurnal period was defined as 07:00–17:59 hours, and the nocturnal period as 18:00–06:59 hours; small mammals and birds visiting during each of these periods respectively were defined as 'inappropriate' visitors. Cages that were destroyed by baboons and/or strong wind ($n = 3$), or by fire (21 July 2014) were replaced with new, undamaged cages in the next changeover period, i.e. within 12 h. During fires before flowering in June 2013 (J. Wesson, pers. comm.) and on 21 July 2014, plants suffered little damage, apart from slight burning of some outer leaves (S. L. Payne, pers. obs.; Arena *et al.* 2015).

Reproductive success

Pollinator contributions were defined using measures of reproductive success, i.e. fruit set, seed set (average seed $fruit^{-1}$), total seed production $plant^{-1}$ and seed $flower^{-1}$. In 2013, reproductive success was measured for only 12 of the plants per treatment, as two racemes went missing in each treatment (possibly destroyed by baboons, S. L. Payne, pers. obs.). In 2014, reproductive success was measured for seven



Fig. 1. (*a*) A flowering *Aloe peglerae* plant, with red buds and greenish-yellow mature flowers, and exserted anthers and stamens; (*b*) a male Cape rock-thrush (*Monticola rupestris*) feeding on *A. peglerae* nectar and likely pollinating the flowers; (*c*) a sengi (*Elephantulus* sp.) standing on an *A. peglerae* plant at night; (*d*) an *A. peglerae* plant that produced fruit, densely packed on the raceme. Photographs: (*a*, *d*:) S. L. Payne photographs; (*b*, *c*) camera trap photographs. All scale bars = 10 cm.

plants in the ALL_{TRT}, five in the $DIUR_{TRT}$ (two racemes missing), six in the $NOCT_{TRT}$ (one raceme missing) and seven in the $NOVIS_{TRT}$. Approximately five weeks after the end of the flowering season (9 September 2013; 5 September 2014), fruit were removed from each of the racemes and counted. A maximum of five whole fruit were randomly selected from each plant, and the remaining fruit were scattered around the site (Arena *et al*. 2013; Payne *et al*. 2016). The number of flowers on each raceme, represented by the number of peduncles (Symes *et al*. 2009; Arena *et al*. 2013; Payne *et al*. 2016) was counted, and

the fruit set of each aloe was calculated by dividing the number of fruit produced by the number of flowers. The mean number of flowers per plant for each treatment was also calculated and compared between treatments and years.

Harvested fruit were returned to a laboratory at the University of the Witwatersrand, and allowed to dehisce for ~5 weeks in separate, open brown paper bags (57 × 85 × 170 mm) at ambient temperature. The number of seeds in each dehisced fruit was then counted and the mean number of seed fruit^{-1} (seed set) determined for each plant. The estimated total seed production plant^{-1} was calculated by multiplying the mean seed set for each plant by the number of fruit produced by that plant. Seeds flower^{-1} was calculated by dividing total seed production by the number of flowers produced per plant.

Germination trials and seed viability

A maximum of 10 seeds from each fruit were randomly selected and pooled for each plant. Seeds were placed in labelled, sterilised Petri dishes on top of two sheets of filter paper and covered with one sheet of filter paper (Boeco Germany, Grade 3 hw, 65 g m^{-2}, 90 mm diameter). Petri dishes were kept saturated with distilled water in an environmental control chamber with a 12-h day-night cycle, with daytime temperatures of 25°C and night-time temperatures of 15°C and RH of 50% (Arena *et al.* 2013). Germinating seeds, identified by a radicle protruding by ≥2 mm (ISTA 2003), were counted every day for 3 weeks, and then three times a week for a further 3 weeks thereafter (Arena *et al.* 2013). Germinated seeds were removed, placed in separate Petri dishes and allowed to grow, with the intention of planting the seedlings back in The Peglerae Conservancy. Cumulative percentage germination was calculated as the percentage of seeds within each treatment that had germinated over the entire 6-week period.

After 6 weeks, seeds that had not germinated were removed from the control chamber. Empty seed coats were removed and these seeds recorded as 'dead', whereas remaining seeds were sliced in half to expose the embryo and immersed in a 1.0% 2,3, 5-triphenyltetrazolium chloride (tetrazolium) solution overnight. The following day, seeds with pale pink embryos were recorded as 'viable', and seeds with embryos that remained white were considered 'dead' (ISTA 2003). Total seed viability per plant (%) was calculated as the sum of all germinated seeds and viable seeds after tetrazolium testing, divided by the number of seeds tested per plant for each treatment.

Mean germination time (MGT) was calculated using the following equation:

$$\mathrm{MGT} = \frac{\Sigma Dn}{\Sigma n} \quad (1)$$

where D is the number of days since the beginning of germination, and n is the number of seeds germinated on day D (Zanjan and Asli 2012).

Data analysis

All data were tested for normality using Shapiro-Wilk normality test. The effect of treatment and year on overall treatment visitation rates and vertebrate guild visitation rates (birds vs small mammals in the $\mathrm{NOCT_{TRT}}$ and $\mathrm{ALL_{TRT}}$ only; visits 1000 plants^{-1} h^{-1}) were separately modelled with generalised linear models (GLM), with visits modelled as count data, with an offset for hours of sampling effort. Treatment or guild, and year were predictor variables, and a Poisson or Quasi-Poisson distribution (to account for over-dispersion), and a log-link function were specified. Although models did not fit data properly due to high variation within each treatment and small samples sizes (particularly in 2014), residuals were random and so GLM analyses were retained. A non-parametric Mann–Whitney U-test was used to test for significant differences between years in visitation rates of the most common diurnal and nocturnal visiting vertebrate species in each treatment.

Average number of flowers plant^{-1} was compared between treatments in each year with a non-parametric Kruskal–Wallis rank sum test, and between years for each treatment with a parametric Student's *t*-test. Fruit set, average seed fruit^{-1} (seed set), total seed production and seed flower^{-1} were compared between treatments in each year using a non-parametric Kruskal–Wallis rank sum test, and between years for each treatment using a non-parametric Mann–Whitney U-test. Kruskal–Wallis multiple comparison *post-hoc* tests in the package 'pgirmess' were performed on datasets with significant differences ($P < 0.05$; Giradoux 2018). GLMs were not used to test for differences in reproductive success between treatments and years, due to the large number of zeros in each treatment (plants that produced no fruit, and therefore no seed), particularly in the poor flowering year, which meant that the models did not fit the data.

To compare cumulative percentage germination, MGT and total viability among treatments for 2013 and 2014, the results for both years were combined for each treatment, as a result of the small sample size for germination trials in 2014 ($n = 1$, $n = 0$ and $n = 2$ plants produced seed in the $\mathrm{NOCT_{TRT}}$, $\mathrm{DIUR_{TRT}}$ and $\mathrm{ALL_{TRT}}$ respectively). Treatments were compared using a non-parametric Kruskal–Wallis rank sum test. Total viability of seeds produced by plants in each treatment was compared between years with a Chi-square test for Independence with Yates' continuity correction. *Post-hoc* tests, if necessary, were performed as above. All statistical analyses were performed in R (ver. 3.5.1; R Core Team 2018), with graphics created using the R package 'ggplot2' (Wickham 2016). Data are presented as mean ± s.e.

Results

Seasonal visitors and visitation rates

A total of 17 species (12 bird, four mammal and one reptile) visited *A. peglerae* plants across the four exclusion treatments in both years (see Supplementary Material table S1, available at the journal's website). Most $\mathrm{ALL_{TRT}}$ plants received both bird and small mammal visits in both years, with the exception of one plant in 2013 and one in 2014 receiving only bird visitors. All $\mathrm{DIUR_{TRT}}$ plants received only bird visitors in both 2013 and 2014, with one plant receiving no visitors in 2014. In 2013, nine of 11 $\mathrm{NOCT_{TRT}}$ plants received small mammal visits and at least one avian visit, and two plants received no visitors. In 2014, three $\mathrm{NOCT_{TRT}}$ plants received visits from both birds and small mammals, one plant received visits from small mammals only, three received only avian visits and one received no visits at all. Camera and exclusion cage changeover times did

Table 1. Visitation rates (visitors 1000 plants^{-1} h^{-1}) for two different pollinator guilds (birds and small, non-flying mammals) for *Aloe peglerae* plants in selective exclusion treatments, in two successive flowering seasons with differing proportions of flowering plants

2013 represents a 'good' flowering year, and 2014 represents a 'poor' flowering year. Visitation rates were converted to visits 1000^{-1} plants h^{-1} to account for rates within the entire *A. peglerae* population. Abbreviations: $NOCT_{TRT}$, $DIUR_{TR}$, and ALL_{TRT} = nocturnal, diurnal, and all visitors treatments respectively. Coefficients of variation (CVs) are presented, and emphasise the high variability within each treatment. Appropriate visitors, i.e. birds on $DIUR_{TRT}$ plants, small mammals on $NOCT_{TRT}$ plants, and both guilds on ALL_{TRT} plants, to each treatment are in bold text. Values are presented as means ± s.e.

	2013 (visits 1000 plants^{-1} h^{-1})	2014 (visits 1000 plants^{-1} h^{-1})
$NOCT_{TRT}$	59.17 ± 20.33	63.56 ± 21.11
Coefficient of variation	94.00%	113.90%
Birds	16.34 ± 3.54	37.64 ± 14.78
Small mammals	42.44 ± 24.86	24.86 ± 14.68
$DIUR_{TRT}$	167.18 ± 23.75	75.18 ± 19.19
Coefficient of variation	49.20%	67.50%
Birds	163.23 ± 23.78[A]	75.18 ± 19.19
Small mammals	0	0
ALL_{TRT}	89.33 ± 13.27	131.58 ± 22.70
Coefficient of variation	44.60%	41.80%
Birds	80.33 ± 13.53	93.05 ± 19.78
Small mammals	7.83 ± 2.45	38.21 ± 10.99

[A]The total visitation rate of the $DIUR_{TRT}$ in 2013 also includes visits from ground agama (*Agama aculeata*), resulting in the difference between the total visitation rate for the treatment and the visitation rate for the bird guild.

not differ significantly between years (Mann–Whitney U-test, $NOCT_{TRT}$ start times: 2013: 16:45 hours, 2014: 16:58 hours; U_{230} = 5876.0, P = 0.730; $NOCT_{TRT}$ end times: 2013: 07:47 hours, 2014: 07:46 hours; U_{230} = 6071.5, P = 0.955). In both years, 'inappropriate' avian visitors to the $NOCT_{TRT}$ plants occurred either during sunrise just before the changeover period, or during the changeover period. To account for the effect birds may have had on $NOCT_{TRT}$ aloe pollination, an 'inappropriate visitors correction' was applied to reproductive output data of plants in this treatment, where the proportion of visits by birds was subtracted from each measure of reproductive success (Payne *et al*. 2016). This did not make any significant difference to the statistical results so the original, uncorrected datasets were used. Cameras focussed on plants in the $NOVIS_{TRT}$, to confirm treatment (cage) effectiveness, did not record any visits to the plants in both 2013 and 2014, and therefore cages were deemed effective in fully excluding all potential pollinators.

Visitation rates were highly variable within each year, and this variability was maintained between years (Table 1). Visitation to the $DIUR_{TRT}$ decreased significantly between years, whereas the other treatments remained unaffected by year (Table 2). A total of 98.1 and 100% of visits to the plants in the $DIUR_{TRT}$ were made by birds, in 2013 (n = 266 visits) and 2014 (n = 70) respectively. A total of 70.2% of visits (n = 131) in 2013 and 33% of visits (n = 75) in 2014 to the $NOCT_{TRT}$ were made by small mammals. Although visitation did not change significantly between years for either guild in the $NOCT_{TRT}$, small mammal visitation to the ALL_{TRT} increased between years (Tables 1 and 2).

Table 2. Results of general linear models (GLMs)

Parameter estimates (β), with standard errors (s.e.), and significance of each factor for overall treatment visitation rates, and guild visitation rates (birds vs small mammals) for the all visitors control treatment (ALL_{TRT}) and the nocturnal visitors treatments ($NOCT_{TRT}$) are presented. Visitation rates (the dependent variable) were modelled as count data, with an offset for hours of sampling effort, with Poisson or Quasi-Poisson distributions (to account for over-dispersion). In the overall treatment visitation rate model, the all visitors control treatment (Treatment (all visitors)) and 2013 (Year (2013)) are the reference categories for treatment and year factors respectively. In the guild visitation rate models, the bird guild (Guild (birds)) and 2013 (Year (2013)) are reference categories for the guild and year factors respectively. Significant differences are indicated: *P < 0.05. Large estimates and standard errors are due to high variation within treatments, and small sample sizes; however, residuals are random and therefore GLM analyses were retained

Predictors	Estimate (β) ± s.e.	P-value
Treatment visitation rates		
Intercept	–352.07 ± 506.86	0.49
Treatment (diurnal visitors)	1860.55 ± 887.87*	0.04*
Treatment (nocturnal visitors)	535.24 ± 935.38	0.57
Year (2014)	0.18 ± 0.25	0.49
Treatment (diurnal visitors): year	–0.92 ± 0.44*	0.04*
Treatment (nocturnal visitors): year	–0.27 ± 0.47	0.57
Guild visitation rates		
All visitors treatment (ALL_{TRT})		
Intercept	–2.55 ± 209.9	0.99
Guild (small mammals)	–1538 ± 503.2*	0.002*
Year (2014)	0.0017 ± 0.1	0.99
Guild (small mammals): year	0.76 ± 0.25*	0.002*
Nocturnal visitors treatment ($NOCT_{TRT}$)		
Intercept	–1493.18 ± 1304.7	0.26
Guild (small mammals)	3258.26 ± 1905.88	0.1
Year (2014)	0.74 ± 0.65	0.26
Guild (small mammals): year	–1.62 ± 0.95	0.1

The Cape rock-thrush (Fig. 1*b*) was the most common diurnal visitor, accounting for ~80% of all visits in the $DIUR_{TRT}$ (2013: n = 214 of 266 visits; 2014: n = 56 of 70 visits) in both years. Although the visitation rate of this species did not decrease significantly between years (ALL_{TRT}: U_{14} = 20, P = 0.46; $DIUR_{TRT}$: U_{16} = 39, P = 0.38), the overall percentage contribution to visitation in the ALL_{TRT} decreased from 68.2% (n = 199 of 292 visits) in 2013 to 52.4% (n = 99 of 189 visits) in 2014 (Supplementary material table S1). The Namaqua rock mouse was the most common nocturnal visitor, accounting for 66.4% in 2013 (n = 87 of 131 visits) and 26.7% in 2014 (n = 20 of 75 visits) of all visits in the $NOCT_{TRT}$ (Supplementary material table S1). Percentage contribution to visitation by the Namaqua rock mouse in the ALL_{TRT} increased from 9.6% (n = 28 of 292 visits) in 2013 to 20.6% (n = 39 of 189 visits) in 2014. Although there was no significant change in Namaqua rock mouse visitation rates to the $NOCT_{TRT}$ between the two seasons (U_{14} = 58, P = 0.25), the percentage contribution by this species to visitation decreased in the $NOCT_{TRT}$.

The visitation rates of the most abundant visitors per guild, i.e. three bird species (Cape rock-thrush, dark-capped bulbul (*Pycnonotus tricolor*), and streaky-headed seed-eater (*Crithagra gularis*)), and two small mammal species (Namaqua rock mouse and sengi (*Elephantulus* sp., likely *E. myurus* or *E. brachyrhynchus*; M. Perrin, H. Smit-Robinson, G. Rathbun,

pers. comm.; Fig. 1*c*) were compared within each treatment between 2013 and 2014. There were no significant changes in visitation rates of these species (Supplementary material table S2), with the exception of the streaky-headed seed-eater, which decreased, seeming to have disappeared altogether, in the $DIUR_{TRT}$ in 2014, from 12.59 ± 2.95 to 0 visits 1000 plants^{-1} h^{-1} (U_{17} = 73.5, P = 0.01; Supplementary material table S2). Despite the presence of mountain wheatear (*Oenanthe monticola*) and mocking cliff-chat (*Thamnolaea cinnamomeiventris*), known to feed on *Aloe* nectar (Symes 2010), they were not recorded feeding on *A. peglerae* nectar in either flowering season.

Insects do not contribute significantly to the pollination of the aloe (Arena *et al.* 2013), and their activity and abundance (especially in the early morning) is low in winter (Symes *et al.* 2008). However, camera trap photographs recorded insects, presumably bees, around the flowers from 10:00 hours, with activity peaking at 12:00 hours and slowly declining from 15:00 hours (Arena *et al.* 2013). This contrasts with the peak visiting times of the Cape rock-thrush, an insectivore, where bird presence on the aloes increases between 07:00 and 08:00 hours, declined between 10:00 and 14:00 hours, and increased again at ~15:00 hours. This suggests that the birds (specifically the Cape rock-thrush) are feeding on nectar in the early morning and late afternoon to supplement their diet when insects, their primary food source, are inactive (Symes *et al.* 2008), switching to their optimal insect diet as it becomes available. However, no camera trap photographs recorded any birds noticeably or actively foraging for insects around the aloe, following the suggestion by Oatley (1964) that nectar is the primary attractant for birds, and the possibility of an insectivore finding prey at the aloe flowers is purely 'fortuitous'. This pattern in other bird species and feeding guilds, dependent on the primary diet of the particular species, requires further investigation.

Streaky-headed seed-eaters were recorded tearing perianths of flowers and consuming flowers (and possibly nectar) in 2013. The Namaqua rock mouse was destructive in both years, removing flowers at night. In the case of one $NOCT_{TRT}$ aloe in 2013, mice removed several flowers each night, until the plant had no flowers left, and the aloe produced no fruit. In 2014, a $NOCT_{TRT}$ aloe received no nocturnal visitors, until fire burnt the surrounding area. Thereafter, small mammals visited the plant and herbivory occurred, resulting in no fruit being produced. Baboons were recorded pulling on – but in these instances not damaging – inflorescences, twice in both years. Two $DIUR_{TRT}$ aloes were visited by a ground agama (*Agama aculeata*) in 2013. The agama sat either on the flowers or leaves, and did not appear to feed on flowers or nectar. In 2014, one Smith's red rock rabbit (*Pronolagus rupestris*) was recorded once sniffing the inflorescence of a $NOCT_{TRT}$ aloe, but no other contact with the aloes was recorded. Seven other mammal species were recorded by the camera traps in both years, but were not associating with the aloes and inflorescences in any way (Supplementary material table S3).

Reproductive success

Some inflorescences went missing or were destroyed in both years, while some aloes were exposed to a second fire during fruiting in 2014 (J. Wesson, pers. comm.) and no whole fruit was available for harvest (Table 3). Many aloes in 2014 showed evidence of fruit production, i.e. dry pieces of fruit capsule, or green peduncles or peduncle scars where fruit had been removed (Table 3). In these cases, fruit set could be calculated for these aloes, but seed set and total seed production could not.

The mean number of flowers plant^{-1} (2013: 241 ± 10 flowers plant^{-1}, n = 48 plants; 2014: 182 ± 11 flowers plant^{-1}, n = 25

Table 3. Reproductive success for *Aloe peglerae* plants in each selective exclusion treatment in two consecutive flowering seasons of differing flowering success, where 2013 represents a 'good' flowering year (14% of plant flowering), and 2014 represents a 'poor' flowering year (7% flowering)
Abbreviations: $NOVIS_{TRT}$, no visitors treatment; $NOCT_{TRT}$, nocturnal visitors treatment; $DIUR_{TRT}$, diurnal visitors treatment; ALL_{TRT}, all visitors treatment. All values are presented as means ± s.e.

	2013 $NOVIS_{TRT}$	2014 $NOVIS_{TRT}$	2013 $NOCT_{TRT}$	2014 $NOCT_{TRT}$	2013 $DIUR_{TRT}$	2014 $DIUR_{TRT}$	2013 ALL_{TRT}	2014 ALL_{TRT}
No. of plants in treatment	12	7	12	6[A]	12	5[A]	12	7
No. of missing/destroyed inflorescences	2	0	2	1	2	2	2	0
Average no. of flowers plant^{-1}	256 ± 26	183 ± 21	240 ± 15	157 ± 21	243 ± 16	167 ± 12	226 ± 20	214 ± 24
No. of plants with fruit burnt in fire	0	1	0	0	0	0	0	1
No. of plants with evidence of fruit[B]	0	0	0	1	0	0	0	3
No. of plants that produced harvestable fruit[B]	0	0	8	2	11	0	10	5
Average no. of fruit produced plant^{-1}	0	0	23 ± 7.7	9.5 ± 7.9	55 ± 11.1	0	41.5 ± 8.3	40.7 ± 22.2
Fruit set (%)	0	0	8.9 ± 2.7	4.9 ± 4.0	23.3 ± 4.9	0	19.9 ± 4.1	18.1 ± 9.6
Seed set (all flowering plants)	0	0	24.1 ± 5.5	5.0 ± 5.0	42.6 ± 4.3	0	43.4 ± 6.1	11.0 ± 7.2
Seed set (fruit producing plants only)	0	0	36.2 ± 3.2	14.7 ± 9.6	25.4 ± 4.8	0	52.0 ± 1.9	38.6 ± 5.8
Average seed production plant^{-1}	0	0	861 ± 321	242 ± 242	2433 ± 457	0	2129 ± 424	1270 ± 828
Average seed flower^{-1}	0	0	10.4 ± 2.0	1.2 ± 1.2	3.3 ± 1.1	0	10.0 ± 2.0	6.1 ± 4.1

[A]Seven plants were originally assigned to each treatment; however, from the end of field sampling, i.e. flowering period, to the fruit harvest, two racemes in the $DIUR_{TRT}$ and one raceme in the $NOCT_{TRT}$ went missing, presumably removed by baboons. Therefore, these plants could not be used for reproductive success calculations and were removed from the sample for these analyses.

[B]The number of plants with evidence of fruit were those which showed some indication of producing fruit, usually in the form of remaining pieces of fruit capsule on the raceme, or green peduncle scars where fruit has been recently removed. Fruit set, and consequently seed set, could not be calculated for these plants. The number of plants that produced harvestable fruit were those where fruit was still attached to the raceme at the time of harvest and fruit set could be calculated. Whole fruit could be harvested from these plants and seed set could be calculated as well.

plants) did not differ among treatments in each year (2013: $F_{3,44} = 0.37$, $P = 0.77$; 2014: $F_{3,21} = 1.38$, $P = 0.28$). However, inflorescences produced by plants in 2014 produced significantly fewer flowers than in 2013 for plants assigned to the $DIUR_{TRT}$ ($t_{15} = 2.87$, $P = 0.01$) and the $NOCT_{TRT}$ ($t_{16} = 3.25$, $P = 0.01$; Table 3).

In 2013, most plants in each treatment produced fruit (Fig. 1*d*), whereas in 2014 the majority of sampled plants produced no fruit, despite receiving visitors during flowering (Table 3). Plants in the $NOVIS_{TRT}$ produced no fruit in either year and, therefore, no seed could be harvested. Fruit set differed significantly between the ALL_{TRT} (19.9%) and both the $DIUR_{TRT}$ (23.3%) and the $NOVIS_{TRT}$ (0.0%) in 2013 ($H_{3,44} = 23.31$, $P < 0.0001$), but not between the $NOCT_{TRT}$ (9.0%) and the $DIUR_{TRT}$ or the $NOVIS_{TRT}$, as indicated by *post-hoc* testing. Although there was an overall significant difference in mean fruit set among treatments in 2014 ($H_{3,24} = 10.48$, $P = 0.02$), *post-hoc* tests indicated no significant differences among treatments. When comparing between years in each treatment, mean fruit set in the $DIUR_{TRT}$ differed significantly between 2013 (23.3%) and 2014 (0.0%) ($U_{17} = 80.0$, $P = 0.0008$; Fig. 2*a*), but not in the other three exclusion treatments.

A total of 144 whole fruits were collected in 2013 (55 from $DIUR_{TRT}$, 39 from $NOCT_{TRT}$ and 50 from ALL_{TRT}), whereas only 15 whole fruits were collected in total during 2014 (10 from ALL_{TRT} and five from $NOCT_{TRT}$), due to fire, fruit predation and the overall low fruit production in the $DIUR_{TRT}$. Seed set (average seed fruit^{-1}) differed significantly between the $NOVIS_{TRT}$ and both the ALL_{TRT} and $DIUR_{TRT}$ in 2013 ($H_{3,44} = 25.64$, $P < 0.0001$), but not among treatments in 2014 ($H_{3,24} = 4.12$, $P = 0.59$; Fig. 2*b*). Seed set in the $DIUR_{TRT}$ in 2014 was zero (no fruit produced), a significant decrease from 2013 (42.6 ± 4.3 seed fruit^{-1}; $U_{17} = 80.5$, $P = 0.001$). Seed set differed significantly between years in the ALL_{TRT} (from 43.35 ± 6.1 in 2013 to 11.0 ± 7.2 seed fruit^{-1} in 2014; $U_{17} = 75.0$, $P = 0.01$; Fig. 2*b*). In both years, minimal fruit appeared to be parasitised by wasps (or other insect species), and the effect that the parasites may have had on seed set and reproductive output was not considered further.

Total seed production differed significantly between the $NOVIS_{TRT}$ (0 seed plant^{-1}) and both the $DIUR_{TRT}$ (2433 ± 457 seed plant^{-1}) and the ALL_{TRT} (2129 ± 424 seed plant^{-1}) in 2013, but there was no significant difference in total seed produced in the same treatments in 2014 ($H_{3,24} = 3.61$, $P = 0.31$; Table 3). Significantly more seed was produced per plant in the $DIUR_{TRT}$ in 2013 (2433 ± 457 seed plant^{-1}) than in 2014 (0 seed plant^{-1}; $U_{17} = 57.5$, $P = 0.004$; Fig. 2*c*). Seed flower^{-1} differed significantly between the $NOVIS_{TRT}$ (0 seed flower^{-1}) and both the $NOCTVIS_{TRT}$ (10.39 ± 2.02 seeds flower^{-1}) and $ALLVIS_{TRT}$ (10.03 ± 1.99 seed flower^{-1}) in 2013 ($H_{3,44} = 24.31$, $P < 0.001$), but did not differ among treatments in 2014 ($H_{3,21} = 3.61$, $P = 0.31$; Fig. 2*d*; Table 3). Between years, seed flower^{-1} decreased significantly in the $NOCT_{TRT}$ ($U_{16} = 66.5$, $P = 0.004$) and the $DIUR_{TRT}$ ($U_{15} = 50$, $P = 0.026$; Fig. 2*d*, Table 3).

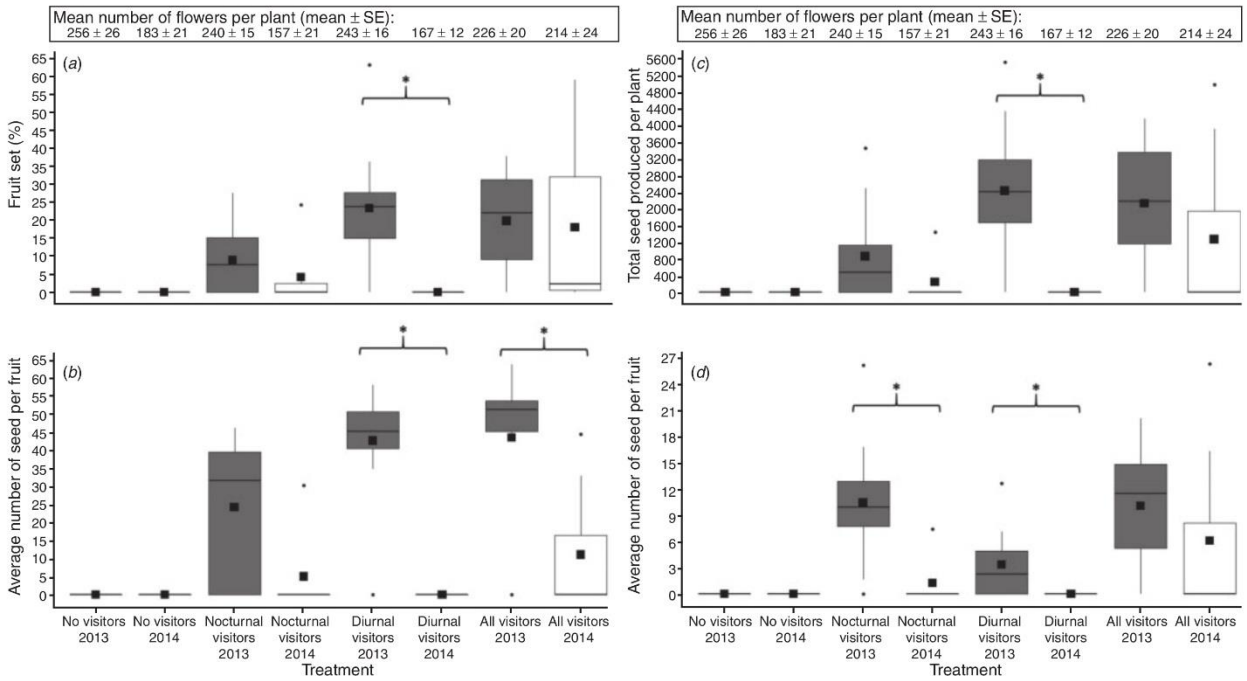


Fig. 2. Reproductive output of *Aloe peglerae* plants in pollinator selective exclusion treatments in two flowering seasons. 2013 (grey boxes) represents a 'good' flowering year and 2014 (white boxes) represents a 'poor' flowering year. (*a*) Fruit set (proportion of flowers that set fruit); (*b*) average number of seed per fruit (seed set); (*c*) total seed produced per plant; (*d*) average seeds per flower (2013: $n = 12$ plants treatment^{-1}; 2014: $n = 7$ plants in 'no visitors' treatment and 'all visitors' treatment six plants in 'nocturnal visitors' treatment and five plants in 'diurnal visitors' treatment. Thick solid lines represent medians, solid squares represent means, outer edges of boxes represent interquartile-ranges, vertical bars represent minimum and maximum values and solid dots represent outliers. Treatments significantly different between years are indicated: $*P < 0.05$.

Germination trials and seed viability

Germination began to slow 10 days after commencing the trials in both years, with the final seed germinating after 19 days in 2013, and 18 days in 2014. Cumulative germination, MGT and total viability results in 2013 and 2014 for each treatment were combined, due to low sample sizes in 2014, for statistical analyses. Cumulative percentage germination and other results for individual treatments in each year are presented in Supplementary material table S4. The cumulative percentage germination did not differ among treatments (90–97%; $H_{2,29}$ = 4.25, P = 0.12). Similarly, MGT showed no significant difference between treatments (range: 6–9 days; $H_{2,29}$ = 2.15, P = 0.14). Total seed viability (germinated seeds plus viable seeds after tetrazolium) was not significantly different between treatments ($H_{2,29}$ = 5.66, P = 0.06), ranging from 90 to 98%. Total viability did not show any significant difference between years in the ALL_{TRT} and the $NOCT_{TRT}$ (χ^2_1 = 0.60, P = 0.44 and χ^2_1 = 1.94, P = 0.16 respectively).

Discussion

Effects of variable flowering on bird (mobile) pollinators

Reproductive output of *A. peglerae* decreased between years, and most noticeably for the $DIUR_{TRT}$, where birds were regular visitors. Although the lower reproductive success in the poor flowering year may be linked to the fewer visits by primary pollinators attracted to the limited nectar supply (fewer flowering plants), we cannot exclude the possibility that poorer overall plant condition played a role in reduced reproductive output. The decrease in bird visitation rates during the poor flowering year suggests a disproportionate decrease in bird abundance, and that the generalist nectar-feeding birds may avoid the site entirely when resources become too low (and dispersed). Because of their vagility, birds are able to easily respond to available resources (e.g. MacNally 1996; Malizia 2001; Kuiper *et al.* 2015), optimising foraging in a way that is different to less mobile non-flying small mammals. The lower flowering proportions in the subpopulation and the smaller inflorescences (fewer flowers), suggest that foraging on this nectar source during a poor flowering year could be uneconomical, and it may be more beneficial to search for richer food sources elsewhere, rather than the same birds feeding on fewer plants more often. Opportunistic and/or migrant nectar feeders do not rely on predictable nectar resources and feed on nectar when it is abundant, and other food resources are not (e.g. Brown and Hopkins 1996; Franklin 1999). Therefore, visitation and feeding by such birds should decrease in poorer flowering years. The decrease in overall visitation rates between years suggests that nectar-feeding may be opportunistic and that the Cape rock-thrush, a winter altitudinal migrant (Johnson and Maclean 1994), uses nectar purely as an energy supplement during resource depleted winters. Indeed, the implied reduction in bird numbers during the poor flowering year suggests that more energetically viable resources are available elsewhere. The transient nature of the birds, and the likelihood of resource tracking, is further reinforced by the lack of recapture of birds ringed in two different flowering seasons (2012 and 2014), where only one male Cape rock-thrush, ringed in 2014, returned to the site only twice within the same flowering season (Payne *et al.* 2016).

Effects of variable flowering on small mammal (sedentary) pollinators

Small mammal visitation remained the same between years, implying that the small mammals, restricted by their limited mobility, are forced to exploit the resources available in their immediate surroundings. The Namaqua rock mouse and the sengi are both restricted to small home ranges (e.g. eastern rock sengi (*Elephantulus myurus*) males have home ranges of ± 3958 m^2; Ribble and Perrin 2005), and, therefore, are restricted to small foraging areas. Resources may be limited in the poor flowering year, resulting in the visitation rates remaining the same or increasing, as small mammals visit the plants more often to optimise the reduced available resources. Since different plants were assigned to the ALL_{TRT} in each year, the increased small mammal visitation in the ALL_{TRT} is likely due to the mobility and territoriality of the small mammals surrounding the individual plants sampled. However, the distance travelled by small mammals between flowering aloes, along with population density fluctuations, requires further investigation.

Competition for nectar between birds and small mammals

Although small mammal territories surrounding sampled plants likely differed between years, possibly contributing to the changes in small mammal visitation rates between years, sampling different territories within the Conservancy should not affect bird visitation, as birds are able to easily move between plants. Therefore, the decrease in bird visitation can be attributed to factors different than those which affect small mammals, the most likely being the availability of nectar resources. It is likely that there is competition between birds and small mammals for nectar resources. This is evident as more birds may be foraging during dusk and dawn for accumulated nectar produced by $NOCT_{TRT}$ plants during sunrise (before cage changeovers) or sunset (after cage changeovers) in a poor flowering year. Approximately 67% of visits in the $NOCT_{TRT}$ were made by birds (although this was not a significant change between years and had no effect on reproductive output after corrections), and these birds are probably depleting nectar resources available for small mammals. While birds are able to move to another area in search of other viable food resources, small mammals are forced to make use of the limited nectar available in their immediate surroundings. To meet energy requirements, small mammals need to: (i) find alternative plants at which to forage, (ii) forage at the same plants more often, and/or (iii) feed at the same plant throughout the night as nectar supplies become replenished. Camera trap footage shows *A. peglerae* flowers opening consistently throughout the night, as with *A. marlothii* (Symes and Nicolson 2008). *Aloe peglerae* nectar is consistently produced over a 24-h period, with nectar concentration remaining unchanged over time (S. L. Payne, unpubl. data), providing ample, good quality nectar for small mammals during the night and, where unconsumed by small mammals, for birds during the early hours of the morning.

Effects of variable flowering and subsequent pollinator visitation on reproductive output

The decrease in reproductive output between years further emphasises the primary role that birds play in the pollination

of *Aloe peglerae*. In the poor flowering year, no fruit was produced in plants pollinated only by birds, whereas plants pollinated by birds or small mammals produced minimal fruit. Plants that received no visits in both years ($NOVIS_{TRT}$) produced no fruit, suggesting that the aloe is reliant on a biotic pollen vector. Small mammals may be acting as alternative, albeit less effective pollinators (especially since they are less effective at moving pollen across greater distances, and between populations; Cunningham 1991), whereas the birds are moving to areas with more abundant food and water resources. Also, smaller floral displays in 2014 likely decreased the attractiveness of the area to potential pollinators (particularly birds) which subsequently lowered visitation rates and reproductive output (Sutherland 1986; Broyles and Wyatt 1990). Although small mammals can be destructive when removing flowers, often to the point where the plant does not produce fruit, the similar quality of seed produced by bird- and small mammal-pollinated plants suggests that small mammals are cross-pollinating to some extent, and further reinforces the suitability of the small mammals as alternative pollinators, in both 'good' and 'poor' flowering years (Payne *et al*. 2016). In both years, fruit showed evidence of frugivory (ALL_{TRT}: 35.2 and 92.0% of fruit produced in 2013 and 2014 respectively), although it is not known whether small mammals or birds (presumably the streaky-headed seed-eater) were feeding on the fruit, as no camera traps were active in the period between flowering and fruit harvest. Nevertheless, the increase in frugivory in the 'poor' flowering season (100% of fruit on some plants showed evidence of frugivory), could be an indication of limited food resources for animals, both per plant and across the entire flowering/fruiting subpopulation, in the Conservancy.

The significant decrease in the number of flowers produced by plants in 2014 decreases the number of flowers available for pollination and fertilisation, which could be resulting in a lower fruit set, as well as lowering the amount of nectar available for pollinators. Small mammals are less efficient at cross-pollination (Faegri and van der Pijl 1979; Cunningham 1991). Consequently, they are likely, to some degree, to contribute to self-pollination on individual inflorescences. Since *A. peglerae* appears to be self-incompatible (Arena *et al*. 2013; Payne *et al*. 2016), transfer of genetically similar pollen by small mammals (and possibly birds as well) may be initiating the self-incompatibility reaction, decreasing reproductive output (e.g. Sutherland and Delph 1984).

Conclusion

Nectar is an important winter food source for birds and mammals alike (e.g. Symes *et al*. 2008, 2011; Schmid *et al*. 2015; Steenhuisen *et al*. 2015). A decrease in the proportion of flowering *A. peglerae* plants and subsequent decline in nectar availability may force vagile birds to avoid the site in search of more energetically viable resources. This was suggested by the decrease in visitation to, and reproductive output of, plants in the $DIUR_{TRT}$. Meanwhile, visitation by small mammals remained the same in the $NOCT_{TRT}$, but increased in the ALL_{TRT}, with reproductive output unaffected between years. This is most likely due to the limited mobility and home ranges of the small mammals, where they are forced to exploit and compete for resources available in their immediate environments.

Along with decreased visitation rates, minimal fruit production could also be as a result of limited resources available to the plants (especially maternal resources), and climatic conditions before flowering, all of which require further investigation. Since an alternative pollinator appears to adequately pollinate the species, this – along with the perennial nature of the plant – may also buffer the species against years of low seed production in poorer flowering years, with low pollinator abundance. *A. peglerae* may be viable in small subpopulations and protected from Allee effects, which could further decrease reproductive output, especially if the primary pollinator is absent from the area. However, the conservation of this endangered and endemic aloe should be prioritised, as the variation in flowering could be detrimental to the persistence of *A. peglerae*, notwithstanding the current effects of anthropogenic disturbance on this species.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix 3.1. Supplementary Information

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Good times, bad times: Inter-annual reproductive output in a montane endemic succulent (*Aloe peglerae*; Asphodelaceae) driven by contrasting visitor responses of small mammals and birds

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Supplementary tables

Table S1. Percentage (%) of visits to *Aloe peglerae* by each species in each exclusion treatments in two different flowering seasons (winter 2013, 14% of plants flowering; winter 2014, 7% flowering); $NOCT_{TRT}$, $DIUR_{TRT}$ and ALL_{TRT} = Nocturnal Visitors, Diurnal Visitors, and All Visitors treatments respectively. Bold values indicate visitors on the correct treatment (i.e. appropriate visitors); values in parentheses are visitation rates (number of visits 1000 plants^{-1} hour^{-1}; mean ± SE). Visitation rates were converted to 1000 plants hour^{-1} to account for rates within the entire *A. peglerae* population. Visitation rates highlighted in grey are significantly different between years (Mann-Whitney U Test, $P < 0.05$). An animal was recorded as a visitor if it touched the plant (e.g. perched on leaves, etc.) or inflorescence in any way. No visitors were recorded inside the exclusion cages of the No Visitors treatment. Taxonomy for birds follows Hockey *et al*. (2005) and Chittenden *et al*. (2014). The 2013 columns of the table have been previously presented in Payne *et al*. (2016) and adjusted to suit this article. [A] Visitors that touched the plant and/or inflorescence and probed flowers; [B] Visitors that touched the plant and/or inflorescence only; [C] Visitors that also fed on flowers; [D] Visitors not previously recorded by Arena *et al*. (2013).

	$NOCT_{TRT}$		$DIUR_{TRT}$		ALL_{TRT}	
	2013	**2014**	**2013**	**2014**	**2013**	**2014**
	(2274 hrs; 11 plants)	**(1168 hrs; 8 plants)**	**(1599 hrs; 12 plants)**	**(827 hrs; 7 plants)**	**(3318 hrs; 9 plants)**	**(1440 hrs; 6 plants)**
BIRDS:						
Cisticolidae						
Wailing Cisticola	0.8	0	**0**	**0**	**0**	**0**
Cisticola lais[B,D]	(0.52 ± 0.52)	0	**0**	**0**	**0**	**0**
Dicruridae						
Fork-tailed Drongo	1.5	0	**0.4**	**0**	**0.7**	**0**
Dicrurus adsimilis[A,D]	(1.12 ± 1.12)	0	**(0.56 ± 0.56)**	**0**	**(0.56 ± 0.56)**	**0**
Fringillidae						
Streaky-headed Seed-eater	3.8	2.7	**7.9**	**0**	**4.1**	**3.2**
Crithagra gularis[A,C]	(1.79 ± 0.96)	(1.67 ± 1.67)	**(12.59 ± 2.95)**	**0**	**(4.29 ± 1.29)**	**(3.96 ± 1.53)**
Lybiidae						
Crested Barbet	0	0	**0.4**	**1.4**	**1.7**	**0**
Trachyphonus vaillantii[A,D]	0	0	**(0.56 ± 0.56)**	**(0.93 ± 0.93)**	**(1.76 ± 1.18)**	**0**

	NOCT$_{TRT}$		DIUR$_{TRT}$		ALL$_{TRT}$	
	2013	**2014**	**2013**	**2014**	**2013**	**2014**
	(2274 hrs; 11 plants)	**(1168 hrs; 8 plants)**	**(1599 hrs; 12 plants)**	**(827 hrs; 7 plants)**	**(3318 hrs; 9 plants)**	**(1440 hrs; 6 plants)**
Motacillidae						
Long-billed Pipit	0	0	**0.4**	**0**	**0**	**0**
Anthus similis[B]	0	0	**(0.54 ± 0.54)**	**0**	**0**	**0**
Muscicapidae						
Cape Rock-Thrush	16.0	42.7	**80.5**	**80.0**	**68.2**	**52.4**
Monticola rupestris[A]	(9.30 ± 3.32)	(24.12 ± 9.12)	**(134.02 ± 22.40)**	**(59.34 ± 15.77)**	**(60.15 ± 7.58)**	**(65.53 ± 12.68)**
Familiar Chat	0	1.3	**1.1**	**1.4**	**1**	**3**
Cercomela familiaris[B]	0	(0.61 ± 0.61)	**(1.87 ± 1.29)**	**(1.36 ± 1.36)**	**(0.66 ± 0.66)**	**(1.75 ± 1.11)**
Mountain Wheatear	0	0	**0.4**	**0**	**0**	**0**
Oenanthe monticola[B,D]	0	0	**(0.69 ± 0.69)**	**0**	**0**	**0**
Nectariniidae						
White-bellied Sunbird	0	0	**0**	**1.4**	**0**	**0**
Cinnyris talatala[A,D]	0	0	**0**	**(1.36 ± 1.36)**	**0**	**0**
Pycnonotidae						
Dark-capped Bulbul	4.6	20.0	**7.1**	**15.7**	**13.7**	**20.0**
Pycnonotus tricolor[A]	(2.43 ± 1.06)	(11.25 ± 5.13)	**(12.40 ± 4.20)**	**(12.19 ± 5.30)**	**(12.91 ± 5.12)**	**(21.81 ± 7.27)**
Sturnidae						
Red-winged Starling	0.8	0	**0**	**0**	**0**	**0**
Onychognathus morio[A,D]	(0.52 ± 0.52)	0	**0**	**0**	**0**	**0**
Zosteropidae						
Cape White-eye	1.5	0	**0**	**0**	**0**	**0**
Zosterops virens[A]	(0.67 ± 0.67)	0	**0**	**0**	**0**	**0**
MAMMALS:						
Cercopithecidae						
Chacma Baboon	0.7	0	**0**	**0**	**1.0**	**1.1**

	$NOCT_{TRT}$		$DIUR_{TRT}$		ALL_{TRT}	
	2013	**2014**	**2013**	**2014**	**2013**	**2014**
	(2274 hrs; 11 plants)	**(1168 hrs; 8 plants)**	**(1599 hrs; 12 plants)**	**(827 hrs; 7 plants)**	**(3318 hrs; 9 plants)**	**(1440 hrs; 6 plants)**
Papio hamadryas ursinus[B,C,D]	(0.38 ± 0.38)	0	**0**	**0**	**(1.18 ± 0.65)**	**(2.13 ± 1.38)**
Leporidae						
Smith's Red Rock Rabbit	0	1.3	**0**	**0**	**0**	**0**
Pronolagus rupestris[B,D]	0	**(1.06 ± 1.06)**	**0**	**0**	**0**	**0**
Macroscelididae						
Sengi	**3.8**	**5.3**	0	0	**0**	**3.2**
Elephantulus Sp.[A,D]	**(2.28 ± 2.28)**	**(3.66 ± 2.44)**	0	0	**0**	**(5.68 ± 4.36)**
Muridae						
Namaqua Rock Mouse	**66.4**	**26.7**	0	0	**9.6**	**20.6**
Micaelamys namaquensis[A,C,D]	**(40.17 ± 18.75)**	**(21.20 ± 13.22)**	0	0	**(7.83 ± 2.45)**	**(30.73 ± 1.95)**
REPTILES:						
Agamidae						
Ground Agama	0	0	**1.9**	**0**	**0**	**0**
Agama aculeata[B,D]	0	0	**(3.95 ± 2.21)**	**0**	**0**	**0**
Total Number of Species	10	7	9	5	8	7
Total Number of Visitors	**92,** 39	**25,** 50	**266,** 0	**70,** 0	**292,** 0	**189**, 0
Mean Visitation Rate	59.17 ± 20.33	63.56 ± 21.11	167.18 ± 23.75	75.18 ± 19.19	89.33 ± 13.27	131.58 ± 22.70

Note: In 2013, a total of 7191 camera hours (~225 hours plant^{-1}) yielded a total of 689 legitimate visits (visits where animals contacted the plant in any way, e.g. touching flowers, sitting/standing on leaves, etc.) across the ALL_{TRT}, $DIUR_{TRT}$ and $NOCT_{TRT}$, while a total of 3395 camera trap hours (~161 hours plant^{-1}) yielded a total of 334 legitimate visits across the same three treatments in 2014. The number of camera hours/plant was less in 2014 because, i) the flowering/sampling period was shorter in 2014 (~33 days in 2013, ~29 days in 2014), because inflorescences were smaller with fewer flowers, and, ii) three full days of observations were lost due to the fire and logistical difficulties accessing the site, requiring the removal of cameras, resulting in only 26 days of sampling.

Table S2. Percentage (%) of visits to *Aloe peglerae* by the most common visitors in the bird and small mammal guilds, in each exclusion treatment in two different flowering seasons (winter 2013, 14% of plants flowering; winter 2014, 7% flowering; $NOCT_{TRT}$, $DIUR_{TRT}$ and $ALLVIS_{TRT}$ = Nocturnal Visitors, Diurnal Visitors and All Visitors treatments respectively.

Values in parentheses are visitation rates (number of visits 1000 plants^{-1} hour^{-1}; mean ± SE). Visitation rates were converted to 1000 plants hour^{-1} to account for rates within the entire *A. peglerae* population. Bold values are significantly different between years (Mann-Whitney U-Test, $P < 0.05$). An animal was recorded as a visitor if it touched the plant (e.g. perched on leaves, etc.) or inflorescence in any way. No visitors were recorded inside the exclusion cages of the No Visitors treatment. Taxonomy for birds follows Hockey *et al*. (2005) and Chittenden *et al*. (2014). The 2013 columns of the table have been previously presented in Payne *et al*. (2016) and adjusted to suit this article.

	$NOCT_{TRT}$			**$DIUR_{TRT}$**			**ALL_{TRT}**		
	2013 (2274 hrs; 11 plants)	**2014 (1168 hrs; 8 plants)**	**Test Statistics**	**2013 (1599 hrs; 12 plants)**	**2014 (827 hrs; 7 plants)**	**Test Statistics**	**2013 (3318 hrs; 9 plants)**	**2014 (1440 hrs; 6 plants)**	**Test Statistics**
BIRDS:									
Fringillidae									
Streaky-headed Seed-eater	3.8	2.7	$U_{17} = 49.0$	7.9	0	$U_{17} = 73.5$	4.1	3.2	$U_{13} = 28.0$
Crithagra gularis	(1.79 ± 0.96)	(1.67 ± 1.67)	$P = 0.60$	(12.59 ± 2.95)	0	$P = 0.005$	(4.29 ± 1.29)	(3.96 ± 1.53)	$P = 0.95$
Muscicapidae									
Cape Rock-Thrush	16	42.7	$U_{17} = 29.0$	80.5	80	$U_{17} = 39.0$	68.2	52.4	$U_{13} = 20.0$
Monticola rupestris	(9.30 ± 3.32)	(24.12 ± 9.12)	$P = 0.23$	(134.02 ± 22.40)	(59.34 ± 15.77)	$P = 0.38$	(60.15 ± 7.58)	(65.53 ± 12.68)	$P = 0.66$
Pycnonotidae									
Dark-capped Bulbul	4.6	20	$U_{17} = 30.0$	7.1	15.7	$U_{17} = 43.0$	13.7	20	$U_{13} = 17.0$
Pycnonotus tricolor	(2.43 ± 1.06)	(11.25 ± 5.13)	$P = 0.21$	(12.40 ± 4.20)	(12.19 ± 5.30)	$P = 0.97$	(12.91 ± 5.12)	(21.81 ± 7.27)	$P = 0.26$
MAMMALS:									
Macroscelididae									
Sengi	3.8	5.3	$U_{17} = 38.0$	0	0	-	0	3.2	$U_{13} = 18.0$
Elephantulus Sp.	(2.28 ± 2.28)	(3.66 ± 2.44)	$P = 0.48$	0	0	-	0	(5.68 ± 4.36)	$P = 0.09$
Muridae									
Namaqua Rock Mouse	66.4	26.7	$U_{17} = 58.0$	0	0	-	9.6	20.6	$U_{13} = 13.5$
Micaelamys namaquensis	(40.17 ± 18.75)	(21.20 ± 13.22)	$P = 0.25$	0	0	-	(7.83 ± 2.45)	(30.73 ± 1.95)	$P = 0.13$
Total Number of Visitors to Treatment	**92,** 39	**25,** 50		**266,** 0	**70,** 0		**292,** 0	**189**, 0	
Mean Overall Treatment Visitation Rate	59.17 ± 20.33	63.56 ± 21.11		167.18 ± 23.75	75.18 ± 19.19		89.33 ± 13.27	131.58 ± 22.70	

Table S3. Additional mammal species recorded by camera traps, on one or more occasions, during two consecutive flowering seasons (2013 and 2014) of *Aloe peglerae*.

Presence of the species in each flowering season is indicated with an "X". These species were recorded in the vicinity of the aloes, but did not interact with the aloes in any way, with the exception of one Smith's Red Rock Rabbit (*Pronolagus rupestris*), which sniffed one *A. peglerae* raceme in 2014, but no other contact was recorded.

	2013	2014
Bovidae		
Mountain Reedbuck *Redunca fulvorufula*	-	X
Canidae		
Black-backed Jackal *Canis mesomelas*[A]	X	-
Felidae		
Caracal *Caracal caracal*[A]	-	X
Herpestidae		
Slender Mongoose *Galerella sanguinea* [A,B]	X	-
Hystricidae		
Cape Porcupine *Hystrix africaeaustralis*	X	-
Leporidae		
Smith's Red Rock Rabbit *Pronolagus rupestris*	X	X
Viverridae		
Genet *Genetta* Sp.[A,C]	X	X

[A] Species that may be predators of small mammals such as mice and shrews/sengis.
[B] Cape grey Mongoose (*Galerella pulverulenta*) has been recorded feeding on *Protea canaculilata* and *Protea recondita* nectar (Steenhuisen *et al*. 2015).
[C] Large-spotted Genet (*Genetta tigrina*) has been recorded feeding on *Protea pendula* and *Protea scabra* nectar (Steenhuisen *et al*. 2015; Zoeller *et al*. 2016).

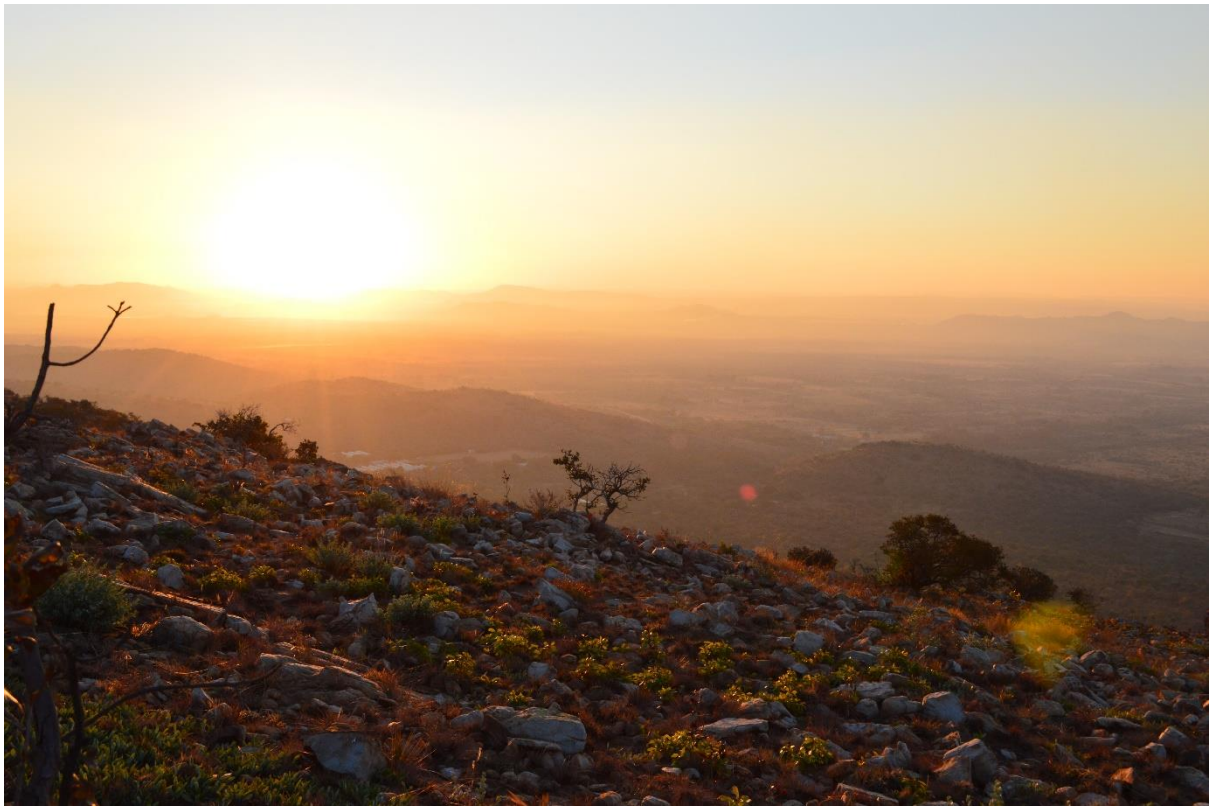
Table S4. Seed germination and viability (tetrazolium trial) data for *Aloe peglerae* plants in each selective exclusion experiment, for two consecutive years of different flowering proportions, where 2013 represents a "good" and 2014 a "poor" flowering year.

$NOCT_{TRT}$ = Nocturnal Visitors treatment, $DIUR_{TRT}$ = Diurnal Visitors treatment and ALL_{TRT} = All Visitors treatment. No fruit (and therefore seed) was produced by plants in the No Visitors treatment. Germination trials took place during 19 October-30 November 2013, and 06 October-17 November 2014. All values are presented as mean ± standard error, where possible

	$NOCT_{TRT}$		$DIUR_{TRT}$		ALL_{TRT}	
	2013	**2014**	**2013**	**2014**	**2013**	**2014**
No. of plants in trial	8	1	11	0	10	2
No. of seeds dish^{-1} in germination trial	46.8 ± 2.3	50	48.6 ± 0.8	0	50.0 ± 0.3	47.5 ± 2.5
No. of days until first seed germinated	1	5	3	0	3	5
No. of days until last seed germinated	18	14	18	0	23	17
Mean Germination Time (MGT; days)	7.3 ± 0.5	8.7	6.4 ± 0.2	0	6.5 ± 0.3	8.5
Cumulative germinated seeds (%)	89.5 ± 4.4	98	92.4 ± 2.1	0	97.2 ± 1.4	94
No. of dead seeds	1.9 ± 1.7	1	2.7 ± 1.1	0	0.3 ± 0.2	1
No. of seeds in tetrazolium trials	2.5 ± 1.0	0	0.9 ± 0.3	0	1.1 ± 0.5	2
No. of viable seeds after tetrazolium	0.3 ± 0.2	0	0.2 ± 0.1	0	0.3 ± 0.2	1
Viable seeds after germination & tetrazolium (%)	90.0 ± 4.1	98	93.0 ± 2.1	0	98.0 ± 1.3	96

CHAPTER FOUR

Rock around the clock: Diurnal-nocturnal visitation patterns to a flowering montane endemic succulent



Sunset at The Peglerae Conservancy, Gauteng. (Maxine Semple)

4.1 Abstract

Nectar is an important attractant and reward for floral visitors which many plants produce in synchrony with peak activity periods of specific pollinator guilds. *Aloe peglerae* is a winter-flowering aloe that is pollinated primarily by diurnal generalist birds, implying that most nectar production should be taking place during the day. However, small mammals make additional contributions to pollination at night, likely visiting aloes to feed on any remaining or freshly produced nectar. Nectar production over a 24-hour period was measured in screened and unscreened plants (n = 10 plants per treatment), and the associated partitioning of visits by diurnal birds and nocturnal small mammals assessed. Visits to aloes by these two guilds was monitored over two different flowering seasons, using camera traps (n = nine plants in 2013; n = six plants in 2014). Visitation patterns by birds and small mammals were separated temporally, with short periods of overlap at dusk and dawn. Nectar is produced constantly over a 24-hour period but at different rates, with peaks in different guild visitation tracking periods when nectar production, and/or availability (standing crop), is high. Nectar volume, and hence availability, is significantly higher early in the morning, just prior to a significant increase in bird visitation. Nectar concentration did not vary over a 24-hour period. *Aloe peglerae* has increased nectar production during the day, when its primary pollinators are most active, but the continual, albeit reduced, production at night attracts small mammals. The vertebrate pollinator guilds of mobile diurnally active birds, and less-mobile, nocturnally active, small mammals, both rely on this nectar resource during dry and cold South African winters and, in turn, are important pollinators for this Critically Endangered *Aloe* species.

4.2 Key words

Aloe peglerae; Bird Pollination; Nectar Availability; Nectar Production; Small mammal Pollination; Temporal Partitioning.

4.3 Introduction

Floral nectar is produced as an attractant and reward for pollinators (Waser 1983). Many selection pressures, both extrinsic and intrinsic, act on plant nectar production and properties (Aizen and Basilio 1998; McDade and Weeks 2004). Selection by pollinator classes/guilds, and competition to attract these pollinators, is suggested to influence nectar production rates (e.g. Waser 1983; Varassin *et al*. 2001; Castellanos *et al*. 2002; Kaczorowski *et al.* 2005). To efficiently attract pollinators with minimal wastage of resources, plants often orchestrate flower opening, anthesis, and nectar production to coincide with the active foraging period of the primary pollinator guild (e.g. Percival 1974; Cruden *et al*. 1983; Crome and Irvine 1986; Varassin *et al*. 2001; Kaczorowski *et al*. 2005). Differences in nectar secretion rates over a 24-hour period may serve to organise the roles and visitation of different pollinator guilds (Cruden *et al*. 1983), facilitating efficient and successful pollination events.

Potential pollinators may alter their foraging behaviour based on the nectar variation within a plant population, by increasing frequency and timing of visits, length of probe time, and the number of flowers probed (Rathcke 1992). Furthermore, some pollinating guilds, and even species, are competitively superior in different times and spaces (e.g. Perrin and Kotler 2005; Abu Baker and Brown 2014). This may result in the temporal and spatial partitioning of different, coexisting guilds and/or species visiting a plant in search of resources, provided that resources and/or rewards are available during the time of foraging (e.g. Crome and Irvine 1986; Abu Baker and Brown 2014; Zoeller *et al*. 2016).

Most species within the genus *Aloe* flower in the winter (~55%; Van Wyk and Smith 2014), and produce generous amounts of nectar (e.g. Hoffman 1988; Nicolson and Nepi 2005; Johnson *et al.* 2006; Symes *et al*. 2008; Botes *et al*. 2009a, b; Forbes *et al*. 2009). For example, *Aloe marlothii* is a bird pollinated aloe, visited mostly by generalist nectar feeders

(Oatley 1964; Symes *et al*. 2008, 2009). When in flower it produces copious amounts of dilute nectar, which attracts a large variety of opportunistic nectarivores (Symes *et al*. 2008). Although these bird visitors are active during the day, the aloe produces nectar throughout a 24-hour period (Symes and Nicolson 2008). The constantly available nectar is thought to be an important food and water resource for small mammals such as mice and mongooses, as was found in *Protea* (Symes and Nicolson 2008; Schmid *et al*. 2015; Steenhuisen *et al*. 2015), although the consistency and timing of their presence at the aloes, and their potential role in pollination, has yet to be quantified. Chacma Baboon (*Papio hamadryas ursinus*) are known to climb onto *A. marlothii* rosettes and damage inflorescences during the day, sucking the nectar from the open flowers (Symes and Nicolson 2008), while no nocturnal small mammal visits have been recorded.

Another winter-flowering aloe, *Aloe peglerae*, which co-occurs with *A. marlothii* along the edge of its distribution (Van Wyk and Smith 2014), receives both diurnal and nocturnal visitors (Payne *et al*. 2016; Chapter 2). Although the Cape Rock-Thrush (*Monticola rupestris*), is the primary pollinator of this *Aloe* species, assisted by other generalist nectarivorous birds (Arena *et al*. 2013; Chapter 2), small mammals, mainly the Namaqua Rock Mouse (*Micaelamys namaquensis*), have been shown to play an important role in the pollination of this *Aloe* species (Chapter 2). The small mammals are consistently visiting the aloes at night, suggesting that there is some benefit or reward available. Generalist-pollinated aloes produce large amounts of nectar when their primary pollinators are active (Hoffman 1988; Nicolson and Nepi 2005; Johnson *et al*. 2006), and possibly throughout the day and night as with *A. marlothii* (Symes and Nicolson 2008). Therefore, it is likely that remaining nectar, or fresh nectar secreted at night, is an important attractant and resource for the nocturnal small mammals. This study aimed to assess how *Aloe peglerae* nectar production patterns and the activity of the primary pollinators (diurnal birds) are coordinated, as well as

to assess the availability of nectar at night as an attractant for nocturnal small mammals visiting the aloes. *Aloe peglerae* nectar production and properties were measured over a 24-hour period. The temporal partitioning of visitation to, and nectar resources of, *A. peglerae* by diurnal birds and nocturnal small mammals, was also investigated.

4.4 Methods and Materials

Study site and species

Aloe peglerae is a Critically Endangered aloe that is endemic to the Magaliesberg Mountain Range (MMR; Pfab *et al*. 2016). The Peglerae Conservancy, ~25km east of Pretoria in the MMR (25˚41' S, 27˚59' E), South Africa, was established to facilitate the monitoring of the species' conservation, as well as to protect it from habitat fragmentation and the illegal harvest of seed and whole plants (Wesson 2007). The site is classified as Gold Reef Mountain Bushveld (Rutherford *et al*. 2006), with shallow soils dominated by grasses, shrubs and scattered trees (Rutherford *et al*. 2006; Arena *et al*. 2013, 2015). Rainfall is seasonal, falling mostly in summer, but varies between years (530 mm in 2013, and 289 mm in 2014; South African Weather Service 2016).

Aloe peglerae is a stemless aloe, and adult plants (> 20 cm in diameter) flower in winter (June – July), producing a single, densely packed, raceme with tubular, scarlet flowers that turn yellowish-green with deep purple stamens and exserted anthers (± 25 mm past the mouth of the flower) as they mature and open (Scholes 1988; Van Wyk and Smith 2014; Figure 1a in Chapter 2). Flowers open over a period of approximately 15 days, starting at the base and opening sequentially upwards (Chapter 2). Flowering is annual at a population level, but highly variable in individual plants (Scholes 1988; Arena *et al.* 2013; Payne *et al*. 2019; Chapter 3), with the variable proportions of flowering plants in the population each year

significantly affecting visitation by primary pollinators, and subsequently, reproductive success (Chapter 3).

Seasonal visitors and visitation rates

Visits by diurnal and nocturnal animals to the aloes were recorded during the flowering seasons of 2013 and 2014, with the use of camera traps. In 2013, 14% of all *A. peglerae* plants flowered, while 2014 was a much poorer flowering year, when only 7% flowered (Chapter 3). Indeed, these are both poor flowering years, as an average of 29% of plants have flowered per year over five years, with a maximum of 63% of plants sampled in 2010, declining in each subsequent year (Craig Symes, *unpubl. data*; Gina Arena, *unpubl. data*; Chapter 3). In previous studies of the same population, a total of 609 plants were numbered with metal tags (Arena *et al*. 2013; Chapter 2), and the GPS location of each plant recorded (Garmin, GPSMAP 60Cx, USA). In both years, flowering plants (2013: n = nine plants; 2014: n = six plants) were randomly selected prior to flower opening and were left exposed for the entire duration of the flowering season, to allow visitation by both diurnal and nocturnal visitors. The same plants could not be sampled in both years due to individual plant flowering variability. Camera traps (Bushnell, models 119456 and 119477, China) were positioned on tripods, placed approximately one metre away from plants, and set on high sensitivity to take two photographs when activated by movement, with three second intervals between activations (Chapter 2). Cameras were left on for the entire duration of the sampling period, with batteries and memory cards (SanDisk 2GB and 4GB) changed twice daily, within the hour after dawn (06:45 – 07:45), and within the hour before dusk (16:30 – 17:30). Sampling lasted for four to five weeks in both years (25 June – 27 July 2013; 02 July – 30 July 2014). A fire of unknown origin started on the afternoon of 21 July 2014, and cameras were removed from aloes that were in close proximity to the fire, and returned the following

morning. Apart from the slight burning of the outer leaves, the aloes suffered little damage (Arena *et al*. 2015; Chapter 2).

An animal was considered a visitor if it touched the plant in any way (e.g. perched on the inflorescence and/or leaves, probed flowers, etc.), as this action was sufficient to implicate the animal as a potential visitor. Visitation rates at each hour of a 24 hour period, for each visitor guild (i.e. birds and small mammals), and for each visitor species, were calculated over the entire duration of both sampling periods. Rates were calculated as the mean number of visits per hour per 1000 plants (visits/1000 plants/hour). The diurnal period was defined as 07:00 – 16:59 (sunrise: ~ 06:55) and the nocturnal period as 17:00 – 06:59 (sunset: ~ 17:35).

Nectar production

Nectar standing crop was measured over a 24-hour period during 21 – 22 July 2016. One day prior to sampling (20 July 2016), 20 flowering plants were randomly selected, and 10 plants were screened with exclusion cages, while the remaining 10 plants were left exposed (unscreened). Exclusion cages were constructed of a bamboo tripod frame (height = ~0.9 m, basal area = ~0.3 m^2), covered in pale brown nylon mesh (1mm^2 holes), which excludes all visitor types, including insects (Chapter 2). The cages are large enough not to touch the flowers, and do not alter microhabitat within the cage (Chapter 2). Temperature data loggers (Thermocron iButtons, Australia, accurate to 0.5°C), were placed in the rosette of each aloe.

Nectar was extracted with disposable haematocrit tubes (75 µL) from one flower removed from each plant, every two hours (n = 20 flowers per sampling session; N = 240 flowers in total; flowers were destructively sampled), over 24-hours, starting at 07:00 on 21 July 2017. Nectar volume (µL) was recorded and nectar concentration (% w/w) was

measured using a handheld refractometer (Bellingham & Stanley Eclipse Refractometer, Model 45-81, Tunbridge Wells, UK). Sampled flowers were completely open, with anthers newly dehisced and stigmas were receptive, but not yet fully exserted past the stamens (stage 2 of development; Figure 2 in Symes and Nicolson 2008). Screened plants remained covered for the duration of the 24-hour sampling period. Sampling was undertaken on a warm, clear day (average maximum temperature = 35.8°C at 14:00), and at night, skies were clear with a full moon (average minimum temperature = 9.2°C at 06:00). Exclusion cages and iButtons were removed on 22 July 2016, at the end of the final sampling session (05:00).

Data analysis

Visitation rates for each guild were modelled as count data, with time and guild as predictor variables and an offset for hours of sampling effort, using a Generalised Linear Model (GLM) with Poisson distribution and a log-link function. Years were pooled together to test for significance of time of day on visitation rate, rather than between years. Nectar volumes were modelled using a simple linear regression, after data had been log-transformed, with time and treatment as predictor variables. Nectar concentration was modelled using a GLM, with time and treatment as predictor variables, a binomial distribution and a logit-link function. Spearman rank correlations were performed to assess whether nectar volume and concentration were correlated to temperature for each treatment. All statistical analyses were performed in R (Version 3.5.0; R Core Team 2018), and all graphics were produced using the R packages "ggplot2", "extrafontdb" and "extrafont" (Chang 2012, 2014; Wickham 2016). Data are presented as mean ± standard error.

4.5 Results

Seasonal visitors and visitation rates

A total of six bird and two small mammal species were recorded visiting the sampled aloes over both years (Table 4.1). An additional six bird species were recorded on other *Aloe peglerae* plants (Table 4.2 in Appendix 4.1). Birds contributed 89.2% of visits in 2013 (*n* = 257 of 288 total visits), while only contributing 72.7% in 2014 (*n* = 120 of 165 total visits). Small mammal contributions to visitation increased from 9.7% in 2013 to 26.1% in 2014.

In 2013, six bird species visited the aloes, while in 2014, four bird species were recorded (Table 4.1). The Cape Rock-Thrush (*Monticola rupestris*; Figure 4.1 insert B), the primary pollinator of *A. peglerae* (Arena *et al*. 2013), made up 76.3% and 70.0% of all bird visits in 2013 and 2014 respectively. The Dark-capped Bulbul (*Pycnonotus tricolor*) made up a bulk of the remaining visits (15.2% in 2013 and 23.3% in 2014), with the other species combined contributing the remaining 8.6% (*n* = four species in 2013) and 6.7% (*n* = two species in 2014). Twenty-eight small mammal visits were recorded in 2013, while 43 visits were recorded in 2014. The Namaqua Rock Mouse (*Micaelamys namaquensis*; Figure 4.1 insert A), contributed 100% of visits in 2013; while in 2014, 86.1% were Namaqua Rock Mouse visits, while the remaining 14.0% were visits by sengi (*n* = six visits; *Elephantulus* sp., likely *E. myurus* or *E. brachyrhynchus*; Prof. Michael Perrin, Dr Hanneline Smit-Robinson, Dr Galen Rathbun, Prof. Neville Pillay, *pers. comm.*).

Visitation rates differed significantly between guilds over a 24-hour period ($\beta = 2.98 \pm 0.26$, $P < 0.001$), revealing temporal partitioning between visiting guilds. Rates decreased significantly with time ($\beta = -1.53 \pm 0.12$, $P < 0.001$), and were significantly different between guilds, with small mammals visiting less frequently than birds ($\beta = -1.61 \pm 0.20$, $P < 0.001$). Birds dominated visitation during daylight hours (from 06:00 to 17:00), while small

mammals became active at night (from 17:00 to 06:00; Figure 4.1). There were overlaps in visitation by the two guilds in both years, between 05:00 and 07:00 (dawn) and again between 17:00 and 18:00 (dusk), during transitions between day and night and *vice versa*.

Table 4.1. Percentage of total visits to *Aloe peglerae* by each species during two flowering seasons (July – August, 2013 and 2014). Visitation rates for each species for the duration of each of the flowering seasons are in parentheses (number of visits/1000 plants/hour; mean ± SE). An animal was recorded as a visitor if it touched the plant (leaves and/or raceme) in any way. Contributions and visitation rates of dominant visitors for each guild are in bold. Bird taxonomy follows Hockey *et al.* (2005) and Chittenden (2014), mammal taxonomy follows Skinner and Chimimba (2005). Both columns been similarly presented in Payne *et al*. (2016, 2019), and have been adjusted accordingly.

Guild and Species	**2013 (9 plants; 3251.3 hrs)**	**2014 (6 plants; 1323.4 hrs)**
BIRDS		
Dicruridae		
Fork-tailed Drongo	0.7	0
Dicrurus adsimilis	(0.6 ± 0.6)	0
Fringillidae		
Streaky-headed Seed-eater	4.2	3.6
Crithagra gularis	(4.4 ± 1.3)	(3.9 ± 1.4)
Lybiidae		
Crested Barbet	1.7	0
Trachyphonus vaillantii	(1.8 ± 1.2)	0
Muscicapidae		
Cape Rock-Thrush	**68.1**	**50.9**
Monticola rupestris	**(60.6 ± 7.5)**	**(62.3 ± 10.1)**
Familiar Chat	1.0	1.2
Cercomela familiaris	(0.7 ± 0.7)	(1.3 ± 0.8)
Pycnonotidae		
Dark-capped Bulbul	13.5	17.0
Pycnonotus tricolor	(13.0 ± 5.1)	(20.7 ± 7.4)
MAMMALS		
Cercopithecidae		
Chacma Baboon	1.0	1.2
Papio hamadryas ursinus	(1.2 ± 0.7)	(2.5 ± 1.6)
Macroscelididae		
Sengi	0	3.6
Elephantulus sp.	0	(6.2 ± 4.6)
Muridae		
Namaqua Rock Mouse	**9.7**	**22.4**
Micaelamys namaquensis	**(8.0 ± 2.5)**	**(32.0 ± 13.3)**
Total Number of Species	8	7
Total Number of Visitors	288	165
Overall Visitation Rate	90.2 ± 13.2	128.8 ± 25.2

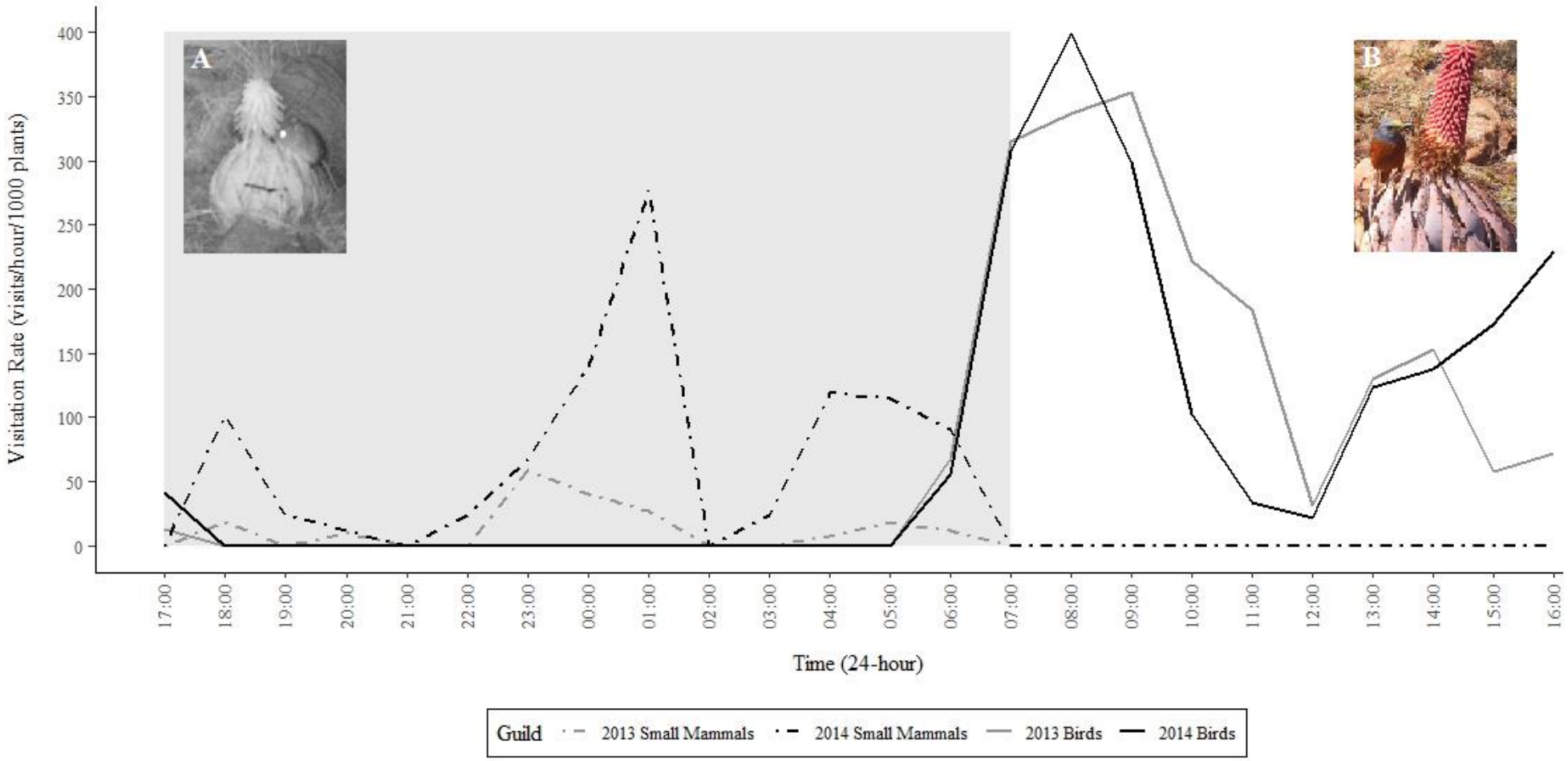


Figure 4.1. Mean hourly visitation rates (visits/1000 plants/hour) by bird (solid line) and small mammal (dashed line) guilds for the duration of two flowering seasons to *Aloe peglerae* (July – August 2013: *n* = 9 plants, 3251 camera hours); July – August 2014: *n* = 6 plants, 1323 camera hours). The nocturnal period was defined as 17:00 – 06:59 (represented by the shaded region; sunset: ~17:35), and the diurnal period as 07:00 – 16:59 (sunrise: ~06:55). Insert A: The Namaqua Rock Mouse (*Micaelamys namaquensis*) is the most common nocturnal visitor. Insert B: The Cape Rock-Thrush (*Monticola rupestris*) is the most common diurnal visitor, and the primary pollinator of *Aloe peglerae*. Inserts A and B: Camera trap photographs by Stephanie Payne.

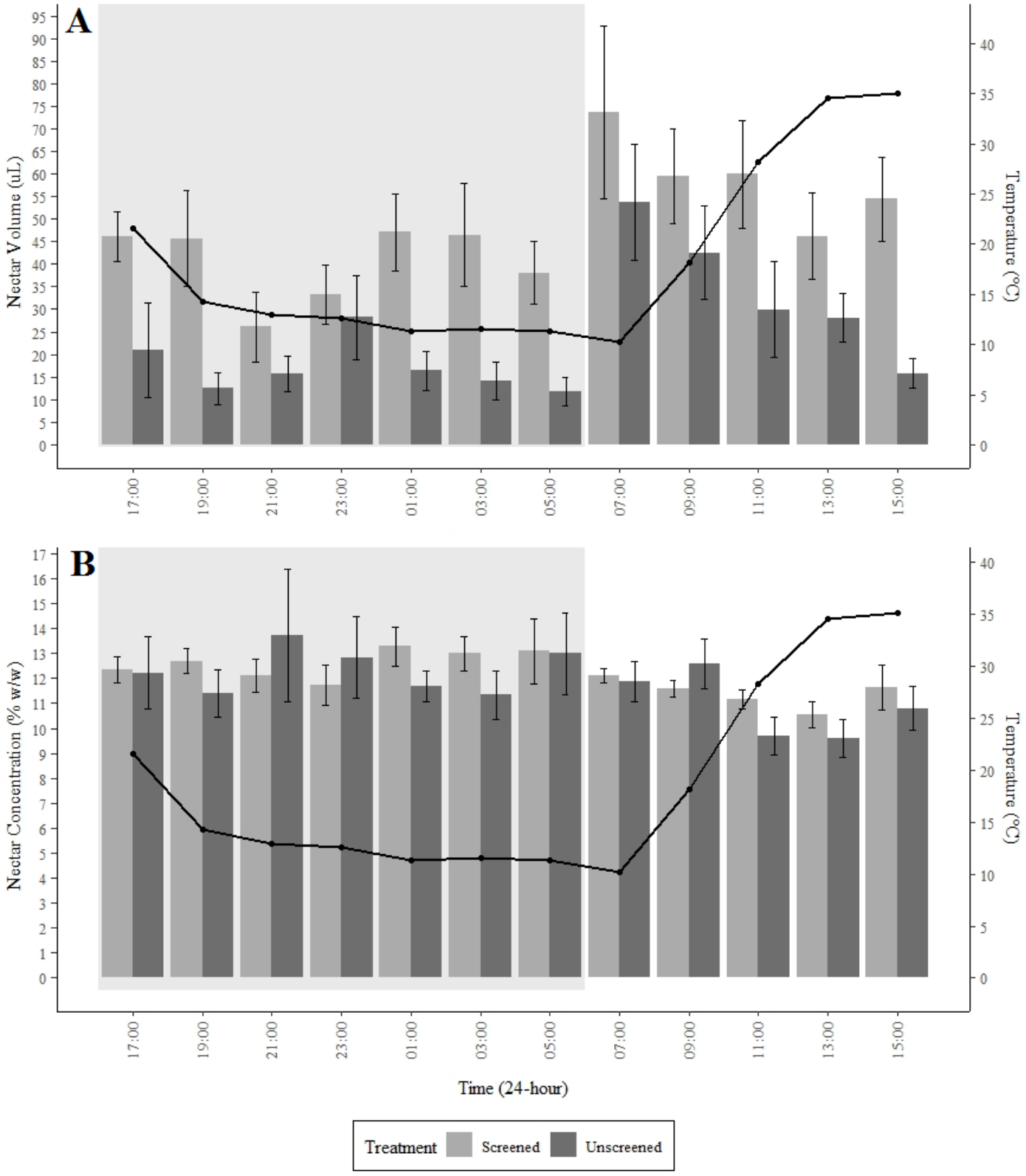


Figure 4.2. A) *Aloe peglerae* nectar volumes (µL) and B) nectar concentrations (% w/w; mean ± S.E.) of screened and unscreened plants (n = 10 plants per treatment), measured at two-hour intervals throughout a 24-hour period. The nocturnal period (17:00 – 06:59) is represented by the shaded region (Sunrise ~ 06:55; Sunset ~ 17:35). Mean temperature (°C) at each two-hour interval is represented by the solid black line. Standard errors of mean hourly temperature were <1.0°C and could not be plotted on the graphs.

Multiple peaks in visitation were apparent for both guilds. Bird activity peaked early in the morning, just after sunrise (08:00 – 10:00), and again later in the afternoon, prior to sunset (14:00 – 16:00). Small mammal activity peaked soon after sunset (18:00) and then again at 01:00 and 04:00, just before the transition between night and day begins, and birds start visiting the aloes. There appeared to be no temporal partitioning between individual diurnal species, or between individual nocturnal species. Peaks in visitation by both guilds were at the same time in both years, but small mammals rates were higher in 2014 (Chapter 3), while bird visitation rates appeared to decline earlier in 2014.

Nectar production

Aloe peglerae produced nectar constantly over a 24-hour period, with production higher during the day than at night (Figure 4.2A). Nectar volumes for both treatments were equally affected by time of day (β = -0.01 ± 0.08, P = 0.93), with volumes decreasing significantly into the night (β = -0.18 ± 0.05, $P < 0.001$). Unscreened plants had significantly less nectar than screened plants (β = -0.39 ± 0.05, $P < 0.001$). Screened plant nectar volumes peaked at 07:00 (mean volume = 73.6 ± 19.2 μL), and were lowest at 21:00 (mean volume = 26.1 ± 7.7 μL). Unscreened nectar volumes were lower than screened volumes, with maximum volumes also recorded at 07:00, (mean volume = 53.8 ± 12.9 μL), while minimum volumes were recorded at 05:00 the following day (mean volume = 11.8 ± 3.1 μL). Mean maximum screened volume was 2.8 times larger than mean minimum screened volume, while mean maximum unscreened volume was 4.6 times greater than the mean unscreened minimum. Due to nectar consumption by visitors, the available nectar is lower in unscreened plants – maximum volumes = 1.4 times lower, minimum volumes = 2.2 times lower – and is much more variable throughout the course of the day (unscreened Coefficient of Variation (CV) = 108%, while screened CV = 70%).

Nectar concentrations did not differ among times (β = 0.03 ± 0.04, *P* = 0.514), nor between treatments (β = -0.06 ± 0.04, *P* = 0.145; Figure 4.2B), as each treatment was equally affected by time (β = 0.04 ± 0.06, *P* = 0.485). Maximum concentrations for screened plants were recorded at 01:00, coinciding with lowest nectar volumes (mean concentration = 13.3 ± 0.8 % w/w), and minimum concentrations recorded at 09:00, when volumes were high (mean concentration = 11.6 ± 0.4 % w/w). Nectar concentration for unscreened plants were at a maximum at 21:00 (mean concentration = 13.3 ± 0.8 % w/w) and at a minimum at 13:00 (mean concentration = 9.6 ± 0.7 % w/w).

Temperature and nectar volume were not correlated for either treatment (Screened: R_S = 0.045, *P* = 0.63; Unscreened: R_S = 0.005, *P* = 0.96). However, nectar concentration was weakly, negatively correlated with temperature (Screened: R_S = -0.35, *P* = 0.0001; Unscreened: R_S = -0242, *P* = 0.008). At lower temperatures, nectar may be more concentrated (Figure 4.2B).

4.6 Discussion

Visitation Rates and Temporal Partitioning

Both bird and small mammals are important for the pollination of *Aloe peglerae* (Chapter 2). Guild visitation to *A. peglerae* is separated temporally, with multiple bird species, visiting during daylight hours, and small mammals at night, with only short overlaps at dusk and dawn. Each guild is further dominated by one individual species, the diurnal Cape Rock-Thrush and the nocturnal Namaqua Rock Mouse. These two species are usually considered generalist feeders, but from the standpoint of *A. peglerae*, they are its "specialised pollinators", each representing more than half the visitors for their relevant time periods.

There appears to be no spatial separation as birds and small mammals forage at the same individual plants, likely due to the constant production of nectar over the 24-hour

period. There is also no partitioning, either spatial or temporal, between individual species within each guild. The Namaqua Rock Mouse is a pollinator of many *Protea* species (e.g. Fleming and Nicolson 2002; Biccard and Midgley 2009; Zoeller *et al*. 2016), and is known to visit the same inflorescences as other small mammal species (Zoeller *et al*. 2016). The Cape Rock-Thrush has been recorded visiting many *Aloe* species (and individual plants) that are frequently visited by other bird species as well (e.g. Oatley and Skead 1972; Johnson *et al*. 2006; Botes *et al*. 2008; Symes *et al*. 2008; Forbes *et al*. 2009; Symes 2010).

Nectar production is higher during the day than the night, and visitation patterns follow a similar trend to that of nectar production, with initiation and/or increased production (and standing crop) of nectar reflecting the activity of pollinators (Percival 1974; Cruden *et al*. 1983). Peak nectar production in *A. peglerae* occurs just prior to the peak in bird visitation, reinforcing the important role of birds in the pollination of this species (Arena *et al*. 2013; Chapter 2). The birds visiting these aloes are primarily insectivores and are likely supplementing their diets with the abundant nectar, until the insects become active later in the morning, as the day warms up (Symes *et al*. 2008).

Nectar Production and Pollinators

New flowers open consistently throughout the day, and at a slower rate at night (Stephanie Payne, *pers. obs.*), ensuring that there is always a fresh supply of nectar available throughout the course of 24 hours. Overnight nectar production, and active nectar secretion in the morning, is common in plants adapted for bird pollination (Cruden *et al*. 1983; Pleasants 1983), as nectar resources are replenished for early morning bird visitors. Although small mammals may take advantage of the overnight nectar production and deplete nectar volumes by midnight, active secretion begins again before dawn, to accommodate the early morning bird visitors. These dawn visitors reduce the nectar standing crop by midday, but by late

afternoon, standing crop (available nectar) has been replenished to accommodate the second influx of birds.

Aloes commonly produce nectar throughout the day (e.g. *A. castanea*, Nicolson and Nepi 2005; *A. greatheadii* var. *davyana*, Human and Nicolson 2008), and some species are known to produce nectar through the night as well (*A. marlothii*, Symes and Nicolson 2008). The cost of this constant nectar production is balanced by the low concentrations of these nectars (Nicolson and Nepi 2005), and visitors are forced to make multiple and/or longer visits to meet their energy requirements, thereby positively influencing pollen transfer and plant reproductive success (Rathcke 1992). Although peaks in visitation are at similar times each year, bird visitation rates begin to decline earlier in 2014, when fewer plants are flowering and fewer nectar resources are available, than in 2013. The birds are foraging at the nectar resource early in the morning, but due to their ability to move over large distances easily, are moving to areas with more abundant and energy-efficient resources (Chapter 3). Small mammals are less mobile and forced to stay in a smaller area, and are making use of this energy-rich resource in the resource-limited winter months (Chapter 3). Their increased reliance on these limited resources is evident in the increase in visitation rates in 2014 compared to 2013.

Nectar Replenishment

Nectar concentrations reflect preferences of generalist nectar feeders at all times of the day (Johnson and Nicolson 2008). Nectar concentration remained the same throughout the 24-hour period, suggesting that there is no reabsorption of unconsumed nectar taking place (Nicolson and Nepi 2005). Volume decreases over time are likely linked to consumption by visitors, or slower production, rather than evaporation which is often counteracted by the tubular shape of the flowers (Pleasants 1983; McDade and Weeks 2004).

Nectar replenishment after removal has been documented in *Aloe castanea*, and flowers where nectar is repeatedly removed produce cumulatively more nectar, albeit at a lower concentration, than those which have nectar removed less frequently (Nicolson and Nepi 2005). Additionally, nectar volumes and concentrations are most affected by floral age (Aizen and Basilio 1998; McDade and Weeks 2004; Nicolson and Nepi 2005). In species with hermaphroditic flowers, nectar production may be driven by selection pressures for the individual male and female functions to be successful, particularly when pollinators are scarce (Aizen and Basilio 1998). Nectar volumes are often higher, with faster secretion/replenishment rates occurring during the male (staminate) phase, with little to no nectar being produced in the female (pistillate) phase (Aizen and Basilio 1998; Castellanos *et al*. 2002; McDade and Weeks 2004). In *Aloe*, nectar volumes vary throughout the different stages of floral development, with the staminate (male) phase producing higher volumes of nectar than other stages, and concentrations increasing in the pistillate (female) phases (e.g. Nicolson and Nepi 2005; Human and Nicolson 2008; Symes and Nicolson 2008).

The changes in nectar volume and concentration throughout the floral development of *Aloe peglerae* were not measured in this study, but the trend is likely similar to that of other *Aloe* species. It is also unknown whether *A. peglerae* replenishes consumed nectar. Due to the tubular shape of *A. peglerae* flowers, complete sampling of individual flowers is destructive, and repeat samples to measure replenishment are difficult to perform. Further study into the number of probes required for successful pollen transfer may indicate whether replenishment is necessary to attract additional visitors once a flower has been probed for the first time (e.g. hummingbird-pollinated flowers require multiple probes; Mitchell and Waser 1992). Should few visits be required for successful pollen transfer, nectar replenishment may not be necessary, and remaining nectar could serve as an attractant for visitors to neighbouring

pistillate flowers with less nectar (Castellanos *et al*. 2002; McDade and Weeks 2004; Nicolson and Nepi 2005).

Influences on Nectar Production, Standing Crop and Replenishment

Aloe castanea has more open and campanulate flowers compared to other *Aloe* species, exposing the nectar to the elements. As temperature decreases and relative humidity increases, nectar volumes increase and concentrations decrease (Nicolson and Nepi 2005), Similar changes, although not as marked, are observed in *A. greatheadii* var. *davyana* and *A. marlothii*, which have more tubular flowers, similar to those of *A. peglerae* (Human and Nicolson 2008; Symes and Nicolson 2008). Although not compared between different weather conditions, it appears that *A. peglerae* nectar concentrations may be affected by lower temperatures in a similar way to the latter species, while standing crop is unaffected. Nectar production is possibly a result of a circadian rhythm of the species; this remains to be tested.

Although pollinator selection may play an important role in the nectar production rate of plants (Aizen and Basilio 1998), including *A. peglerae*, nectar production may be constrained by phylogeny and production costs (McDade and Weeks 2004), along with other selection pressures that were outside the scope of this study. Rathcke (1992) suggests that nectar production rates may be plastic, owing to the variability and resource allocation within a plant itself, and the influence of environmental factors. Specific nectar properties, such as sugar and amino acid concentrations may be heritable, and/or constrained by physiological processes within the plant (Rathcke 1992; McDade and Weeks 2004). Environmental factors, such as air temperature, sunlight, relative humidity, soil moisture and season (Pleasants 1983; Waser 1983; Rathcke 1992; Aizen and Basilio 1998) may indirectly affect nectar production through plant growth and resource allocation within the plant (Rathcke 1992). Other selection

factors that may require consideration include flower density, plant morphology, plant habitat, breeding system and nectar thieving (Cruden *et al*. 1983; Pleasants 1983; Kaczorowski *et al*. 2005).

4.7 Conclusion

Aloe peglerae produces nectar constantly over 24-hours, with higher production during the day than at night, and is consequently visited throughout the day and night by two different pollinator guilds – diurnal birds and nocturnal small mammals. Nectar production and/or standing crop peaks early in the morning, just prior to a peak in visitation by birds, (the primary pollinators of the aloe) after their nocturnal "fast", and remains abundant during daylight hours, while birds are most active. However, small mammals are taking advantage of the remaining and freshly secreted nectar at night and, consequently, providing an additional pollination service for the aloes.

This may represent a dual-guild of "specialist" pollinating species for *A. peglerae* – the diurnal bird guild, dominated by the Cape Rock-Thrush, and the nocturnal small mammals, particularly the Namaqua Rock Mouse. Hence there is a dual-guild pollination system, with temporal division of visitation and resources between these two "specialist" pollinating species, into time periods where each guild is competitively superior. We are unaware of other studies that have found this same set of circumstances, so presently this appears to be unique.

Nectar is an important resource for both birds and mammals (e.g. Fleming and Nicolson 2002; Kaczorowski *et al*. 2005; Symes *et al*. 2008, 2011; Schmid *et al*. 2015; Steenhuisen *et al*. 2015). The constant production and availability of this resource, while supplementing diets of visitors in the resource-limited winter, also ensures pollination events for the *Aloe* species, and safeguards the persistence of this Critically Endangered species.

Other nectar properties of *A. peglerae* are poorly studied (e.g. sugar and amino acid constituents, phenolics, etc.), and further studies may aid in the understanding of how this unique species is able to successfully entice a wide variety of visitors throughout a 24-hour period.

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4.10 Appendix 4.1

Table 4.2. Additional species recorded perching and/or feeding on other *Aloe peglerae* plants that were not sampled during the present study. Visitors to aloes were recorded over two consecutive flowering seasons (July – August 2013 and 2014). Bird taxonomy follows Hockey *et al*. (2005) and Chittenden (2014). Small mammal taxonomy follows Skinner and Chimimba (2005).

Guild and Species	2013	2014
BIRDS		
Cisticolidae		
Wailing Cisticola (*Cisticola lais*)	X	
Motacillidae		
Long-billed Pipit (*Anthus similis*)	X	
Muscicapidae		
Mountain Wheatear (*Oenanthe monticola*)	X	
Nectariniidae		
White-bellied Sunbird (*Cinnyris talatala*)		X
Sturnidae		
Red-winged Starling (*Onychognathus morio*)	X	
Zosteropidae		
Cape White-eye (*Zosterops virens*)	X	
MAMMALS		
Leporidae		
Smith's Red Rock Rabbit (*Pronolagus rupestris*)*		X
REPTILES		
Agamidae		
Ground Agama (*Agama aculeata*)	X	

*One Smith's Red Rock Rabbit was recorded sniffing one raceme, but probably did not pollinate the aloe. No other contact with any other aloes by this species was recorded.

CHAPTER FIVE

Co-dominant specialist and generalist (opportunistic) nectar-feeding bird guilds partition visitation in a summer-flowering grassland *Aloe*



A patch of flowering *Aloe reitzii* var. *reitzii* plants on Klipbankspruit Farm, Mpumalanga. (Stephanie Payne)

5.1 Abstract

Aloe reitzii var. *reitzii* is a summer-flowering (February – March) succulent, endemic to a very restricted range within the grasslands of eastern South Africa. Floral structure and nectar properties suggest specialist nectarivorous bird-pollination. This study investigated the community of biotic visitors that visit, and potentially pollinate, this aloe. Temporal partitioning of visitation events, and nectar resources by specialist and generalist nectar-feeding birds were also assessed. Although a previous short term study (~3 days) in 2014 revealed that Cape Weavers (*Ploceus capensis*), which are generalist (opportunistic) nectarivores, were the dominant visitors, the use of extensive camera observations in the present study during the 2017 flowering season (23 days) showed that both specialist and generalist nectarivorous birds (*n* = four and five species respectively) are regular aloe visitors during the day, with hawkmoths visiting at night. The specialist Malachite Sunbird (*Nectarinia famosa*) accounted for 49% of all visits, while generalist Cape Weavers made up 41% of all visits; these species appear to co-dominate visitation, and both may be important for pollination. Although guild visitation rates were similar, visitation by each guild was separated temporally, with generalists visiting earlier in the day than specialists. Coleoptera and Orthoptera were observed perched on the flowers and likely had no effect on pollen transfer, while honeybees drank nectar and collected pollen. Nectar is produced throughout the day and properties (i.e. volume and concentration) range across both generalist and specialist preferences, which may explain the co-dominance of both guilds. Fruit set was low, with an average of 12.1 ± 2.4% fruit set/plant, with many fruit appearing to have a bacterial or viral infection, or evidence of insect predation. The role of both insects (diurnal and nocturnal) and birds in pollination remains to be quantified through exclusion experiments. Birds of multiple guilds, especially generalists, potentially playing a crucial role in *A. reitzii* pollination is unusual, as many summer-flowering aloes are primarily insect- or sunbird-

pollinated. Some generalist species, especially weavers are considered nectar addicts, feeding regularly on nectar, along with their usual diets; this may be the case in *A. reitzii*. Insect visitors are likely important for the pollination of the aloe. *Aloe reitzii* may be a true "generalist aloe", attracting insects and members of multiple bird guilds. This likely ensures successful pollination events in its limited distribution within a heavily transformed habitat with low bird abundance/densities.

5.2 Key words

Aloe reitzii; Cape Weaver; Floral Syndromes; Grassland; Malachite Sunbird; Temporal Partitioning.

5.3 Introduction

Floral characteristics are often used to predict the potential pollinators of a plant species, and create testable hypotheses for field experiments (Hargreaves *et al*. 2004; Ollerton *et al*. 2009). The most commonly utilised characteristics are flower shape and colour, flowering season, scent, and nectar properties (Faegri and van der Pijl 1979; Dafni 1992; Ollerton *et al*. 2009), which can vary within genera. The genus *Aloe* consists of approximately 350 species in southern and eastern Africa alone, with a wide variety of growth forms and floral characteristics (Cousins and Witkowski 2012; Van Wyk and Smith 2014), which has enabled predictions of primary pollinators (e.g. Hargreaves *et al*. 2008; Symes *et al*. 2008; Botes *et al*. 2009a, b; Arena *et* al. 2013). However, these predictions are not always accurate as visitors from multiple guilds are often attracted to the same flowers, despite some guilds not being predicted by floral characteristics (e.g. Hoffman 1988; Botes *et al*. 2009a; De Merxem *et al.* 2009; Ollerton *et al.* 2009; Symes *et al*. 2009; Cousins *et al*. 2013).

Fifty-five percent of South African aloes (*N* = 158 aloes) flower in winter, and 40% in summer (Van Wyk and Smith 2014). Many winter flowering aloes have short to medium length, brightly coloured tubular flowers, which are tightly packed on the racemes, and are pollinated by both generalist and specialist nectar-feeding birds (Hoffman 1988; Johnson *et al*. 2006; Botes *et al*. 2008; Symes *et al*. 2008, 2009; Forbes *et al*. 2009; Hargreaves *et al*. 2012; Arena *et al*. 2013; Kuiper *et al*. 2015), and small, non-flying mammals (Payne *et al*. 2016; Chapter 2). Insects are less active in winter, and usually play a negligible role in pollination of these species (e.g. Stokes and Yeaton 1995; Symes *et al*. 2008, 2009; Arena *et al*. 2013; but see Symes *et al.* 2009 for *A. greatheadii* var. *davyana*). Insect-pollinated species tend to have small flowers with pale pink, green or cream flowers, which reflect insect preferences (Faegri and van der Pijl 1979; Botes *et al*. 2009b). Summer-flowering aloes display different floral characteristics to their winter-flowering counterparts, with shorter tubular flowers that are more loosely packed on the raceme (Botes *et al*. 2009b; Wilson *et al*. 2009; Hargreaves *et al*. 2010; Van Wyk and Smith 2014). Flowers are either pale or brightly coloured, sometimes borne on large racemes (Van Wyk and Smith 2014). Most of these aloes are visited by multiple guilds (i.e. both insects and specialist birds), and are primarily insect- and/or sunbird pollinated, with generalist nectarivore birds having no significant effect on pollination (Botes *et al*. 2009b; Wilson *et al*. 2009; Hargreaves *et al*. 2010). Insects that pollinate these aloes include solitary bees (Megachillidae), sweat bees (Halictidae), membrane bees (Colletidae), honeybees, allodapine and amegilla bees (Apidae; Botes *et al*. 2009b; Wilson *et al*. 2009; Hargreaves *et al*. 2010). Although efficient pollinators, these insects can also act as nectar and pollen thieves, decreasing plant reproductive output (Hargreaves *et al*. 2010). Sunbirds, such as the Malachite Sunbird (*Nectarinia famosa*), are primary pollinators of the summer-flowering *Aloe maculata*, and bees are pollen thieves

(Hargreaves *et al*. 2010); generalist nectarivores are not common visitors to, and/or pollinators of, summer-flowering aloes.

Aloe reitzii Reynolds var. *reitzii* (hereafter referred to as *Aloe reitzii*) is a summer-flowering aloe that displays a typical bird-pollination syndrome, with long, scarlet, tubular flowers that produce substantial amounts of nectar (Faegri and van der Pijl 1979; Johnson and Nicolson 2008; Symes 2017). A short-term (~ three days) study by Symes (2017) revealed that multiple pollinating guilds – generalist and specialist nectivorous birds, and insects – visit these aloes when they are flowering, and might be contributing to pollination. Three bird species were recorded regularly visiting the aloes – two specialist and one generalist nectar-feeding species (Symes 2017). The Cape Weaver (*Ploceus capensis*) contributed 60% of all visits, with the Malachite Sunbird and Greater Double-collared Sunbird (*Cinnyris afer*) making up the remainder of the visits (27% and 13% respectively; Symes 2017).

Although visitation rates were not measured, honeybees (*Apis mellifera*), and ants (*Camponotus* sp.) were observed visiting the flowers, drinking nectar and removing pollen (Symes 2017). Beetles (*Porphyronata* sp. and *Dischista* sp.) and grasshoppers (*Maura* sp.), were observed perching on the flowers or inflorescence stems (Symes 2017). Since honeybees are primary pollinators of many summer-flowering aloes, it is likely that they may play a role, along with the birds, in the pollination of *A. reitzii* (Botes *et al*. 2009b; Wilson *et al*. 2009; Duffy *et al*. 2014; Symes 2017).

Aloe reitzii produces moderate volumes (36 ± 27 µL) of concentrated (16.5 ± 1.7% w/w) nectar, suggesting specialist nectarivore (sunbird) pollination (specialist preferences: volume: 10 – 30 µL, concentration: 15 – 25% w/w), but narrowly extend into generalist nectarivore ranges (volume: 40 – 100µL, concentration: 8 – 12% w/w; Johnson and Nicolson

2008). With these nectar properties, visual and structural features of the flowers (i.e. long, narrow floral tubes), it is surprising that generalist birds dominate visitation.

This study aimed to further investigate the contributions of specialist and generalist nectar-feeding birds to visitation, and potentially pollination, events, during the peak flowering season (February – March 2017) of *Aloe reitzii* var. *reitzii*. The following questions were addressed: 1) What is the population structure of the assessed sub-population? 2) Which species visit, and dominate visitation to, the aloes during the peak of the flowering period? 3) Do nectar resources (a distinguishing feature used to separate specialist and generalist bird-pollinated plants) change throughout the day? 4) Do the specialist- and generalist-nectar feeding birds partition their visits to the aloes, and the nectar resource, temporally?

5.4 Methods and Materials

Study species and site

Aloe reitzii Reynolds var. *reitzii* (Asphodelaceae; Figure 5.1A) is a stemless, Near Threatened aloe that is endemic to regions around Roossenekal, Belfast, and Dullstroom, Mpumalanga, South Africa (Siebert *et al*. 2005; Van Wyk and Smith 2014). The green leaves are long and broad (up to 120 mm at the base), with reddish teeth along the margins, forming a large rosette. The aloes flower in February – March, and can produce three racemes on average, but plants are known to produce between one and 10 racemes (Jeppe 1969; Van Wyk and Smith 2014; Symes 2017). The long, tubular flowers are red in bud, turning yellow as they open and mature (Van Wyk and Smith 2014). The aloe is distinguished from other similar species (e.g. *A. aculeata*) by its distinctly curved, downward pointing flowers (Jeppe 1969; Van Wyk and Smith 2014). The Critically Endangered spring-flowering (August – September) form, *A. reitzii* Reynolds var. *vernalis* is endemic to a very restricted range in Vryheid, northern Kwazulu-Natal (Victor and Van Wyk 2007; Van Wyk and Smith 2014).

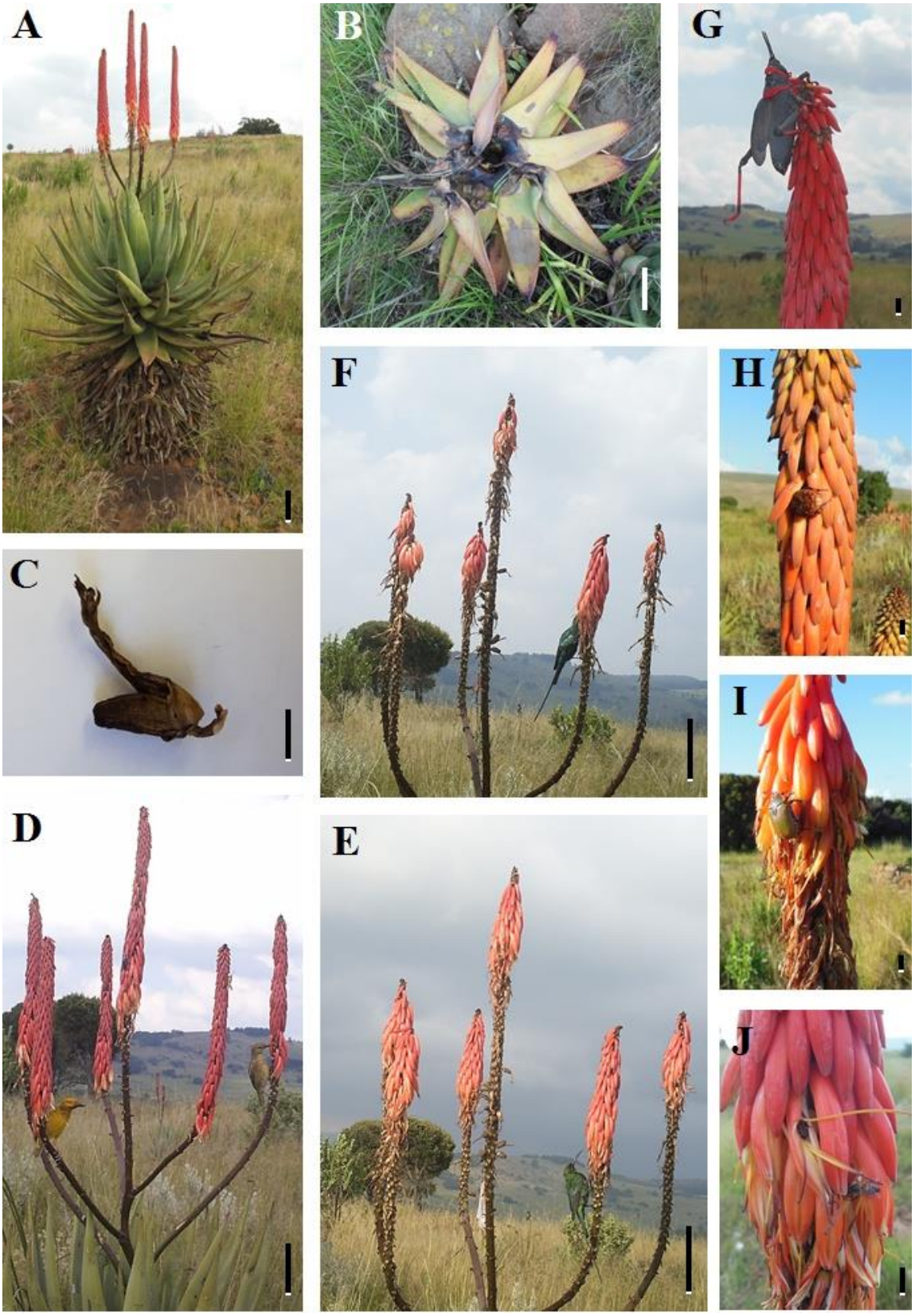

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Figure 5.1. Visitors to *Aloe reitzii* var. *reitzii*: A) A flowering, established (adult) *A. reitzii* var. *reitzii* plant; B) A dead and rotting *A. reitzii* var. *reitzii* plant; C) A damaged/infected *A. reitzii* var. *reitzii* fruit, with dried perianth still attached; D) Male and female Cape Weavers (*Ploceus capensis*) perched; E) Male Malachite Sunbird (*Nectarinia famosa*) perched; F) Male Malachite Sunbird feeding on nectar; G) Koppie Foam Grasshopper (*Dictyophorus spumans*); H) Fruit chafer (*Porphyronota* sp.); I) Fruit chafer (*Dischista* sp.); J) Honeybee (*Apis mellifera*). Scale bars: Photographs A-B, D-F = 10 cm; Photographs C, G-J = 1cm. Photographs A-J by Stephanie Payne.

Aloe reitzii is abundant on Klipbankspruit Farm, approximately 30 km north of Dullstroom, Mpumalanga (~1 930 m a.s.l.). The farm is privately owned and is used for commercial beef farming and grass-baling, mainly of *Eragrostis* and *Digitaria* species (Landowner, *pers. comm*.). Cattle, donkeys, and sheep regularly graze through the sites where the aloes are predominantly found. The farm is situated in Sekhukhune Montane Grassland, which is comprised of various habitat types, such as hills, rocky slopes and open grasslands with clusters of trees and shrubs (Mucina *et al*. 2006). The region received 121.4 mm of rain during the peak of the flowering season (February 2017; South African Weather Service 2018).

Population structure

During the sampling period (11 February – 05 March 2017), 674 *Aloe reitzii* plants along a wandering transect (~1.8 km) through the farm were measured. Aloes were classified as flowering or non-flowering "established" aloes, seedlings (aloes where a full, round rosette had not yet formed) or dead/rotting (Figure 5.1B). The following morphological measurements were taken for each established plant (n = 533 plants; 79% of measured plants; in cm): Diameter at the widest point of the rosette (d), rosette height (living + dead leaves), inflorescence height, number of racemes, and individual raceme lengths. Rosette circumference (C; in cm) was calculated as $C = \pi d$, and total plant height was calculated as the sum of rosette height and inflorescence height. Leaf height (cm) was measured, and the

number of leaves were counted for seedlings (n = 57 seedlings; 9% of measured plants). No measurements were taken for dead/rotting aloes (n = 84 plants; 12% of measured plants).

Visitor Observations

Visitors to the aloes over the peak of the flowering season in 2017 were recorded by motion-sensitive camera traps (Bushnell HD Model No. 119537 and Bushnell Natureview Model No. 119740, China). Over the course of 23 days (11 February 2017 – 05 March 2017), 34 flowering *Aloe reitzii* plants were observed. These aloes were randomly selected, but selections represented the different habitats found along the farm (i.e. rocky slopes, flat grassland, rocky grassland, and wooded, rocky outcrops). Camera traps on tripods (height: ~ 1.5 m) were placed approximately one metre away from the aloe, facing southwards. Cameras were set to take two photographs when triggered by motion, at three-second intervals; memory cards (SanDisk 2GB, 4GB, or 16GB) and batteries were changed as required (every one – two days). Cameras were set for the entire sampling period, with the exception of two days (16 and 17 February), where strong winds and heavy rain caused all cameras to fall over. Anecdotal researcher observations of visitors to any flowering aloes, during field sampling, were also recorded.

An animal was recorded as a visitor if it touched the aloe in any way (i.e. probed flowers, perched on racemes and/or leaves, etc.). Although an animal touching the plant may not be a legitimate pollinator, any contact with the plant was classified as an action sufficient to implicate the animal as a potential visitor, as it may inadvertently pick up pollen (e.g. by brushing the raceme in flight; Chapter 2). If a visitor was photographed in successive frames, either on the same raceme, or a different raceme of the same aloe, it was considered the same visitor. Visitors that left the aloe, and then appeared to return the same aloe, were recorded as new visitors, as were visitors that were recorded at least 30 seconds apart, unless it was clear

that it was the same individual (e.g. aberrant feather positioning, position on the aloe remained the same, etc.). Visitation rates for both the generalist and specialist pollinating guilds were calculated for each hour in a 24-hour period, for the duration of the sampling period. Overall visitation rates, and the percentage contribution to these rates, for each visiting species, and for each guild over the entire sampling period were calculated. Visitation rates were calculated as the mean number of visits/1000 plants/hour.

Nectar Production

Nectar properties were measured during daylight on 01 March 2017. One day prior, on 28 February 2017, ten flowering aloes were randomly selected, each with a minimum of two flowering racemes. On each aloe, one raceme was labelled as "screened" and covered with a pale pink fine mesh bag, and one was left uncovered and labelled as the "unscreened" raceme. Temperature data loggers (Thermocron iButtons, Australia, correct to 0.5°C) were placed on each sampled raceme. Nectar was extracted using disposable haematocrit tubes (75 µL) from one flower per raceme (N = 56 screened flowers; N = 47 unscreened flowers; sampling was destructive) every two hours, starting at 07:00. Nectar volume was recorded, and nectar sugar concentration (% w/w) was measured using a handheld refractometer (Bellingham & Stanley Eclipse Refractometer, Model 45-81, Tunbridge Wells, UK). Flowers sampled were fully open, and anthers newly dehisced. The stigma was receptive, but was not yet exserted past the stamens. *Aloe* nectar is most abundant during this stage of floral development (Human and Nicolson 2008; Symes and Nicolson 2008). Since only one specific stage was sampled, some racemes were not sampled in some flowering sessions as a suitable flower was not available. Labels, iButtons, and bags were removed after the final sampling session at 17:00. Sampling was undertaken on a hot day with clear skies in the morning (minimum temperature = 16.6°C (05:00); maximum temperature = 26.8°C (12:00)); a heavy thunderstorm began at approximately 17:30.

Reproductive Output

Reproductive output of the aloes sampled for vertebrate visitation with camera traps was measured approximately five weeks later (11-12 April 2017). Eight sampled aloes (24%) had missing or broken racemes, and these aloes were excluded from reproductive output analyses. Three racemes were randomly selected from each of the remaining 25 sampled aloes, and the number of fruit produced was counted. The number of flowers per raceme, represented by the pedicels (Symes *et al*. 2009; Chapter 2), was counted, to calculate the fruit set (proportion of flowers that set fruit) of the racemes and the aloe plant. Once counted, fruit was scattered at the site. The fruit of three sampled aloes (9%), had the appearance of being burnt (Figure 5.1C), but there had been no fires in the time between flowering and fruiting (Landowner, *pers. comm.*). The fruit may have been damaged by a bacterial or viral infection (Prof. Ben-Erik van Wyk, *pers. comm.*), or predated on by insects prior to fruit development (Prof. Glynis Goodman-Cron, *pers. comm.*). These damaged fruit were hard and could not be opened, and seed set could not be obtained for these aloes. Therefore, no fruit were collected and no seed set was calculated for this sub-population. Number of flowers per plant, fruit set per plant (%), and damaged fruit set per plant (%) were calculated as follows:

$$\text{Number of flowers/plant} = (\text{mean no. flowers/raceme}) \times (\text{No. racemes})$$

$$\text{Fruit set/plant (\%)} = \frac{(\text{mean no. fruit/raceme}) \times (\text{No. racemes})}{\text{No. flowers/plant}} \times 100$$

$$\text{Damaged fruit set/plant (\%)} = \frac{(\text{mean no. damaged fruit/raceme}) \times (\text{No. racemes})}{\text{No. flowers/plant}} \times 100$$

To evaluate the frequency of plants with damaged fruit within the sub-population, 100 aloes that had flowered in 2017 were visually assessed along a wandering transect (~0.5 km) and classified as “no fruit”, “normal fruit”, or “damaged fruit”. If any fruit on the aloe

showed evidence of damage, the aloe was classified as having “damaged fruit”. Camera trap sampled aloes were included in this count.

Data analysis

Size-class distribution of the *Aloe reitzii* population by rosette diameter and rosette height was tested with a Kolmogorov-Smirnov Test. A Mann-Whitney *U*-Test was applied to test for differences in rosette diameter and height between flowering and non-flowering plants. Visitation rates for generalist and specialist nectar-feeding guilds, were modelled as count data, with time and guild as predictor variables and an offset for hours of sampling effort, using a Generalised Linear Model (GLM) with Poisson distribution and a log-link function. Visitation rates for the dominant species from each feeding guild were modelled as count data, using a GLM with time and species as predictor variables and an offset for hours of sampling effort, with Poisson distribution and log-link function. Only daylight hours when birds visited (05:00 – 19:00), were included in visitation rates analyses.

Nectar volumes were modelled using a simple linear regression, with log-transformed data, and time and treatment as predictor variables. Nectar concentration was modelled using a GLM, with time and treatment as predictor variables, a binomial distribution and a logit-link function. Spearman rank correlations assessed whether nectar volume and concentration were correlated to temperature. All statistical analyses were performed in R (Version 3.5.0; R Core Team 2018), and all graphics were produced using the R packages “ggplot2”, “extrafontdb” and “extrafont” (Chang 2012, 2014; Wickham 2016). Data are presented as mean ± standard error.

5.5 Results

Population Structure

Most plants were classified as established plants (79%, N = 674), 12% of plants were dead and/or rotting, and 9% classified as seedlings. Established plant and seedling size-class distributions by rosette diameter did not follow a normal distribution (D = 0.08, P = 0.002), but does appear to have a more-or-less bell-shaped structure (Figure 5.2A). Eleven percent of plants are < 10 cm in diameter (n = 67 plants), while 15% of plants are in the 70-79.9 cm size class (n = 88 plants). When seedlings are removed, the established plant population does follow a normal, bell-shaped size structure (D = 0.06; P = 0.05). Rosette height does not follow a normal distribution, both when seedlings are included (D = 0.09, P = 0.0001; Figure 5.2B), or excluded (D = 0.07, P = 0.001), with some size classes not represented (e.g. 150.0 – 169.9 cm classes). Diameter is the best measure of *Aloe reitzii* population structure. Only 29% of the established plant population was flowering, and produced an average of 3.2 ± 0.1 racemes per plant (range = 1 – 10 racemes; N = 154 plants; Table 5.1). Flowering aloes were significantly larger than those plants that did not flower, with aloes greater than 30 cm in rosette diameter beginning to flower (diameter: U_{531} = 51280, P < 0.001; height: U_{531} = 45407, P < 0.001; Figure 5.2A), although only from a much larger size of ≥ 100 cm did all plants flower, indicating inconsistent annual flowering in plants between 30-100 cm in diameter. Seedlings had an average of six leaves per plant.

Visitor Observations

A total of nine bird species (five generalist, and four specialist nectar feeding species) were recorded visiting *Aloe reitzii* inflorescences over the sampling period (7383 camera hours; Table 5.2). Fifteen individual birds could not be identified to species, due to poor light

or the position of the bird on the inflorescence, and were broadly identified as generalists (*n* = six birds) or specialists (*n* = nine birds), based on body and/or bill shape or size.

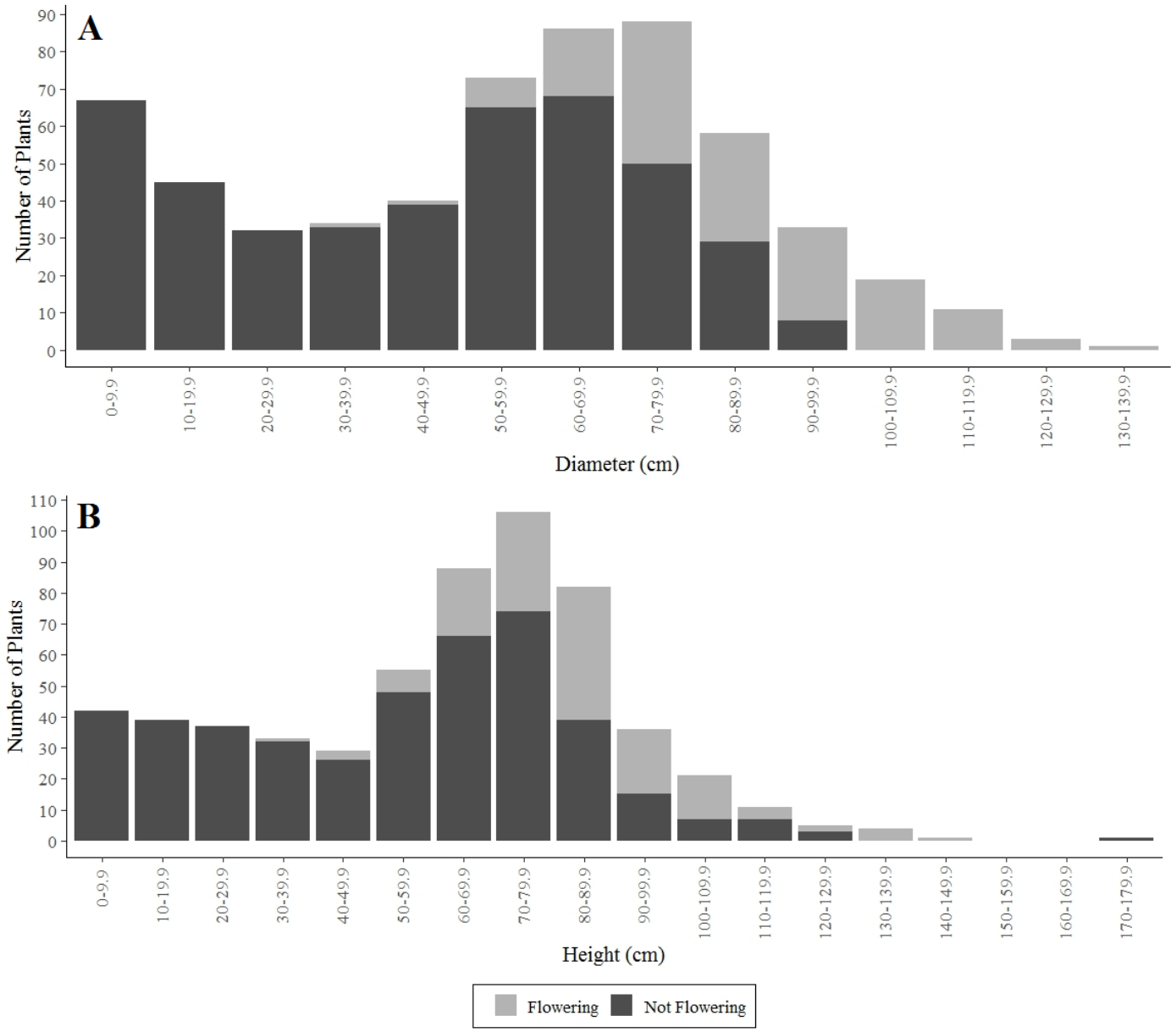


Figure 5.2. A) Rosette diameter (cm), and B) Rosette height (cm) size structure of 590 flowering and non-flowering *Aloe reitzii* var. *reitzii* plants and seedlings (plants that had not yet formed a round rosette), at Klipbankspruit Farm, Dullstroom, Mpumalanga, in 2017. Dead and/or rotting plants were excluded from the analyses (*n* = 84 plants). Within the 0 – 9.9 cm size classes, 85% of plants were classed as seedlings (plants where a round rosette had not yet formed; *n* = 57 of 67 plants). Size-class distributions for both diameter and height do not follow normal distributions (Kolmogorov-Smirnov Test; $P < 0.05$), with the exception of diameter, which follows a normal, bell-shaped curve once seedlings are removed from analysis (Kolmogorov-Smirnov test: $D = 0.06$, $P = 0.05$).

Table 5.1. Mean (± S.E.) morphological measurements for *Aloe reitzii* var. *reitzii* individuals (*N* = 674 plants) at Klipbankspruit Farm, Dullstroom, Mpumalanga. Established plants are plants where a full, round rosette had formed, and were further split into flowering and non-flowering plants. Seedlings are plants that had not yet formed a round rosette. No measurements were taken for dead/rotting plants. Total plant height is calculated as the sum of rosette and inflorescence heights.

Morphological Measurement	**Established Plants**		**Total Established Plants (flowering + non-flowering)**	**Seedlings**	**Dead**
	Flowering plants	**Non-flowering plants**			
Number of Individuals	154	379	533	57	84
Rosette Diameter (cm)	84.7 ± 1.4	50.7 ± 1.2	60.6 ± 1.2	-	-
Estimated Rosette Circumference (cm)	226.0 ± 4.4	134.9 ± 3.2	161.2 ± 3.2	-	-
Rosette Height (cm)	83.4 ± 1.5	59.8 ± 1.3	66.6 ± 1.1	8.2 ± 0.5	-
Number of Racemes	3.2 ± 0.12	-	-	-	-
Raceme Length (cm)	31.2 ± 0.6 (*n* = 478)	-	-	-	-
Inflorescence Height (cm)	76.1 ± 1.8	-	-	-	-
Total Plant height (cm)	159.5 ± 2.6	59.8 ± 1.3	88.6 ± 2.3	8.2 ± 0.5	-
Number of leaves	-	-	-	6.0 ± 0.2	-

Visitation to the aloes was co-dominated by two bird species from different feeding guilds. The Malachite Sunbird (*Nectarinia famosa*) was the most common specialist and overall visitor, accounting for 49% of all bird visits (Figures 5.1E, 5.1F; Figure 5.3; Table 5.2). The Cape Weaver (*Ploceus capensis*) accounted for 41% of all bird visits, and was the most common generalist visitor (Figure 5.1D; Figure 5.3; Table 5.2). Forty percent of the sampled plants that received bird visits were visited by both species (along with other bird species), while 24% were visited only by Malachite Sunbirds only, and 16% only by Cape Weavers (amongst others). Forty-eight percent of plants received only Malachite Sunbird and/or Cape Weaver visits, and the remaining 52% were visited by other bird species, along with Malachite Sunbirds and/or Cape Weavers.

A single sunbird (species unknown) was the earliest visitor to the aloes at 05:56. Visitation to the aloes by both generalist and specialist guilds increased from 07:00, and began decreasing from 15:00 onwards (Figures 5.3A and 5.3B, Figure 5.4). Although the Cape Weaver was the most frequent visitor between 06:00 and 08:00, the Malachite Sunbird became more regular from 08:00 onwards, and was more consistent for the rest of the day (Figures 5.3A and 5.3B). Three generalist individuals (Cape Weaver, Dark-capped Bulbul (*Pycnonotus tricolor*) and Southern Fiscal (*Lanius collaris*)) visited the aloes between 18:00 and 18:20, with the final visit at 19:04 by a Southern Fiscal. *Cisticola* sp. were observed perching on the racemes, but were not recorded on cameras (Stephanie Payne, *pers. obs.*). A Gurney's Sugarbird (*Promerops gurneyi*) was observed trying to feed on nectar, but was chased by a male Malachite Sunbird. The sugarbird was later recorded feeding on another aloe.

Table 5.2. Percentage contribution (%; top value) to visitation and mean visitation rates (visits/hour/1000 plants; mean ± S.E; bottom value) of all generalist and specialist nectarivorous bird species visiting *Aloe reitzii* var. *reitzii* plants (*N* = 33 plants), recorded by camera traps during the February – March 2017 flowering season. Percentage contributions are calculated as percentage of the total number of visits by that species' guild, with contributions of the most regular visitor in bold text. Each species' activity was noted, as "feeding only", "perching only" or "both". References are provided for known *Aloe* visitors in Southern Africa. Bird taxonomy follows Hockey *et al*. (2005).

Species	Contribution and Visitation Rate	Feeding and/or Perching	Known *Aloe* Visitor (References)
GENERALIST NECTAR FEEDERS			
LANIIDAE			
Southern Fiscal	2.3	Perching only	Yes
Lanius collaris	0.61 ± 0.39		7
MALACONOTIDAE			
Bokmakierie	0.6	Perching only	No
Telophorus zeylonus	0.32 ± 0.32		-
PLOCEIDAE			
Cape Weaver	**87.9**	Both	Yes
Ploceus capensis	**19.40 ± 6.76**		2,3,6,7,8,11,16
PYCNONOTIDAE			
Dark-capped Bulbul	5.2	Both	Yes
Pycnonotus tricolor	1.53 ± 0.82		2,4,10,14,17
STURNIDAE			
Pied Starling	0.6	Perching only	Yes
Spreo bicolor	0.50 ± 0.50		2,16
UNKNOWN	3.4	Both	-
Unknown Generalist Sp.	1.45 ± 1.04		-
Total Number of Generalist Visitors	174	-	-
Total Generalist Rate	24.67 ± 7.36	-	-
SPECIALIST NECTAR FEEDERS			
NECTARINIIDAE			
Amethyst Sunbird	1.0	Perching only	Yes
Chalcomitra amethystina	0.22 ± 0.22		2,3,5,9,10,12,14
Greater Double-collared Sunbird	3.9	Feeding only	Yes
Cinnyris afer	0.95 ± 0.52		2,4,5,16
Malachite Sunbird	**90.2**	Both	Yes
Nectarinia famosa	**23.86 ± 8.10**		1,5,6,8,10,11,13,15,16
PROMEROPIDAE			
Gurney's Sugarbird	0.5	Feeding only	Yes
Promerops gurneyi	0.07 ± 0.07		2,11
UNKNOWN	4.4	Both	-
Unknown Sunbird Sp.	2.60 ± 1.65		-
Total Number of Specialist Visitors	203	-	-
Total Specialist Rate	26.84 ± 8.71	-	-

References: [1]Skead 1967; [2]Oatley and Skead 1972; [3]Hoffman 1988; [4]Johnson *et al*. 2006; [5]Botes *et al*. 2008; [6]Symes and Nicolson 2008; [7]Symes *et al*. 2008; [8]Forbes *et al*. 2009; [9]Wilson *et al*. 2009; [10]Hargreaves *et al*. 2010; [11]Symes 2010; [12]Duffy and Johnson 2011; [13]Hargreaves *et al*. 2012; [14]Arena *et al*. 2013; [15]Cousins *et al*. 2013; [16]Kuiper *et al*. 2015; [17]Payne *et al*. 2016.

Honeybees (*Apis mellifera*, Hymenoptera: Apidae) were observed visiting flowers, removing nectar and pollen (Figure 5.1J). Koppie Foam Grasshoppers (*Dictyophorus spumans*; Figure 5.1G) and Elegant Grasshoppers (*Zonocerus elegans*; both Orthoptera: Pyrogomorphidae) were recorded perching on racemes, sometimes moving between racemes and plants. Coleoptera, *Dischista* sp. and *Porphyronota* sp. (Scarabaeidae: Cetoniinae) were often perched on racemes, or burrowed between individual flowers (Figures 5.1H and 5.1I). Camera traps recorded hawkmoths, likely *Argius* sp. (Nic Venter, *pers. comm.*), visiting the aloes from 18:10, and continued visiting regularly (n = 148 total records) throughout the night until sunrise. One Striped Hawkmoth (*Hyles livornica*) was recorded at one aloe during the day. Common Duiker (*Sylvicapra grimmia*), Common Reedbuck (*Redunca arundinum*), and Serval (*Leptailurus serval*) were observed walking in the area of the aloes, but did not interact with the plants in any way.

Generalist and specialist nectar-feeding bird visitation to the aloes does not differ significantly ($\beta = 0.07 \pm 0.11$, $P = 0.50$), but visitation time periods for each guild differed, indicating that visitation is separated temporally by guild ($\beta = -0.70 \pm 0.16$, $P < 0.001$), with generalists dominating visitation in the early morning (Figure 5.4). Overall bird visitation rates did not differ throughout the day ($\beta = 0.09 \pm 0.11$, $P = 0.40$), as the specialists consistently visit the aloes from the mid-morning into the early evening (Figure 5.4). Malachite Sunbird and Cape Weaver visitation rates were not significantly different ($\beta = -0.07 \pm 0.12$, $P = 0.54$), with both species contributing equally to visitation. Overall visitation rates by these species did change over time ($\beta = -0.67 \pm 0.11$, $P < 0.001$), but were temporally separated ($\beta = 0.73 \pm 0.16$, $P < 0.001$), with Malachite Sunbirds dominating visitation from the late morning onwards.

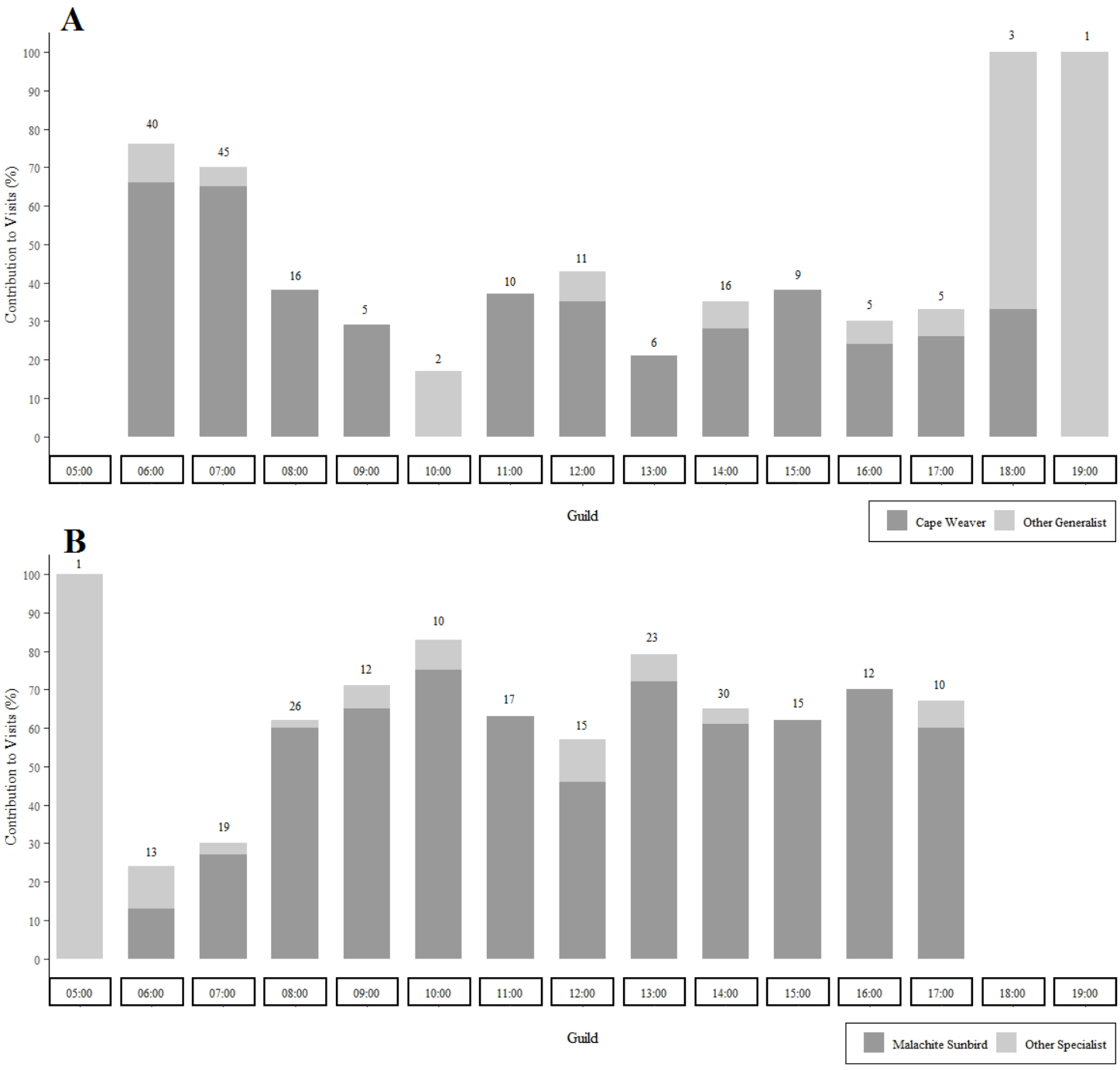


Figure 5.3. Percentage contribution to visitation at each daylight hour (05:00 – 19:00) to *Aloe reitzii* var. *reitzii* plants by A) generalist (opportunistic) nectar-feeding birds, and B) specialist nectarivorous birds, during the February – March 2017 flowering season. Generalists are split into Cape Weaver (*Ploceus capensis*), and other generalist species, while specialists are split into Malachite Sunbird (*Nectarinia famosa*), and other specialist species. The number of individual birds recorded for each guild, at each hour are presented above each stacked bar. Specialist visits at 05:56 consist of a single unidentified sunbird individual (mean sunrise time = 05:49), while a single generalist visit at 19:04 was made by a Southern Fiscal (*Lanius collaris*; mean sunset time = 18:35).

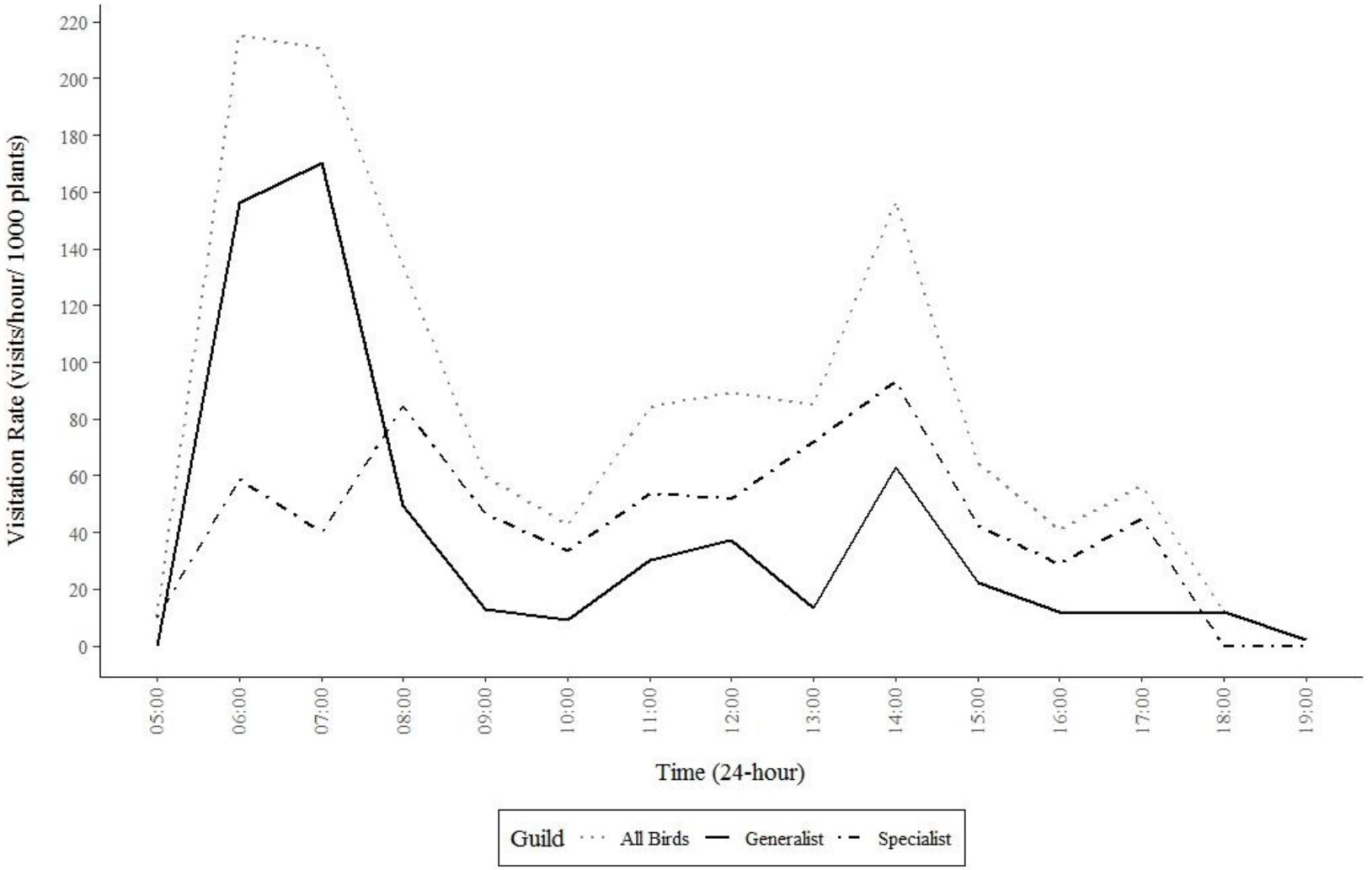


Figure 5.4. Mean hourly visitation rates (visits/plant/1000 hours), during daylight hours (05:00 – 18:59) for generalist (opportunistic) nectar-feeding birds (solid black line) and specialist (dashed black line) nectarivorous birds on *Aloe reitzii* var. *reitzii* plants during the February – March 2017 flowering season (*N* = 33 plants, 7383 camera hours). All bird visitation rates (generalist + specialist visitation rates) are represented by the dotted grey line. No vertebrate visitors were recorded visiting the aloes at night (after 19:05 – 04:59).

Nectar Production

Nectar is produced continually throughout daylight hours (Figure 5.5A). Nectar volumes for screened and unscreened flowers were equally affected by time (β = -12.98 ± 10.95; P = 0.24), with no significant changes in volume over time (β = -0.06 ± 7.44; P = 0.99). Nectar volumes in unscreened plants were significantly lower than screened plants (β = -32.93 ± 8.19; $P < 0.01$). Mean screened nectar volumes peaked at 07:00 (100 ± 20.3 µL, n = 10 flowers), and were lowest at 17:00 (65.9 ± 11.9 µL, n = 10 flowers). Mean unscreened nectar volumes were highest at 11:00 (70.1 ± 16.7 µL; n = 8 flowers) and lowest at 15:00 (36.6 ± 5.6 µL; n = 8 flowers).

Nectar concentration did not differ between treatments (β = 0.02 ± 0.06; P = 0.74), nor did it change over time (β = 0.12 ± 0.59; P = 0.83; Figure 5.5B). Mean screened nectar concentrations were highest at 15:00 (11.3 ± 0.3% w/w; n = 6 flowers), and lowest at 07:00 (9.7 ± 0.6% w/w; n = 10 flowers). Mean unscreened nectar concentrations peaked at 11:00 (11.5 ± 0.5% w/w; n = 8 flowers), and the lowest concentrations measured at 07:00 (9.1 ± 0.6% w/w).

Temperature and nectar volume were not correlated for either the screened or unscreened treatments (Screened: R_S = -0.10, P = 0.48; Unscreened: R_S = -0.04, P = 0.81). Unscreened nectar concentration was not correlated with temperature (R_S = 0.26, P = 0.08), while screened nectar concentration was weakly, positively correlated with temperature (R_S: 0.31, P = 0.02). At lower temperatures, when not directly exposed to environmental elements, nectar may be more concentrated (Figure 5.5B).

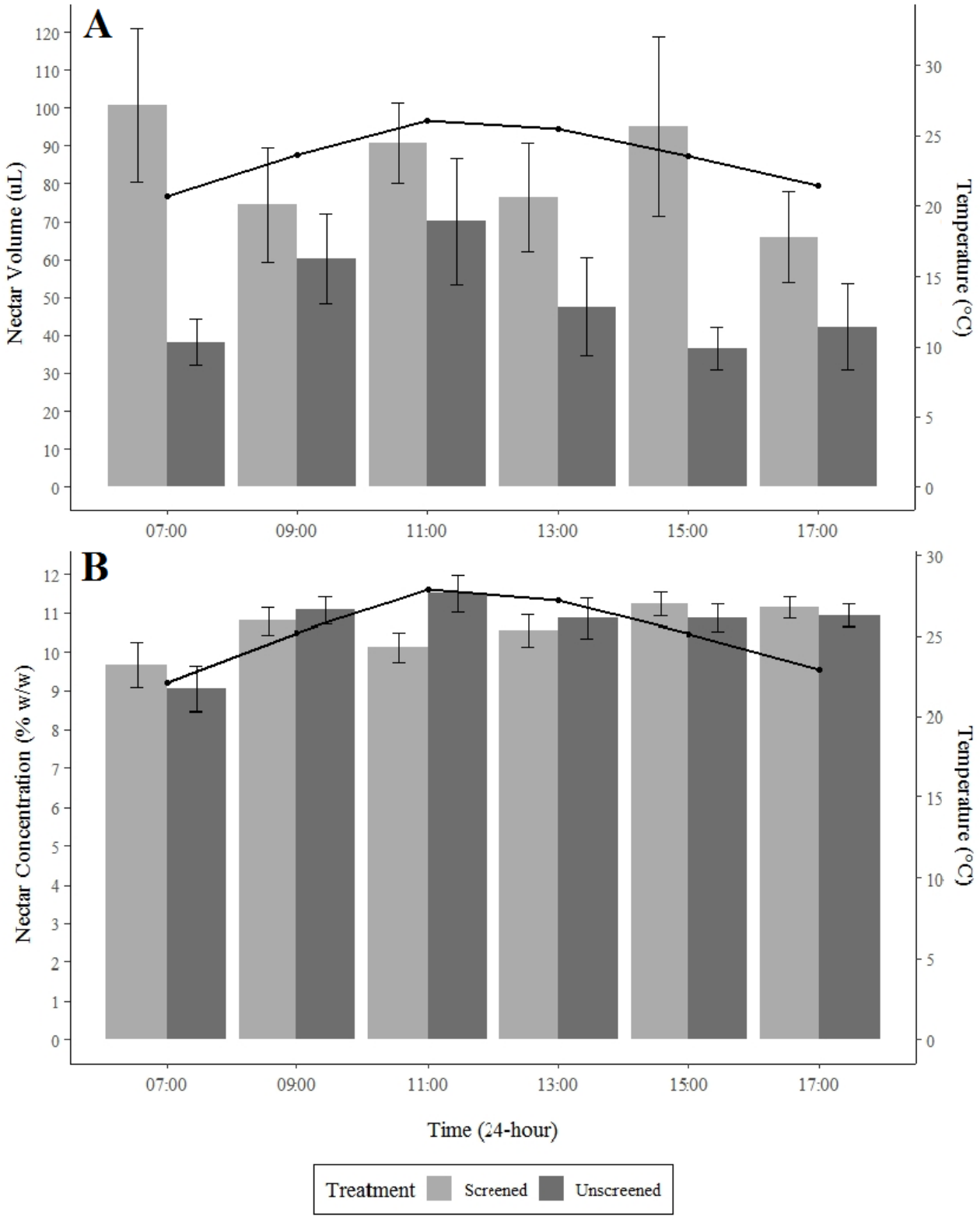


Figure 5.5. A) *Aloe reitzii* var. *reitzii* nectar volumes (µL; mean ± S.E.) and B) concentrations (% w/w; mean ± S.E.) of screened and unscreened plants ($n \leq 10$ flowers per time period per treatment; $N = 10$ plants per treatment), measured at two-hour intervals during daylight hours. Mean temperature (°C) is represented by the solid black line. Standard errors for mean hourly temperature were too small to plot (< 1°C).

Reproductive Output

Sampled plants have an average of 1 212 ± 118 flowers/plant (274 ± 16 flowers/raceme; *n* = 25 plants). Of the 25 plants sampled, three individuals did not produce fruit on any racemes. Mean fruit production/plant was 152.3 ± 33.6 fruit/plant, resulting in a low mean fruit set of 12.1 ± 2.4% per plant (mean fruit set/raceme = 12.3 ± 2.4%; *n* = 25 plants). Each of the 22 fruiting plants produced fruit that showed evidence of damage, with 43.3 ± 5.9% of all fruit counted affected (range = 15 – 100%; *n* = 22 plants). This resulted in a mean damaged fruit set of 5.8 ± 1.2% per plant (range = 0.03 – 15.7%). The wandering transect to assess the extent of the damage revealed that 31% of flowering plants produced normal fruit, 30% produced damaged fruit, while the remaining 39% of plants did not produce any fruit (*N* = 100 plants).

5.6 Discussion

Role and partitioning of specialist and generalist avian visitors

Aloe reitzii var. *reitzii* displays characteristics typical of an ornithophilous (bird) pollination syndrome, with long (~50 mm; Van Wyk and Smith 2014), tubular red flowers that produce large amounts of dilute nectar throughout the day (Faegri and van der Pijl 1979; Johnson and Nicolson 2008). Symes (2017) found that the nectar properties were more suited to specialist pollinators, although volumes and concentrations did extend into the preferred range for generalist pollinators, as was found in this study (Johnson and Nicolson 2008). This broad range of nectar properties may explain the similar visitation rates, and co-dominance, of specialist sunbirds and generalist weavers. The Malachite Sunbird was the more dominant visitor (49% of bird visits), and was more frequent than previously recorded in Symes (2017), where it only comprised 27% of all visits. Both species appeared to partition the nectar resource temporally, with the Cape Weaver dominating early morning visitation, while the

Malachite Sunbird dominated visitation from the late morning into the afternoon. The potentially shared role of both the Cape Weaver and the Malachite Sunbird in the pollination of this aloe is reinforced (Symes 2017), although assessments of the pollen-loads of the individual species, and/or pollen tracking-experiments with fluorescent dyes, are required to quantify the individual contributions of each of these species to effective cross-pollination.

The Cape Weaver is a regular visitor to winter-flowering aloes, including *Aloe ferox* (*A. candelabrum*), *A. arborescens, A. spectabilis* and *A. marlothii* (Oatley and Skead 1972; Hoffman 1988; Stokes and Yeaton 1995; Symes and Nicolson 2008; Symes *et al*. 2008; Forbes *et al*. 2009; Kuiper *et al*. 2015). Although insectivorous, most weavers are opportunistic nectar-feeders and nectar addicts, regularly feeding on the abundant nectar of many other flowering species, such as *Erythrina* and *Schotia*, amongst others (Oatley 1964; Jacot Guillarmod *et al*. 1979; Symes and Yoganand 2013; Craig 2014); the Cape Weaver is likely the same, feeding on the abundance of *Aloe* nectar during peak flowering (Kuiper *et al*. 2015). Weavers, along with some other generalist nectar-feeding species (e.g. mousebirds, bulbuls, white-eyes, etc.), may be more reliant on nectar than previously thought (Oatley 1964).

Malachite Sunbirds are commonly territorial (Skead 1967) and are known to chase other birds from food and breeding resources (Hargreaves *et al*. 2004; Geerts and Pauw 2009a). Therefore, there is likely competition between the species for nectar resources, as suggested by the high proportion of plants that were visited by the Malachite Sunbird only, as compared to the Cape Weaver. This could also explain temporal partitioning between the two species and more regular visitation by the Malachite Sunbird compared to the weaver, as well as absence of other sunbird species and sugarbirds (Geerts and Pauw 2009a). Furthermore, *Aloe reitzii* is summer flowering and insects are more active, especially at the aloe flowers,

which might explain the regular visitation throughout the day, rather than peaks in visitation at specific times.

In the Cape provinces of South Africa, many plants have evolved a "Malachite Sunbird Pollination Syndrome", where flower tubes have elongated and become exclusively pollinated by Malachite Sunbirds (Geerts and Pauw 2009a). Such flowers usually have a floral tube length ranging from 35 – 58 mm, which excludes shorter-billed birds from accessing the nectar and/or nectaries (Geerts and Pauw 2009a). Malachite Sunbird bill lengths range from 29 mm (female) to 32 mm (male; Dowsett-Lemaire 1989), and up to 51 mm with the tongue extended (Geerts and Pauw 2009a); *Aloe reitzii* floral tubes are approximately 50 mm long (Van Wyk and Smith 2014), and Malachite Sunbirds can likely easily access and drink the nectar. Floral length, along with exclusion of smaller sunbird species, may be resulting in the aloe tending towards a long-billed bird, such as the Malachite Sunbird, specialised pollination syndrome (Geerts and Pauw 2009a). However, these birds might not be effectively transferring large amounts of pollen, as bright yellow *A. reitzii* pollen was not visible on the feathers of the Malachite Sunbirds, and these birds might only be picking up small amounts of pollen on their long, narrow bills (Geerts and Pauw 2009b), or even bypassing the pollen-laden anthers altogether (Dowsett-Lemaire 1989). In *Protea*, Malachite Sunbirds tend to pick up pollen on the crown of their heads, while *Aloe boylei* pollen is transferred to the chin and bill (Hargreaves *et al*. 2004); similar *A. reitzii* pollen deposition sites are likely. Conversely, Cape Weavers forced their short bills into the flowers (from the mouth of the flower) to access nectar, and their faces and foreheads became covered in pollen (Stephanie Payne, *pers. obs.*). Although this behaviour might destroy the flower perianth (e.g. Geerts and Pauw 200b), weavers may be more effective at transferring larger amounts of *Aloe* pollen than Malachite Sunbirds (Kuiper *et al*. 2015). Exclusion experiments, pollen swabs, and detailed observations of feeding positions and behaviours are

required to assess the efficacy of each of these species at *A. reitzii* pollen transfer, and consequently, pollination.

Aloe ferox, a winter-flowering aloe, is predominantly visited by both Malachite Sunbirds and *Ploceus* weavers, both of which track the seasonal nectar resources and respond to the increase in nectar availability (Kuiper *et al*. 2015). Bird densities often increase in winter months when large aloes are in flower (Symes *et al*. 2008; Forbes *et al*. 2009; Kuiper *et al*. 2015), with sunbirds, in particular, often following a regular route (Craig and Hulley 1994). The Malachite Sunbird is a "tourist", following a regular route, but can also act as a "shopper", opportunistically feeding at seasonally available, resource rich sites (Craig and Hulley 1994; Symes *et al*. 2001). These mixed strategies of the Malachite Sunbird movements largely depend on the seasonality and proportion of plants flowering in a population (Symes *et al*. 2001). A greater proportion of *Aloe reitzii* plants were flowering in 2018 (29% of established plants), compared to 2014 (18.9%; Symes 2017), and this increase in resource availability might explain the considerable increase in Malachite Sunbird visitation between years. Larger plants are more likely to flower, signalling greater nectar rewards to visitors (e.g. Willson and Rathcke 1974; Hoffman 1988), particularly in this fragmented habitat where resources may be scarce.

Cape White-eye (*Zosterops capensis*), Fork-tailed Drongo (*Dicrurus adsimilis*), Cape Rock-Thrush (*Monticola rupestris*), Red-winged Starling (*Onychognathus morio*) and Southern Masked Weaver (*Ploceus velatus*) are well-known *Aloe* visitors and "nectar addicts" (Oatley 1964; Skead 1967; Johnson *et al*. 2006; Botes *et al*. 2008, 2009a; Forbes *et al*. 2009; Symes *et al*. 2009; Hargreaves *et al*. 2012; Arena *et al*. 2013; Patrick *et al*. 2018; Chapter 2), often tracking the increased production of nectar during peak flowering (Symes *et al*. 2008; Kuiper *et al*. 2015; Payne *et al*. 2019; Chapter 3). These species were not seen on the farm or feeding at these aloes, but have been observed in the region during January –

April (South African Bird Atlas Project 2 2018). Their absence is of interest, particularly in the case of the Cape White-eye. However, these species are primarily insectivores, and mostly feed on *Aloe* nectar in winter to supplement their diets when insects are less active (Symes *et al*. 2008). Thus, nectar may be less important in summer, and they do not rely on summer-flowering aloes as much as winter-flowering species. Their absence may also be due to the fragmentation of the aloe habitat for farming, or they are being chased away by the territorial Malachite Sunbirds (Skead 1967). Black "suikerbekkies", later identified as Amethyst Sunbirds (*Chalcomitra amethystina*) when shown a picture, have been seen feeding on *Aloe reitzii* nectar (Landowner, *pers. comm.*), but were not recorded as regular visitors in this study.

Diurnal and nocturnal insect visitors

Insects, particularly honeybees and solitary bees, are the primary pollinators of many summer-flowering aloes (Botes *et al*. 2009a, b; Wilson *et al*. 2009; Duffy *et al*. 2014), with birds playing a minor role in pollination (Botes *et al*. 2009a). Although their contribution was not quantified, honeybees were regularly recorded on *Aloe reitzii*, and were often seen on the filaments of the stamens, which protrude past the mouth of the flower, presumably drinking nectar that was running out of the flowers (Stephanie Payne, *pers. obs.*; Figure 5.1J). Exclusions are required to assess if these insects are acting as pollinators as they contact the filaments, or are nectar robbers, as they miss the pollen on the anthers. Bees are known pollen thieves in the sunbird pollinated, summer-flowering, *A. maculata*, negatively affecting fecundity (Hargreaves *et al*. 2010); a similar scenario may be occurring in *A. reitzii*, where bees are removing nectar, but not successfully transferring pollen. Beetles were usually perched on the closed flowers, possibly feeding on flower material and/or piercing flowers to gain access to the nectar (Dr James du G. Harrison, *pers. comm.*), and are likely not

influencing pollination at all. Grasshoppers are not known to pollinate *Aloe* flowers and were likely using the racemes as perches.

Symes (2017) recorded moths visiting the aloes at night, but only for approximately two hours early in the evening. In this study, hawkmoths were recorded visiting the aloes throughout the night and into the early hours of the morning. Moth-pollinated flowers are usually white, and heavily scented with nocturnal anthesis (Baker 1961; Faegri and van der Pijl 1979). *Aloe reitzii* is a scentless aloe, and although flowers do not close at night, anthesis does become slower at night, as with other *Aloe* species (Symes *et al*. 2008; Chapter 2). Although hawkmoths are important pollinators of long-tubed flowers in Africa (Johnson and Raguso 2016), moth pollination is not recorded in *Aloe*. However, the sheer abundance of moths recorded visiting these aloes is of interest, and may suggest either a role in pollination, or nectar thieving, highlighting the need for nocturnal exclusions.

Small mammal visitors

Anecdotal reports describe "rats with long, pointed snouts" visiting the aloes, on the ridge "where the big, flat rocks are" (Landowner, *pers. comm.)*. This description suggests that sengi (*Elephantulus* sp.) visit the aloes, but camera trapping on the ridge (n = 4 plants, ~824 hours), did not record any small mammal visitors. *Aloe peglerae*, a winter-flowering aloe endemic to the Magaliesberg Mountain Range, is successfully pollinated by the Namaqua Rock Mouse (*Micaelamys namaquensis*) and sengi at night, along with its primary pollinator, the diurnal Cape Rock-thrush (*Monticola rupestris*; Arena *et al*. 2013; Chapter 2). Food resources are often scarce in winter, and the small mammals may be relying on the aloe nectar as an energy source (Wiens *et al*. 1983; Johnson 2004; Chapter 2), whereas in summer, food resources are more readily available (particularly insects for the insectivorous sengi), and small mammals may not be as reliant on nectar. Also, *A. peglerae* is a smaller aloe that is

closer to the ground, allowing for more ground cover, than *A. reitzii* (*A. peglerae* height: ~ 40 cm; *A. reitzii* height: ~ 1.5 m; Jeppe 1969), and accessing the more exposed *A. reitzii* nectar may be a greater predation risk (e.g. Jensen *et al*. 2003).

5.7 Conclusion

The abundance of diurnal birds, along with diurnal and nocturnal insects suggest that multiple guilds are attracted to, and rely on, *Aloe reitzii*. This aloe produces nectar constantly throughout the day, and this is likely the major attractant for visitors, as bird visitation trends follow nectar production. Specialist and generalist nectar-feeding birds co-dominate visitation to *A. reitzii*, but do partition the nectar resource temporally, likely due to competition for resources between guilds, or territoriality. Within each of these visiting guilds, there appears to be a "specialist" visitor that dominates visitation events. Insect, bird and day-night exclusions are required to assess and quantify the contribution of each of these guilds (and species) to the pollination of this aloe. The abundance of birds from different guilds visiting *A. reitzii* is unusual, as many summer-flowering aloes are primarily insect-pollinated. Therefore, this aloe may be a true "generalist aloe", with floral characteristics (floral tube length and colour, nectar properties) that appeal to visitors from multiple feeding guilds, at all times of the day and night. This may ensure successful pollination events in its limited distribution within a heavily transformed habitat with low bird abundance/densities.

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CHAPTER SIX

Does colour matter? Pollinator visitation and reproductive output in a polymorphic endemic, *Aloe petricola*



The orange-yellow morph of *Aloe petricola* at Pullen Nature Reserve, Mpumalanga. (Stephanie Payne)

6.1 Abstract

Flower colour polymorphism is common in angiosperms, and is thought to be driven by pollinator-mediated selection for specific flower colours. This, in turn, can have consequences on reproductive output and selective pressures driving evolution. *Aloe petricola* is a polymorphic aloe, endemic to Mpumalanga province, South Africa. The red-white morph (red buds, white open flowers) and the orange-yellow morph (orange buds, yellow open flowers) are most commonly reported, with a red-yellow morph (red buds, yellow open flowers) observed in a sampled population at Pullen Nature Reserve, Mbombela. This study investigated pollinator-mediated selection of visitors to different morphs, by assessing flower visitors to, and reproductive output of, each morph. There were no morphometric or nectar differences among morphs, and generalist and specialist avian nectarivores did not preferentially select among morphs, with similar species richness and diversity indices for each morph. All three morphs were equally affected by insect parasitism with 47% of fruit predated, resulting in the destruction of seed. Reproductive output did not differ among morphs, with sub-population reproductive output as follows: 20.4% fruit set/plant, 26 seeds/fruit/plant, 2980 seeds/plant, 5.2 seeds/flower/plant (weighted means). Avian visitors do not appear to be driving the polymorphism within *A. petricola*, but are rather foraging at all available food resources during the winter flowering season. Interactions and functional trade-offs between biotic (e.g. pollinators, floral pigments) and abiotic factors (e.g. clinal differences, spatial and temporal variation in environment and visitors (birds, small mammals and insects), resource availability and use) may be driving and maintaining polymorphism, and ultimately optimising fitness, in this species.

6.2 Key words

Aloe petricola; Flower Colour; Pollination; Pollinator-mediated Selection; Polymorphism.

6.3 Introduction

Floral traits are suggested to reflect the preferences and/or adaptations of plant primary pollinator(s), as a result of selection pressure for successful pollination events (Ollerton *et al*. 2009). These traits serve either to attract potential pollinators, or to filter out inefficient pollinators (Schemske and Horvitz 1984; Thomson and Thomson 1992; Castellanos *et al*. 2004). Many studies have found that pollinators, particularly insects, select flowers based on colour (e.g. Mogford 1974; Waser and Price 1981; Stanton *et al*. 1989; Comba *et al*. 2000; Jones and Reithel 2001), while pollinator selection, particularly by birds, based on floral tube shape, nectar rewards, and floral display size is less explored (e.g. Odell *et al*. 1999; Strauss and Whittal 2006; Smith *et al*. 2008).

Different flower colour phenotypes within a species is commonly recorded in angiosperms (e.g. Smith *et al*. 2008; de Jager and Ellis 2014; Heystek *et al*. 2014; Ng and Smith 2016a, b). This is often due to a shift in the biochemical pathways that drive pigment compositions and combinations, namely anthocyanin (red, pink, purple, or blue pigments) and carotenoid (red, yellow, or orange pigments) production in the petals, causing the colour (hue) of the corolla to change (Tanaka *et al*. 2008; Ng and Smith 2016a, b). This can result in flower colour polymorphism, where individuals of the same species display different corolla colours, either coexisting in the same population or occurring in separate, geographically isolated, colour populations ("monomorphic" populations; e.g. de Jager and Ellis 2014).

Many studies advocate that directional selection for corolla colour is driven by "pollinator-mediated selection" which, in turn, maintains polymorphism within a population and/or species (e.g. Mogford 1974; Stanton 1987; Stanton *et al*. 1989; Jones and Reithel 2001). Strict preference for one specific colour morph, termed flower constancy, can result in reproductive isolation (Waser 1986; Grant 1994; Chittka *et al*. 1999), which further drives floral divergence, particularly in geographically isolated populations (Grant 1994). These

preferences may be innate, or learned, as pollinator preferences are often shaped by previous experiences (Chittka *et al*. 1999; Jones and Reithel 2001; Heystek *et al*. 2014). Larger plants and/or inflorescences (with more brightly coloured flowers) often signal greater rewards, and may encourage visitation by potential pollinators (Willson and Rathcke 1974; Willson and Price 1977; Hoffman 1988), especially in geographically isolated/fragmented populations (Kunonga *et al*. 2019).

The succulent genus *Aloe* occurs throughout Africa, into the western Indian Ocean Islands, the Arabian Peninsula and Madagascar (Grace *et al*. 2009; Carter *et al*. 2011; Cousins and Witkowski 2012). South Africa has the highest concentration and diversity of *Aloe* species (Cousins and Witkowski 2012). Aloes produce single or multiple raceme inflorescences during their flowering seasons [55% of 158 species recorded by Van Wyk and Smith (2014) flower during winter (June – August)]. These flowers are either uniform in colour throughout their lifespan (e.g. *A. pruinosa* produces flowers that remain reddish throughout their lifespan; Wilson *et al.* 2009), or they change colour as they mature and open (e.g. *A. reitzii* produces red buds that become orange or pale-yellow as they open; Van Wyk and Smith 2014; Symes 2017). Only *A. mutans* flowers change from pink to yellow once pollinated (Van Wyk and Smith 2014). However, floral colour polymorphism does exist within the genus. Thirty-seven of 158 species (23%) recorded by van Wyk and Smith (2014) display multiple colour forms/morphs within a species. This polymorphism is common in 20 species, and is a rare occurrence in 17 species (Van Wyk and Smith 2014), but remains unstudied in the genus, especially with respect to potential pollinators and pollinator-mediated selection.

The stemless aloe, *Aloe petricola* Pole-Evans is a polymorphic aloe with a single, rounded rosette of long, inwardly curved, green-grey leaves with brown, triangular teeth along the leaf margins (Jeppe 1969; Van Wyk and Smith 2014). Mature individuals produce

between one and six long, narrow racemes branched in a single inflorescence during winter (late June – early August; Jeppe 1969; Van Wyk and Smith 2014). Occasionally, two inflorescences are produced (Stephanie Payne, *pers. obs.*). Racemes are densely packed with tubular flowers, which can be up to 30 mm long. The racemes are distinctly bicoloured and two different colour morphs have been recorded. The common morph, hereafter referred to as "red-white", has buds that are red and become white as they open (Jeppe 1969; Van Wyk and Smith 2014; Figure 6.1C). A less common morph has orange buds, which become yellow as they mature and open ("orange-yellow"; Jeppe 1969; Van Wyk and Smith 2014; Figure 6.1B). There is a third morph identified, where buds are red, becoming yellow as they mature and open ("red-yellow"; Stephanie Payne, *pers. obs.*; Duncan McKenzie, *pers. comm.*; Figure 6.1D). Flower characteristics are suggestive of a bird pollination syndrome (i.e. red, orange or yellow tubular flowers with copious amounts of nectar), suggesting that the aloes are visited and/or pollinated by generalist (opportunistic) and/or specialist nectar feeding birds (Faegri and Van der Pijl 1979: Rebelo 1987).

This study investigated pollinator-mediated selection influences with respect to the proportions and maintenance of different floral polymorphs in *Aloe petricola*. The following questions were addressed: 1) Are there differences in morphometrics (apart from flower colour) and nectar properties among the three morphs? 2) What visits the aloe flowers, and do visiting species or guilds preferentially visit one colour morph over the others? 3) Does floral colour selection by visitors confer a reproductive advantage? If pollinators are showing a colour preference, then reproductive success (fruit set, mean seed per fruit, total seed production and seeds per flower) should be higher for preferentially selected morphs.

6.4 Methods and Materials

Study site and species

Aloe petricola is classified as Least Concern, despite being endemic to a small region (Extent of Occurrence: 3700 km^2 in 2009) in Mbombela (formerly Nelspruit) and immediate surrounds (Sabie, Barberton, Schoemanskloof, Krokodilpoort), Mpumalanga province, South Africa (Foden and Potter 2009; Van Wyk and Smith 2014). The species grows on sandstone slopes and granite outcrops, often in shallow, well-drained and acidic soils (Figure 6.1A; Jeppe 1969; Weisser and Deall 1989; Van Wyk and Smith 2014). This study was conducted at Pullen Nature Reserve (25°34'19" S, 31°10'53" E; 910 m a.s.l.), 35 km east of Mbombela, Mpumalanga, South Africa. This is a mountainous savanna landscape, with Malelane Mountain Bushveld vegetation (Rutherford *et al*. 2006). The area experiences summer rainfall (October – April) and annual precipitation in 2017 was 772 mm (South African Weather Service 2018). *Aloe petricola* flowers during winter (June – August), a period of low resource availability for many bird (pollinator) species. The proportion of each of the three colour morphs is unbalanced, with the uncommon orange-yellow morph being more dominant than both the red-white and red-yellow morphs (55% orange-yellow, 22% red-white and 22% red-yellow morphs). Seven sites on granite outcrops with *A. petricola* sub-populations within an altitudinal range of 171 m, were studied (Table 6.1). Sites were > 50 m apart, and/or were separated by a patch of dense bush and/or trees.

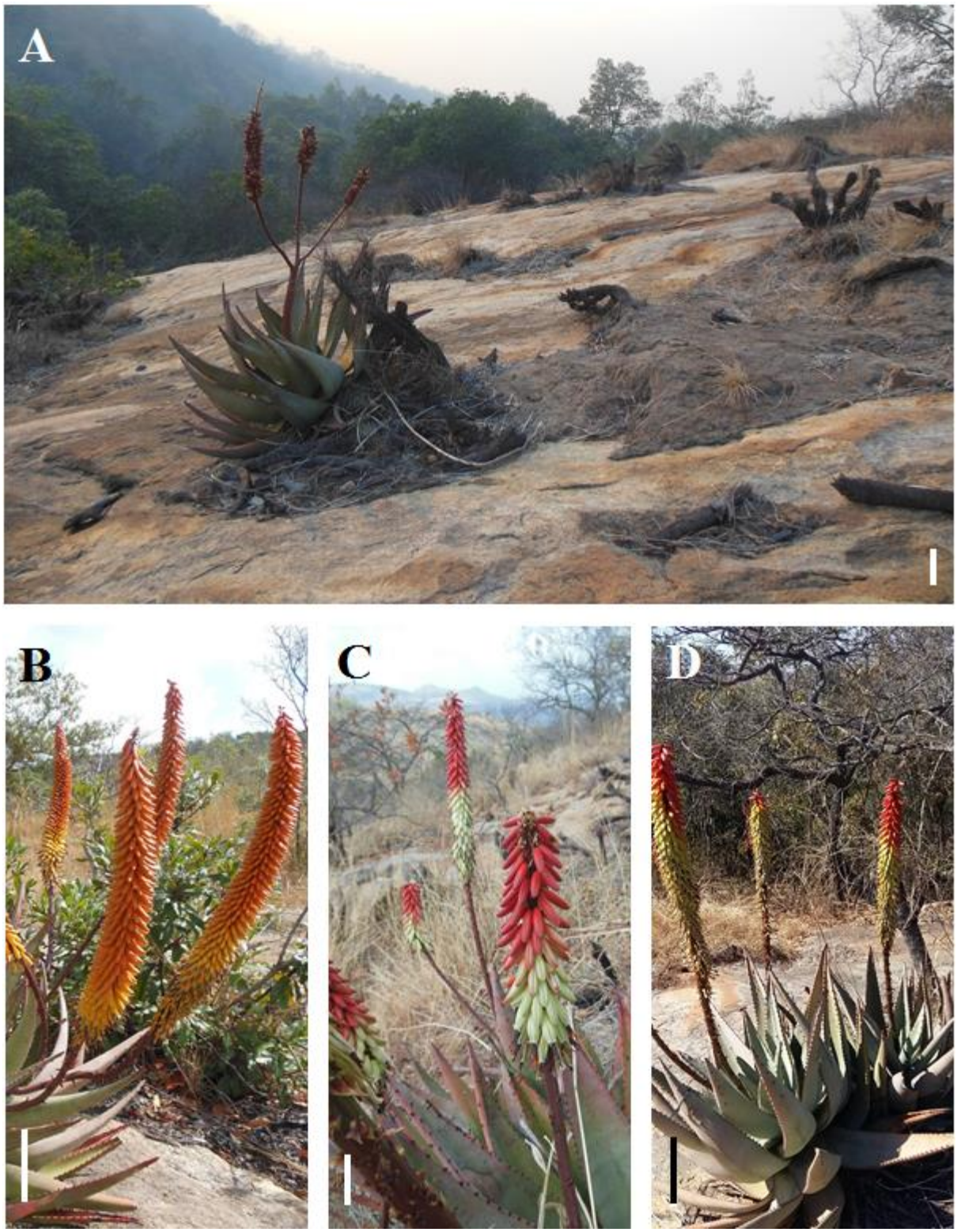


Figure 6.1. A) A fruiting, adult *Aloe petricola* plant, growing on a granite outcrop at Pullen Nature Reserve; B) The less frequently reported orange-yellow *A. petricola* morph. Buds are orange and become yellow as they mature; C) Racemes of a red-white *A. petricola* morph. This is the most commonly reported morph, with red buds that become white as they mature and open; D) A third red-yellow morph is apparent, with red buds that become yellow as they mature. Scale bars = 10 cm. Photographs A-D: Stephanie Payne.

Plant Morphometrics

Across the Nature Reserve, the location of each individual *Aloe petricola* plant was recorded using a handheld GPS (N = 115 plants; Garmin GPSMAP 60Cx, USA). Plants were classified as flowering or non-flowering, and the number of racemes was counted for flowering plants. Morph colour (orange-yellow, red-white, or red-yellow) was recorded where colour could be visually confirmed. The following measurements (in centimetres) were taken for each plant: i) mean rosette diameter (three measurements were taken at different angles across each plant); ii) diameter at the widest point of the rosette (d); iii) leaf height (from the base to top of the tallest leaf); iv) inflorescence height (from the base of the branch of the inflorescence to the top of the tallest raceme) and; v) individual raceme length (from the base of the flowers to the top of the raceme). Total plant height was calculated as the sum of leaf and inflorescence heights. Rosette circumference (C; in cm) was calculated as: $C = \pi d$. For seedlings (plants where a round rosette had not yet formed), rosette height was measured, and the number of leaves was counted.

Floral Visitors

Visitors to the three *Aloe petricola* morphs (orange-yellow, n = 14; red-white, n = six; and red-yellow, n = seven) at seven granite outcrop sites (out of nine total identified sites) were recorded during the peak of the July – August 2017 flowering season (Table 6.1), with the use of camera traps (Bushnell, models 119456, 119477, 119537 and 119740, China). Cameras, attached to tripods or strapped to trees, were placed a minimum of one metre away from the plant, facing south to minimise sun glare, and downwards to minimise vegetation movement triggering the sensors. Cameras operated day and night for the entire duration of the sampling period (15 days; 30 July 2017 – 13 August 2017), to record all visitors. Memory Cards (SanDisk 2GB, 4GB, 16GB) and batteries were checked regularly (every one – three

days) and changed as required. Cameras motion sensors were set on high sensitivity to take two photographs, when triggered, with three-second intervals between activations (Payne *et al*. 2016; Chapter 2).

An animal was considered a visitor if it touched the plant or inflorescence in any way (e.g. perching/sitting on the leaves/racemes, probing flowers, etc.), since such an action was considered sufficient to classify the animals as a potential visitor with the potential to transfer pollen (Chapter 2). Each visitor was identified to genus and, where possible, to species, and the behaviour of the visitor was classified as flower probing, or perching only. The duration of each visit was recorded, as were the movements of each visitor between racemes, on each plant. Each visit by an animal to each raceme was recorded and used to calculate visitation rate/100 racemes/hour. When calculating visitation rate/100 plants/hour, visitors were recorded as the same visitor if the individual moved between racemes in successive photographs, where ≤ 30 seconds had lapsed between photographs, or if it was in a similar position or a characteristic had remained the same after successive three-second activation intervals, (e.g. aberrant feather positioning, pollen deposition patterns; Chapter 2). Overall mean visitation rate per 100 racemes per hour (visits/100 racemes/hour) and per 100 plants per hour (visits/100 plants/hour) were calculated for each morph. Mean visitation rates for each visiting species for each morph were also calculated (visits/100 racemes/hour and visits/100 plants/hour). Mean vertebrate (bird and small mammal) species richness, Simpson's diversity, Shannon-Wiener diversity and E-evenness indices were calculated and compared among each morph.

Temperature data loggers (accurate to 0.5°C, Thermocron iButtons, Australia), were placed on tree branches, approximately at chest height, (~1.2 m) at sites two and six (n = three and four data loggers respectively) to record temperature at each site at hourly intervals throughout the sampling period.

Nectar Characteristics

Nectar volumes (μL) and concentrations (% w/w; handheld refractometer, Bellingham & Stanley Eclipse Refractometer, Model 45-81, Tunbridge Wells, UK) were compared between screened racemes of each of the three morphs. One day prior to sampling (01 August 2017), nine plants were randomly selected (*n* = two, three, and four orange-yellow, red-white, and red-yellow morphs respectively), and one raceme on each plant was covered with a fine mesh bag to exclude all visitors, including insects. Temperature data loggers (iButtons), recording temperature at 30-minute intervals, were placed on each of the sampled racemes (*N* = nine racemes). Nectar was collected from one flower per raceme (*N* = 54 screened flowers in total; sampling was destructive) in disposable haematocrit tubes (75 μL) at two hour intervals during daylight (07:00 to 17:00) on 02 August 2017. Sampled flowers had anthers which were newly dehisced, and the stigma was not fully exposed (Stage 2 of development; Symes and Nicolson 2008). Nectar extraction was conducted on a clear day, with temperatures ranging from 11.6°C (06:30) to 32.7°C (12:00). iButtons and mesh bags were removed after the final sampling session (from 17:00 onwards).

Reproductive Success

Reproductive success was represented by i) fruit set (%; proportion of flowers that set fruit), ii) mean seed/fruit, iii) total seed production and, iv) seeds/flower. Approximately four weeks after flowering (10 – 16 September 2017), a maximum of three racemes were randomly selected from each sampled plant. The number of flowers, (represented by the number of pedicels), and the number of fruit produced were counted. A maximum of five fruit were randomly selected from each plant (across the three harvested racemes), and each fruit was placed in individual, labelled envelopes and stored at ambient temperatures in a laboratory at the University of the Witwatersrand, to dehisce. Once fruit had dehisced, the

number of seeds in each fruit was counted. In some fruit, predation by insects was apparent (holes in the capsule, pupae found in the locule) and the seed in these predated locules were destroyed by the insects, to the point where the seed had disintegrated. Therefore, only whole, seeds (i.e. non-disintegrated) were counted. Number of flowers/plant, fruit set/raceme, fruit set/plant, mean seed/fruit/raceme, mean seed/fruit/plant, total seeds/raceme, total seeds/plant, seeds/flower/raceme, seed/flower/plant, and percentage fruit predated/plant were calculated as follows (Witkowski and Wilson 2001):

Number of flowers/plant = (mean flowers/raceme) × No. racemes;

$$\text{Fruit set/raceme (\%)} = \frac{\text{No. fruit harvested from raceme}}{\text{No. of flowers on raceme}} \times 100;$$

$$\text{Fruit set/plant (\%)} = \frac{(\text{mean no. fruit/raceme}) \times (\text{No. racemes})}{\text{No. flowers/plant}} \times 100;$$

Seed/fruit/raceme = (total no. of seed harvested from raceme) × (No. fruit harvested from raceme);

$$\text{Seed/fruit/plant} = \frac{\text{total no. of seed harvested from plant}}{\text{No. fruit harvested per plant}};$$

Total seed/raceme = (seed/fruit/raceme) × (No. fruit per raceme);

Total seed/plant = (seed/fruit/raceme) × (No. fruit/raceme) × (No. racemes);

$$\text{Seed/flower/raceme} = \frac{\text{Total seed/raceme}}{\text{No. flowers/raceme}};$$

$$\text{Seed/flower/plant} = \frac{\text{Total seed/plant}}{\text{No. flowers/plant}}; \text{ and}$$

$$\text{Fruit predated/plant (\%)} = \frac{\text{No. predated (harvested) fruit/plant}}{\text{No. fruit harvested/plant}} \times 100.$$

Data analysis

All datasets were tested for normality using a Shapiro-Wilks normality distribution test. Normally distributed datasets (Shapiro-Wilks $P > 0.05$) were tested among morphs using a one-way Analysis of Variance (ANOVA; see Table 6.4 in Appendix 6.1 for a detailed list of analyses), and Tukey Honest Significant Difference *post-hoc* tests were performed on datasets that were significantly different ($P < 0.05$). A non-parametric Kruskal-Wallis rank sum test was used to test for differences among morphs in datasets that were not normally distributed (Shapiro-Wilks $P < 0.05$; Table 6.4 in Appendix 6.1). Kruskal-Wallis multiple comparison *post-hoc* tests, from the R package "pgirmess" (Giradoux 2018), were performed on datasets with significant differences ($P < 0.05$). Daily minimum and maximum temperatures between sites two (938 m a.s.l.) and six (836 m a.s.l.) were compared using a Mann-Whitney *U* – test (Shapiro-Wilk normality distribution test, $P < 0.05$).

Nectar volumes of screened plants were modelled using a Generalised Linear Model (GLM) with time and colour morph as predictor variables, a Quasi-Poisson distribution to account for over-dispersion, and logit-link function. Nectar concentrations were modelled as a GLM with time and colour morph as predictor variables, a binomial distribution and a logit-link function. Altitudinal effects on the number of racemes produced by each morph were modelled with a GLM with a Poisson distribution and logit-link function, with colour morph and altitude as predictor variables. All statistical analyses were performed, and graphics created, in R (Version 3.5.1.; R Core Team, 2018), and results are presented as mean ± standard error (S.E.). Graphics were created with the packages "ggplot2", "extrafont" and "extrafontdb" (Chang 2012, 2014; Wickham 2016), and diversity indices were calculated with "BiodiversityR" (Kindt and Coe 2005).

6.5 Results

Plant Morphometrics

A total of 115 *Aloe petricola* plants were sampled (n = 83 adult plants and n = 32 seedlings) across Pullen Nature Reserve. Of the entire adult population, 70% of plants were flowering (n = 58 plants). At the time of sampling, 22 flowering aloes (38%) were almost finished flowering, and flower colours could not be visually confirmed; these aloes were excluded from further analyses. Of the remaining 36 flowering aloes, 20 aloes (56% of the known flowering population) had orange-yellow flowers, eight (22%) had red-white flowers and eight (22%) had red-yellow flowers (Table 6.2). The red-yellow aloes had significantly longer racemes (31.4 ± 2.2 cm) than orange-yellow aloes (24.5 ± 1.2 cm; $H_{2,84}$ = 6.33; P = 0.04), and had significantly more flowers than both the orange-yellow and the red-white aloes ($F_{2,25}$ = 6.90, P = 0.002; Table 6.2). The three aloe morphs did not differ significantly in any other morphological measurements (P > 0.05; Table 6.2). Seedlings have 10.2 ± 0.9 leaves per plant, with a mean diameter at the widest point of 16.38 ± 1.55 cm, and a mean leaf height of 15.50 ± 1.61 cm.

Site Five, at a higher altitude (~100 m higher; ridge slope) than Site One (valley), experienced greater fluctuations between daily minimum and maximum temperatures. Minimum daily temperatures were significantly cooler at Site Five (Site 5: 9.88 ± 0.28°C; Site 1: 11.68 ± 0.25°C; U_{75} = 276, P < 0.001), and maximum daily temperatures were significantly higher at the same site (Site 5: 30.63 ± 0.99°C; Site 1: 26.52 ± 0.67°C; U_{75} = 1021, P = 0.002). The number of racemes/plant does not differ among morphs at different altitudes (β = 0.001 ± 0.058, P = 0.99; β = -0.002 ± 0.010, P = 0.82).

Table 6.1. Seven sites (of nine identified sites; in order of altitude) within Pullen Nature Reserve were selected for visitor, nectar and reproductive success sampling. Sites were > 50 m apart and/or separated by patches of dense shrubs and/or trees. Values in parentheses are the number of aloes sampled in each site. Some flowering aloes were not suitable for sampling since they were either about to complete flowering or were damaged (n = 15 out of 48 flowering aloes in the seven selected sites; e.g. broken inflorescences). Mean ± S.E. altitude is presented.

Site	**Elevation (m a.s.l.)**	**Number of aloes**	**Number of flowering aloes**	**Orange-Yellow Aloes**	**Red-White Aloes**	**Red-Yellow Aloes**
1	836	17	13 (6)*	7 (5)	2 (1)	0
2	847	19	6 (6)	1 (1)	5 (5)	0
3	847	6	6 (5)*	6 (5)	0	0
4	868	10	4 (4)	2 (2)	0	2 (2)
5	938	23	6 (4)*	1 (0)	0	5 (4)
6	949	1	1 (1)	1 (1)	0	0
7	1007	17	12 (1)*	0	0	1 (1)
Total	899 ± 25	93	48 (27)	18 (14)	7 (6)	8 (7)

*The sum of Red-White, Orange-Yellow and Red-Yellow aloes does not equal the total number of flowering aloes, since the morph colours of some of the flowering aloes could not be visually confirmed as the aloes had completed flowering (i.e. most flowers had wilted) or the racemes were broken and/or aborted.

Table 6.2. Morphological characteristics (mean ± S.E., where possible) of the three colour morphs of flowering *Aloe petricola* plants within Pullen Nature Reserve. "Flowering individuals" are those individuals whose morph colour could be positively identified (n = 36 plants); the colour of other flowering plants could not be visually confirmed at time of sampling (n = 22 plants; N = 58 flowering plants in total).

	Orange-Yellow	**Red-White**	**Red-Yellow**
Number of flowering individuals	20	8	8
Proportion of flowering population (%)	56	22	22
Rosette diameter* (cm)	57.4 ± 4.6	60.1 ± 5.5	68.6 ± 3.9
Rosette diameter at widest point (cm)	69.4 ± 4.0	59.8 ± 8.3	72.3 ± 3.3
Rosette circumference (cm)**	218.0 ± 12.4	187.8 ± 26.1	227.2 ± 10.4
Total height (leaf height + inflorescence height; cm)	111.3 ± 4.1	108.1 ± 3.9	117.6 ± 6.7
Number of racemes per plant	2.6 ± 0.4	2.6 ± 0.3	2.1 ± 0.6
Raceme length (cm)	24.5 ± 1.2	25.8 ± 2.9	31.4 ± 2.2
Number of flowers per raceme[#]	197.1 ± 21.8 (n = 14)	167.2 ± 20.5 (n = 6)	323.7 ± 41.7 (n = 7)
Number of flowers per plant[#]	448.9 ± 49.5 (n = 14)	481.0 ± 65.6 (n = 6)	645.9 ± 168.9 (n = 7)
Nectar volume[₮] (µL)	109.0 (n = 2)	104.3 ± 28.1 (n = 3)	83.3 ± 6.1 (n = 4)
Nectar concentration[₮] (% w/w)	8.8 (n = 2)	9.3 ± 0.9 (n = 3)	8.3 ± 1.3 (n = 4)

*Rosette diameter is calculated as the average of three measurements taken at three different diameters of the plant.
**Rosette circumference is calculated as $C = \pi d$ where d is the rosette diameter at the widest point.
[#] Number of flowers was only counted for plants that were sampled with camera traps for visitors. The numbers of sampled plants are in parentheses.
[₮] Mean measurements of nectar volumes and concentrations are of plants screened with fine mesh bags, sampled at 07:00, which is the period of peak bird visitation (see later). The numbers of sampled plants are in parentheses.

Floral Visitors

Visitation rates per raceme were significantly higher on red-white racemes (3.39 ± 0.92 visits/100 racemes/hour) than both the orange-yellow (1.28 ± 0.34 visits/100 racemes/hour), and the red-yellow (1.23 ± 0.43 visits/100 racemes/hour) morphs ($H_{2,62}$ = 9.02; P = 0.01; Kruskal-Wallis Multiple Comparison, $P < 0.05$). However, visitation rates per 100 plants did not differ between morphs ($H_{2,24}$ = 3.77, P = 0.15; Table 6.3), possibly as a result of fewer red-white individuals flowering overall.

Six generalist (opportunistic) bird species, and four specialist nectar-feeding bird species were recorded visiting the three morphs (N = 343 visits overall). Dark-capped Bulbul (*Pycnonotus tricolor*) was the most common visitor at all three morphs, contributing 43.2% of all visits across the three morphs (Table 6.3; Figure 6.2A). Other generalist species recorded include Cape White-eye (*Zosterops capensis*), Black-headed Oriole (*Oriolus larvatus*), Cape Glossy Starling (*Lamprotornis nitens*), and Red-headed Weaver (*Anaplectes rubriceps*; Table 6.3; Figure 6.2B, 6.2C and 6.2E). *Ploceus* weavers were recorded visiting red-white and red-yellow aloes on three separate occasions, but the birds could not be identified to species from photographs (Table 6.3).

White-bellied Sunbird (*Cinnyris talatala*) was the most common specialist nectar feeding species, contributing 10.2% (n = 35 visits) of all visits across the three morphs (Table 6.3). Other nectarivores recorded include the Amethyst Sunbird (*Chalcomitra amethystina*), and one other sunbird species that could not be identified further (Table 6.3; Figure 6.2E). One Collared Sunbird (*Hedydipna collaris*) was recorded perching, but not probing on one orange-yellow aloe (Table 6.3). Mice (*Micaelamys* sp.) were recorded moving around among the leaves of five aloes, and were recorded climbing along, and potentially feeding on the nectar of, two aloe racemes (Table 6.3; Figure 6.2D). All mice visits occurred at aloes at the

edges of the granite outcrops, where aloes grew next to shrubs (Stephanie Payne, *pers. obs.*). Although excluded from further analyses, honeybees (*Apis mellifera*) and butterflies, particularly the Citrus Swallowtail butterfly (*Papilio demodocus*), visited the aloes.

More specialist nectar feeders were recorded at the orange-yellow morph (five species) compared to the other two morphs (three species each; Table 6.3). Conversely, more generalist birds visited the red-white morph (six species), compared to both the orange-yellow (four species), and the red-yellow morphs (two species). Certain bird species do appear to preferentially visit one morph over the others. The White-bellied Sunbird contributed 69.8% of specialist visits to the red-yellow morph, while contributing < 3% of visits to either of the other two morphs (Table 6.3). The Black-headed Oriole did not visit the red-yellow morph at all, but regularly visited the other two morphs, while the Red-headed Weaver only visited the red-white morph (Table 6.3). However, overall visitor species richness did not differ among morphs ($H_{2,21}$ = 2.74, P = 0.25), and both Simpson's and Shannon-Wiener diversity indices were similar among morphs ($H_{2,21}$ = 6.1, P = 0.05; $F_{2,21}$ = 2.09, P = 0.15 respectively; Table 6.3). E-evenness was significantly higher in the orange-yellow morph than both the red-white and red-yellow morphs ($F_{2,21}$ = 6.87, P = 0.005; Table 6.3), likely due to the larger number of orange-yellow plants sampled. Honeybees and butterflies, were observed visiting the plants. Butterflies appeared to visit the red-white colour morphs more than the other morphs (70% of all recorded butterfly visits; N = 60 visits across all three morphs). However, since insects are known to play a negligible role in the pollination of winter flowering aloes (e.g. Botes *et al*. 2009a; Symes *et al*. 2009; Arena *et al*. 2013), and due to the difficulty of identifying insects on camera traps photographs, the contributions of insects were not considered further.

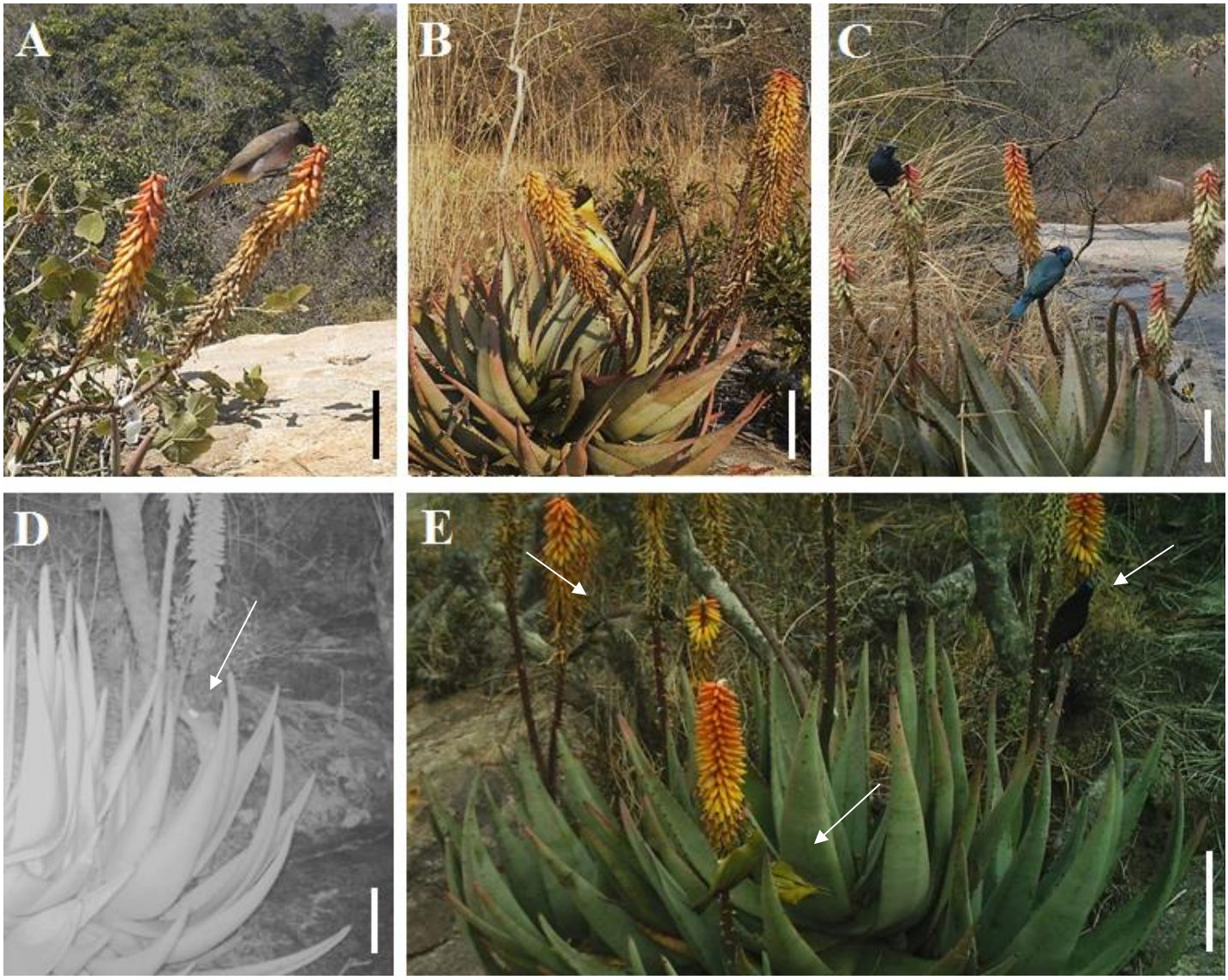


Figure 6.2. Vertebrate visitors to *Aloe petricola*. A) Dark-capped Bulbul (*Pycnonotus tricolor*) on an orange-yellow *A. petricola* colour morph. This species was the most commonly recorded visitor; B) Black-headed Oriole (*Oriolus larvatus*) perched on an orange-yellow raceme; C) Cape Glossy Starlings (*Lamprotornis nitens*) each perched on an orange-yellow and red-white *A. petricola* morph; D) A mouse (*Micaelamys* sp.) sitting on the leaves of an *A. petricola* plant, and reaching up to the raceme (indicated by arrow); E) Dark-capped Bulbul (third raceme from left), Cape White-eyes (*Zosterops capensis*; bottom front raceme) and Amethyst Sunbird (*Chalcomitra amethystina*; far right raceme) feeding on a cluster of five orange-yellow *A. petricola* plants simultaneously (indicated by arrows). Scale bars = 10 cm. Photographs A-E: Camera trap photographs by Stephanie Payne.

Table 6.3. Percentage visits to three *Aloe petricola* morphs (orange-yellow, red-white and red-yellow), during the flowering season of 2017. Values in parentheses are visitation rates (visits/100 plants/hour; mean ± S.E.). An animal was recorded as a visitor if it touched the plant or inflorescence in any way (e.g. perched on the leaves, fed on nectar, etc.). Significant differences in specific species' visitation rates among morphs are indicated by superscript letters (Kruskal Wallis Rank Sum test, $P < 0.05$). Overall visitation rates did not differ between morphs (Kruskal Wallis Rank Sum test $P > 0.05$). Bird taxonomy follows Hockey *et al*. (2005), and small mammal taxonomy follows Skinner and Chimimba (2005). Diversity indices are presented (mean ± S.E.); significant differences among morphs are indicated with superscript letters (ANOVA, $P < 0.05$).

	Orange-yellow	**Red-white**	**Red-yellow**
Total Camera hours	3789	1856	1898
Number of plants sampled	14	6	7
BIRDS			
NECTARINIIDAE			
Amethyst Sunbird	2.5	8.3	9.3
Chalcomitra amethystina	(0.06 ± 0.05)	(0.69 ± 0.42)	(0.18 ± 0.09)
Collared Sunbird	0.8	0	0
Hedydipna collaris	(0.02 ± 0.02)	(0)	(0)
White-bellied Sunbird	2.5	1.1	69.8
Cinnyris talatala	(0.14 ± 0.10)	(0.11 ± 0.07)	(1.14 ± 0.63)
Sunbird spp.	0.84	2.21	2.33
Sunbird spp.	(0.02 ± 0.02)	(0.21 ± 0.11)	(0.06 ± 0.06)
ORIOLIDAE			
Black-headed Oriole	12.6	13.3	0
Oriolus larvatus	$(0.33 \pm 0.14)^{ab}$	$(1.01 \pm 0.39)^{a}$	$(0)^{b}$
PLOCEIDAE			
Red-headed Weaver	0	7.2	0
Anaplectes rubriceps	(0)	(0.69 ± 0.52)	(0)
Weaver spp.	0	0.55	4.65
Ploceus spp.	(0)	(0.06 ± 0.06)	(0.12 ± 0.08)
PYCNONOTIDAE			
Dark-capped Bulbul	38.7	53.0	14.0
Pycnonotus tricolor	(1.32 ± 0.66)	(4.46 ± 2.74)	(0.26 ± 0.17)
STURNIDAE			
Cape Glossy Starling	5.9	1.1	0
Lamprotornis nitens	(0.16 ± 0.11)	(0.11 ± 0.07)	(0)
ZOSTEROPIDAE			
Cape White-eye	15.2	13.3	0
Zosterops capensis	(0.61 ± 0.24)	(1.22 ± 0.64)	(0)
MAMMALS			

	Orange-yellow	Red-white	Red-yellow
MURIDAE			
Micaelamys sp.	21.0 (0.56 ± 0.22)	0 (0)	0 (0)
Species richness	3.77 ± 0.46	5.50 ± 0.85	3.80 ± 0.80
Simpson's diversity index	0.54 ± 0.05	0.58 ± 0.07	0.34 ± 0.09
Shannon-Wiener diversity index	1.06 ± 0.13	1.17 ± 0.17	0.66 ± 0.18
E-evenness index	0.80 ± 0.04[a]	0.63 ± 0.04[b]	0.57 ± 0.05[b]
Total number of visits	**119**	**181**	**43**
Overall mean visitation rate	**3.237 ± 0.834**	**8.552 ± 4.499**	**1.870 ± 0.838**

Nectar Characteristics

Nectar volumes do not differ among morphs (β = 0-0.12 ± 0.18, $P = 0.50$; β = -0.21 ± 0.17, $P = 0.22$), and do not change over time (β = 0.15 ± 0.18, $P = 0.42$; Figure 6.3A). There is also no significant interaction between nectar volumes for each morph and time, suggesting that each morph is equally affected by time (β = -0.16 ± 0.24, $P = 0.51$; β = -0.03 ± 0.23, $P = 0.88$). Nectar volumes at peak bird visitation times for the aloe species (all three morphs combined) were 96.0 ± 9.6 µL at 07:00 and 72.3 ± 11.3 µL at 15:00.

Nectar concentrations do not differ among the three morphs (β = 0.49 ± 0.13, $P = 0.71$; β = -0.02 ± 0.13, $P = 0.86$), at any time of the day (β = -0.10 ± 0.14, $P = 0.46$; Figure 6.3B). Nectar concentrations for each morph are equally affected by time, as there is no significant interaction between nectar concentration and time (β = -0.07 ± 0.18, $P = 0.72$; β = 0.03 ± 0.17, $P = 0.85$). Combined nectar concentrations were 8.7 ± 0.6 % and 9.6 ± 0.4 % at 07:00 and 15:00 respectively.

Reproductive Success

Although red-yellow plants had significantly more flowers per raceme than both orange-yellow and red-white plants, number of flowers/plant did not differ among morphs ($F_{2,24}$ = 1.26, P = 0.30; Table 6.2). Fruit set/raceme (weighted mean: 18.6%) and fruit set/plant (weighted mean: 20.5%) did not differ among the three morphs ($H_{2,55}$ = 0.76, P = 0.68 and $F_{2,24}$ = 0.03, P = 0.97 respectively; Figure 6.4A). All sampled red-white plants produced fruit (n = six plants), while only one of each of the sampled orange-yellow and red-yellow plants did not produce fruit (n = 14 and n = seven plants respectively).

Fruit showed evidence of predation and insects found in the fruit and in the envelopes included Diptera and Hymenoptera. However, the percentage of predated fruit/plant did not differ among morphs (48%, 57% and 47% predation per plant in orange-yellow, red-white, and red-yellow morphs respectively; $H_{2,24}$ = 0.92, P = 0.63). All fruit produced similar numbers of intact seed, at a raceme level, with mean seed/fruit/raceme not differing among morphs ($H_{2,55}$ = 1.09, P = 0.52); Figure 6.4B). At a plant level, mean seed/fruit/plant differed between morphs ($H_{2,24}$ = 6.16, P = 0.045), but *post-hoc* testing revealed that there were no significant differences among morphs (Kruskal-Wallis Multiple Comparison Test, $P > 0.05$). Total seed/raceme and total seed/plant did not differ among morphs ($H_{2,55}$ = 2.22, P = 0.33; $F_{2,24}$ = 0.54, P = 0.59 respectively; Figure 6.4C). There was no difference among morphs between seeds/flower/raceme and seeds/flower/plant ($H_{2,55}$ = 0.018, P = 0.62; $F_{2,24}$ = 0.003, P = 0.99 respectively; Figure 6.4D).

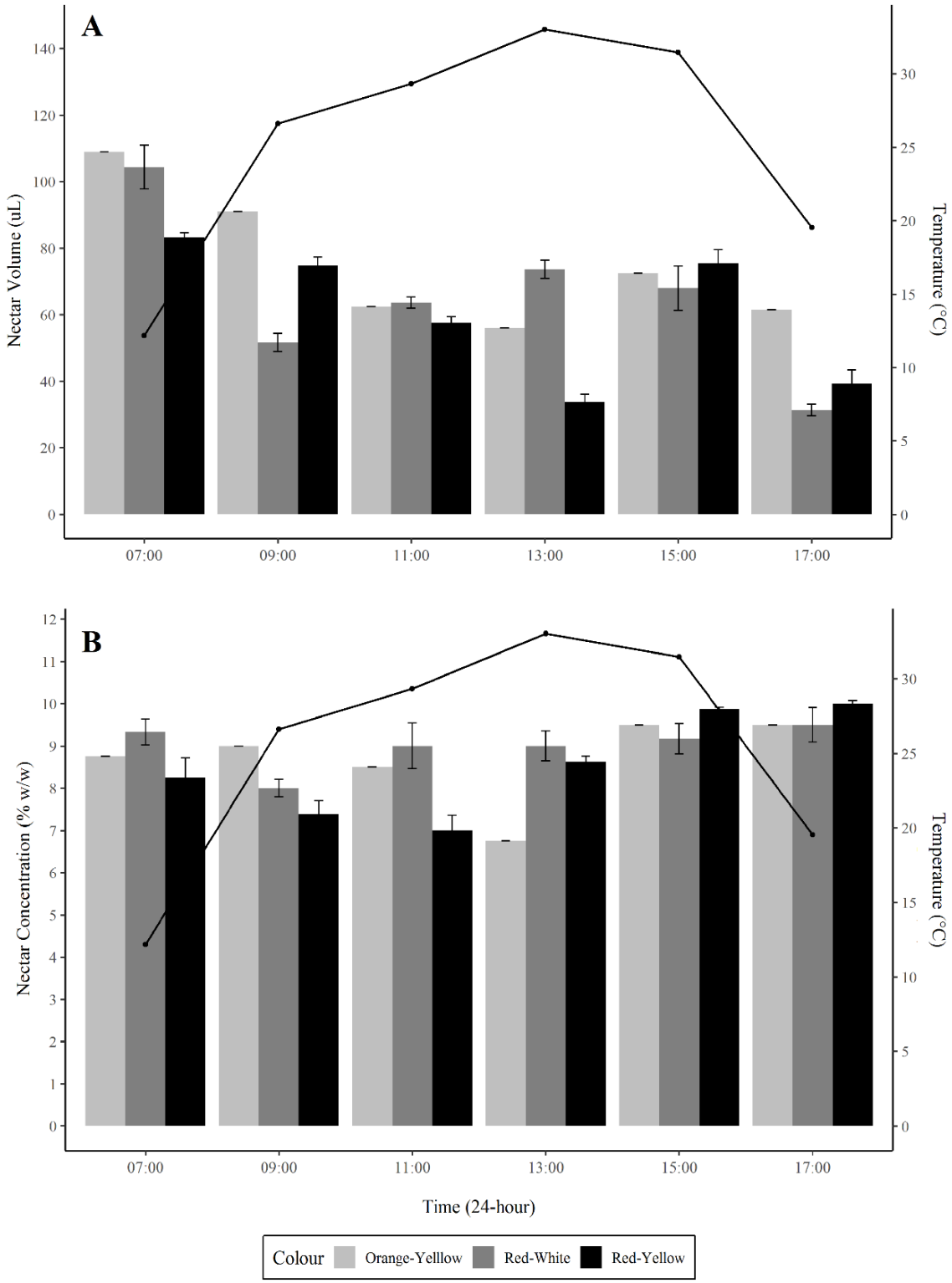


Figure 6.3. A) Nectar volume (µL), and B) Nectar Concentration (% w/w) (mean ±S.E., where possible) of three *Aloe petricola* morphs, sampled every two hours over a 12-hour daylight period on 02 August 2017 (*N* = two, three and four orange-yellow, red-white, red-yellow *A. petricola* morphs respectively). Plants were covered with fine mesh bags to exclude all visitors one day prior to, and during, sampling. Nectar volumes and concentrations did not differ among morphs or among time periods (Generalised Linear Models, $P > 0.05$). Mean temperature (°C) is represented by the solid black line. Standard errors for mean hourly temperatures were < 1°C, and could not be plotted on the graphs.

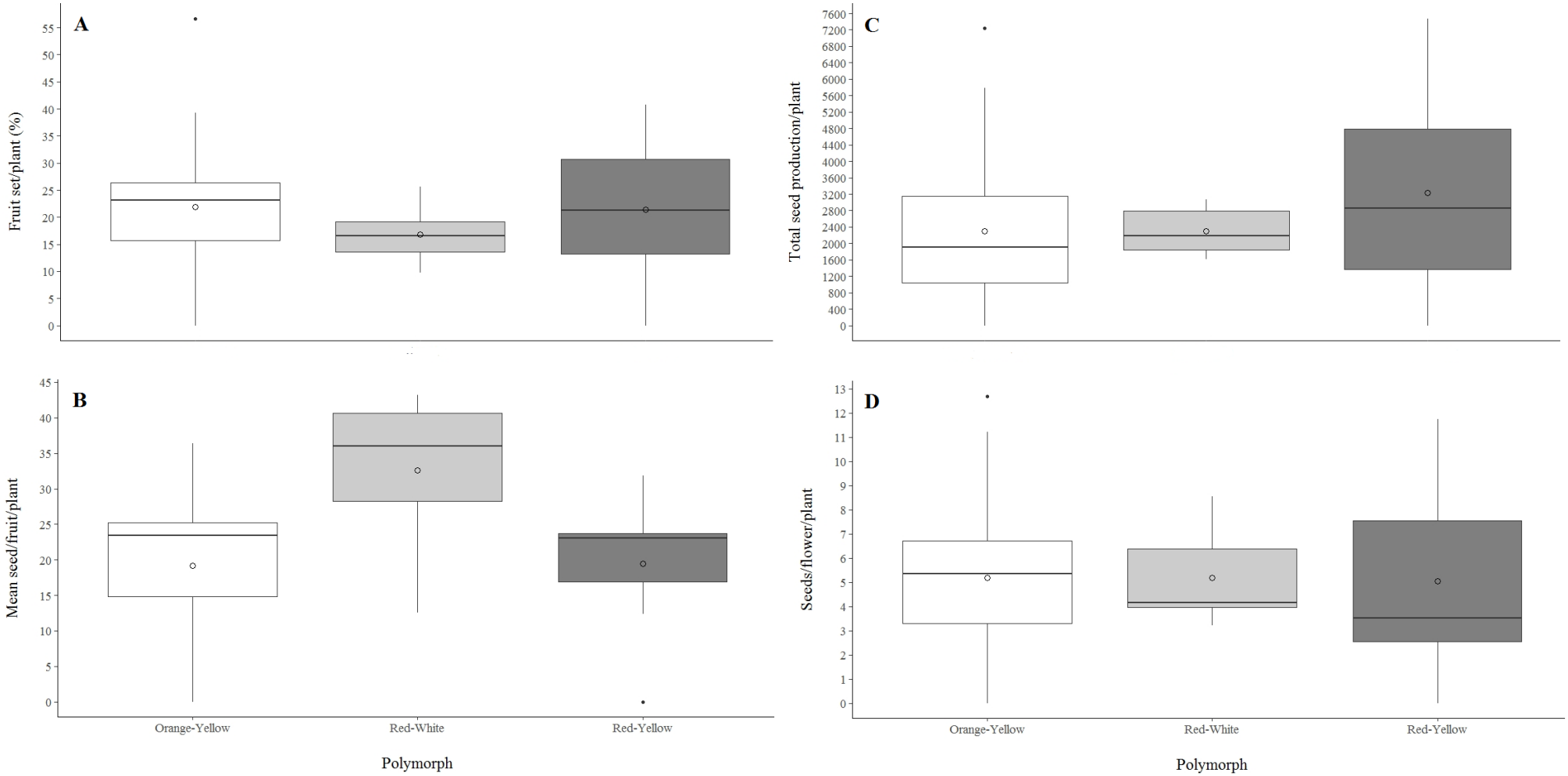


Figure 6.4. Reproductive success of three *Aloe petricola* morphs (n = 14 orange-yellow plants, six red-white and seven red-yellow), as measured by: A) Fruit set/plant (%; proportion of flowers that set fruit); B) mean seed/fruit/plant; C) total seed/plant; and D) seeds/flower/plant. Boxes represent median and interquartile range, while bars represent minimum and maximum values. Solid circles represent outliers, and open circles represent means. There were no significant differences among morphs for any measure of reproductive success (Kruskal Wallis Rank Sum Test or One-way Analysis of Variance $P > 0.05$), except for mean seed/fruit/plant ($H_{2,24} = 6.16$, $P = 0.045$), but *post-hoc* testing showed no significant difference (Kruskal-Wallis Multiple Comparison test, $P > 0.05$).

6.6 Discussion

Floral visitors to plant morphs

Plant morphometrics and nectar characteristics did not differ among the three *Aloe petricola* morphs, with the exception of the perianth colour, and the longer racemes with more flowers in the red-yellow morph. In *Iochroma* (nightshades, Solanaceae), display size, rather than corolla colour, was positively linked to pollinator visitation, suggesting that a larger display is indicative of a greater reward, which may serve to attract pollinators more than colour would (Smith *et al.* 2008). However, bird species richness, diversity and visitation did not differ between the morphs, suggesting that the birds are not selecting based on colour, inflorescence size, or reward. Although overall species richness did not differ among morphs, different bird guilds appear to preferentially visit different plant colour morphs.

Nectar is an important dietary resource for nectarivorous birds, particularly generalist bird species, and birds may simply be foraging for available food resources in the resource-limited winter, when free-standing water is minimal (Symes *et al*. 2008, 2011). Generalist insectivores may also supplement their diets with nectar in the early mornings and late afternoons, particularly in the resource-limited winter months, switching to feed on insects as the day warms up and the insects become more active (Symes *et al*. 2008). The red and orange flower colours are a signal of high caloric reward, and birds are easily able to distinguish such colours (Raven 1972), especially when these colours are in stark contrast to the surrounding brown, winter grassland vegetation. The aloes are likely the main nectar resource in the area, as the only other nectar-rich plants flowering at the same time were the trees *Erythrina lysistemon* and *Dombeya rotundifolia* (Stephanie Payne, *pers. obs.*).

Mice were recorded moving around the aloes and on the leaves on multiple occasions, but only contacting the aloe racemes of separate orange-yellow plants twice. On both occasions, the plants were near to bushes and shrubs, which may have offered the mice protection (Kotler *et al*. 1991; Hughes *et al*. 1994). The Namaqua Rock Mouse (*Micaelamys namaquensis*) and sengi (*Elephantulus* sp.) have been recorded visiting, and successfully pollinating, the grassland endemic, *Aloe peglerae* (Chapter 2), while the Round-eared Elephant Shrew (*Macroscelides proboscideus*) visits *A. claviflora* at night (Arena 2018). These small mammals are likely visiting the aloes in search of additional food resources in winter (Chapter 2). Small mammals may play a role in the pollination of *A. petricola*, but not to the same extent as that of other *Aloe* species, since *A. petricola* is more exposed along the sparsely vegetated granite outcrops, and is taller than the other *Aloe* species (i.e. combined mean plant height for all flowering *A. petricola* morphs is 102.8 ± 3.1 cm, while *A. peglerae* has a mean flowering plant height of only 39.9 ± 0.6 cm; Gina Arena, *unpubl. data)*, posing a greater predation risk for the small mammals (e.g. Jensen *et al*. 2003). Small mammals also have smaller home-ranges (e.g. Ribble and Perrin 2005), and some of the aloes may be outside their foraging and/or home-range.

Insects play a negligible role in the pollination of many winter-flowering *Aloe* species, especially those that display flowers typical of a bird pollination syndrome, as with *A. petricola* (e.g. *A. divaricata*, Ratsirarson 1995; *A. vryheidensis*, Johnson *et al*. 2006; *A. africana, A. speciosa, A. ferox,* Botes *et al.* 2009a; *A. marlothii,* Symes *et al*. 2009; *A. peglerae*, Arena *et al*. 2013). However, insects, particularly nectar- and pollen-collecting honeybees, are efficient pollinators of *A. minima, A. linearifolia* (Botes *et al*. 2009b), *A. greatheadii* var. *davyana* (Symes *et al*. 2009), *A. tenuior* (Duffy *et al*. 2014) and *A. thraskii* (Patrick *et al*. 2018), with *A. inconspicua* exclusively pollinated by insects (Hargreaves *et al*. 2008), despite some of these species displaying flowers suggestive of a bird-pollination

syndrome. Butterflies have not previously been shown to play a role in the pollination of aloes, but since butterflies are attracted to brightly coloured flowers (Faegri and van der Pijl 1979), they may be more important for the pollination of this *Aloe* species than assumed. Since genetic flower colour polymorphism is frequent in insect-pollinated species (Ernst 1987), exclusion experiments are required to assess the relative contributions of insects (both bees and butterflies) and birds to the pollination - and subsequent persistence of polymorphism - of each of the *A. petricola* morphs.

Although not different among morphs at any time of the day, nectar was dilute and produced in copious amounts, indicative of a generalist bird pollination syndrome (40 – 100 µL, 8 – 12 %; Johnson and Nicolson 2008). Indeed, six generalist bird species, the most common being the Dark-capped Bulbul (a regular visitor to numerous *Aloe* species; Oatley and Skead 1972; Johnson *et al*. 2006; Arena *et al*. 2013; Kuiper *et al*. 2015; Chapter 2), were recorded visiting *A. petricola*, supporting the generalist bird pollination system of this aloe. Three specialist species were identified and recorded visiting the aloes. These three species and two additional species, Greater Double-collared Sunbird (*Cinnyris afer*) and Scarlet-chested Sunbird (*Chalcomitra senegalensis*) were recorded visiting nearby *Erythrina lysistemon* trees (*n* = seven trees) that flower at the same time (Stephanie Payne, *pers. obs.*). Since *Erythrina* nectar is highly attractive to specialist nectar feeders, with high sugar concentrations (Symes *et al*., *unpubl. data*.), the specialist nectar feeders may have been drawn to the preferable *Erythrina* nectar resource.

Effect of plant reproductive output on polymorphism

Reproductive output (fruit set, mean seed/fruit, total seed production, and seed/flower) did not differ among morphs. This may be as a result of similar visitation rates among morphs. Ernst (1987) found that seed quality differed between red and white *Digitalis*

purpurea morphs, with the rare white morph producing fewer seeds that took longer to germinate than those produced by the red morph. White seedlings developed slower than red ones, and were subsequently outcompeted (Ernst 1987). Only a small sample of fruit was harvested from the sampled *A. petricola* plants (4.2%, 7.4%, and 3.3% of all fruit produced by orange-yellow, red-white, and red-yellow morphs respectively). Were more fruit harvested for seed collection, a difference in seed production among the morphs may have been apparent; the success and persistence of the morphs may lie in the seeds, rather than pollinator selection for floral colour (Ernst 1987; Wolfe 1993). Seed germination trials are required to test the viability of seed from each of the morphs.

Overall, 46% of harvested fruit were predated by insects, resulting in the damage and loss of seed – and subsequently a decrease in the calculated seed/fruit, total seed produced and seeds/flower – in the predated ovules. Fruit (and seed) predation by insects is not uncommon within the genus *Aloe*, and has been reported in *A. pretoriensis* (Van den Bosch *et al*. 2019), *A. peglerae* and *A. reitzii* (Stephanie Payne, *pers. obs*.). Wasp parasitoids (*Eurytoma* and *Mesopolobus* spp.), and Dropsophilid flies are the most common parasites of aloe fruit and seeds, and result in a significant reduction in the reproductive output of the aloes (Van den Bosch *et al*. 2019). There may be antagonistic selection for flower colour, where pollinators, florivores, and seed predators select plants based on the same traits (Strauss and Whittal 2006). De Jager and Ellis (2014) suggest that antagonistic selection maintains polymorphism in a population, as the morphs less favoured by predators/florivores persist since they do not experience any ovule and/or seed predation.

Other influences

Given that visitors to the aloes do not appear to be discriminating among *Aloe petricola* plants based on colour, pollinator-mediated selection is not likely to be driving and

maintaining the polymorphism within the species and population. Multiple other factors, both intrinsic and extrinsic, may be at play. Many *Aloe* species are self-incompatible and reliant on external pollen vectors for successful pollination events (e.g. *Aloe ferox,* Hoffman 1988; *A. pluridens, A. lineata* var. *muirii, A. africana, A. speciosa*, Botes *et al*. 2009a; *A. marlothii* and *A. greatheadii* var. *davyana*, Symes *et al*. 2009; *A. pruinosa*, Wilson *et al*. 2009). *Aloe thraskii* is fully self-compatible, but still reliant on pollinators for seed set (i.e. allogamous; Patrick *et al*. 2018), while *A. vryheidensis* and *A. peglerae* may be somewhat self-compatible, but fecundity is reduced when selfed (Johnson *et al*. 2006; Arena *et al*. 2013). Although breeding system experiments were not performed on *A. petricola*, inbreeding depression may be resulting in the dominance of one morph over the other (and hence the under-representation of the other morphs), and polymorphism may be genetically controlled (Wolfe 1993), should the species be self-compatible (allogamous). Self-compatible species may be more responsive to abiotic (non-pollinator) factors, as they are less subjected to pollinator-mediated selection (Strauss and Whittal 2006).

Spatial and temporal changes, as well as variation in visitor/pollinator abundance, and visitation rates, across the range of *A. petricola* may generate differences in species traits (e.g. flower colour), as well as differences in morph proportions (Jones and Reithel 2001; Schemske and Bierzychudek 2001; Strauss and Whittal 2006; Arista *et al*. 2013; de Jager and Ellis 2014). At Pullen Nature Reserve, sampled *A. petricola* plants had an altitudinal range of 171 m, with temperatures more consistent and slightly warmer at lower altitudes. Although there was no change in the proportion of each morph, nor the number of racemes produced, across the altitudinal range of the sampled sites, altitudinal and spatial changes in habitat types (i.e. high rocky outcrops *versus* valleys with trees and shrubs) across the Reserve itself may be affecting the composition of visitor communities over time. Temporal and spatial changes (regional morph selection by visitors) in visiting/pollinating species may maintain

polymorphism within the plant species (Hannan 1981; Jones and Reithel 2001), but changes in visitor communities over multiple flowering seasons has not been recorded in this species. A long-term study may prove valuable, as aloes are long-lived, recruitment is slow, and flowering is very erratic (Scholes 1988; Weisser and Deall 1989; Arena *et al*. 2015), and seasonal variation in the proportion of plants flowering affects pollinator presence (Payne *et al*. 2019; Chapter 3). Geographical variation may also exist in antagonistic herbivores/florivores (e.g. ovule predation; de Jager and Ellis 2014).

Environmental factors may cause shifts in the colour compositions of polymorphic populations (Arista *et al*. 2013). An 11-year study by Schemske and Bierzychudek (2001) suggested that floral genotype (and consequently, phenotype) of *Linanthus parryae* was linked to environmental factors, most likely precipitation. Plant tissue of the rare white *L. parryae* morph contained more toxic magnesium and selenium cations than the common blue morph (Schemske and Bierzychudek 2001). In years with low precipitation and soil moisture (i.e. high water stress), high concentrations of these cations in the white flowers would reduce the fitness of these morphs, causing the blue morph to be more successful in that flowering season (Schemske and Bierzychudek 2001). However, in seasons with high rainfall, the cation concentrations are diluted and affect the white morphs less, while blue morphs may have lower fitness, since their adaptation to exclude toxic cations impedes their absorption of favourable minerals (Schemske and Bierzychudek 2001). Such a long-term study of *A. petricola* would be able to assess fluctuations in the proportions of each morph, in relation to abiotic factors, over time.

Anthocyanins, as well as being herbivore deterrents (Fineblum and Rausher 1997; de Jager and Ellis 2014), are also associated with drought resistant species (e.g. Warren and Mackenzie 2001). Warren and Mackenzie (2001) found that anthocyanin-containing morphs (within floral and vegetative tissue) of *Cirsium palustris* and *Digitalis purpurea*, amongst

other species, were more tolerant of drought and/or heat, making them more successful in years of high abiotic stress (Strauss and Whittal 2006). Different morphs may cope better under different environmental conditions (e.g. Schemske and Bierzychudek 2001; Warren and Mackenzie 2001; Arista *et al*. 2013), allowing for the dominance of one morph, particularly those with higher anthocyanin concentrations, over the others within the sampled populations. The proportions of the three morphs may be different in other *A. petricola* populations that experience different environmental conditions. Anthocyanin and/or carotenoid concentrations, and the biochemical pathways of the pigment production, both of floral and vegetative tissue of the different *A. petricola* morphs remain to be tested.

6.7 Conclusion

Apart from flower perianth colour, individuals of the three different *Aloe petricola* morphs did not differ. Flower colour polymorphism may not confer a reproductive advantage; pollinator-mediated selection for flower colour did not occur in the sampled population and, overall, visitors across the study population did not discriminate between colour morphs, but rather foraged at all available food resources. Consequently, reproductive success did not differ among the morphs either. Breeding system and exclusion experiments are required to determine the species' dependence on external pollinators. Floral colour traits may not be adapted to attract pollinators at all ("non-adaptation"), or the floral traits have been slow to respond to shifts in pollinator systems ("non-equilibrium"; Smith *et al*. 2008). Other biotic or abiotic factors (non-pollinator mediated selection), and the interactions thereof, may be playing a role in the divergence of the morph colours and the dominance of the uncommon form over the common ones. Clinal differences, both within the sampled population, and across the species' range, may indicate which abiotic factors may be driving, and maintaining, polymorphism within *A. petricola*.

Many studies have isolated pollinator-mediated selection as the main driver behind polymorphism (e.g. Mogford 1974; Stanton *et al*. 1989; Odell *et al.* 1999; Jones and Reithel 2001), without considering other influencing factors. Factors driving and maintaining floral polymorphism cannot be regarded as discrete entities, but rather the "pluralistic effect" of multiple factors needs to be taken into account (Ernst 1987; Wolfe 1993; Strauss and Whittal 2006; Smith *et al*. 2008). Flower colour may not be an isolated trait driven by one selecting force, but rather the result of a series of complex interactions of both biotic and abiotic factors, as well as functional trade-offs, occurring at different life stages of the plant, in order to optimise the fitness of the species (Ernst 1987; Smith *et al*. 2008).

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6.10 Appendix 6.1

Table 6.4. Statistical tests performed on *Aloe petricola* plant morphometric, visitation rates, reproductive output, and diversity indices datasets after Shapiro-Wilks normality testing.

Analysis of Variance (ANOVA)	**Kruskal Wallis Rank Sum Test**
Data normally distributed (Shapiro-Wilks *P* > 0.05)	**Data not normally distributed (Shapiro-Wilks *P* < 0.05)**
Mean rosette diameter (3 measures)	Rosette height
Rosette diameter at widest point	Individual raceme lengths
Rosette circumference	Number of racemes/plant
Total plant height	Visitation rates/100 racemes/hour
Number of flowers/raceme	Visitation rates/100 plants//hour
Number of flowers/plant	Individual species visitation rates/100 plants/hour
Inflorescence height	Fruit set/raceme
Fruit set/plant	Seed/fruit/raceme
Seed/fruit/plant	Seed/fruit/plant
Total seed produced/plant	Total seed produced/raceme
Seeds/flower/plant	Seeds/flower/raceme
Shannon-Wiener diversity Index	Number of fruit predated/plant
E-evenness diversity Index	Percentage predated fruit/raceme
	Percentage predated fruit/plant
	Species richness
	Simpson’s diversity index

CHAPTER SEVEN

Gender expression in *Aloe*: A theoretical review



Aloe peglerae flowers with exserted anthers and stamens, and dehisced pollen, in The Peglerae Conservancy, Gauteng (Stephanie Payne)

7.1 Abstract

The diverse genus *Aloe* is represented by a range of growth forms and inflorescence structures, with flowering occurring across all seasons. Although annual at a population level, flowering and fruit production is erratic at an individual level. Over four flowering seasons, several *Aloe peglerae* plants did not produce fruit, despite receiving multiple pollinating visits from birds, small mammals and insects. Plants that did set fruit, produced very little viable fruit in proportion to the available flowers on the plant (i.e. low fruit set; 18 – 40%). Low fruit set is common within the genus *Aloe*; 60% of aloe studies report fruit set of ≤ 30%. This suggests that plants are producing "surplus" flowers that do not develop into fruit after pollination. Various theories explain surplus flowers and low fruit set (~22%) in plants with hermaphroditic flowers, and the most common theories are reviewed in the context of *Aloe*, by analysing reproductive output data from published studies (16 species), and raw data provided (seven species). Excess flowers may be produced as additional pollinator attractants (conspicuous colours, pollen and nectar rewards), and to compensate for transfer of self-pollen (most *Aloe* species are self-incompatible), florivory, or fluctuations in pollinator densities. Resources available to the plants are finite and often limited (e.g. soil nutrients/minerals, water); some plants may be "functionally male", allocating resources to producing pollen, rather than to the more resource-intensive female function of producing fruit and seed. Surplus flowers may be acting as pollen donors, following the pollen donation (male function) hypothesis. Maximum reproductive output (100%) may never be achieved, due to space limitations on *Aloe* inflorescences, and resource limitation during flowering and fruiting. Therefore, there may be a trade-off between male and female function, to optimise plant overall fitness. A large scope for extensive research into gender expression in the genus *Aloe* exists, as specific experiments across a broad range of *Aloe* species are required to explain low fruit set, and the production of "surplus" flowers in the genus.

7.2 Key words

Aloe; Functional gender; Gender expression; Pollen donation; Reproductive output; Resource allocation.

7.3 Introduction

Aloe Linnaeus (Asphodelaceae: Alooideae) is a large, diverse genus, comprising > 550 accepted species (Newton and Rowley 2001; Lüthy 2007). The greatest diversity of species (± 350 taxa) is concentrated in southern and eastern Africa, and Madagascar (Klopper and Smith 2007); > 150 of these species are found in South Africa (Klopper and Smith 2007; Klopper *et al*. 2009; Van Wyk and Smith 2014). Aloes can be found in more temperate regions, but usually inhabit dry environments that are water-limited, even during the rainfall season (e.g. South African savanna; Van Jaarsveld 1989; Scholes and Walker 1993; Cousins and Witkowski 2012). Many *Aloe* species have shallow root systems and grow in shallow, rocky soils that are nutrient poor (e.g. calcium, magnesium, potassium, phosphorus and nitrogen; Scholes 1988; Weisser and Deall 1989; Cousins and Witkowski 2012).

Twelve different *Aloe* growth forms are recognised in South Africa, ranging from tall tree aloes (e.g. *Aloidendron* (*Aloe*) *barberae*: ~18 m in height) to small bulbous aloes (e.g. *Aloe bowiea*: ~140 mm); Reynolds 1950; Jeppe 1969; Van Wyk and Smith 2014). In addition, a wide variety of floral morphologies are exhibited, usually as brightly coloured racemose inflorescences, but some species also form spikes, panicles and compound racemes (Reynolds 1950; Jeppe 1969; Holland 1978; Klopper *et al*. 2010; Van Wyk and Smith 2014; Figure 7.1).



Figure 7.1. Different inflorescence types within the genus *Aloe*. A) *Aloe reitzii* var. *reitzii* produces multiple racemes during February – March; B) *Aloe striata*, flowers during July – October; C) *Aloe ecklonis* produces an in November – January; D) *Aloe peglerae* produced a single raceme in June-July; E) *Aloiampelos (Aloe) striatula*, flowers during November – January; and F) *Aristaloe (Aloe) aristata*, produces an inflorescence during November. Photographs A and D: Stephanie Payne; photographs B, E and F: Duncan McKenzie; photograph C: Craig Symes.

These floral displays attract visitors from multiple guilds for pollination. The majority of South African *Aloe* species display floral properties that suggest ornithophilous (bird) pollination, with bright red, orange or yellow (or shades thereof) tubular flowers (Faegri and

van der Pijl 1979; Cousins and Witkowski 2012). These species are regularly visited, and pollinated, by both specialist (e.g. sunbirds and sugarbirds) and generalist nectarivorous birds (e.g. weavers, thrushes, white-eyes, bulbuls; Hoffman 1988; Johnson *et al*. 2006; Botes *et al*. 2008, 2009a; Symes *et al*. 2009; Forbes *et al*. 2009; Hargreaves *et al.* 2010, 2012; Arena *et al*. 2013; Cousins *et al*. 2013; Payne *et al*. 2016; Chapter 2), with birds often tracking the *Aloe* nectar resources as they become more abundant during peak flowering (Oatley 1964; Symes *et al*. 2008; Kuiper *et al.* 2015; Payne *et al*. 2019; Chapter 3). Insects, usually pollen and nectar-collecting bees, regularly visit "ornithophilous" aloes but do not pollinate the aloes, but are rather nectar and/or pollen thieves (e.g. Hoffman 1988; Botes *et al.* 2008, 2009a; Symes *et al*. 2008; Hargreaves *et al*. 2010; Arena *et al.* 2013). However, some species are primarily pollinated by halictid bees (Halictidae), *Amegilla* bee species (Apidae), allodapine bees (Apidae) and honeybees (Apidae; Hargreaves *et al* 2008; 2012; Botes *et al*. 2009b; Wilson *et al* 2009; Duffy *et al*. 2014). These *Aloe* species usually have smaller floral displays with pink, white or green flowers (Jeppe 1969; Van Wyk and Smith 2014); although, the single-stemmed *A. thraskii* has bright yellow flowers, suggestive of bird pollination, but is successfully pollinated by both birds and honeybees (Patrick *et al*. 2018).

Of the A*loe* species tested for self-compatibility through hand-pollination experiments (n = 18 species), only *A. thraskii* is self-compatible (allogamous; Patrick *et al*. 2018), while the other 17 species are reliant on biotic pollen vectors for successful pollen transfer (Hoffman 1988; Johnson *et al*. 2006; Botes *et al*. 2009a, b; Hargreaves *et al*. 2008, 2010, 2012; Symes *et al*. 2009; Wilson *et al*. 2009; Duffy *et al*. 2014). *Kumara* (*Aloe*) *plicatilis* and *A. peglerae* plants that were completely excluded from visitors produced no fruit and seed, suggesting that these species are unable to autonomously self-pollinate, and may be self-incompatible, although not tested through hand-pollination experiments (Arena *et al*. 2013; Cousins *et al*. 2013; Chapter 2).

Aloe plants are slow-growing but long-lived, and once plants reach reproductive size, annual flowering and fruiting is inconsistent, varying between years (e.g. Scholes 1988; Weisser and Deall 1989; Symes *et al*. 2009; Kuiper *et al*. 2015). Fruiting in *A. peglerae* is highly variable (Scholes 1988), and despite receiving many bird, insect and small mammal visitors throughout the flowering season, *A. peglerae* plants produced little to no fruit (Arena 2013; Arena *et al*. 2013; Chapter 2; Chapter 3). Fruit set (the proportion of flowers that set fruit) ranged from 7% to 40% over four consecutive years in the same population (Table 7.1). Reproductive output (fruit set, mean seed per fruit, and total seed production) is not correlated with bird and small mammal visitation rates (Figure 3.2 in Arena 2013; Figure 7.2A; Arena 2013). Low fruit set (~22%), despite visitation by pollinators, is common in plants with hermaphroditic flowers (a category into which *Aloe* falls; Willson and Rathcke 1974; Stephenson 1980; Sutherland and Delph 1984). Within *Aloe*, pollinator visitation and reproductive output has been studied in 23 South African species (Tables 7.2 and 7.3). In each of these studies, less than 50% of flowers set fruit, with 60% of species having an average fruit set of < 30%.

Table 7.1. Visitation rates of birds and small mammals (visits/plant/hour) to, and fruit set (the proportion of flowers that set fruit; %) of, *Aloe peglerae* plants over four consecutive years, within the same population. Plants were assigned to the "All Visitors" (open) control treatment in exclusion experiments. Where possible, data are presented as mean ± S.E. Visitation rate data are not available for 2012.

Year	Visitation rate (Visits/plant/hour)	No. of flowers per plant	Fruit Set (%)	Reference
2011	0.14	200 ± 15	40.0 ± 6.0	Arena *et al*. 2013
2012	-	159 ± 13	7.0 ± 2.0	Arena 2013
2013	0.09 ± 0.01	226 ± 20	19.9 ± 4.1	Payne *et al*. 2016
2014	0.13 ± 0.02	214 ± 24	18.1 ± 9.6	Payne *et al*. 2019

Hermaphroditic plants perform both male (pollen production and dispersal) and female (ovule production, fertilisation and seed maturation) reproductive functions. Female functions require more nutrient and energy resources than male functions, and resources are

allocated accordingly (Bateman 1948; Charnov 1979; Sutherland and Delph 1984). Indeed, in nutrient-poor environments, above-ground allocation of the most limiting nutrients may be as high as 48% of P and 24% of N to seed production, with seeds representing only 0.5% of biomass (Witkowski and Lamont 1996). Assuming resources are finite (Sutherland and Delph 1984; Ashman 1994), then resource allocation to one function would mean that fewer resources are available for allocation to the other function. Therefore, a trade-off is made with function to maximise both male and female fitness and, ultimately, overall plant fitness (Charnov 1979; Charlesworth and Charlesworth 1981; Sutherland and Delph 1984; Sutherland 1986a; Ashman 1994). This disproportionate allocation of resources may result in hermaphroditic plants expressing a "functional gender", where the gender of the plant is determined by the relative contribution to fitness by resource and energy investments into the individual male and female functions (Lloyd 1979, 1980; Barrett and Hough 2013). Therefore, a plant may be "functionally male", and produce pollen and few fruit, or "functionally female", producing both pollen and many fruit (Stephenson 1981). This may explain the production of seemingly "excess" flowers in many hermaphroditic plants (Stephenson 1981; Sutherland 1986b, 1987), including *Aloe*.

Various theories relating to resource allocation and functional gender have been proposed and tested to explain this disparity between flower and fruit production in hermaphrodites (e.g. *Asclepias syriaca*: Willson and Rathcke 1974; Sutherland and Delph 1984; Sutherland 1986a, b; *Agave mckelveyana*: Sutherland 1987; *Asclepias exaltata*: Broyles and Wyatt 1990; *Grevillea* sp.: Hermanutz *et al.* 1998). This paper reviews data on pollinator visitation, and flower and fruit production from published studies on *Aloe* species, and discusses the production of "surplus" flowers, and low fruit set. Five of the most commonly explored theories linked to low fruit set and gender expression in hermaphrodites are discussed in the context of *Aloe*. The following specific questions are addressed: 1) Given

that fruit production is resource intensive, and most of the flowers produced do not set fruit, why do plants allocate resources to the production of "excess flowers", rather than to fruit production? 2) What function do these excess flowers perform, if any?

7.4 Methods and Materials

Data collection and analysis

Relevant information extracted from published pollinator visitation and/or reproductive output studies of South African *Aloe* species (Tables 7.2 and 7.3). A request was made for raw data from these studies and, where provided, raw data was used (*n* = seven species), rather than extracted values (*n* = 16 species). Table 7.2 provides basic morphological characteristics, flowering seasons, breeding systems, nectar properties, and regular visiting guilds (i.e. specialist and generalist birds, insects, and small mammals), for the aloe species reviewed.

Available visitation rate data of visiting guilds were reviewed. Reproductive output, including fruit set (the proportion of flowers that set fruit; %), mean number of seed per fruit and total seed production were extracted. The mean number of flowers, per raceme and/or per plant were extracted, where available. Only data from natural fruit and seed set experiments, and/or open (control) treatments from pollinator exclusion experiments were reviewed. Reproductive output of the reviewed species is summarised in Table 7.3.

Where necessary, statistical analyses on raw data were performed in R (Version 3.5.1; R Core Team 2018), after Shapiro-Wilks Normality Testing. Graphics were produced in R using the packages "ggplot2", "extrafont" and "extrafontdb" (Chang 2012, 2014; Wickham 2016). Where possible, data are presented as mean ± standard error (S.E.), unless otherwise specified.

Table 7.2. Morphological and nectar characteristics of 23 *Aloe* species, arranged by growth form, for which pollination studies have been published. All visitor guilds recorded visiting, but not necessarily pollinating the aloes, are presented. Approximate values (preceded by ~) are extracted from figures. Where possible, values are presented as mean values (exact or approximate), or range of values (minimum – maximum). Specialist birds = specialist nectarivorous birds (e.g. sunbirds and sugarbirds), and Generalist birds = opportunistic nectar-feeding birds (e.g. weavers, thrushes, bulbuls, etc.). S-I = Self-Incompatible, and S-C = Self-Compatible breeding systems.

Species	Flowering Period[#]	Inflorescence Structure[#]	Flower Colour[#]	Mean Nectar Volume (µL)	Mean Nectar Concentration (%)	Pollinators and Visitors	Breeding System	Reference
Tree Aloes								
Kumara (*Aloe*) *plicatilis*	Aug. - Oct.	Loose	Scarlet	Not tested	28.1	Honeybees Specialist birds	Not tested	18, 23
Single-stemmed Aloes								
Aloe africana	Jul. - Sep.	Tight	Yellow-Orange	~ 110	~ 13	Honeybees Specialist birds	S-I	3, 8
Aloe ferox	May - Aug.	Tight	Orange-Red	~ 80 - 180	8.6 - 12.5	Honeybees Specialist birds Generalist birds	S-I	1, 3, 8, 10, 16, 20, 24
Aloe lineata var. *muirii*	Jun. - Nov.	Loose	Pink to Red	~ 25	~ 17	Honeybees Specialist birds	S-I	3, 8
Aloe marlothii	May - Sep.	Tight	Orange-Red	248	12.1	Specialist birds Generalist birds	S-I	6, 7, 11, 14,16, 25
Aloe pluridens	May - Jul.	Loose	Orange	~ 75	~ 15	Honeybees Specialist birds	S-I	3, 8
Aloe speciosa	Jul. - Sep.	Tight	Red to Greenish-white	~ 170	~ 8	Honeybees Specialist birds Generalist birds	S-I	3, 8

Species	Flowering Period[#]	Inflorescence Structure[#]	Flower Colour[#]	Mean Nectar Volume (µL)	Mean Nectar Concentration (%)	Pollinators and Visitors	Breeding System	Reference
Aloe thraskii	Jun. - Jul.	Tight	Yellow	Not tested	Not tested	Solitary/Honeybees Generalist birds	S-C	22
Aloe vryheidensis	Jul. - Aug.	Tight	Reddish-brown	27 - 70	5.6 - 17	Generalist birds	S-I	2
Multi-stemmed Aloes								
Aloe arborescens	May - Jul.	Loose	Red to Orange	39.6	12.1	Honeybees Specialist birds	S-I	16
Rambling Aloes								
Aloe tenuior	Jan. - Dec.	Loose	Yellow to Red	1.06 - 1.2	29.6 - 34.3	Allodapine/Halictid /Honeybees	S-I	16, 19
Stemless Aloes								
Aloe peglerae	Jul. - Aug.	Tight	Red to yellow	73.6	12.1	Honeybees Generalist birds Small mammals	Not tested	17, 21, 26
*Aloe petricola**	Jul. - Aug.	Tight	Red to White; Red to Yellow; Orange to Yellow	RW: 104.34 RY: 83.25 OY: 109	RW: 9.34 RY: 8.25 OY: 8.75	Honeybees Specialist birds Generalist birds	Not tested	26
Aloe reitzii var. *reitzii*	Feb. - Mar.	Tight	Red	95.2	113	Honeybees Specialist birds Generalist birds	Not tested	26
Spotted Aloes								
Aloe greatheadii var. *davyana*	Jun. - Jul.	Loose	Dull Red	30.7	23.5	Honeybees	S-I	5, 11, 25
Aloe maculata	Jun. - Sep.	Loose	Red to Yellow	32.2	16.6	Honeybees Specialist birds	S-I	13, 15, 16

Species	Flowering Period[#]	Inflorescence Structure[#]	Flower Colour[#]	Mean Nectar Volume (µL)	Mean Nectar Concentration (%)	Pollinators and Visitors	Breeding System	Reference
Aloe pruinosa	Feb. - Mar.	Loose	Dark Pink	14.77	19.72	Membrane/Honeybees Specialist birds	S-I	12
Slender Aloes								
Aloe boylei	Jan. - Feb.	Loose	Salmon Pink	19.4	22.1	*Amegilla*/Honeybees Birds	S-I	16
Aloe dominella	Jul. - Oct.	Loose	Yellow	4.5	20.5	Honeybees	-	16
Aloe kraussii	Nov. - Feb.	Loose	Yellow	2.2	19.1	*Amegilla*/Honeybees Specialist birds	S-I	16
Aloe linearifolia	Feb. - Mar.	Loose	Yellow	3.0	26.6	Allodapine/Honeybees	S-I	9
Grass Aloes								
Aloe minima	Feb. - Mar.	Loose	Dull Pink	1.1	25.9	Allodapine/Honeybees	S-I	9
Bulbous Aloes								
Aloe inconspicua	Nov.	Loose	Greenish-white	0.097	Not presented	*Amegilla* bees	S-I	4, 16

Table 7.2 and 7.3 Notes:
[#] Flowering period, inflorescence structure (flowers loosely or tightly arranged) and flower colour are according to Van Wyk and Smith (2014).
*****_Aloe petricola_ is polymorphic, with three colour morphs co-occurring in the same sub-populations. RW = Red-white morph, RY = Red-yellow morph and OY = Orange-yellow morph. Reproductive output does not differ significantly between the three colour morphs (Kruskal-Wallis Rank Sum Test, $P > 0.05$; Chapter 6).

References: [1]Hoffman 1988; [2]Johnson *et al*. 2006; [3]Botes *et al*. 2008; [4]Hargreaves *et al*. 2008; [5]Human and Nicolson 2008; [6]Symes and Nicolson 2008; [7]Symes *et al*. 2008; [8]Botes *et al*. 2009a; [9]Botes *et al*. 2009b; [10]Forbes *et al*. 2009; [11]Symes *et al*. 2009; [12]Wilson *et al*. 2009; [13]Hargreaves *et al*. 2010; [14]Symes 2010; [15]Duffy and Johnson 2011; [16]Hargreaves *et al*. 2012; [17]Arena *et al*. 2013; [18]Cousins *et al*. 2013; [19]Duffy *et al*. 2014; [20]Kuiper *et al*. 2015; [21]Payne *et al*. 2016; [22]Patrick *et al*. 2018; [23]Stephen Cousins, raw data supplied; [24]Timm Hoffman, raw data supplied; [25]Craig Symes, raw data supplied; [26]Stephanie Payne, *unpubl. data*.

Table 7.3. Reproductive output of 21 *Aloe* species, arranged by growth form, for which detailed pollination studies have been conducted and published. Approximate values (preceded by ~) are extracted from figures. Where possible, available values are presented as the mean values (exact or approximate), or range of values (minimum - maximum). Where studies were conducted over multiple years, data for all years are presented individually. Not presented = data were not available/published for these reproductive output measures. No reproductive output values were presented for *A. tenuior* and *A. dominella*.

Species	No. of Flowers	Fruit Set (%)	Seeds per fruit	Total Seed Produced	Reference
Tree Aloes					
Kumara (*Aloe*) *plicatilis*	22.1	21.6	14.9	92.4	18, 23
Single-stemmed Aloes					
Aloe africana	Not presented	~ 42	~ 38	Not presented	3, 8
Aloe ferox	459	27.3	~ 42	Not presented	1, 3, 8, 10, 16, 20, 24
Aloe lineata var. *muirii*	Not presented	~ 22	~ 40	Not presented	3, 8
Aloe marlothii	2006: 232 2007: 278	14.3 19.3	76.9 65.8	Not presented	6, 7, 11, 14,16, 25
Aloe pluridens	Not presented	~ 38	~ 22	Not presented	3, 8
Aloe speciosa	Not presented	~ 45	~ 42	Not presented	3, 8
Aloe thraskii	Not presented	2010: 62.8 - 97.1 2011: 58.4 - 93.2	2010: 0 - 45.6 2011: 0 -49.0	Not presented	22
Aloe vryheidensis	483	Not presented	6	Not presented	2
Multi-stemmed Aloes					
Aloe arborescens	168 (median)	Not presented	Not presented	3219	16

Species	No. of Flowers	Fruit Set (%)	Seeds per fruit	Total Seed Produced	Reference
Stemless Aloes					
Aloe peglerae	2013: 226.3 2014: 213.6	2013: 19.9 2014: 18.1	2013: 43.35 2014: 11.0	2013: 2129.3 2014: 1270.4	17, 21, 26
*Aloe petricola**	RW: 167.2 RY: 323.69 OY: 197.10	RW: 16.9 RY: 21.4 OY: 21.9	RW: 32.6 RY: 19.4 OY: 19.2	RW: 2302 RY: 3235.1 OY: 2298.7	26
Aloe reitzii var. *reitzii*	275.56	12.05	Not presented	Not presented	26
Spotted Aloes					
Aloe greatheadii var. *davyana*	2003: 47.8 2004: 55.2	2003: 44.8 2004: 55.2	2003: 37.8 2004: 46.6	Not presented	5, 11, 25
Aloe maculata	34 (median)	~ 11	~ 35	Not presented	13, 15, 16
Aloe pruinosa	Not presented	40.8	34.02	Not presented	12
Slender Aloes					
Aloe boylei	40 (median)	Not presented	Not presented	10.7	16
Aloe kraussii	67 (median)	Not presented	Not presented	2.8	16
Aloe linearifolia	Not presented	19.4	13.5	Not presented	9
Grass Aloes					
Aloe minima	Not presented	28.8	10.0	Not presented	9
Bulbous Aloes					
Aloe inconspicua	23 (median)	Not presented	~ 3	Not presented	4, 16

7.5 Results and Discussion

Five of the most commonly considered hypotheses/theories pertaining to gender expression and resource allocation are discussed, in the context of *Aloe*. Data relevant to each hypothesis are presented within the appropriate sections.

Pollinator (pollen) limitation

Low fruit set may be an indication of low pollination success during the flowering season (e.g. Schemske 1980; Willson and Schemske 1980; Bierzychudek 1981; Sutherland 1987). This is either due to a shortage of pollen available for transfer (pollen limitation), or low pollinator density in the plant's habitat (Sutherland and Delph 1984; Sutherland 1987). Conversely, an increase in pollinator density would increase fruit set (Sutherland and Delph 1984). Supplemental hand-pollination experiments, where flowers naturally pollinated by pollen vectors have additional pollen mechanically added to the stigmas, are performed to assess pollen limitation (Sutherland 1987). Should pollen-supplemented flowers produce similar numbers and/or quality of fruit to those that are naturally pollinated, the flowers are not pollinator and/or pollen limited (Willson and Schemske 1980; Knight *et al*. 2005).

In three populations of *Aloe pruinosa*, supplemental hand-pollination of flowers did not significantly increase the mean number of fruits per plant, or the mean number of seeds per flower, as compared to naturally pollinated plants (Wilson *et al*. 2009). Similar results were recorded in *A. inconspicua* and *A. maculata*, where supplemental hand-pollination did not significantly increase fruit or seed set (Hargreaves *et al*. 2008, 2010). When comparing outcrossed (and bagged) hand-pollination experiments in many *Aloe* species, fruit and seed set in such experiments is often higher than natural fruit or seed set (see Table 7.4), suggesting that a lack of efficient pollinators may be limiting fruit production. However, only a few flowers per

raceme were manipulated in these experiments and, in some cases, un-manipulated flowers removed (e.g. Patrick *et al*. 2018). Therefore, the amount of resources available for, and allocated to, fruit maturation in manipulated flowers may be greater than under natural conditions (Stephenson 1981).

Table 7.4. Comparison of fruit set (%; proportion of flowers that set fruit) between outcrossed, bagged hand-pollination experiments and open, naturally-pollinated control plants for 16 *Aloe* species. Fruit set results are separated into those species where outcrossed, hand-pollinated experiments produced more fruit than naturally-pollinated controls, or *vice versa*.

Outcrossed Hand-Pollination > Open Control		**Outcrossed Hand-Pollination < Open Control**	
Species	**Reference**	**Species**	**Reference**
Aloe africana	Botes *et al.* 2009a	*Aloe arborescens*	Hargreaves *et al.* 2012
Aloe boylei	Hargreaves *et al*. 2012	*Aloe greatheadii* var. *davyana*	Symes *et al*. 2009
Aloe ferox	Hoffman 1988	*Aloe minima*	Botes *et al*. 2009b
Aloe inconspicua	Hargreaves *et al*. 2008	*Aloe thraskii*	Patrick *et al*. 2018
Aloe kraussii	Hargreaves *et al*. 2012		
Aloe linearifolia	Botes *et al.* 2009b		
Aloe lineata var. *muirii*	Botes *et al.* 2009a		
Aloe marlothii	Symes *et al.* 2009		
Aloe pluridens	Botes *et al.* 2009a		
Aloe pruinosa	Wilson *et al*. 2009		
Aloe speciosa	Botes *et al.* 2009a		
Aloe vryheidensis	Johnson *et al*. 2006		

Arena (2013) found that diurnal (insect and bird) and nocturnal (small mammals) visitation rates are not significantly correlated to fruit set, seed set or total seed production in *Aloe peglerae*, with some plants producing no fruit (and therefore seed), despite receiving multiple visits from pollinators in the 2012 flowering season (Figure 3.2 in Arena 2013; Figure 7.2A). In 2013, overall visitation rate (birds and small mammals combined) and fruit set were not correlated (Spearman Rank Correlation: R_S = -0.16, P = 0.59). A similar result was found for both *A. reitzii* var. *reitzii* (Spearman Rank Correlation: R_S = 0.01, P = 0.95) and *A. petricola* (Spearman Rank Correlation: R_S =0.08, P = 0.70), both of which are regularly visited by insects

and specialist and generalist nectar feeding birds (Chapter 5, 6; Figure 7.2B and 7.2C). This suggests that potential pollinator limitation may not be resulting in low fruit set in these species.

Birds, in particular, are known to track resources, both temporally (between flowering/fruiting seasons) and spatially (between plant populations), moving to areas where energetically viable resources are most abundant (e.g. Brown and Hopkins 1996; Malizia 2001; Cotton 2007; Las-Casas *et al.* 2012). Specialist and generalist bird densities and abundance increase during peaks in *Aloe* flowering seasons, in response to the increase in nectar availability during these periods (Oatley 1964; Symes *et al*. 2008; Forbes *et al*. 2009; Kuiper *et al*. 2015; Chapter 3). Oatley (1964) noted a substantial increase in the number of bird species present and feeding at a stand of *A. marlothii* flowering plants during proliferous flowering years, compared to years when fewer plants flowered. A similar trend has been recorded in *A. peglerae* (Chapter 3). In *A. ferox*, this influx of bird visitors is annual, with Malachite Sunbirds (*Nectarinia famosa*) and weavers (*Ploceus* spp.) flocking to stands with flowering plants (Kuiper *et al*. 2015). The Malachite Sunbird is a regular *Aloe* visitor, and efficient pollinator (Botes *et al*. 2008; Symes *et al*. 2008; Forbes *et al*. 2009; Hargreaves *et al*. 2010; Symes 2010; Cousins *et al* 2013; Kuiper *et al*. 2015; Chapter 5), and is a known nomadic species, moving to areas where resources are abundantly available. It does so, either in a predictable route, or moving "haphazardly", selecting areas where resources are more readily available than in others (Craig and Hulley 1994; Symes *et al*. 2001).

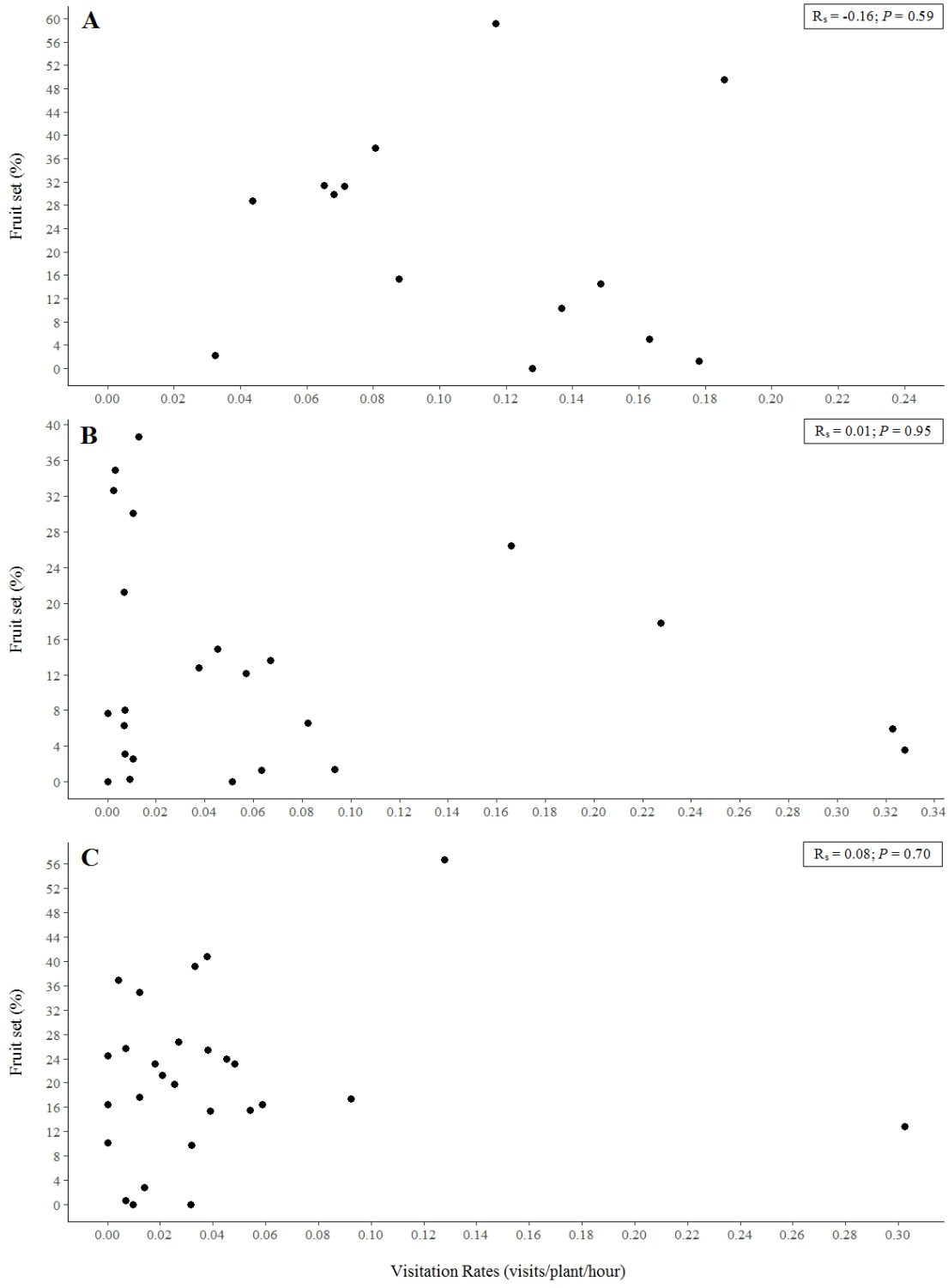


Figure 7.2. The effect of animal visitation (visits/plant/hour) on fruit set (%; proportion of flowers that set fruit) in open control plants for A) *Aloe peglerae*, B) *A. reitzii* var. *reitzii*, and C) *A. petricola.* Most plants received regular visitation from birds (with Namaqua Rock Mouse (*Micaelamys namaquensis*) and Sengi sp. (*Elephantulus* sp.). visits recorded in *A. peglerae*), but set little fruit. There is no correlation between visitation rate and fruit set in any species (Spearman Rank Correlations: $P > 0.05$; results in boxes in top right-hand corner of graph). Data supplied by Stephanie Payne.

Aloe populations that have become isolated, due to habitat fragmentation caused by poor mining and farming practices, and fire (and the misapplication thereof), may be subject to fluctuations in pollinator visitation and densities (e.g. Kremer-Köhne 2018; Patrick *et al*. 2018; Kunonga *et al*. 2019). *Aloe lettyae* and *A. ortholopha* reproductive output decreases with decreasing population size (as pollinator visitation rates decline), and plants appear to be subject to an Allee effect and/or genetic bottlenecks (Kremer-Köhne 2018; Kunonga *et al*. 2019). Although the opposite appears true in *A. thraskii*, where proportion fruit and seed set increased with population isolation, overall fecundity is negatively affected by declining plant population size (and isolation; Patrick *et al.* 2018). Bird and bee visitation rates increased with increasing plant height, plant population size, and population density, while smaller, isolated populations received fewer visits (Patrick *et al.* 2018). Smaller plants with fewer flowers in more isolated populations may be more subject to pollinator limitation than larger plants in bigger populations, reinforcing the necessity for larger, more conspicuous inflorescences to attract pollinators (Patrick *et al.* 2018; Kunonga *et al*. 2019).

However, since the tracking of *Aloe* nectar resources, along with the regular visitation by multiple guilds and species, is well-documented in *Aloe*, it appears that visitation to, and subsequent pollination of, *Aloe* plants by biotic pollen vectors is frequent enough to produce fruit, when compared to supplemental hand-pollination experiments. Although visitation rates in the occasional, extremely poor flowering years can decrease significantly, or in geographically isolated populations, resulting in a decrease in reproductive output (Kremer-Köhne 2018; Patrick *et al*. 2018; Chapter 3), annual fruit production in *Aloe* may not be pollen limited. Changes in pollinator densities should not affect overall reproductive output in large, stable populations;

rather, natural reproductive output is likely close to the physiological maximum that is possible (Wilson *et al*. 2009).

Pollinator attraction

Larger inflorescences signal larger pollinator rewards, such as pollen and nectar, and visitors may preferentially visit plants that offer greater rewards (Willson and Rathcke 1974; Willson and Price 1977; Stephenson 1980; Sutherland 1987; Pyke 1991; Burd 1995). Plants with higher nectar production per flower will have a higher standing crop (Pyke 1991), while also having more pollen rewards available. Aloes have racemose inflorescences with many small, complete flowers either loosely or tightly arranged, on one or more racemes (Reynolds 1950; Jeppe 1969; Van Wyk and Smith 2014; Table 7.2). Larger racemes have more flowers, and therefore have more conspicuous displays (Hoffman 1988), forming a stark colour contrast with the plants' surrounding environment, particularly in dry, resource limited winters, when a majority (~55%) of *Aloe* species flower (Van Wyk and Smith 2014). Kunonga *et al*. (2019) suggest that larger inflorescences with more flowers may be important in small, geographically isolated populations, which have been separated by habitat fragmentation (e.g. mining and farming practices), such as in the Zimbabwean endemic, *A. ortholopha*. The larger inflorescences may make visits more worthwhile for nectar-feeding visitors, increasing pollination success and potentially preventing genetic bottlenecks (Kunonga *et al*. 2019).

Hoffman (1988) recorded that despite larger *Aloe ferox* inflorescences producing more flowers, relative fruit production did not increase with size. Similarly, in *A. reitzii* var. *reitzii*, *A. peglerae* and *A. petricola*, visitation rate and the number of flowers per plant are not significantly correlated (Spearman's Rank correlations: *A. reitzii* var. *reitzii*: $R_S = 0.14$, $P = 0.50$; *A. peglerae*: $R_S = 0.43$, $P = 0.12$; *A. petricola*: $R_S = -0.06$, $P = 0.76$; Stephanie Payne, *unpubl. data*),

suggesting that more conspicuous floral displays may not be attracting more pollinators, based on inflorescence size alone. More important attractants may be nectar and pollen rewards provided by mature flowers.

Most aloes, particularly those that are primarily pollinated by birds, produce copious amounts of nectar (volumes ranging from 10 – 100 µL per flower), with sugar concentrations ranging from highly concentrated to dilute (8 – 25% w/w; Johnson and Nicolson 2008; Table 7.2). *Aloe greatheadii* var. *davyana, A. castanea, A. reitzii* var. *reitzii,* and *A. petricola* produce nectar steadily throughout the day (Nicolson and Nepi 2005; Human and Nicolson 2008; Chapter 5; Chapter 6), while *A. marlothii* and *A. peglerae* have been shown to produce nectar consistently over 24-hours (Symes and Nicolson 2008; Chapter 4). This constant nectar production ensures that nectar rewards are always readily available, for both birds and insects during the day, and small mammals and moths at night (Symes and Nicolson 2008; Chapter 4; Chapter 5). Where unconsumed by nocturnal visitors, nectar is immediately available for dawn visitors (Chapter 4).

Aloe pollen is an important attractant for bees (*A. maculata:* Hargreaves *et al*. 2010; Duffy and Johnson 2011; *A. tenuior*: Duffy *et al*. 2014), and plants may benefit from increased bee visitation when pollen is exposed and available in large amounts (Duffy and Johnson 2011). In the sunbird-pollinated *A. maculata*, bees are suitable pollinators in the absence of birds (Duffy and Johnson 2011). However, substantially increased bee visitation may increase pollen theft and removal, but decrease pollen deposition, reducing overall fecundity (Hargreaves *et al*. 2010; Duffy and Johnson 2011; Duffy *et al*. 2014). Transfer of self-pollen is also more likely, initiating the self-incompatibility reaction, and increasing the number of aborted seeds (Duffy *et al.* 2014).

Large inflorescences may become too attractive to pollinators. Plants with large, conspicuous displays and copious amounts of rewards on offer, may receive increased and prolonged visitation, but this also results in an increased movement between flowers on a single plant (Klinkhamer *et al*. 1994). This increases the chances of the pollinator becoming quickly saturated in self-pollen, increasing the probability of transfer of self-pollen and the initiation of the self-incompatibility reaction, decreasing overall reproductive output (Hoffman 1988; Klinkhamer *et al*. 1994; Pandit and Choudhury 2001).

Therefore, plants need to achieve a balance between visual pollinator attraction, nectar production and pollen presentation, to optimise pollination and reproductive success. This may be achieved through the asynchronous opening of *Aloe* flowers along a raceme (e.g. *A. peglerae* flowers open sequentially from the base of the raceme towards the apex over a period of ~15 days; Chapter 2), which allows access to limited nectar and pollen rewards each day, while the bright, flamboyant displays of unopened flower buds visually advertise the rewards to potential pollinators, while minimising self-pollination (Hoffman 1988). The floral displays enhanced by infertile flowers and/or buds for attraction of pollinators (and the balance thereof) likely plays a major role in the reproductive output within the genus *Aloe*.

Bet-hedging

More flowers may be produced than can be sustained as fruit. The production of surplus flowers may compensate for variation in available resources (usually more consequential in self-compatible plants), or pollinator densities (self-incompatible plants; Stephenson 1980; Sutherland 1987). This is usually tested by measuring the reproductive output of inflorescences where a portion of the flowers (e.g. half a raceme) have been excluded from pollinator visitation (Sutherland 1987). Stephenson (1981) proposed that the production of surplus flowers may

enable plants to take advantage of years where resources are abundant and produce more fruit, while Willson and Rathcke (1974) suggest that surplus flowers increase the plant's contribution to male fitness rather than female fitness (see later).

To our knowledge, no such experiments have been performed on *Aloe*. However, pollinator densities can vary between flowering seasons, as bird pollinators track resources, moving to areas where resources are more abundant (Oatley 1964; Symes *et al*. 2008; Forbes *et al*. 2009; Kuiper *et al*. 2015; Chapter 3). These excess flowers, serving to enlarge floral displays (and rewards), may ensure that there is some degree of pollination success when pollinators are few (Stephenson 1981). In *Aloe*, honeybees, birds (e.g. Dark-capped Bulbul *Pycnonotus tricolor*; Streaky-headed Seed-eater *Crithagra gularis*) and small – medium mammals (e.g. Chacma Baboon *Papio hamadryas ursinus*; Namaqua Rock Mouse *Micaelamys namaquensis*) can destructively visit the plants, piercing or removing whole flowers, in an attempt to gain access to the nectar and/or flower materials (e.g. Botes *et al*. 2008; Symes and Nicolson 2008; Hargreaves *et al*. 2010; Arena *et al*. 2013; Chapter 2). Excess flowers may compensate for the removal of flowers or destruction of floral reproductive organs, to ensure that some flowers are successfully pollinated.

Selective Abortion

Fruit set is highly resource limited (Stephenson 1981; Galen *et al*. 1995), and the resources available to a plant will often determine female success (Bateman 1948). If pollination success is high, and more fruit and seed are initiated than the plant can sustain (i.e. due to resource limitation), only those of a high quality are maintained (Charnov 1979; Stephenson 1980, 1981). Plants will selectively abort fruits of a lower quality (e.g. few fertilized ovules, poor genetic constitution, etc.; Sutherland 1987), known as "fruit-drop", and resources are redirected

to the remaining fruit to enhance seed quality (Stephenson 1980). If flowers are pruned from an inflorescence, and fruit set of the pruned inflorescence is less than that of un-manipulated racemes, this suggests that the plant may not be able to selectively abort poor quality fruits, and is rather forced to maintain all fruits, regardless of quality (Stephenson and Winsor 1986; Sutherland 1987). The amount of extra resources available for reallocation after abortion depends on the amount of resources that have already been invested into fruit maturation (Sutherland 1980), and the balance of resources may not be enough to greatly increase seed quality.

No selective pruning experiments have been performed on *Aloe*, and to our knowledge, no fruit abortion or "fruit-drop" has been reported. Raceme abortion has been observed in *A. peglerae*, *A. reitzii* var. *reitzii*, and *A. petricola*, where racemes are produced but shrivel up soon after development, and do not produce any functional flowers (Melville 1988; Scholes 1988; Stephanie Payne, *pers. obs.*; Figure 7.3A). In *A. maculata*, and *A. tenuior*, plants with more flowers and pollen, received more pollinator visits (i.e. more pollen deposition), and aborted more seeds, compared to those that received fewer visits (Duffy and Johnson 2011; Duffy *et al*. 2014). In *A. maculata*, each fruit contains approximately 70 ovules, and only ~50% of these ovules are matured into seed (Duffy and Johnson 2011).This is likely due to the poor genetic constitution of the seeds, as a result of self-pollination and inbreeding (Duffy and Johnson 2011; Duffy *et al*. 2014). In a recent study, 33% (N = 100 plants) of *A. reitzii* var. *reitzii* plants produced fruit that were shrivelled and hard (Chapter 5). These fruit produced little seed, and it is not known whether this is a fungal or bacterial infection (Prof. Ben-Erik Van Wyk, *pers. comm*.), insect parasitism of the fruit prior to maturation (Prof. Glynis Goodman-Cron, *pers. comm*.; Van den Bosch *et al*. 2019), or if this could be evidence of fruit abortion in *Aloe.*

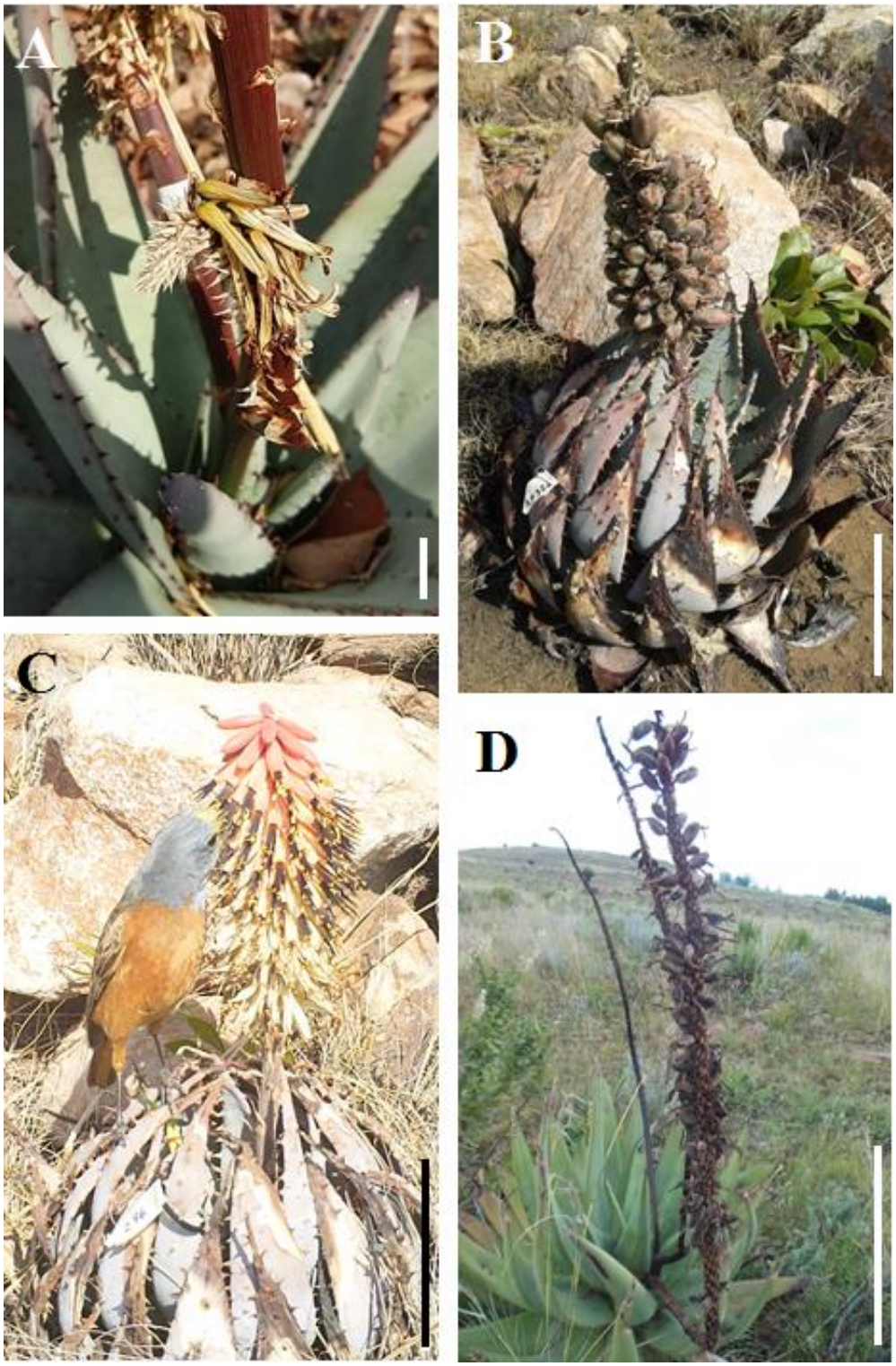


Figure 7.3. A) An *Aloe petricola* raceme that aborted soon after development and did not receive any visitors; B) *A. peglerae* with a raceme tightly packed with fruit. This aloe has a fruit set of 25%; C) Male Cape Rock Thrush (*Monticola rupestris*) reaching up to feed on the nectar of male flowers on an *A. peglerae* plant. Anthers and stigmas are contacting the bird's throat and breast feathers, likely transferring pollen; and D) A fruiting *A. reitzii* var. *reitzii* raceme, with large spaces between fruits where flowers did not develop into fruit. Scale bars: A = 1 cm; B, C and D = 10 cm. Photographs A-D: Stephanie Payne.

Hand-pollinated flowers with outcrossed pollen often produce more fruit than open, naturally-pollinated controls (see Table 7.4). This might suggest that the plant is selecting to mature genetically stronger seeds in the outcrossed plants, while the naturally-pollinated flowers are subject to self-pollination, and produce fruit that is genetically weaker (Sutherland 1987).

Kumara (*Aloe*) *plicatilis* and *A. peglerae* set low amounts of fruit, with few seeds produced per fruit (*K. plicatilis*: fruit set: 21.6%, seed set: 14.9 seeds/fruit; *A. peglerae*: fruit set: 18.1 - 40%, seed set: 11.0 – 43.3 seeds/fruit; Cousins *et al.* 2013; Arena *et al*. 2013; Chapter 3). However, 86% of *K. plicatilis* seeds germinated during germination trials (after 18 months of storage; Cousins *et al*. 2013), and up to 97% of *A. peglerae* seeds germinated (Chapter 2). Tetrazolium testing of un-germinated seeds showed that 100% and 98% of *K. plicatilis* and *A. peglerae* seeds respectively, were viable (ISTA 2003; Cousins *et al*. 2013; Chapter 2). Therefore, although these species produce limited numbers of fruit, most of the seed produced are of a high quality with high germination potential, suggesting that cross-pollination by pollen vectors are producing fruit of a sufficient quality, and selective abortion may not be necessary.

Pollen donation (male function) hypothesis

This hypothesis suggests that excess flowers will be produced if the gain in male fitness outweighs the loss in female fitness, experienced by preferentially allocating more resources to male function rather than to female function (Willson and Rathcke 1974; Sutherland and Delph 1984). Therefore, the functional gender of the plant (i.e. pollen-producing male, or fruit-producing female) will be determined by the number of functionally male flowers acting as pollen donors, and male fitness will contribute more to overall plant reproductive success (Lloyd 1979; Sutherland and Delph 1984). This is the only hypothesis that suggests that male pollen donation, and subsequent “access” to female flowers dictates fruit production; the other four

hypotheses suggest that reduced female function due to resource limitation results in low fruit set (Bateman 1948; Sutherland and Delph 1984).

This may explain the large number of *Aloe peglerae, A. reitzii* var. *reitzii* and *A. petricola* plants that produced little to no fruit, despite regular visitation (Figure 7.2A-C), as well as the low fruit set reported in other *Aloe* species (Table 7.3). These plants may be functionally male, producing large amounts of pollen, and very little fruit due to resource limitation. There may be a threshold where fruit production cannot be increased, regardless of the number of visits to the plant. This could be due to too many pollinators transferring self-pollen onto the stigmas, initiating the self-incompatibility reaction (Hoffman 1988; Klinkhamer *et al*. 1994; Pandit and Choudhury 2001).

There may also be a physical, structural limitation to fruit production (Collins and Rebelo 1987). One *A. peglerae* raceme produces over 200 individual, complete flowers with the potential to mature into fruit after fertilisation (Table 7.3). If racemes are approximately 17 cm in length (the portion of the raceme where flowers are present; Stephanie Payne, *unpubl. data*.), there is not enough space for over 200 fruit capsules to successfully mature on the raceme. Therefore, the "low" fruit set produced (~18 – 40%), may actually be the maximum amount of fruit that could be produced (i.e. 100% possible fruit production), due to space limitations on the raceme (Figure 7.3B). The excess flowers produced might be acting as pollen donors, while as many of the remaining flowers that can be accommodated as fruit are retained to perform the female function (Rebelo and Rourke 1986; Collins and Rebelo 1987). What appears to be low fruit set in the genus, may be a relatively high proportion of flowers that set fruit, possibly even reaching close to the maximum that could be physically achieved on a raceme. However, this

may be species specific and seasonally variable, as in other species, such as *A. reitzii* var. *reitzii*, there is still space available on the raceme for fruit production (Figure 7.3D).

The excess "male" flowers produced may be performing a dual-function: producing and donating pollen, and attracting potential pollinators, by increasing inflorescence size and producing attractive rewards (e.g. Willson and Price 1977). In *Aloe*, flowers in the staminate phase (anthers exserted, stigmas not yet receptive) produce substantially more nectar and pollen than flowers in the pistillate phase (anthers wilted, stigma exserted and receptive; Nicolson and Nepi 2005; Human and Nicolson 2008; Symes and Nicolson 2008). Flowers in the staminate phase, and functioning as males, may be supplementing nectar and pollen rewards when neighbouring flowers are in the pistillate phase, ensuring pollinator attraction and pollen transfer (Castellanos *et al*. 2002; McDade and Weeks 2004; Nicolson and Nepi 2005). Also, since *Aloe* flowers open asynchronously, staminate flowers are higher up along the raceme than pistillate flowers. Therefore, animals have to reach or move along the raceme to access the nectar, and are likely picking up pollen, and transferring it to the lower flowers as they do so (Figure 7.3C).

Additional influences

The Predator Satiation Hypothesis, linked to mast fruiting, suggests that producing massive crops in one year – to satiate predators and reproduce successfully – and then producing minimal fruit in the following seasons, allows for accumulation of resources for reproduction, and prevents mass seed predation each year (Silvertown 1980). Mast fruiting has been well studied in North American Oak trees (*Quercus* spp.; e.g. Sork *et al.* 1993; Koenig *et al*. 1994; Clotfelter *et al*. 2007). Indeed, near-crop failures have been reported in non-mast years, probably due to fertilisation failure (possibly as a result of pollinator limitation) and lack of resources (Sork *et al*. 1993; Koenig *et al*. 1994). Although species dependent, it has also been found that

mast years are linked to climatic conditions (Silvertown 1980; Sork *et al*. 1993), sometimes at least two years prior to the mast year (Clotfelter *et al*. 2007). Although data to support mast-fruiting events in *Aloe* are not available and further investigation is required, mast-fruiting could also be occurring, with the plants yielding a large amount of fruit in one year satiating 'predators' and then storing resources for another large crop in later years, resulting in the low fruit set observed.

7.6 Recommendations

Many of the experiments required to accurately assess the factors influencing reproductive output have not been conducted for this genus. Experiments required include:

i) Supplemental hand pollination experiments to test for pollen limitation (ideally on an entire raceme, or on sections of racemes; e.g. Sutherland 1987; Knight *et al*. 2005);

ii) Assessments of the relationship between pollinator visitation rates and reproductive output, as well as the relationship between pollinator density and population size (e.g. Kunonga *et al*. 2019);

iii) Assessments of the relationship between inflorescence size, pollinator visitation and reproductive output (e.g. Hoffman 1988), as well as the effect of a decrease in floral display (through the removal of flowers) on pollinator visitation (e.g. Klinkhamer *et al*. 1994);

iv) Comparisons between reproductive output of excluded portions of racemes (e.g. top exposed only, middle exposed only, or bottom exposed only) to test for bet-hedging, and the position of flowers likely functioning as females (e.g. Sutherland 1987);

v) Comparisons between reproductive output of pruned and non-pruned inflorescences (e.g. some flowers removed) to test for selective abortion (e.g. Stephenson and Winsor 1986; Sutherland 1987); and

vi) Evaluations of floral phase (e.g. staminate or pistillate) during peak nectar production, to determine whether plants are actively increasing plant attractiveness during the male or female phase of the flowers (e.g. Sutherland 1987). This could give some indication of increased (or decreased) male function in these plants.

These experiments should be conducted on as many *Aloe* species of multiple growth forms, across as wide a range of habitats and flowering seasons as possible. At minimum, species that have been well studied and/or with large populations should be considered first. Such species may include, but are not limited to: *A. africana*, *A. arborescens*, *A. ferox*, *A. greatheadii* var. *davyana*, *A. maculata*, *A. marlothii*, *A. peglerae*, *A. tenuior*, *A. thraskii* and *A. vryheidensis*.

7.7 Conclusion

Plants may never be able to optimally maximise reproductive output due to resource limitation, and so a trade-off between male and female fitness occurs (Charnov 1979). In doing so, the advantage of the gain in male fitness, through the production of surplus flowers and pollen donation must ultimately outweigh the loss in female fitness (Stephenson 1981).

The hypotheses discussed are in no way mutually exclusive, and a combination of theories may be at play (Sutherland and Delph 1984). In conjunction with these theories, other environmental and seasonal variables need to be considered. These may include resource availability (nutrients and/or water), both prior to and during the flowering season, time of

flowering and location of the individual plant (e.g. in a rock crevice *versus* in soil, degree of disturbance, etc.; Kunonga *et al*. 2019). In particular, resource use and storage in *Aloe* is poorly understood, and requires further investigation. In *Aloe*, there may be an optimal number of visitors that are required to produce optimal fruit set, and fruit set might be further limited by the plant's structure, and space on the infructescence. Any flowers that do not set fruit may serve purely as attractants, pollen donors, or as surplus flowers to compensate for those flowers affected by the self-incompatibility reaction, or florivory. This would suggest that a combination of the male function hypothesis (due to resource limitation), pollinator attraction, and bet-hedging hypotheses may be able to explain low fruit set in *Aloe*, along with external influences, such as environmental conditions. Aloes are heavily reliant on pollinators, and so the production of excess flowers aids in pollinator attraction, especially where mobile and/or migratory birds are important visitors (e.g. Oatley 1964; Kuiper *et al*. 2015; Chapter 3)

However, fruit set differs between flowering seasons, and excess flowers may be performing different functions in different seasons/years, depending on the resources available. This may result in mast-fruiting in some years, and the effects of fruit production in mast years may be reducing fruit set in following years, if the plants rely on stored resources for fruit production. The occurrence of mast-fruiting, as well as the source and storage of resources in the genus *Aloe* requires further investigation.

Reproductive output and persistence of *Aloe* species may be reliant on factors other than pollinator visitation and successful pollen transfer, both intrinsic and extrinsic to the plant. This review highlights the need for further experimentation and research into the genus *Aloe*. The data available are seemingly conflicting, as some species' data support certain theories, while data from other species do not fit the same theory. Specific experiments are required, on multiple

species of varying growth forms within the genus, to ascertain which theory, or combination of theories, may be influencing low fruit set and the production of seemingly surplus flowers in the genus.

7.8 Acknowledgments

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CHAPTER EIGHT

General Discussion and Conclusions



Two different floral colour morphs of *Aloe ferox* at Vernon Crookes Nature Reserve, KwaZulu Natal (Craig Symes)

8.1 General discussion

Plant-pollinator Interactions

Aloe floral displays play an important role in pollinator attraction; visitation by biotic vectors is vital as most aloe species are self-incompatible, and reliant on pollen vectors for successful pollen transfer (e.g. Hoffman 1988; Johnson *et al*. 2006; Botes *et al*. 2009a, b; Hargreaves *et al*. 2008, 2010, 2012; Symes *et al*. 2009; Wilson *et al*. 2009; Duffy *et al*. 2014). In return, flowering aloes supply important food rewards and resources (nectar and pollen) for birds (specialist nectarivores and generalist (opportunistic) nectar-feeders), insects and small mammals alike, as most of these aloes flower in winter (June – August; ~55% of 158 species flower in winter; Van Wyk and Smith 2014), a period of food resource limitation.

Bright red, yellow and orange floral colours are synonymous with bird pollination (Faegri and van der Pijl 1979). It appears that birds do not discriminate between these specific perianth colours in a polymorphic *Aloe* (Chapter 6). Instead, they are likely drawn to the nectar rewards offered by the plants. Since *Aloe* flowers are often one of the few (and in some cases, the only) abundant nectar resources available in their immediate surroundings, the birds flock to these resources in large numbers (Payne *et al*. 2019; Chapters 3, 6; Oatley 1964; Symes *et al*. 2008; Forbes *et al*. 2009; Kuiper *et al*. 2015;). These nectar rewards are produced consistently throughout the day and night (Nicolson and Nepi 2005; Human and Nicolson 2008; Symes and Nicolson 2008), and are offered to visitors (birds, small mammals and insects) at all times of the day (Payne *et al*. 2016; Chapters 2, 3, 4, 5, and 6; Figure 8.1). This consistent production, while serving to attract multiple guilds of visitors and/or pollinators, also allows for visitation to be partitioned temporally throughout a 24-hour period (Chapter 4; Zoeller *et al*. 2016).

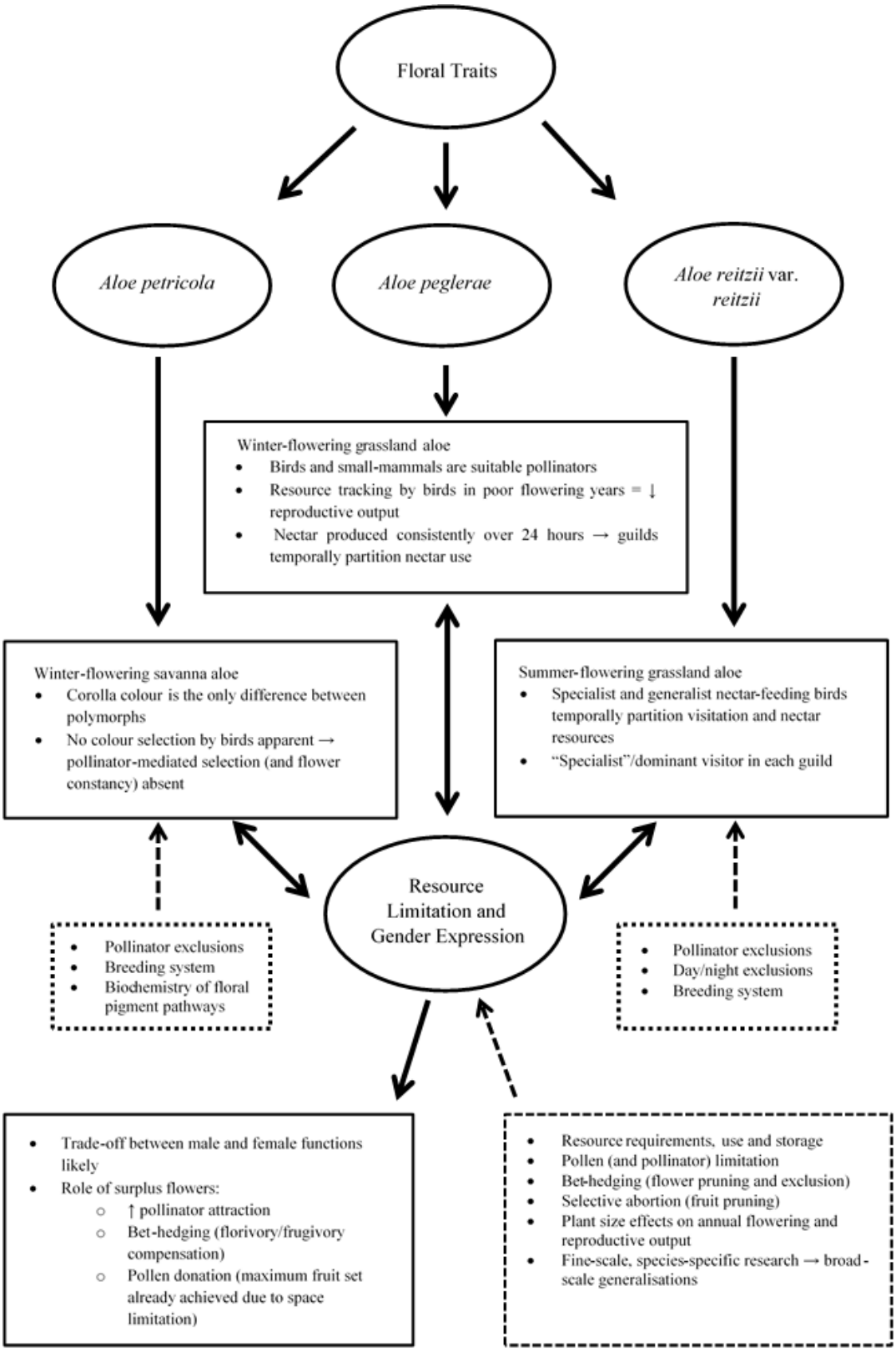


Figure 8.1. A broad diagrammatic synthesis of the results of this study. Main themes and study species are in solid, oval shapes. Solid arrows and boxes represent conclusions from this study. Dashed arrows and boxes represent areas of recommended research.

These resources are further partitioned within guilds, with specialist and generalist nectar feeding birds also appearing to partition the resource temporally during daylight hours (Chapter 5; Figure 8.1). This may increase the probability of successful pollination events, as the plants are being visited at all times of the day, by different visitors with different pollinating abilities – a benefit to *Aloe* in particular, as their flowers (stigmas) are only receptive for approximately three days (Stephanie Payne, *unpubl. data*).

Pollinators track the production of these resources and flock to stands of flowering aloes during the peak of the flowering season (Chapter 3; Symes *et al*. 2008; Forbes *et* al. 2009; Kuiper *et al*. 2015). In some species (e.g. *Aloe peglerae*), it appears that the proportion of plants flowering is highly variable between flowering seasons, and nectar resource abundance may differ significantly between years (Chapter 3; Scholes 1988). Mobile pollinators, i.e. birds, will usually select foraging areas based on reward/food resource availability, moving between sites as resources become depleted in one site, and available in another (Craig and Hulley 1994; Symes *et al*. 2001). Poor flowering seasons often cause significant fluctuations in mobile pollinator presence, which, in turn, decreases reproductive output of the aloes (Chapter 3). However, less mobile visitors, such as small mammals which are restricted to limited foraging areas (Chapters 2, 3; Ribble and Perrin 2005), will be forced to make use of these limited resources. Although they may not be as effective as bird pollinators, small mammals may be suitable, alternative pollinators in periods when fewer primary pollinators (birds) are present, aiding in the persistence of some *Aloe* species (Chapters 2, 3). The quantification of small mammal pollination in the primarily bird-pollinated *A. peglerae* is a first for the genus (Chapter 2).

Floral characteristics may prove useful in predicting specific pollinators but, in fact, appear to cater for multiple guilds (Figure 8.2). Nectar properties are often used to separate

specialist nectarivorous bird pollinated plants from those that are pollinated by generalist (opportunistic) pollinators (Johnson and Nicolson 2008). Flower colour, structure, and scent distinguish bird-pollinated plants from insect-pollinated plants (Faegri and van der Pijl 1979). In the genus *Aloe*, however, this seems to be less distinct as visitors of multiple guilds are attracted to the same flowering plants (Figure 8.1). Yet, within each of these visiting guilds, there appears to be one species that dominates visitation. In *A. peglerae,* the Cape Rock-Thrush (*Monticola rupestris*) is the most regular diurnal bird visitor, while the Namaqua Rock Mouse (*Micaelamys namaquensis*) is the most common nocturnal visitor (Chapters 2, 3). In *A. reitzii* var. *reitzii*, specialist nectarivore bird visitation is dominated by the Malachite Sunbird (*Nectarinia famosa*), while the Cape Weaver (*Ploceus capensis*) is the most regular generalist, nectar-feeding bird visitor (Chapter 5). These dominant animal species may be acting as "specialist pollinators" that are most suited to successfully pollinating their respective *Aloe* species, despite the aloes appearing to display broad pollination syndromes, that attract a wide diversity of visitors. This may eventually result in the an aloe species naturally tending towards a more specialised pollination syndrome over many generations, such as *A. reitzii* var. *reitzii* which may be tending to the Malachite Sunbird pollination syndrome (Chapter 5; Geerts and Pauw 2009). Visitation by multiple guilds may also buffer the aloes against seasons of low seed production in years of poor flowering success, where pollinator abundance (particularly of the "specialist" and/or primary pollinators), may be lower (Chapter 3).

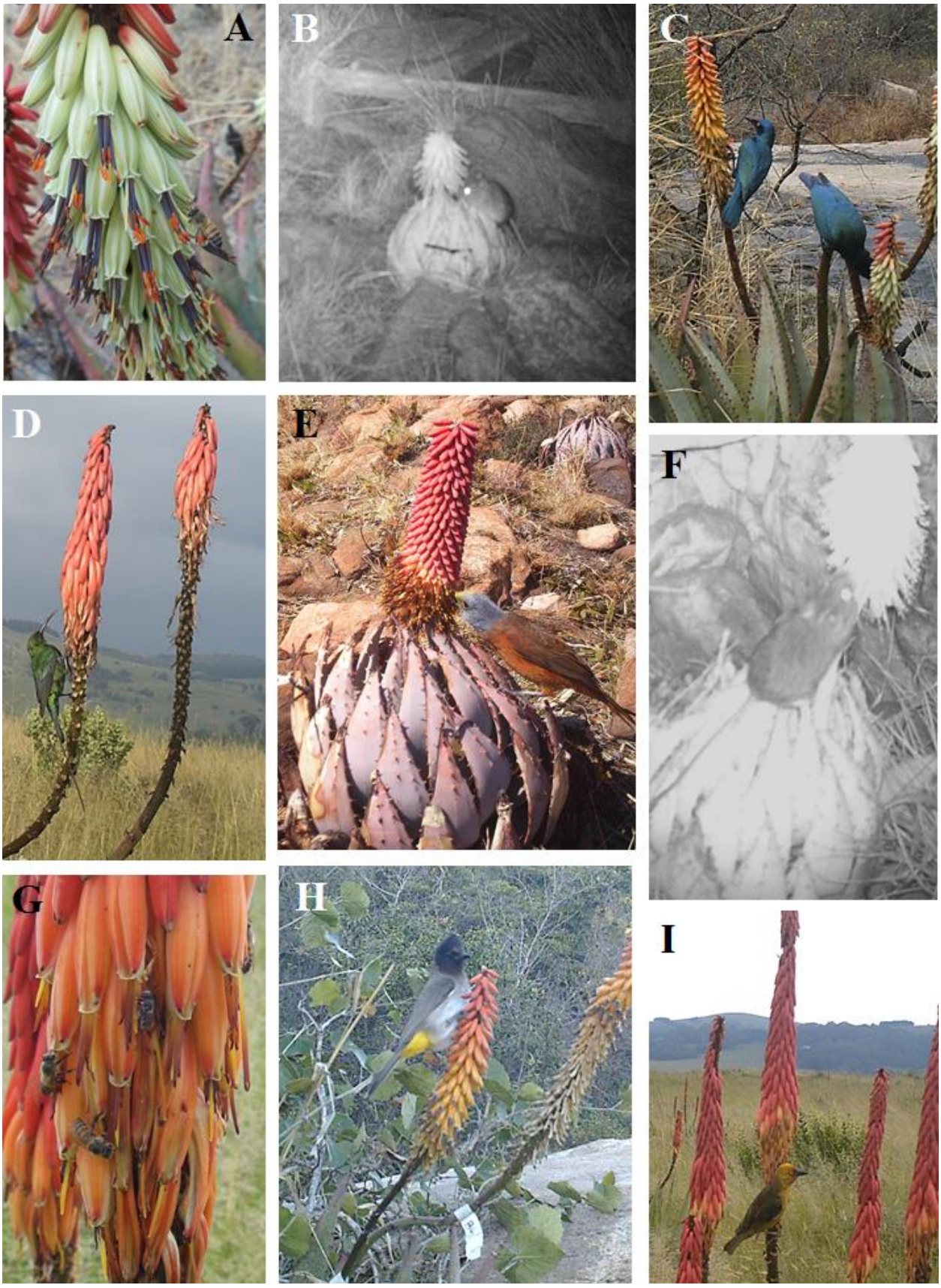

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Figure 8.2. Floral visitors to *Aloe* species. A) A honeybee (*Apis mellifera*) visiting a red-white inflorescence of the polymorphic *A. petricola*; B) A Namaqua Rock Mouse (*Micaelamys namaquensis*) standing on an *A. peglerae* plant at night; C) Cape Glossy Starlings (*Lamprotornis nitens*) perched and feeding on two separate orange-yellow and red-white morphs of *A. petricola*, which are growing close together; D) A male Malachite Sunbird (*Nectarinia famosa*) perched on an *A. reitzii* var. *reitzii* raceme; E) A male Cape Rock-Thrush (*Monticola rupestris*) feeding on *A. peglerae* nectar; F) A Namaqua Rock Mouse feeding on *A. peglerae* nectar at night; G) Honeybees on *A. reitzii* var. *reitzii*, likely feeding on nectar; H) A Dark-capped Bulbul (*Pycnonotus tricolor*) perched on an orange-yellow *A. petricola* morph; and I) A male Cape Weaver (*Ploceus capensis*) perched on an *A. reitzii* var. *reitzii* raceme. Photographs A – I: Camera trap photographs by Stephanie Payne.

These pollinator attractants play an important role in complex plant-pollinator interactions in the genus (Figure 8.1). However, the production and presentation of pollinator attractants requires resources, as does the production of fruits and seeds to ensure the reproductive success of the individual. Therefore, a trade-off between resources allocated to pollinator attraction, (e.g. pollen and/or nectar production, "male function"), and fruit and seed production ("female function") is necessary (Charnov 1979; Charlesworth and Charlesworth 1981).

Resource limitation and plant fitness

Floral displays and rewards are successful in attracting pollinators, and *Aloe* plants often experience high rates of visitation from floral visitors. However, it is not uncommon for regularly visited plants to produce little to no fruit (i.e. low fruit set; proportion of flowers that set fruit; ≤ 30%; Chapter 7). *Aloe* fruit production does not appear to be pollen and/or pollinator limited (e.g. Hargreaves *et al*. 2008, 2010; Wilson *et al*. 2009), but plants may never be able to optimally maximise reproductive output (i.e. 100% fruit set), due to resource limitation (Charnov 1979). Therefore, these seemingly "surplus" flowers that do not set fruit, may perform other functions apart from fruit production (Chapter 7; Figure 8.1).

The excess flowers may be acting purely as pollinator attractants, making the inflorescence larger and more conspicuous to visitors (Willson and Rathcke 1974; Willson and Price 1977; Stephenson 1980; Sutherland 1987; Pyke 1991). Excess flowers and larger displays may be important to ensure pollinator visitation at small, geographically isolated *Aloe* populations, to make visits more worthwhile for nectar-feeders (e.g. *A. orthopha*, Kunonga *et al*. 2019). Nectar and pollen produced by these excess flowers, particularly in the staminate phase, are serving as additional attractants for neighbouring flowers in the pistillate phase, where nectar production is decreased (Nicolson and Nepi 2005; Human and Nicolson 2008; Symes and Nicolson 2008). Aloes are subject to florivory by insects, birds and small mammals (Chapter 2; Botes *et al*. 2008; Hargreaves *et al*. 2010; Arena *et al*. 2013), and fruit predation by insects (Van den Bosch *et al*. 2019) and birds (e.g. Streaky-headed Seed-eater *Crithagra gularis*; Chapter 2). Surplus flowers likely compensate for the damage to plant reproductive organs, and removal of these flowers and fruit (bet-hedging), ensuring that high quality fruit and seed are still produced.

However, these pollinator attractants (large inflorescences and abundant nectar/pollen rewards) may be rendering the inflorescence too attractive which, although increasing visitation to the inflorescence, may increase the probability of the transfer of self-pollen between flowers on the raceme (Hoffman 1988; Klinkhamer *et al*. 1994; Pandit and Choudhury 2001). This will initiate the self-incompatibility reaction, where flowers either do not develop into fruit, or fruit of a poor-quality, i.e. self-fertilised fruit, are aborted (selective abortion; Stephenson 1981; Sutherland 1987).

The low fruit set reported on these racemes may be the actual maximum that the plant can physically support (e.g. Collins and Rebelo 1987). *Aloe* racemes often have large numbers of flowers tightly packed onto a single raceme (e.g. *A. peglerae* has > 200 flowers per raceme

which are, on average, only 17 cm long; Chapters 3, 7), and the same number of fruit capsules cannot fit on the raceme. This may also be dictated by raceme structure – loosely packed racemes may be able to fit and produce more fruit on average (e.g. *A. greatheadii* var. *davyana* mean fruit set: ~ 55%), while tighter packed racemes may be able to fit as many fruit relative to the number of flowers (e.g. *A. marlothii* mean fruit set: ~19%; Symes *et al*. 2009). Excess flowers may be acting as "male flowers", performing the less resource-intensive male function of producing pollen and ensuring gene flow through the population (Pollen donation hypothesis; Willson and Rathcke 1974; Lloyd 1979; Sutherland and Delph 1984; Rebelo and Rourke 1986; Collins and Rebelo 1987).

8.2 Furthering *Aloe* research

Considering the number of *Aloe* taxa found in South Africa alone, there is a dearth of information on individual species within the genus, many of which are of ecological, horticultural and medicinal importance (Holland 1978; Grace *et al*. 2009; Parker and Bernard 2009). Studies on *Aloe* floral traits, floral visitors, pollination systems, breeding systems and/or reproductive success have only been conducted on 21% of South African species (n = 33 out of 158 species listed in Van Wyk and Smith 2014; see Table 8.1 in Appendix 8.1). An understanding of pollination systems within and between species, plant-pollinator interactions, as well as the effects of resource limitation on plant fitness, is vital to understanding the genus. Specific experiments are required. Experiments to investigate the roles of specific visitors may include pollinator exclusions (including day and night exclusions) and breeding system experiments, especially in the case of *A. reitzii* var. *reitzii* and *A. petricola* (Figure 8.1). Biochemical pathways of flower colour, especially in polymorphic aloes, will also provide valuable information on flower colour selection by pollinators, and persistence of polymorphism.

Since information on *Aloe* resource use and storage is not readily available, research targeted at nutrient requirements, storage and limitation (e.g. Pate *et al.* 1990; Witkowski 1990; Witkowski and Lamont 1996), would be valuable (Figure 8.1). The effect of plant size and age on flowering and fruiting, over multiple seasons, is currently poorly understood within the genus (e.g. Hoffman 1988; Scholes 1988), and such information may aide in conservation planning, and for the granting of permits for sustainable research and/or harvesting.

Specific experiments, over multiple flowering seasons, that can provide valuable information relating to the effect of resource limitation on "surplus" flower production, the role of these flowers and their effect on overall reproductive output should be undertaken. Such experiments may include supplemental hand-pollinations to test for pollinator limitation, and flower and fruit pruning to test for the role of flowers in pollinator attraction, bet-hedging, and selective abortion (Figure 8.1). Measuring male function and female function in individual flowers (and subsequently, in individual plants), can be achieved by quantifying primary attractants (pollen and nectar), since these costs are not shared in protandrous flowers (Sutherland 1987). This may also explain the role of surplus flowers, as well as the low fruit set, despite regular pollinator visitation.

While short term studies may be useful as pilot studies to improve experimental design and optimise research resources, longer studies provide more detailed information about visiting species and the frequency of their visits (e.g. Chapter 5). Recently, camera trap technology has enabled non-invasive research to be carried out while overcoming human limitations (e.g. observer effort; Bahaa-El-Din and Cusack 2018; Krauss *et al*. 2018). This same technology has enabled extensive testing of pollination syndromes, and identification of unexpected, nocturnal and/or shy visitors and/or pollinators, in multiple plant genera (e.g. *Aloe*: Chapters 2, 5; Arena *et*

al. 2013; Symes 2017; *Banksia*: Krauss *et al*. 2018; *Erica*: Turner *et al*. 2011; Lombardi *et al*. 2017; *Protea*: Melidonis and Peter 2015; Steenhuisen *et al*. 2015). However, researcher observations are still required to supplement camera trap photographs as animal behaviour and activity on a plant cannot always be interpreted from photographs or even short films that are recorded (e.g. Chapter 5; Krauss *et al*. 2018). Detailed accounts of visitor and/or pollinator activity when visiting *Aloe* plants will provide vital information in pollination experiments, and assist in conservation practices, particularly for Vulnerable, Threatened and Endangered *Aloe* species.

8.3 Concluding remarks

Although enabling testable hypotheses to be formed, pollination syndromes are highly generalised (e.g. Hingston and McQuillan 2000). *Aloe* is not exempt from these broad generalisations, as visitors that were not expected (e.g. nocturnal moths and small mammals) were recorded along with the expected diurnal insects, and specialist and/or generalist nectar feeding birds. Researchers are quick to make generalisations about the genus *Aloe* based on the knowledge of only a few species (e.g. *A. ferox*, *A. marlothii*, *A. peglerae*; Table 8.1 in Appendix 8.1), while there are large gaps in the information available for many other species. Fine-scale studies on pollination systems, reproductive output and resource use on more *Aloe* species are required to make broad generalisations.

In *Aloe*, plant-pollinator interactions are complex, and are further confounded by factors intrinsic to the plant itself and external factors such as climate variability; these biological systems influence each other, both directly and indirectly. Long term-studies on flowering and fruiting variability between flowering seasons (e.g. Scholes 1988; Pfab and Scholes 2004), and the effect of resource availability and usage (e.g. Pate *et al*. 1990; Witkowski 1990), may prove

invaluable in understanding the complexities of these interactions. Although simpler, further studies should avoid treating extrinsic (e.g. pollinators, resource availability) and intrinsic (e.g. plant size and age, maternal control/gender expression) factors that affect overall plant fitness as mutually exclusive entities. Rather, the interactions and relationships between these factors should be taken into consideration. Resources are required to produce visual and food attractants for pollinators, which are required to successfully pollinate plants, ensuring successful reproduction for the plants. However, efforts of pollinators may be futile, if sufficient resources are not available for fruit maturation. Should resources be inadequately allocated, the overall fitness of the plant could be jeopardised. Illegal harvesting, habitat transformation (farming and mining), and land degradation are serious threats to *Aloe* species (e.g. Pfab and Scholes 2004; Siebert *et al*. 2005; Foden and Potter 2009; Pfab *et al*. 2016; Kunonga *et al*. 2019). Reduced fitness, a result of many complex factors and interactions, may severely impact the species' persistence and, ultimately, have serious implications for conservation and management strategies.

8.4 References

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APPENDICES

Appendix 8.1

Table 8. 1. List of South African aloes (*N* = 33 species; 21% of S.A. aloes), where plant properties relating to visitor attraction (flower traits, nectar rewards, pollen rewards) and/or pollination have been examined/studied. *Aloe* species are arranged by growth form, according to Van Wyk and Smith (2014).

Species	Available Information	Reference
Tree Aloes		
Aloidendron (Aloe) barberae	Floral traits	Van Jaarsveld 2010
Aloidendron (Aloe) dichotoma	Floral traits Visitor predictions	Tribe and Johannsmeier 1996 Van Jaarsveld 2010
Aloidendron (Aloe) pillansii	Floral traits Visitor predictions	Tribe and Johannsmeier 1996
*Aloidendron (Aloe) ramosissimum**	Floral traits Visitor predictions	Tribe and Johannsmeier 1996
Aloidendron (Aloe) tongaense[#]	Floral traits	Van Jaarsveld 2010
Kumara (*Aloe*) *plicatilis*	Fire survival Nectar properties Plant size vs. inflorescence production Pollinator exclusion Seed banks Seed dispersal Seed storage and germination Visitor observations	Cousins *et al*. 2013, 2014, 2015
Single-stemmed Aloes		
Aloe africana	Breeding system Flower phenology Nectar properties Pollinator exclusion Visitor observations	Botes *et al*. 2008, 2009a
Aloe ferox	Breeding system Flower phenology Nectar properties Plant size vs. inflorescence production Pollinator exclusion Resource tracking Visitor observations	Hoffman 1988 Stokes and Yeaton 1995[Ŧ] Botes *et al*. 2008, 2009a Forbes *et al*. 2009 Hargreaves *et al*. 2012 Kuiper *et al*. 2015

Species	Available Information	Reference
Aloe lineata var. *muirii*	Breeding system Flower phenology Nectar properties Pollinator exclusion Visitor observations	Botes *et al*. 2008, 2009a
Aloe marlothii	Breeding system Flower phenology Nectar isotopic analysis Nectar properties Nectar secretion patterns Pollinator exclusion Seed banks Seed dispersal Seed germination Visitor observations	Symes and Nicolson 2008 Symes *et al*. 2008, 2009, 2011 Symes 2010, 2012 Hargreaves *et al*. 2012
Aloe pretoriensis	Breeding system (autonomy only) Pollinator exclusion Seed predation Visitor observations	Shibanda 2016 Van den Bosch *et al.* 2019
Aloe pluridens	Breeding system Flower phenology Nectar properties Pollinator exclusion Visitor observations	Botes *et al*. 2008, 2009a
Aloe speciosa	Breeding system Flower phenology Nectar properties Pollinator exclusion	Botes *et al*. 2008, 2009a
Aloe thraskii	Breeding system Fecundity and population isolation Pollinator exclusion Visitor observations	Patrick *et al*. 2018
Aloe vryheidensis	Breeding system Flower traits Nectar phenolics Nectar properties Pollinator response to nectar phenolics Pollinator exclusion Visitor observations	Johnson *et al.* 2006 Hargreaves *et al*. 2012

Species	Available Information	Reference
Multi-stemmed Aloes		
Aloe arborescens	Flower traits Nectar properties Pollinator exclusion Visitor observations	Hargreaves *et al*. 2012
Aloe castanea	Nectar secretion patterns	Nicolson and Nepi 2005
Aloe claviflora	Visitor observations	Arena 2018
Rambling Aloes		
Aloe tenuior	Breeding system Flower traits Nectar properties Pollen tube growth Pollinator exclusion Temporal partitioning Visitor observations	Hargreaves *et al*. 2012 Duffy *et al*. 2014
Stemless Aloes		
Aloe peglerae	Inflorescence height vs. reproductive output Long term population monitoring Microhabitat Nectar properties Nectar secretion patterns Pollinator exclusion Population ecology and structure Resource tracking Seed germination Seed harvesting Temporal partitioning Visitor observations	Melville 1988; Scholes 1988 Pfab and Scholes 2004 Arena *et al*. 2013, 2015 Phama *et al*. 2014 Payne *et al.* 2016 (Chapter 2) Payne *et al.* 2019 (Chapter 3) Chapter 4 (this study)
Aloe petricola	Nectar properties Visitor observations	Chapter 6 (this study)
Aloe reitzii	Nectar properties Population structure Visitor observations	Symes 2017 Chapter 5 (this study)
Spotted Aloes		
Aloe greatheadii var. *davyana*	Breeding system Flower phenology Flower traits Nectar properties Pollen nutritional content Pollinator exclusion Visitor observations	Human and Nicolson 2006, 2008 Symes *et al*. 2009

Species	Available Information	Reference
Aloe lettyae	Microhabitat Nectar properties Pollinator exclusion Population structure Seed germination Seed predation Visitor observations	Kremer-Khöne 2018
Aloe maculata	Breeding system Emasculation effect on fecundity Flower traits Pollen deposition Pollen theft Pollinator exclusion Visitor observations	Hargreaves *et al*. 2010, 2012 Duffy and Johnson 2011
Aloe pruinosa	Breeding system Nectar properties Pollen loads Pollinator exclusion Seed production vs. population size Visitor observations	Wilson *et al*. 2009
Slender Aloes		
Aloe boylei	Breeding system Flower traits Nectar properties Pollinator exclusion Visitor observations	Hargreaves *et al*. 2012
Aloe dominella	Flower traits Nectar properties	Hargreaves *et al*. 2012
Aloe kraussii	Breeding system Flower traits Nectar properties Pollinator exclusion Visitor observations	Hargreaves *et al*. 2012
Aloe linearifolia	Breeding system Flower scent Nectar properties Pollinator exclusion Visitor observations	Botes *et al.* 2009b

Species	Available Information	Reference
Grass Aloes		
Aloe minima	Breeding system Flower scent Nectar properties Pollinator exclusion Visitor observations	Botes *et al*. 2009b
Bulbous Aloes		
Aloe inconspicua	Breeding system Flower traits Nectar properties Pollinator exclusion Visitor observations	Hargreaves *et al.* 2008, 2012
Aloe modesta	Flower scent	Van der Riet 1977

**Aloidendron ramosissimum* was previously identified as *Aloe ramosissima* (Tribe and Johannsmeier 1996; Van Wyk and Smith 2014).
[#]*Aloidendron tongaense* was previously identified as *Aloe tongaensis* (Van Jaarsveld 2010; Van Wyk and Smith 2014)
[T]*Aloe candelabrum* (Stokes and Yeaton 1995) was previously classified as the KwaZulu-Natal form of *A. ferox* and, consequently, has been grouped with *A. ferox*, until its recent reinstating (Smith *et al*. 2016).
Note: Pollination studies and reproductive output have been conducted on *Aloe divaricata* (Madagascar; Ratsirarson 1995), *A. mayottensis* (Mayotte; Pailler *et al*. 2002), *A. saponaria* (Brazil; Corredor-Prado *et al*. 2016) and *A. ortholopha* (Zimbabwe; Kunonga *et al.* 2019). These species have been excluded from this table, as they are not indigenous to South Africa.

Appendix 8.2

Payne, S., Witkowski, E. and Symes, C. 2017. The delectable *Aloe peglerae*. *Veld & Flora* 103(2):65-69.



A male Cape Rock-Thrush (*Monticola rupestris*) probing an *Aloe peglerae* plant in The Peglerae Conservancy, Gauteng (Camera trap photograph, Stephanie Payne)

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THE DELECTABLE *ALOE PEGLERAE*

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High on the Magaliesberg Mountains in South Africa, one can find the unique Magaliesberg Aloe (*Aloe peglerae*). For most of the year, *A. peglerae* is nothing more than a small ball of fleshy, spiny, grey-green leaves hidden in the long grass. However, in winter these aloes come alive, producing hundreds of tightly packed, scarlet-red flowers, that turn a greenish-yellow as they mature. Together with the bright yellow pollen, which contrasts with the deep purple filaments of the stamens, they signal their presence to pollinators. Flowering plants produce copious amounts of dilute nectar, the reward to these visitors for providing the aloe with a valuable service – pollination.



A flowering adult *Aloe peglerae* plant. Produced on a single raceme, flowers are dull red in the bud stage, becoming greenish-yellow as they mature and open, presenting bright yellow pollen. Photo by Maxine Hulett.

A SPLENDID DIVERSITY

Abundant in the arid- and semi-arid regions of sub-Saharan Africa, the highest concentration and variety of aloes is found in southern Africa. Most aloes (including the newly recognised genera that were previously considered to belong to the genus *Aloe*) are succulents with diverse growth forms ranging from tall tree aloes (e.g. *Aloidendron barberae*), to medium-sized multi- and single-stemmed aloes (e.g. *A. arborescens* and *A. marlothii*), to smaller stemless aloes (e.g. *A. aculeata*), to grass aloes (e.g. *A. minima*).



Aloe peglerae **flowers. Buds are dull red, becoming greenish-yellow as they open and mature. Bright yellow pollen is presented on deep purple stamens that are exserted past the mouth of the open flowers. Photo by Craig Symes.**

RECENT TAXONOMIC CHANGES TO THE *ALOE* FAMILY

A series of fairly recent reviews of the taxonomy of the plant family Asphodelaceae have resulted in an extensive revision of the *Aloe* genus. Some of the changes at generic level include:

- ***Aloe***: the 'typical' aloes – retains most of the roughly 500 species;
- ***Aloidendron***: the tree aloes – five species including *Aloidendron barberae* (better known as *Aloe bainesii*) and *Aloidendron dichotomum* (better known by its old name – *Aloe dichotoma*, the Kokerboom or Quiver Tree);
- ***Aloiampelos***: the scrambling aloes such as *Aloe ciliaris* (now *Aloiampelos ciliaris*) – a genus with seven species and several recognised varieties of some of these species;
- ***Aristaloe***: a single species – previously *Aloe aristata*;
- ***Gonialoe***: three species – including the species previously known as *Aloe variegata*; and
- ***Kumara:*** two species, *K. plicatilis* and *K. haemanthifolia*.

Members of the genus *Chortolirion* have ended up in the *Aloe* genus as part of this extensive taxonomic revision. Taxonomic revisions are ongoing and further changes may ensue.

HOW ALOES ATTRACT BIRD POLLINATORS

Over half (55%) of southern African aloes flower in winter, with most displaying a bird pollination syndrome, i.e. floral characteristics that are suited to attracting avian visitors. Bird pollinated aloes tend to have long, tubular flowers with a range of vivid colours like bright yellow to orange and red. Depending on the species, the flowers produce either copious amounts (40 – 100µL) of dilute (8 – 12% weight by weight (w/w)) nectar or small amounts (<30µL) of concentrated, hexose-rich nectar (15 – 25% w/w), and have no discernible scent. Numerous studies on *Aloe* species have shown birds – both those that feed only on nectar (specialist nectarivores) and those that feed mostly on fruit, seeds or insects, but which also feed on nectar when it is available (generalist nectarivores) – to be the primary pollinators.

A THREATENED SPECIES

The stemless Magaliesberg Aloe, once abundant along the Magaliesberg Mountain Range, is endemic to South Africa. Today, this species is categorised as Critically Endangered, mostly due to the illegal harvesting of seeds and whole plants that has reduced its distribution to a few small subpopulations. For young seedlings to become established they need optimal microhabitat conditions, i.e. rocky ground with reduced grass cover to reduce fire impacts. Recruitment (the ability to successfully produce new generations through germination and successful establishment of seedlings) of these scattered populations is low.



A Dark-capped Bulbul (*Pycnonotus tricolor*) landing on a flowering *Aloe peglerae* plant. Camera trap photo by Stephanie Payne.

The *A. peglerae* population density has been estimated to have decreased by approximately 43% over 10 years and the Peglerae Trail in Kgaswane Nature Reserve, Rustenburg – named for the historic abundance of Magaliesberg Aloes along the trail – sadly now has very few, if any, aloes remaining along the once densely-populated route. The species is also threatened by herbivory, erosion, fire, and White Scale – an insect pest that infests the plant's roots, stems and leaves, causing infested plants to rot and ultimately perish. Of concern is that, despite the rapid population decline, very little is known about this charismatic species.

POLLINATION RESEARCH

Over the past five years, detailed research at the University of the Witwatersrand has attempted to understand more about the Magaliesberg Aloe, especially its pollination system. An important research tool has been the use of 24-hour camera traps, images from which have revealed the identity of a variety of plant visitors. By conducting selective pollinator-exclusion experiments – that allowed access by some pollinators but excluded others – the contribution of different pollinator guilds (broad groups that use a resource in a similar fashion) to pollination and reproductive output of the plants, i.e. fruit and seed production, could be ascertained.

AVIAN VISITORS

Numerous bird species, including those characteristic of the areas in which the plants grow, were identified as regular visitors to plants. These included Dark-capped Bulbul (*Pycnonotus tricolor*), Streaky-headed Seed-eater (*Crithagra gularis*), Cape White-eye (*Zosterops virens*), Familiar Chat (*Cercomela familiaris*), Fork-



An Eastern Rock Sengi (*Elephantulus myurus*) feeding on *Aloe peglerae* nectar. While the sengi is feeding on nectar, it is likely that pollen is being transferred to the fur on the chest and abdomen, as well as between flowers on the plant. Camera trap photo by Gina Arena.



A male Cape Rock-thrush (*Monticola rupestris*) perches on top of an *Aloe peglerae* raceme. The racemes are often used as vantage points by birds. Camera trap photo by Stephanie Payne.

tailed Drongo (*Dicrurus adsimilis*), Crested Barbet (*Trachyphonus vaillantii*), and Red-winged Starling (*Onychognathus morio*). However, the Cape Rock-thrush (*Monticola rupestris*), which generally occurs at low densities, and is often transient at sites where the aloes flower, was by far the most regular visitor, making up 68% and 52% of visits in 2013 and 2014 respectively, and hence is considered to be the primary pollinator of this endemic plant species.

MAMMALIAN VISITORS

Without the assistance of technology, observations at night might have been near-impossible and our camera traps identified the Namaqua Rock Mouse (*Micaelamys namaquensis*) and the Eastern Rock Sengi (*Elephantulus myurus*) as nocturnal visitors, feeding on nectar and flowers. Visitations by these night-time visitors were found to occur less often and their contribution to pollination was subsequently found to be significantly less than that of birds (10% in 2013 and 24% in 2014). The nocturnal visits by the Eastern Rock Sengi were particularly interesting though, since it is a diurnal species that feeds primarily on insects. Although birds are the most efficient pollinators of *A. peglerae*, small mammals may thus be suitable alternative pollinators, should the bird community in the area change.

A PAUCITY OF SPECIALIST NECTAR-FEEDERS

Of considerable interest has been the absence of specialist nectar-feeding bird species. In >1,000 records of bird visitors, over five flowering seasons, only two individual sunbirds, an Amethyst Sunbird (*Chalcomitra amethystina*) and a White-bellied Sunbird (*Cinnyris talatala*), were photographed feeding on *Aloe peglerae*. By association, sunbirds have often been implicated as the primary pollinators of many *Aloe* species and it is only recently that their role as pollinators has been quantified. Generally, the dichotomy between specialist and generalist nectarivore pollinated aloes seems to be determined by nectar characteristics, although other factors like distribution, flowering season and growth form, may be important. Therefore, in the instance of *A. peglerae*, that produces copious amounts (approximately 47 µL) of very dilute (approximately 10% w/w) nectar, the primary visitors are generalist nectar-feeding bird species. The study to date has therefore highlighted the important role of generalist nectar-feeding birds in the pollination of *A. peglerae*. In addition, it has also highlighted the importance of the *A. peglerae* nectar – much like many other *Aloe* species – as a food (and water) resource, for birds and small mammals alike, in dry winters when resources and free-standing water are limited.

MORE ABOUT MAMMALIAN PLANT POLLINATORS

This, to our knowledge, is the first time that small mammal visitation to *Aloe* plants has been quantified. It is expected that small mammal visitation to, and consequently contribution to the pollination of, aloes may be more common than originally thought. Small mammals, mostly rodents, have been recorded as the main pollinators of many southern African plants, which include the

Redelinghuis Pincushion (*Leucospermum arenarium*), approximately 14 species of *Protea*, the Pagoda Lily (*Massonia bifolia*; previously *Whiteheadia bifolia*) and African Lily (*Massonia depressa*), as well as *Colchicum scabromarginatum*. Grey Mongoose and Large-spotted Genet have been recorded feeding on *Protea* nectar. Since all these plants flower in winter and are near to the ground – characteristics in common with *A. peglerae* and a few other aloes – it is not surprising that small mammals were recorded visiting *A. peglerae* and it is likely that small mammals are visiting other aloes similar to *A. peglerae*, such as *A. claviflora*, *A. melanacantha*, *A. petricola*, *A. pratensis* and *A. reitzii*. There are also various anecdotal records of Sengi on *A. reitzii* and of other mammals – such as Chacma Baboon, Vervet Monkey, Slender Mongoose, Four-striped Grass Mouse, Namaqua Rock-mouse and *Rattus* species – visiting *A. marlothii* and *A. ferox* plants.

A PLEA FOR CITIZEN SCIENTIST INVOLVEMENT

Future studies aim to delve deeper into the role that a diversity of potential pollinators play in pollinating members of the genus *Aloe*. To assist in understanding the complex mutualistic relationships between plants and animals, we are requesting assistance from the general public. Specifically we are looking for anecdotal observations (including photographs if possible) of visitors to *Aloe* species that flower near to the ground, especially our target species for further research which are: *A. claviflora, A. peglerae, A. petricola* and *A. reitzii*.

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