

**THE IMPORTANCE OF SUNBIRDS AS POLLINATORS
OF THE SOUTHERN AFRICAN PLANTS *ALBERTA*
MAGNA AND *STREPTOCARPUS DUNNII***

CALLY JULIA JANSEN

221022144

Submitted in fulfilment of the academic requirements for the degree
Master of Science in Biological Sciences

School of Life Sciences
University of KwaZulu-Natal,
Pietermaritzburg
South Africa

February 2023



PREFACE

The experimental work described in this dissertation was carried out in the Republic of South Africa under the auspices of the School of Life Sciences, University of Natal, Pietermaritzburg, from March 2021 to February 2023 under the supervision of Professor Steven D. Johnson and co-supervision of Dr Ruth J. Cozien.

This thesis represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

Δ



Cally Jansen

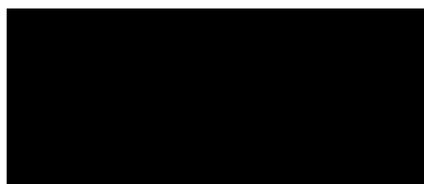
9 February 2023

I certify that the above statement is correct



Professor Steven D. Johnson (supervisor)

As the candidate's supervisor I have approved this thesis for submission.



Professor Steven D. Johnson (supervisor)



Dr Ruth J. Cozien (co-supervisor)

DECLARATION 1 - PLAGIARISM

I, Cally Julia Jansen declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
 - a. Their words have been re-written but the general information attributed to them has been referenced
 - b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed:

A solid black rectangular box redacting the signature of the author.

Cally Jansen, 9 February 2023

ABSTRACT

Bird pollination is important for plant reproduction and is found in about 65 flowering plant families. I studied putative bird pollination systems of two southern African plant species, *Alberta magna* (Rubiaceae), which grows above cliffs and *Streptocarpus dunnii* (Gesneriaceae), which occurs among boulders in open grassland. A special emphasis of this study was to test the utility of close-focusing motion-activated camera traps for documenting bird pollination of plant species that are rare and occur in habitats that present challenges for conventional bird pollination studies. The aims of this project were to 1) quantify floral traits for comparison with other bird-pollinated species; 2) establish the breeding systems, including reliance on pollinators for reproduction; 3) identify floral visitors and measure their contribution to reproductive success; and 4) determine if seed production is pollen limited. Floral traits of *S. dunnii*, including large volumes of dilute nectar and long tube lengths, also aligned with an ornithophilous pollination syndrome. In naturally-pollinated plants, overall fecundity was high, with 80% of flowers developing fruits with large numbers of seeds. Malachite sunbirds (*Nectarinia famosa*) were the only legitimate visitor to flowers of *S. dunnii*. Greater double-collared sunbirds (*Cinnyris afer*), which have shorter bills than malachite sunbirds, occasionally rob flowers of nectar. *Streptocarpus dunnii* was found to be fully self-compatible but does not self-autonomously. Selective exclusion experiments showed that the species is almost entirely reliant on birds for seed production. The species did not experience pollen limitation, indicating that sunbirds are effective pollinators. Measured floral traits of *A. magna*, including large volumes of dilute nectar and long tube lengths, were consistent with an ornithophilous pollination syndrome. Motion triggered cameras and observations showed that southern double-collared sunbirds (*Cinnyris chalybeus*) and olive sunbirds (*Cyanomitra olivacea*) were the most common visitors to *A. magna*, while amethyst sunbirds (*Chalcomitra amethystina*) were occasional visitors. Selective exclusion experiments showed that bird visitors are important for reproductive success which was found not to be pollen-limited. The low levels of fecundity in *A. magna* indicate that there may be other potential factors that limit seed production in this species and the degree of self-compatibility in this species was not firmly established. I conclude that both species are specialized for pollination by sunbirds and that close-focusing camera traps are effective for documenting bird pollination systems of rare plant species which occur in habitats that are challenging for conventional human observations.

ACKNOWLEDGMENTS

I want to thank God for guiding me, strengthening me and giving me this opportunity. I had no intention of coming to KZN to do my Masters but God directed me here and I know now that I was meant to end up in Pietermaritzburg.

To my family, daddy, mommy and Liza, I would be nowhere without your support and love. I cannot put into words how grateful I am for all you have done for me. I have accomplished what I have because of you, this is our degree.

A special thank you to my connect group I started out with, where I was pushed to grow and ended up meeting some of my closest friends. Thank you to my connect group of the past year, Thursday nights was always something to look forward to. Nolwazi AKA mngani, you are the most amazing friend I could have asked for in this time. Thank you for constantly praying for me, reminding me to pray and for motivating me to keep going.

To my labmates (local and international): Greg, Oageng, Viren, Luhlomelo, Bonolo, Lindani, Joris, David; you are all brilliant, thank you for making things more interesting in the lab. Thank you to my friends in Cape Town who checked on me and prayed for me. Thanks to “my” cat, Coconut Ice, for always being there when I got home from hiking.

Thank you to Greg, Oageng, Viren, Daniel, Joris, Liza and Michael for joining in the field and helping me with set-ups. Thank you to Professor van der Niet for helping with set ups and for helping with graphics. Thank you to Professor Steve Johnson for allowing me to take on this project.

Finally, a very special thank you to Doctor Ruth Cozien for always helping me get the equipment I need, guiding me on our fieldtrips, working through stats with me for hours and pretty much showing me the ropes in everything. I would have been lost without your guidance.

Thank you to the following people for helping me gather information on *A. magna*: John Roff for showing us where populations could be found in Karkloof Canopy Tours and Kai for allowing us to work in the area. Dave and Guy for allowing me to work in Mbona Private Nature Reserve. Special thanks to Ronnie and Peter Ritchie for allowing us work in their backyard. Anne Skelton for guiding us to trees at Umtamvuna Nature Reserve. Maggie for providing accommodation and allowing us to work in the tree in her backyard. Conrad at Laager Farm for showing me to the trees. Special thanks to Mpho at Pietermaritzburg

Botanical Gardens for assisting me and organizing a tractor so we could reach flowers. Shane for driving the tractor.

Thank you to the following people for helping me gather information on *S. dunnii*:

Frans Krige for directing us to populations at Verloren Vallei Nature Reserve. Shirley for allowing us to work in the reserve. Gregg Williams and Gavin Botha for allowing us to work at Field and Stream (Tonteldoos) farm. Delia at Mountainlands Nature Reserve for allowing us to place cameras. Malolotja Nature Reserve for letting us do fieldwork.

Thank you to the NRF for funding this project.

TABLE OF CONTENTS

PREFACE	i
DECLARATION: PLAGIARISM.....	ii
ABSTRACT.....	iii
ACKNOWLEDGMENTS.....	iv
TABLE OF CONTENTS.....	vi
Chapter 1: INTRODUCTION.....	1
1.1 Introduction	1
1.2 Bird pollination.....	1
1.3 The use of camera traps in pollination biology	3
1.4 Methods to assess pollinator importance.....	5
1.5 Pollination syndromes	6
1.6 Pollination failure	8
1.7 Study species	10
1.8 Outline of dissertation structure.....	14
1.9 References.....	16
Chapter 2: DONE SPECULATING: CAMERA TRAPS AND EXPERIMENTAL EVIDENCE CONFIRM THE LONG-SUSPECTED IMPORTANCE OF SUNBIRDS AS POLLINATORS OF <i>STREPTOCARPUS DUNNII</i> (GESNERIACEAE).....	28
2.1 Abstract	29
2.2 Introduction	30
2.3 Materials and methods	33
2.3.1 Study site and species	33

2.3.2 Floral traits.....	33
2.3.2.1 Measurement of flower and inflorescence traits.....	34
2.3.2.2 Scanning electron microscopy.....	34
2.3.2.3 Nectar volume and concentration	35
2.3.2.4 Flower colour analysis.....	35
2.3.3 Breeding system experiments.....	36
2.3.4 Visitor observations and camera trapping.....	37
2.3.5 Selective exclusion experiments	37
2.3.6 Pollen limitation	38
2.3.7 Pollen and ovule production	39
2.3.8 Data analyses	39
2.4 Results	40
2.4.1 Floral traits.....	40
2.4.1.1 Flower measurements	40
2.4.1.2 Scanning electron microscopy	43
2.4.1.3 Nectar volume and concentration.....	43
2.4.1.4 Flower colour analysis	44
2.4.2 Breeding systems	45
2.4.3 Pollinator observations	47
2.4.4 Pollen and ovule production	53
2.4.5 Selective exclusion experiments	53
2.4.6 Pollen-supplementation experiments.....	55
2.5 Discussion	57

2.6 Conclusion.....	63
2.7 References	64
Chapter 3: EVIDENCE FOR SUNBIRD POLLINATION IN THE CLIFF-DWELLING SOUTH AFRICAN ENDEMIC TREE <i>ALBERTA MAGNA</i>	71
3.1 Abstract	72
3.2 Introduction	73
3.3 Materials and methods	75
3.3.1 Study site and species	75
3.3.2 Floral traits.....	75
3.3.2.1 Measurement of flower and inflorescence traits.....	75
3.3.2.2 Scanning electron microscopy	76
3.3.2.3 Nectar volume, concentration and composition.....	76
3.3.3.4 Flower colour analysis	77
3.3.3 Breeding system experiments.....	77
3.3.4 Pollinator observations.....	78
3.3.5 Selective exclusion experiments.....	78
3.3.6 Pollen limitation.....	79
3.3.7 Pollen and ovule production.....	79
3.3.8 Data analyses.....	80
3.4 Results	80
3.4.1 Floral traits	80
3.4.1.1 Flower measurements	80
3.4.1.2 Scanning electron microscopy.....	82

3.4.1.3 Nectar volume, concentration and composition.....	82
3.4.1.4 Flower colour analysis	83
3.4.2 Breeding systems.....	84
3.4.3 Pollinator observations.....	86
3.4.4 Pollen-ovule ratios.....	90
3.4.5 Selective exclusion experiments.....	92
3.4.6 Pollen-supplementation experiments	92
3.5 Discussion	94
3.6 Conclusion	99
3.7 References	100
Chapter 4: DISCUSSION AND CONCLUSION.....	106
4.1 Discussion.....	106
4.2 Conclusions.....	112
4.3 References	113

CHAPTER 1

1.1 INTRODUCTION

Pollination is an important ecosystem process, which supports biodiversity and ecosystem functioning (Vanbergen, 2014; Van Etten et al., 2015). Pollination is required for plant sexual reproduction which requires the transfer of genetic material from one plant to the next (or within plants in the case of self-compatible species) via pollen (Hein, 2009). Pollination is also key for food production and maintenance of biodiversity and is thus an important ecosystem service for humans (Rodger et al., 2004; Hein, 2009). Nectar and pollen produced serve as food for animals and are usually required in order for pollination to take place (Lunau, 2000; Gardener and Gillman, 2002; Hargreaves et al., 2009). Despite the recognition of the importance of pollination services, pollination systems face threats from anthropogenic activities such as habitat fragmentation and expansion of industries (Obute, 2010; Biesmeijer et al., 2006; Potts et al., 2016). Pollen transfer takes place through the use of wind, water or animals such as insects, mammals, birds and reptiles which act as pollinators (Regan et al., 2015; Potts et al., 2016). Ollerton et al. (2011) determined that about 87.5% of all angiosperms are animal-pollinated and Rodger et al. (2021) estimated that around a third of flowering plants would produce no seeds without pollinators.

Most plants are pollinated by insects but vertebrates have also been found to be important pollinators for many angiosperm species (Fleming et al., 2009). The exclusion of vertebrate visitors has been shown to reduce the production of seed and fruit, indicating their importance as contributors to reproductive success of many plant species (e.g. Amorim et al., 2022; Arizaga et al., 2000; Cozien et al., 2019 reviewed by Ratto et al., 2018). Lizards are rare pollinators and visit only a few plant species (Olesen and Valido, 2003; Godínez-Alvarez, 2004). Bats are the most common mammal pollinators, pollinating species in about 250 plant genera (Renner and Ricklefs, 1995). Other less common mammal pollinators include primates, rodents and marsupials (Carthew and Goldingay, 1997). Birds, the most common pollinator after insects, pollinate species in about 500 plant genera (Nabhan and Buchmann, 1997; Sekercioglu, 2006; Krauss et al., 2017). Compared to insects, birds and bats are larger and able to carry more pollen and are described as more reliable visitors, particularly in extreme climates (Fleming et al., 2009).

1.2 Bird pollination

Pollination by birds plays an important role in many ecosystems globally (Abrahamczyk, 2019) and occurs in about 65 flowering plant families (Cronk and Ojeda, 2008). Regan et al. (2015) identified 1089 bird species that are known to function as pollinators. Birds act as pollinators by transferring plant pollen from the anther to the stigma and the suites of floral adaptations for bird pollination are termed ornithophily (Stiles, 1978). Pollen is most often found to be placed on the throat or crown feathers of birds (Geerts and Pauw, 2009) but is also placed on birds' feet (Frost and Frost, 1981; Johnson and Brown, 2004), bills (Johnson, 1996; Hargreaves et al., 2019) or tongues (Pauw, 1998). The nectar found in flowers serves as the lure for birds to feed on (Stiles, 1978). Most birds feed on floral nectar from a perched position, using plant stems, branches or stalks as a perch, while the very large group of hummingbirds in the New World hover when feeding and do not generally land on the plant (Cronk and Ojeda, 2008), though some hummingbirds do regularly perch while feeding if a perch is available (Miller, 1985).

Birds are good pollinators because they are able to travel long distances and have good visual acuity (Cronk and Ojeda, 2008; Krauss et al., 2017). The extensive distances birds travel allows for substantially more gene flow and/or cross pollination than pollination by insects would allow (Symes and Nicolson, 2008; Pauw, 2019). Moreover, birds have larger body surfaces which can carry larger pollen loads and they are more effective at pollen outcrossing than insects (Carpenter, 1976; Robertson, 1992; Paton, 1993; Richardson et al., 2000). Additionally, they actively pollinate in colder periods (Kunitake et al., 2004), higher altitude environments (Cruden, 1972; Pi et al., 2020) and on some isolated islands, whereas insects are generally inactive or rarely found in these conditions (Feinsinger et al., 1982; Barrett et al., 1996). Thus, birds offer many advantages as pollinators to plant species.

Birds can be either specialist or generalist pollinators. Plants tend to show specialization either for pollination by specialist nectarivorous birds or for pollination by more generalist omnivorous birds (Johnson and Nicolson, 2008). Pollination by generalist birds is common in the tropics and subtropics and has been found in 124 plant families (Abrahamczyk, 2019). Generalist bird pollination has evolved in angiosperms multiple times independently, likely from insect or specialist bird pollination (Abrahamczyk, 2019). The ecological factors, such as habitat preference, biogeographical distribution and reproductive systems of plant species, that play a role in the evolution of pollination by generalist and specialist birds, differ (Abrahamczyk, 2019). Flower visitation by generalist birds is found in independent bird

lineages whereas flower visitation by specialist birds typically involves closely related bird lineages (Abrahamczyk, 2019).

Specialist bird pollination is common in regions where flowers and nectar are accessible all year, particularly in aseasonal tropical and subtropical locations (Cronk and Ojeda, 2008). Regions which experience a lengthy dormant season normally lack bird pollination or it is rare (Cronk and Ojeda, 2008). Nectariniidae (the sunbirds and spiderhunters) and Promeropidae are bird families, along with hummingbirds and honeyeaters, which have evolved as flower specialists (Cronk and Ojeda, 2008; Regan et al., 2015). The Nectariniidae family are important bird pollinators in Africa and Asia (Cronk and Ojeda, 2008). In Africa, sunbird species are dominant and visit over 320 genera and 460 plant species (Fleming and Muchhala, 2008; Newmark et al., 2020). Sunbirds visit a variety of plant families including Rubiaceae, Fabaceae, Loranthaceae, Lamiaceae, Bignoniaceae and Aloaceae (Fleming and Muchhala, 2008). Southern Africa, in particular, is rich in plant species and diverse and specialised pollination systems (Johnson and Steiner, 2000; Johnson and Steiner, 2003; Johnson, 2004). Nectar-feeding birds found in South Africa include species such as sugarbirds and sunbirds (Johnson, 2004). In the Cape floral region plant families such as Asphodelaceae, Iridaceae, Ericaceae and Proteaceae include ornithophilous species (Rebello, 1987). Bird pollination is less well known from the summer rainfall regions of South Africa, but are represented in the Asphodelaceae (e.g. Diller 2019), Amaryllidaceae (Butler et al., 2022), Iridaceae (Goldblatt et al., 1999) and Orchidaceae (van der Niet et al., 2015).

1.3 The use of camera traps in pollination biology

Over time, different techniques have developed and have been proven useful to investigate plant-pollinator interactions, from direct human observations to the use of camera traps (Yamaji and Ohsawa, 2016). The use of a combination of techniques is often most effective to obtain highly detailed data on different aspects of pollination interactions (Krauss et al., 2017). Anderson et al. (2016) identified three main approaches used to assess the importance of bird pollinators, namely recording flower visitation, investigating fruit and seed set using selective exclusions, and identifying floral syndromes or morphological adaptations.

Human observations through direct sightings have proven useful to study ecology, allowing for recording flower visitation behaviour in real time (Aguiar and Moro-Rios, 2009).

Observers are able to adapt their method of observation based on their experience, for example changing the distance between themselves and the subject (Aguiar and Moro-Rios,

2009). However, human observations have limitations, for example the presence of an observer may influence pollinator visits or behaviour (Aguilar and Moro-Rios, 2009; Johnson and van der Niet, 2019; Ortmann and Johnson, 2020), hence observations must often be from a distance. Additionally, human observations on plant species which are rare or which grow in secluded spaces can be challenging (Johnson and van der Niet, 2019). Humans are also restricted to doing observations in the daytime and cannot observe for very long periods (Yamaji and Ohsawa, 2016; Krauss et al., 2018). Thus, different or additional methods of observation, not subject to these limitations could be useful to derive more details of bird-flower interactions.

The use of camera trapping as a means to observe wildlife has increased over recent years (Cutler and Swann, 1999; Brown and Gehrt, 2009; Rovero et al., 2013; Melidonis and Peter, 2015). Camera trapping involves using remotely triggered cameras to automatically take pictures or videos of subjects passing by (Brown and Gehrt, 2009; Rovero et al., 2013). Cameras which can run without requiring human control, known as remote cameras, allow for the possibility of recording interactions (Johnson and van der Niet, 2019). Remote cameras not activated by motion can continuously record (Johnson and van der Niet, 2019). Motion-activated cameras only record when detecting movement and can thus be operational for a long period of time (Krauss et al., 2018), however this might be limiting if cameras only detect the movement of larger pollinators but not smaller ones (Brown and Gehrt, 2009; Johnson and van der Niet, 2019 but see Ortmann and Johnson, 2020). Therefore, in order to record all possible pollinators in a system, additional methods of recording pollinator interactions can be used but direct observations are still useful (Johnson and van der Niet, 2019; Krauss et al., 2017). There are two kinds of motion-activated cameras used in ecology: video motion detection (VMD) and passive infrared (PIR) (Steen, 2017; Ortmann and Johnson, 2020). PIR camera traps are able to detect subjects even in limited lighting, can be left at selected sites for long periods and are not too expensive (Ortmann and Johnson, 2020). PIR depends on heat emitted from endotherms in order for them to be detected (Swann et al., 2004; Rovero et al., 2013). VMD recording is triggered by the motion of a subject and is thus not dependent on temperature, allowing for ectotherms to be captured as well (Steen, 2017). Remote-camera models have shown to be useful to collect an array of information pertinent to vertebrate pollination (Zoeller et al., 2016). Camera traps can dramatically increase observation time relative to human observations (Krauss et al., 2017; Krauss et al., 2018) and review of footage allows insight into details of the pollination process which may have been

overlooked before, such as behaviour and number of visits (Zoeller et al., 2016; Krauss et al., 2017; Krauss et al., 2018). Rare or shy pollinators or those visiting at night can be detected using camera technology (Melidonis and Peter, 2015; van der Niet et al., 2015; Zoeller et al., 2016; Krauss et al., 2017). Remote camera resolution has advanced over time and camera footage has been shown to provide enough clarity to identify vertebrate pollinators to the species level (Zoeller et al., 2016; Steen, 2017; Krauss et al., 2018). Cameras with close-focussing lenses can record details of interactions at distances of 25 to 60 cm from focal flowers, allowing high levels of detail to be observed (e.g. van der Niet et al. 2022). When using cameras where the flash was activated, Krauss et al. (2018) found that visitors seemed unaffected by the presence of the camera and the flash. Video footage provides more detailed information than images, however it requires a large amount of memory (Brown and Gehrt, 2009; Rovero et al., 2013; Krauss et al., 2018;). Moreover, in some cases many cameras are needed to accurately determine visitation rates (Krauss et al., 2018).

1.4 Methods to assess pollinator importance

Most experimental approaches to quantify the importance of pollinators for plant reproduction utilise fruit and/or seed set as a measure of successful pollination. The breeding system of plants can be determined using controlled hand-pollination experiments (Jones and Little, 1983; Lozada-Gobilard et al., 2019) which have proven useful to assess capacity for autonomous autogamy and determine self-compatibility (Schoen and Lloyd, 1992; e.g. Hargreaves et al., 2004; Ford and Johnson, 2008; Butler et al., 2022). Breeding system experiments involve hand pollinations using cross-pollen, self-pollen and an autonomous treatment, which is bagged to prevent visitors from accessing and pollinating flowers. The bagging of flowers, whereby pollinator-exclusion bags are used to exclude all visitors, are useful to test for self-pollination and autonomous autogamy (Socias et al., 2004; Yamaji and Ohsawa, 2016). The reproductive success, measured from fruit and seeds that are produced from self-pollen, relative to that from cross-pollinated control, indicates whether the plant species is self-compatible. Production of fruits and seeds in flowers which are not accessible to pollinators indicates capacity to produce seed independently of pollinators. Thus, breeding system experiments help indicate the extent to which plant species rely on pollinators for seed production.

Pollinator exclusion experiments have proven useful to identify the primary animal groups responsible for pollinating a certain plant species and to quantify the contributions of

different pollinator types to pollination success (Kleizen et al., 2008; Botes et al., 2009; Yamaji and Ohsawa, 2016; Johnson and van der Niet, 2019). The use of netting or mesh cages, with a certain mesh aperture, to cover a flower or inflorescence can effectively exclude target species from pollinating (Botes et al., 2009; Johnson and van der Niet, 2019). Fruit and seed production often declines if the primary pollinator is excluded from a plant species, for example, bees have been found to be ineffective as pollinators when visiting bird-pollinated aloes, where birds were excluded using cages (Botes et al., 2009). When bees were found to contribute to aloe fruit set, it was less than the fruit set produced when birds and bees had access to pollinate. In another study, Geerts (2016) found that short-billed sunbirds could not act as substitute pollinators for the long-billed malachite sunbird as visitors of *Chasmanthe floribunda*. Seed set was significantly lower in flowers visited by short-billed visitors. Successful pollinator exclusion experiments require that attention is paid to careful positioning of the mesh cages or bags and to choice of appropriate mesh aperture diameter (Yamaji and Ohsawa, 2016). If the mesh aperture size is too small, more non-target pollinating species may be excluded such as large moths or bees, if for example only birds are meant to be excluded. If the mesh aperture size is too big, smaller target pollinating species may be able to fit through it or reach flowers with their beaks or tongues. If mesh cages are not positioned well, flowers may stick out the aperture, allowing pollinators to have access. Moreover, cages which exclude birds, may also exclude similarly sized butterflies, making it hard to separate the respective contributions of either group (e.g. Butler et al., 2022). The presence of mesh cages may also disturb the natural behaviour of pollinators (Keys et al., 1995; Celebrezze and Paton, 2004; Fernández de Castro et al., 2017). Potential disturbance of natural pollinator behaviour can be tested beforehand by observing the control population (where mesh cages are not present) and the caged population to assess whether pollinators not meant to be excluded behave differently (Fernández de Castro et al., 2017).

1.5 Pollination syndromes

Flowers pollinated by birds are characterised by similar traits such as a lack of scent, dilute nectar, a large volume of nectar at the floral tube base, deep floral tubes and bright colours, often red (van der Pijl, 1961; Grant, 1966; Raven, 1972; Cronk and Ojeda, 2008; Johnson and van der Niet, 2019). A pollination syndrome is a set of characteristics that display floral phenotypic uniformity adapted for pollination by a particular group of animals (Müller and Delpino, 1871; Baker, 1963; Faegri and van der Pijl, 1979; Fenster et al., 2004). These specific floral traits, including features such as scent, nectar and flower morphology, which

are associated with bird pollinators specifically, is known as bird pollination syndrome (Vogel, 1954; Faegri and van der Pijl, 1966; Cronk and Ojeda, 2008; Ollerton et al., 2009). Pollination syndromes have been used to infer what animal group pollinates a plant species prior to conducting experiments and observations (Ollerton et al., 2009; Rosas-Guerrero et al., 2014). By investigating plant floral traits and the species which pollinate through observations and experiments (van der Niet, 2021), it can be confirmed if plant species traits and effective pollinators align with a pollination syndrome.

Flowers pollinated by birds are often red and orange, which contrasts against background vegetation making the flowers visible from considerable distances (Grant, 1966; Raven, 1972). In a community context, red can be learned as a good indicator of a reward high in calories for birds, while this reward is not obvious to insects (Raven, 1972). Red is the least striking colour to other potential insect pollinators which may have acted as competitors for nectar (Grant, 1966). The red colour of bird-pollinated flowers has been said to act as an exclusion mechanism for insects, to prevent the interference of pollination and nectar robbing (Cronk and Ojeda, 2008). Bees, in particular, have been found to not visit flowers with traits which are present in bird-pollinated flowers, such as red flowers and those lacking nectar guides (Bergamo et al., 2016; Wester et al., 2020).

Bird pollinators are relatively large and warm-blooded and thus require more energy than most insect pollinators (Cronk and Ojeda, 2008). Consequently, bird-pollinated flowers have larger volumes of nectar (compared to bee-pollinated flowers) and larger flowers to cater for birds (Cronk and Ojeda, 2008; Johnson and Nicolson, 2008). When ingesting such high volumes of dilute nectar, birds ingest large amounts of water (McWhorter et al., 2003). Birds have different ways of dealing with this, for example Palestine sunbirds (*Nectarinia osea*) have been found to only absorb some of their water intake and thus avoid having to eliminate the ingested water (McWhorter et al., 2003). Nectar is composed of three common sugars including glucose, fructose and sucrose in different concentrations (Stiles and Freeman, 1993). Sugar concentrations have been found to differ according to which pollinator visits a plant (Percival, 1961). Sucrose accounts for 40 to 60% of total sugar in nectar in flowers adapted to specialized passerine visitors while sucrose content in flowers adapted to generalized bird visitors is only 0 to 5% (Johnson and Nicolson, 2008). Nectar concentrations of flowers adapted for specialized passerine nectivores typically range from 15 to 25% versus 8 to 12% for flowers adapted to generalized bird pollinators (Johnson and Nicolson, 2008) while bee-pollinated flowers have a concentration of around 35% (Pyke and Waser, 1981). It

has been suggested that dilute nectar could play a bigger role as an insect deterrent than a bird attractant (Symes and Nicolson, 2008).

The lack of odour found in bird-pollinated flowers is not surprising as most birds have a poor sense of smell (Delph and Lively, 1985; Hayden, 2014 but see Whittaker, 2022). This lack of floral scent in bird-pollinated species in contrast to insect-pollinated relatives is exemplified in the *Protea* genus, where beetle-pollinated proteas produced an amount of scent ten times more than bird-pollinated proteas and the number of scent compounds produced was much higher in the beetle-pollinated species (Steenhuisen et al., 2012). Further experiments showed that beetles were more attracted to the *Protea* species with the fruitier scent than the faint scent of the bird-pollinated *Protea* species.

The morphology of bird-pollinated flowers also shows evidence of adaptation to the morphology of bird pollinators. Long floral tubes, sturdy axes, protruding stigmas and anthers allow easier access and support for birds to reach nectar while effectively transferring pollen (Symes and Nicolson, 2008). Narrow floral tubes may restrict access to birds with specialised, elongated feeding mouthparts and increase the accuracy of pollen placement (Hargreaves et al., 2019), whereas flowers pollinated by generalist birds, with shorter bills, are often characterised by floral openings with a larger diameter (e.g. Kiepiel et al., 2022). Birds which pollinate flowers are also often characterised by adaptive traits which enable efficient nectar feeding, including long bills, brush tongues and the ability to hover (Stiles, 1978).

1.6 Pollination failure

Pollination failure puts plant population sustainability at risk by depressing seed production and increases the possibility of extinction of rare plants (Wilcock and Neiland, 2002). Factors which could play a role in plant extinction include the probability of dispersal or pollinator failure, the degree of reproductive dependence on the plant-pollinator relationship and the significance of seeds in the demography of the plant (Bond, 1994). For example, Robertson et al. (1999) found that mistletoe populations endemic to New Zealand have shown declines associated with the decline of bird pollinators over time. Habitat fragmentation has impacted populations of *Brunsvigia litoralis* in South Africa as malachite sunbirds, which are sensitive to anthropogenic change, are absent in the urban population and seed set is low (Geerts and Pauw, 2012). In rural areas, malachite sunbirds are present and seed set was significantly higher (Geerts and Pauw, 2012). Pollination failure can be caused by pollen, pollination or

pollinator limitation (Wilcock and Neiland, 2002); concepts which often overlap or are linked. Wilcock and Neiland (2002) distinguishes pollination limitation as the absence of pollen reaching the stigma and pollen limitation where pollen is not sufficient or not of good quality (also see Aizen and Harder, 2007). A plant species can be described as pollen-limited when fewer fruits or seeds are produced under natural pollination conditions than when receiving supplemental pollen (Bierzychudek, 1981; Stephenson, 1981; Knight et al., 2005; Knight et al., 2006). Pollen limitation, which leads to the reduction of fruit and seed production, could result in a decline in plant population growth (Wesselingh, 2007; Law et al., 2010; van Etten et al., 2018) but is a common phenomenon among plant species (Burd, 1994; Knight et al., 2005). Pollen limitation levels have been found to be higher in bird than in insect-pollinated plants (Anderson et al., 2016) and thus bird-pollinated plants may be more vulnerable. Pollen supplementation experiments have been used to determine if a plant species is pollen-limited. The experiment involves manual addition of cross-pollen to flowers and using unmanipulated, naturally-pollinated flowers as a control to compare fruit and seed production (Knight et al., 2006). If more fruits or seeds are produced in hand-pollinated flowers, the plant species is considered to be pollen-limited. Pollen supplementation experiments are useful in identifying whether pollinator activity contributes to suboptimal reproductive success. If seed production does not increase with hand-pollinations, it could be due to other factors such as the plant having limited access to resources, the number of ovules produced or herbivory (Lee, 1988).

The knowledge and understanding of what causes pollination failure, leading to extinction, can be useful to conservationists to mitigate future extinctions (Wilcock and Neiland, 2002). Although extinction is a process commonly found in nature, anthropogenic activities increase the number of small plant populations that could go extinct (Wilcock and Neiland, 2002). Anthropogenic activities that threaten plant-pollinator interactions include the dispersal of non-native plants (Vanbergen, 2013), the increase in agricultural activities (Scott-Dupree and Winston, 1987; Kearns and Inouye, 1997; Dupont et al., 2018), urbanization (Pauw and Louw, 2012) and habitat fragmentation (Kearns and Inouye, 1997; Gibson et al., 2006; García and Chacoff, 2007; Kolb, 2008). The loss of pollinator diversity and abundance could impact the maintenance of plant diversity and the overall stability of the ecosystem (Regan et al., 2015). Regan et al. (2015) found that one bird pollinator species moves one Red List level closer to extinction every year. Other species which did not change Red List categories likely also experienced population declines and range shrinkage (Regan et al., 2015). Land-use

change, including agricultural and infrastructure expansion, and invasive alien species are the main contributors to bird pollinator declines (Regan et al., 2015). To prevent the loss of pollination services due to pollinator and plant decline, more information on the pollinator species and pollinator distribution is needed to inform policy and implement effective conservation practises (Rodger et al., 2004; Regan et al., 2015; Knight et al., 2018).

1.7 Study species

The field of pollination ecology has advanced in its research over the past two decades and is continuing to grow (Knight et al., 2018), however there are still plant-pollinator interactions which are yet to be studied, particularly in South Africa where pollination studies are mainly concentrated in the Cape region (Rodger et al., 2004) and inferences of pollination systems are still often based only on syndrome traits, without empirical confirmation (Johnson and Wester, 2017, also see van der Niet, 2021) . A recent review found that only approximately a third of syndrome-based predictions of pollination systems for almost 1000 species in the South African flora, have been verified with empirical evidence more than half a century later (Vogel, 1947; Johnson and Wester, 2017). Although predictions of ornithophily were accurate (in more than 90% of cases), only half of the syndrome-based predictions had been verified (Johnson and Wester, 2017). Bird pollination may be particularly hard to document, due to difficulties in closely observing birds, as well as in capturing birds to verify pollen loads (Johnson and van der Niet, 2019; van der Niet et al., 2022). Indeed, even in widespread and relatively accessible species, bird pollination remains inferred based on traits rather than tested. This is exemplified in *Aloe*, a genus hypothesized to largely bird-pollinated, yet very few studies actually document bird pollination inferred from ornithophilous traits (Hoffman, 1988; Stokes and Yeaton, 1995, Johnson et al., 2006). Two species for which bird pollination has long been assumed based on traits, but for which detailed observation have proved elusive, are *Alberta magna* (E. Mey.) (Rubiaceae) and *Streptocarpus dunnii* (Hook.f.) (Gesneriaceae). Current research on the pollination of *A. magna* is restricted to a few personal observations and assumptions based on ornithophilous pollination syndrome (Vogel, 1954; Puff et al., 1984). The research available on the pollination biology of *S. dunnii* also includes only a small number of personal observations (Möller pers. obs. in Hughes et al., 2007; Krige and Möller pers. obs. in de Villiers, 2008) and the association with ornithophilous pollination syndrome based on plant traits (Harrison et al., 1999; Viljoen and Velembu, 2013). In these cases, the difficulty associated with gathering data on their pollinators in their respective

habitats and finding populations to work with may be the reason for their rarity in the literature.

The genus *Streptocarpus* includes 176 species distributed in Africa and Madagascar (Nishii et al., 2015). *Streptocarpus* forms two clades, the first clade includes the caulescent *Streptocarpus* species, *Hovanella*, *Schizoboea*, *Acanthonema* and *Trachystigma*. The second clade is comprised of acaulescent *Streptocarpus* species, *Colpogyne* and *Linnaeopsis* (Nishii et al., 2015). The *Streptocarpus* genus contains a wide range of morphological diversity and is associated with different pollination syndromes (Harrison et al., 1999). The evolution of the variety of flower types in the genus is likely in response to the various pollinators the species has been exposed to (Harrison et al., 1999). *Streptocarpus dunnii* is unique as it looks different to all other species found in *Streptocarpus*. *Streptocarpus dunnii* is a small herbaceous plant which is patchily found in rocky habitats in Mpumalanga and eSwatini (Fig. 1A; Hilliard and Burtt, 1971; Hughes et al., 2007; Hook et al., 2018; Weber et al., 2021). The species produces red tubular flowers, which are hypothesized to be bird-pollinated, mainly based on pollination syndromes and some direct observations (Fig. 1B; Vogel, 1954; Harrison, et al. 1999; Hughes et al., 2007; de Villiers, 2008; Möller et al., 2019; Weber et al., 2021). The dense inflorescence of *S. dunnii*, with its curved tubular flowers and cylindrical corolla tube, is consistent with the syndrome of sunbird pollination (Hughes et al., 2007). The sister species of *S. dunnii*, *S. pole-evansii* and *S. denticulatus*, both have shorter corolla tubes than *S. dunnii* and range from light magenta to pale violet, making these species more suited to insect pollination (Fig. 1C, D; Hilliard and Burtt, 1971). The foliage of *S. pole-evansii* is highly similar to that of *S. dunnii*, with a difference being that *S. pole-evansii* corollas are short-tubed and bilabiate while *S. dunnii* has large, more arcuate flowers (Hilliard and Burtt, 1971). *Streptocarpus dunnii* plants which carry a single, big leaf are most likely useful for perching by birds (Cronk and Ojeda, 2008) but populations with multiple leaves have been observed (Hilliard and Burtt, 1971). *Streptocarpus pole-evansii* possesses a rosette of grey leaves while *S. denticulatus* also carries a solitary leaf (Hilliard and Burtt, 1971). Inflorescences with many tightly packed flowers, such as those of *S. dunnii*, are often associated with perching birds pollinating in the Old World (Cronk and Ojeda, 2008). The lack of data surrounding the pollination biology of *S. dunnii* may be due to the difficulty of observing birds amongst its rocky habitat, where it is found under rocks and within crevices (Fig. 1A; Hook et al., 2018).

The genus *Alberta* forms part of the tribe Alberteae and consists of one species, *Alberta magna*, found only in South Africa (Kainulainen et al., 2009). The two other genera in the Alberteae tribe consist of *Nematostylis* and *Razafimandimbisonia* (previously *Alberta*) and occur naturally in Madagascar (Kainulainen et al., 2009). *Alberta magna* grows into a small to large tree found on cliffs where grassland meets forest (Fig. 1E) and carries bright red, long and tubular flowers (Fig. 1F; van Staden et al., 1990). The *Nematostylis* genus consisting of, *Nematostylis anthophylla*, also shows floral traits commonly found in butterfly-pollinated plant species including narrow and cylindrical flowers with copious nectar (Fig. 1G; Puff et al., 1984). The Madagascan *Razafimandimbisonia* species, which appears morphologically different to *A. magna*, are presumed to be butterfly-pollinated as there are no obvious perches for birds, corollas are much shorter than that of *A. magna* and they do not have colours typically found in bird-pollinated plants as they are usually pink or white (Fig. 1H; Puff et al., 1984; Kainulainen et al., 2009). *Alberta magna* flowers show typical traits of bird-pollinated flowers, but very few observations of sunbirds visiting have been reported and these are all anecdotal (Vogel, 1954; Puff et al., 1984; Weber et al., 2021). Butterfly pollination of the species cannot be ruled out as a possibility when looking at its Madagascan relatives. *Alberta magna* is found specifically in the Eastern Cape (Transkei) and KwaZulu-Natal (Karkloof) (van Staden et al., 1990) and other individuals of the species are usually found singly or in pairs in areas where they were likely planted. Their highly specialised habitats and limited distributions may be why the pollination biology of this species has not been studied in detail. With the advances made in pollination ecology, the pollination biology of species which have not previously been studied, such as those of *A. magna* and *S. dunnii*, can be investigated.



Figure 1. Growth forms and habitat of the two study species, flowers of each study species and a congener or close relative. *Streptocarpus dunnii* in rocky grassland (A) and a close view of a plant in a typical rock cleft (B). *Streptocarpus pole-evansii*(C), and *Streptocarpus denticulatus* (D), the sister species to *Streptocarpus dunnii*. *Alberta magna* trees along a cliff edge in Umtamvuna Nature Reserve (E). Flowers of *Alberta magna* (F). *Nematostylis anthophylla* (G) and *Razafimandimbisonia minor* (H) (Picture: Kainulainen et al., 2009) the Madagascan relatives of *Alberta magna*.

1.8 Outline of dissertation structure

The main objectives for this thesis are:

1. To determine if floral traits of *Alberta magna* and *Streptocarpus dunnii* align with the floral syndrome associated with bird pollination
2. To determine what are the breeding systems of *Alberta magna* and *Streptocarpus dunnii* and if seed production in these species depend on pollinators
3. To determine if birds are the primary pollinators of *Alberta magna* and *Streptocarpus dunnii* and if so, which species act as pollinators
4. To determine if the production of seeds in *Alberta magna* and *Streptocarpus dunnii* is limited by pollen receipt and/or pollinator activity

In Chapter 2, the pollination ecology of *Streptocarpus dunnii* is investigated. The morphology of the flowers are investigated. The breeding system of *S. dunnii* is established through experiments. The primary pollinators of the species is studied through the use of camera traps and visitor exclusion experiments. Pollen limitation experiments conducted determines if the species is pollen-limited. The Chapter determines the reliance of *S. dunnii* on bird pollinators.

In Chapter 3 focusses on the pollination ecology of *Alberta magna*. The morphological traits of flowers are investigated and the breeding system is determined through experiments. The primary pollinators of *A. magna* are discussed with evidence gathered from camera traps and visitor exclusion experiments. Pollen limitation experiments reveals if *A. magna* experiences pollen limitation. This Chapter determines if *A. magna* is reliant on sunbirds for reproductive success.

Chapter 4 makes concluding remarks and shows the differences between the plant-pollinator relationships for *S. dunnii* and *A. magna*. This Chapter makes recommendations for further research.

1.9 REFERENCES

- Abrahamczyk, S., 2019. Comparison of the ecology and evolution of plants with a generalist bird pollination system between continents and islands worldwide. *Biological Reviews* 94, 1658-1671.
- Aguiar, L.M., Moro-Rios, R.F., 2009. The direct observational method and possibilities for neotropical carnivores: an invitation for the rescue of a classical method spread over the primatology. *Zoologia (Curitiba)* 26, 587-593.
- Aizen, M.A., Harder, L.D., 2007. Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology* 88, 271-281.
- Amorim, M.D., Maruyama, P.K., Baronio, G.J., Azevedo, C.S., Rech, A.R., 2022. Hummingbird contribution to plant reproduction in the rupestrian grasslands is not defined by pollination syndrome. *Oecologia* 199, 1-12.
- Anderson, S.H., Kelly, D., Robertson, A.W., Ladley, J.J., 2016. Pollination by birds: a functional evaluation, in: Sekercioglu, Ç., Wenny, D., Whelan, C. (Eds.), *Why Birds Matter*. University of Chicago Press, Chicago, pp. 73-106.
- Arizaga, S., Ezcurra, E., Peters, E., de Arellano, F.R., Vega, E., 2000. Pollination ecology of *Agave macroacantha* (Agavaceae) in a Mexican tropical desert. II. The role of pollinators. *American Journal of Botany* 87, 1011-1017.
- Baker, H.G., 1963. Evolutionary mechanisms in pollination biology: Origins and functions of floral systems are being elucidated by genetical and ecological studies. *Science* 139, 877-883.
- Baker, H.G., 1975. Sugar concentrations in nectars from hummingbird flowers. *Biotropica* 37-41.
- Barrett, S.C.H., Emerson, B., Mallet, J., 1996. The reproductive biology and genetics of island plants. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 351, 725-733.
- Bergamo, P.J., Rech, A.R., Brito, V.L., Sazima, M., 2016. Flower colour and visitation rates of *Costus arabicus* support the 'bee avoidance' hypothesis for red-reflecting hummingbird-pollinated flowers. *Functional Ecology* 30, 710-720.
- Bierzuchudek, P., 1981. Pollinator limitation of plant reproductive effort. *The American Naturalist* 117, 838-840.
- Biesmeijer, J.C., Roberts, S.P., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R.J.M.C., Thomas, C.D., Settele, J., 2006.

- Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* 313, 351-354.
- Bond, W.J., 1994. Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 344, 83-90.
- Botes, C., Johnson, S.D., Cowling, R.M., 2009. The birds and the bees: using selective exclusion to identify effective pollinators of African tree aloes. *International Journal of Plant Sciences* 170, 151-156.
- Brown, J., Gehrt, S.D., 2009. The basics of using remote cameras to monitor wildlife. Ohio State University, Columbus.
- Burd, M., 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *The Botanical Review* 60, 83-139.
- Butler, H.C., Cozien, R.J., Johnson, S.D., 2022. For the birds? Contrasting pollination and breeding systems of the paintbrush lilies *Scadoxus puniceus* and *S. membranaceus* (Amaryllidaceae). *Plant Systematics and Evolution* 308, 1-11.
- Carpenter, F.L. 1976., Plant-pollinator interactions in Hawaii: Pollination energetics of *Metrosideros collina* (Myrtaceae). *Ecology* 57, 1125-1144.
- Carthew, S.M., Goldingay, R.L., 1997. Non-flying mammals as pollinators. *Trends in Ecology & Evolution* 12, 104-108.
- Celebrezze, T., Paton, D.C., 2004. Do introduced honeybees (*Apis mellifera*, Hymenoptera) provide full pollination service to bird-adapted Australian plants with small flowers? An experimental study of *Brachyloma ericoides* (Epacridaceae). *Austral Ecology* 29, 129-136.
- Cozien, R.J., van der Niet, T., Johnson, S.D., Steenhuisen, S.L., 2019. Saurian surprise: lizards pollinate South Africa's enigmatic hidden flower. *Bulletin of the Ecological Society of America* 100, e01554.
- Cronk, Q., Ojeda, I., 2008. Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany* 59,715-727.
- Cruden, R.W., 1972. Pollinators in high-elevation ecosystems: relative effectiveness of birds and bees. *Science* 176, 1439-1440.
- Cutler, T.L., Swann, D.E., 1999. Using remote photography in wildlife ecology: a review. *Wildlife Society Bulletin* 27, 571-581.
- de Villiers, M.J., 2008. Phylogenetic and population genetic studies in the genus *Streptocarpus* Lindl. (Gesneriaceae DC.). PhD Thesis. Stellenbosch University.

- Delph, L.F., Lively, C.M., 1985. Pollinator visits to floral colour phases of *Fuchsia excorticata*. *New Zealand Journal of Zoology* 12, 599-603.
- Diller, C., Castañeda-Zárate, M., Johnson, S.D., 2019. Generalist birds outperform specialist sunbirds as pollinators of an African *Aloe*. *Biology Letters* 15, 20190349.
- Dupont, Y.L., Strandberg, B., Damgaard, C., 2018. Effects of herbicide and nitrogen fertilizer on non-target plant reproduction and indirect effects on pollination in *Tanacetum vulgare* (Asteraceae). *Agriculture, Ecosystems & Environment* 262, 76-82.
- Faegri, K., van der Pijl, L., 1966. *The principles of pollination ecology*, first ed. Pergamon Press, Oxford.
- Faegri, K., van der Pijl, L., 1979. *The principles of pollination ecology*, third ed. Pergamon Press, Oxford.
- Feinsinger, P., Wolfe, J.A., Swarm, L.A., 1982. Island ecology: reduced hummingbird diversity and the pollination biology of plants, Trinidad and Tobago, West Indies. *Ecology* 63, 494-506.
- Fenster, C.B., Armbruster, W.S., Wilson, P., Dudash, M.R., Thomson, J.D., 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* 35, 375-403.
- Fernández de Castro, A.G., Moreno-Saiz, J.C., Fuertes-Aguilar, J., 2017. Ornithophily for the nonspecialist: Differential pollination efficiency of the Macaronesian island paleoendemic *Navaea phoenicea* (Malvaceae) by generalist passerines. *American Journal of Botany* 104, 1556-1568.
- Fleming, T.H., Geiselman, C., Kress, W.J., 2009. The evolution of bat pollination: a phylogenetic perspective. *Annals of Botany* 104, 1017-1043.
- Fleming, T.H., Muchhala, N., 2008. Nectar-feeding bird and bat niches in two worlds: pantropical comparisons of vertebrate pollination systems. *Journal of Biogeography* 35, 764-780.
- Ford, C.M., Johnson, S.D., 2008. Floral traits, pollinators and breeding systems in *Syncolostemon* (Lamiaceae). *Plant Systematics and Evolution* 275, 257-264.
- Frost, S.K., Frost, P.G.H., 1981. Sunbird pollination of *Strelitzia nicolai*. *Oecologia* 49, 379-384.
- García, D., Chacoff, N.P., 2007. Scale-dependent effects of habitat fragmentation on hawthorn pollination, frugivory, and seed predation. *Conservation Biology* 21, 400-411.

- Gardener, M.C., Gillman, M.P., 2002. The taste of nectar – a neglected area of pollination ecology. *Oikos* 98, 552-557.
- Geerts, S., 2016. Can short-billed nectar thieving sunbirds replace long-billed sunbird pollinators in transformed landscapes? *Plant Biology* 18, 1048-1052.
- Geerts, S., Pauw, A., 2009. Hyper-specialization for long-billed bird pollination in a guild of South African plants: the Malachite Sunbird pollination syndrome. *South African Journal of Botany* 75, 699-706.
- Geerts, S., Pauw, A., 2012. The cost of being specialized: pollinator limitation in the endangered geophyte *Brunsvigia litoralis* (Amaryllidaceae) in the Cape Floristic Region of South Africa. *South African Journal of Botany* 78, 159-164.
- Gibson, R.H., Nelson, I.L., Hopkins, G.W., Hamlett, B.J., Memmott, J., 2006. Pollinator webs, plant communities and the conservation of rare plants: arable weeds as a case study. *Journal of Applied Ecology* 43, 246-257.
- Godínez-Alvarez, H., 2004. Pollination and seed dispersal by lizards: a review. *Revista Chilena de Historia Natural* 77, 569-577.
- Goldblatt, P., Manning, J.C., Bernhardt, P., 1999. Evidence of bird pollination in Iridaceae of southern Africa. *Adansonia* 1999, 25-40.
- Grant, K.A., 1966. A hypothesis concerning the prevalence of red coloration in California hummingbird flowers. *The American Naturalist* 100, 85-97.
- Hargreaves, A.L., Harder, L.D., Johnson, S.D., 2009. Consumptive emasculation: the ecological and evolutionary consequences of pollen theft. *Biological Reviews* 84, 259-276.
- Hargreaves, A.L., Harder, L.D., Johnson, S.D., 2012. Floral traits mediate the vulnerability of aloes to pollen theft and inefficient pollination by bees. *Annals of Botany* 109, 761-772.
- Hargreaves, A.L., Johnson, S.D., Nol, E., 2004. Do floral syndromes predict specialization in plant pollination systems? An experimental test in an “ornithophilous” African *Protea*. *Oecologia* 140, 295-301.
- Hargreaves, A.L., Langston, G.T., Johnson, S.D., 2019. Narrow entrance of short-tubed *Aloe* flowers facilitates pollen transfer on long sunbird bills. *South African Journal of Botany* 124, 23-28.
- Hargreaves, A.L., Langston, G.T., Johnson, S.D., 2019. Narrow entrance of short-tubed *Aloe* flowers facilitates pollen transfer on long sunbird bills. *South African Journal of Botany* 124, 23-28.

- Harrison, C.J., Möller, M., Cronk, Q.C., 1999. Evolution and development of floral diversity in *Streptocarpus* and *Saintpaulia*. *Annals of Botany* 84, 49-60.
- Hayden, W.J., 2014. Humming Birds: Pollination Facts and Fancy. *Bulletin of the Virginia Native Plant Society* 33, 5.
- Hein, L., 2009. The economic value of the pollination service, a review across scales. *The Open Ecology Journal* 2, 74-82.
- Hilliard, O.M., Burt, B.L., 1971. *Streptocarpus*: An African plant study. University of Natal Press, Pietermaritzburg.
- Hoffman M.T., 1988. The pollination ecology of *Aloe ferox* Mill. *South African Journal of Botany* 54, 345-350.
- Hook, I., Sheridan, H., Reid, C., 2018. Trichomes and naphthoquinones protect *Streptocarpus dunnii* Hook. f. against environmental stresses. *South African Journal of Botany* 119, 193-202.
- Hughes, M., Möller, M., Edwards, T.J., Bellstedt, D.U., de Villiers, M., 2007. The impact of pollination syndrome and habitat on gene flow: a comparative study of two *Streptocarpus* (Gesneriaceae) species. *American Journal of Botany* 94, 1688-1695.
- Johnson S.D., Hargreaves A.L., Brown M. 2006. Dark, bitter-tasting nectar functions as a filter of flower visitors in a bird-pollinated plant. *Ecology* 87, 2709-2716
- Johnson, S.D. 1996., Bird pollination in south African species of *Satyrium* (Orchidaceae). *Plant Systematics and Evolution* 203, 91-98.
- Johnson, S.D., 2004. An overview of plant-pollinator relationships in southern Africa. *International Journal of Tropical Insect Science* 24, 45-54.
- Johnson, S.D., Brown, M., 2004. Transfer of pollinaria on birds' feet: a new pollination system in orchids. *Plant Systematics and Evolution* 244, 181-188.
- Johnson, S.D., Nicolson, S.W., 2008. Evolutionary associations between nectar properties and specificity in bird pollination systems. *Biology Letters* 4, 49-52.
- Johnson, S.D., Steiner, K.E., 2000. Generalization versus specialization in plant pollination systems. *Trends in Ecology & Evolution* 15, 140-143.
- Johnson, S.D., Steiner, K.E., 2003. Specialized pollination systems in southern Africa. *South African Journal of Science* 99, 345-348.
- Johnson, S.D., van der Niet, T., 2019. Bird pollination in an African *Satyrium* (Orchidaceae) confirmed by camera traps and selective exclusion experiments. *Plant Systematics and Evolution* 305, 477-484.

- Johnson, S.D., Wester, P., 2017. Stefan Vogel's analysis of floral syndromes in the South African flora: An appraisal based on 60 years of pollination studies. *Flora* 232, 200-206.
- Jones, C. E., Little., R.J., 1983. *Handbook of Experimental Pollination Biology*. Scientific and Academic Editions, New York.
- Kainulainen, K., Mouly, A., Khodabandeh, A., Bremer, B., 2009. Molecular phylogenetic analysis of the tribe Alberteae (Rubiaceae), with description of a new genus, *Razafimandimbonia*. *Taxon* 58, 757-768.
- Kearns, C.A., Inouye, D.W., 1997. Pollinators, flowering plants, and conservation biology. *Bioscience* 47, 297-307.
- Keys, R.N., Buchmann, S.L., Smith, S.E., 1995. Pollination effectiveness and pollination efficiency of insects foraging *Prosopis velutina* in south-eastern Arizona. *Journal of Applied Ecology* 32, 519-527.
- Kiepiel, I., Brown, M., Johnson, S.D., 2022. A generalized bird pollination system in *Schotia brachypetala* (Fabaceae). *Plant Biology Journal* 24, 806-814
- Kleizen, C., Midgley, J., Johnson, S.D., 2008. Pollination systems of *Colchicum* (Colchicaceae) in southern Africa: evidence for rodent pollination. *Annals of Botany* 102, 747-755.
- Knight, T., Ashman, T.L., Bennett, J., Burns, J., Passonneau, S., Steets, J., 2018. Reflections on, and visions for, the changing field of pollination ecology. *Ecology Letters* 21, 1282-1295.
- Knight, T.M., Steets, J.A., Ashman, T.L., 2006. A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. *American Journal of Botany* 93, 271-277.
- Knight, T.M., Steets, J.A., Vamosi, J.C., Mazer, S.J., Burd, M., Campbell, D.R., Dudash, M.R., Johnston, M.O., Mitchell, R.J., Ashman, T., 2005. Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution, and Systematics* 467-497.
- Kolb, A., 2008. Habitat fragmentation reduces plant fitness by disturbing pollination and modifying response to herbivory. *Biological Conservation* 141, 2540-2549.
- Krauss, S.L., Phillips, R.D., Karron, J.D., Johnson, S.D., Roberts, D.G., Hopper, S.D., 2017. Novel consequences of bird pollination for plant mating. *Trends in Plant Science* 22, 395-410.

- Krauss, S.L., Roberts, D.G., Phillips, R.D., Edwards, C., 2018. Effectiveness of camera traps for quantifying daytime and nighttime visitation by vertebrate pollinators. *Ecology and Evolution* 8, 9304-9314.
- Kunitake, Y.K., Hasegawa, M., Miyashita, T., Higuchi, H., 2004. Role of a seasonally specialist bird *Zosterops japonica* on pollen transfer and reproductive success of *Camellia japonica* in a temperate area. *Plant Species Biology* 19, 197-201.
- Law, W., Salick, J., Knight, T.M., 2010. The effects of pollen limitation on population dynamics of snow lotus (*Saussurea medusa* and *S. laniceps*, Asteraceae): Threatened Tibetan medicinal plants of the eastern Himalayas. *Plant Ecology* 210, 343–357.
- Lee, T.D., 1988. Patterns of fruit and seed production, in: Doust, J.L., Doust, L.L. (Eds.), *Plant reproductive ecology: patterns and strategies*. Oxford University Press, New York, pp. 179-202.
- Lozada-Gobilard, S., Weigend, M., Fischer, E., Janssens, S., Ackermann, M., Abrahamczyk, S., 2019. Breeding systems in Balsaminaceae in relation to pollen/ovule ratio, pollination syndromes, life history and climate zone. *Plant Biology* 21, 157-166.
- Lunau, K., 2000. The ecology and evolution of visual pollen signals. *Plant Systematics and Evolution* 222, 89-111.
- McWhorter, T. J., Martínez del Rio, C., Pinshow, B., 2003. Modulation of ingested water absorption by Palestine sunbirds: evidence for adaptive regulation. *Journal of Experimental Biology* 206, 659-666.
- Melidonis, C.A., Peter, C.I., 2015. Diurnal pollination, primarily by a single species of rodent, documented in *Protea foliosa* using modified camera traps. *South African Journal of Botany* 97, 9-15.
- Miller, R.S., 1985. Why hummingbirds hover. *The Auk* 102, 722-726.
- Möller, M., Atkins, H., Barber, S., Purvis, D., 2019. The living collection at the Royal Botanic Garden Edinburgh illustrates the floral diversity in *Streptocarpus* (Gesneriaceae). *Sibbaldia: The International Journal of Botanic Garden Horticulture* 155-177.
- Möller, M., Cronk, Q.C.B., 1997. Phylogeny and disjunct distribution: evolution of *Saintpaulia*. *Proceedings of the Royal Society of London* 264, 1827-1836.
- Müller, H., Delpino, F., 1871. Application of the Darwinian Theory to Flowers and the Insects which Visit Them. *The American Naturalist* 5, 271-297.
- Nabhan, G.P., Buchmann, S.L., 1997. Services provided by pollinators. *Nature's Services: Societal Dependence on Natural Ecosystems* 133-150.

- Newmark, W.D., Mkongewa, V.J., Amundsen, D.L., Welch, C., 2020. African sunbirds predominantly pollinate plants useful to humans. *The Condor* 122, 1-9.
- Nishii, K., Hughes, M., Briggs, M., Haston, E., Christie, F., DeVilliers, M.J., Hanekom, T., Roos, W.G., Bellstedt, D.U., Möller, M., 2015. *Streptocarpus* redefined to include all Afro-Malagasy Gesneriaceae: Molecular phylogenies prove congruent with geographical distribution and basic chromosome numbers and uncover remarkable morphological homoplasies. *Taxon* 64, 1243-1274.
- Obute, G.C., 2010. Pollination: a threatened vital biodiversity service to humans and the environment. *International Journal of Biodiversity and Conservation* 2, 001-013.
- Olesen, J.M., Valido, A., 2003. Lizards as pollinators and seed dispersers: an island phenomenon. *Trends in Ecology & Evolution* 18, 177-181.
- Ollerton, J., 1996. Reconciling ecological processes with phylogenetic patterns: the apparent paradox of plant--pollinator systems. *Journal of Ecology* 84, 767-769.
- Ollerton, J., Alarcón, R., Waser, N.M., Price, M.V., Watts, S., Cranmer, L., Hingston, A., Peter, C.I., Rotenberry, J., 2009. A global test of the pollination syndrome hypothesis. *Annals of Botany* 103, 1471-1480.
- Ollerton, J., Winfree, R., Tarrant, S., 2011. How many flowering plants are pollinated by animals? *Oikos* 120, 321-326.
- Ortmann, C.R., Johnson, S.D., 2020. How reliable are motion-triggered camera traps for detecting small mammals and birds in ecological studies? *Journal of Zoology* 313, 202-207.
- Paton, D.C., 1993. Honeybees in the Australian environment. *Bioscience* 43, 95-103.
- Pauw, A., 1998. Pollen transfer on birds' tongues. *Nature* 394, 731-732.
- Pauw, A., 2019. A bird's-eye view of pollination: Biotic interactions as drivers of adaptation and community change. *Annual Review of Ecology, Evolution, and Systematics* 50, 477-502.
- Pauw, A., Louw, K., 2012. Urbanization drives a reduction in functional diversity in a guild of nectar-feeding birds. *Ecology and Society* 17, 27.
- Percival, M.S., 1961. Types of nectar in angiosperms. *New Phytologist* 235-281.
- Pi, H.Q., Quan, Q.M., Wu, B., Lv, X.W., Shen, L.M., Huang, S.Q., 2021. Altitude-related shift of relative abundance from insect to sunbird pollination in *Elaeagnus umbellata* (Elaeagnaceae). *Journal of Systematics and Evolution* 59, 1266-1275.

- Potts, S.G., Imperatriz-Fonseca, V., Ngo, H.T., Aizen, M.A., Biesmeijer, J.C., Breeze, T.D., Dicks, L.V., Garibaldi, L.A., Hill, R., Settele, J., Vanbergen, A.J., 2016. Safeguarding pollinators and their values to human well-being. *Nature* 540, 220-229.
- Puff, C., Robbrecht, E., Randrianasolo, V., 1984. Observations on the SE African-Madagascan genus *Alberta* and its ally *Nematostylis* (Rubiaceae, Alberteae), with a survey of the species and a discussion of the taxonomic position. *Bulletin du Jardin botanique national de Belgique/Bulletin van de Nationale Plantentuin van België* 293-366.
- Pyke, G.H., Waser, N.M., 1981. The production of dilute nectars by hummingbird and honeyeater flowers. *Biotropica* 260-270.
- Ratto, F., Simmons, B.I., Spake, R., Zamora-Gutierrez, V., MacDonald, M.A., Merriman, J.C., Tremlett, C.J., Poppy, G.M., Peh, K.S.H., Dicks, L.V., 2018. Global importance of vertebrate pollinators for plant reproductive success: a meta-analysis. *Frontiers in Ecology and the Environment* 16, 82-90.
- Raven, P.H., 1972. Why are bird-visited flowers predominantly red? *Evolution* 26, 674-674.
- Rebelo, A.G., 1987. Bird Pollination in the Cape Flora, in: Rebelo A.G. (Ed.), *A preliminary synthesis of pollination biology in the Cape flora*. CSIR, Pretoria, pp. 83-108
- Regan, E.C., Santini, L., Ingwall-King, L., Hoffmann, M., Rondinini, C., Symes, A., Taylor, J., Butchart, S.H., 2015. Global trends in the status of bird and mammal pollinators. *Conservation Letters* 8, 397-403.
- Renner, S.S., Ricklefs, R.E., 1995. Dioecy and its correlates in the flowering plants. *American Journal of Botany* 82, 596-606.
- Richardson, M.B., Ayre, D.J., Whelan, R.J., 2000. Pollinator behaviour, mate choice and the realised mating systems of *Grevillea mucronulata* and *Grevillea sphacelata*. *Australian Journal of Botany* 48, 357-366.
- Robertson, A.W., 1992. The relationship between floral display size, pollen carryover and geitonogamy in *Myosotis colensoi* (Kirk) Macbride (Boraginaceae). *Biological Journal of the Linnean Society* 46, 333-349.
- Robertson, A.W., Kelly, D., Ladley, J.J., Sparrow, A.D., 1999. Effects of pollinator loss on endemic New Zealand mistletoes (Loranthaceae). *Conservation Biology* 13, 499-508.
- Rodger, J.G., Balkwill, K., Gemmill, B., 2004. African pollination studies: where are the gaps? *International Journal of Tropical Insect Science* 24, 5-28.
- Rodger, J.G., Bennett, J.M., Razanajatovo, M., Knight, T.M., van Kleunen, M., Ashman, T.L., Steets, J.A., Hui, C., Arceo-Gómez, G., Burd, M., Burkle, L.A., 2021.

- Widespread vulnerability of flowering plant seed production to pollinator declines. *Science Advances* 7, eabd3524.
- Rosas-Guerrero, V., Aguilar, R., Martén-Rodríguez, S., Ashworth, L., Lopezaraiza-Mikel, M., Bastida, J.M., Quesada, M., 2014. A quantitative review of pollination syndromes: do floral traits predict effective pollinators? *Ecology Letters* 17, 388-400.
- Rovero, F., Zimmermann, F., Berzi, D., Meek, P. 2013., " Which camera trap type and how many do I need?" A review of camera features and study designs for a range of wildlife research applications. *Hystrix* 24, 148–156.
- Schoen, D.J., Lloyd, D.G., 1992. Self-and cross-fertilization in plants. III. Methods for studying modes and functional aspects of self-fertilization. *International Journal of Plant Sciences* 153, 381-393.
- Scott-Dupree, C.D., Winston, M.L., 1987. Wild bee pollinator diversity and abundance in orchard and uncultivated habitats in the Okanagan Valley, British Columbia. *The Canadian Entomologist* 119, 735-745.
- Sekercioglu, C.H., 2006. Increasing awareness of avian ecological function. *Trends in Ecology & Evolution* 21, 464-471.
- Socias, R., Alonso, J.M., Aparisi, J.G., 2004. Fruit set and productivity in almond as related to self-compatibility, flower morphology and bud density. *The Journal of Horticultural Science and Biotechnology* 79, 754-758.
- Steen, R., 2017. Diel activity, frequency and visit duration of pollinators in focal plants: in situ automatic camera monitoring and data processing. *Methods in Ecology and Evolution* 8, 203-213.
- Steenhuisen, S.L., Raguso, R.A., Johnson, S.D., 2012. Floral scent in bird-and beetle-pollinated *Protea* species (Proteaceae): Chemistry, emission rates and function. *Phytochemistry* 84, 78-87.
- Stephenson, A.G., 1981. Flower and fruit abortion: proximate causes and ultimate functions. *Annual review of ecology and systematics* 12, 253-279.
- Stiles, F.G., 1978. Ecological and evolutionary implications of bird pollination. *American Zoologist* 18, 715-727.
- Stiles, F.G., Freeman, C.E., 1993. Patterns in floral nectar characteristics of some bird-visited plant species from Costa Rica. *Biotropica* 191-205.
- Stokes C.J., Yeaton R.I. 1995. Population dynamics, pollination ecology and the significance of plant height in *Aloe candelabrum*. *African Journal of Ecology* 33, 101-113.

- Swann, D.E., Hass, C.C., Dalton, D.C., Wolf, S.A., 2004. Infrared-triggered cameras for detecting wildlife: an evaluation and review. *Wildlife Society Bulletin* 32, 357-365.
- Symes, C.T., Nicolson, S.W., 2008. Production of copious dilute nectar in the bird-pollinated African succulent *Aloe marlothii* (Asphodelaceae). *South African Journal of Botany* 74, 598-605.
- van der Niet, T., 2021. Paucity of natural history data impedes phylogenetic analyses of pollinator-driven evolution. *New Phytologist* 229, 1201-1205.
- van der Niet, T., Cozien, R.J., Johnson, S.D., 2015. Experimental evidence for specialized bird pollination in the endangered South African orchid *Satyrium rhodanthum* and analysis of associated floral traits. *Botanical Journal of the Linnean Society* 177, 141-150.
- van der Niet, T., Cozien, R. J., Castañeda-Zárate, M., Johnson, S. D., 2022. Long-term camera trapping needed to identify sunbird species that pollinate the endangered South African orchid *Satyrium rhodanthum*. *African Journal of Ecology* 60, 1278-1282.
- van der Pijl, L., 1961. Ecological aspects of flower evolution. II. Zoophilous flower classes. *Evolution* 15, 44-59.
- van Etten, M.L., Sukkaewmanee, P., Tate, J.A., Robertson, A.W., 2018. Pollinator service affects quantity but not quality of offspring in a widespread New Zealand endemic tree species. *Conservation Genetics* 19, 815-826.
- van Etten, M.L., Tate, J.A., Anderson, S.H., Kelly, D., Ladley, J.J., Merrett, M.F., Peterson, P.G., Robertson, A.W., 2015. The compounding effects of high pollen limitation, selfing rates and inbreeding depression leave a New Zealand tree with few viable offspring. *Annals of Botany* 116, 833-843.
- van Staden, J., Kelly, K., Stormanns, C., 1990. Dormancy and germination of *Alberta magna* seeds. *South African Journal of Botany* 56, 542-545
- Vanbergen, A.J., 2013. Threats to an ecosystem service: pressures on pollinators. *Frontiers in Ecology and the Environment* 11, 251-259.
- Vanbergen, A.J., 2014. Landscape alteration and habitat modification: impacts on plant-pollinator systems. *Current Opinion in Insect Science* 5, 44-49.
- Viljoen, C., Velembu, S., 2013. Kirstenbosch National Botanical Garden. SANBI PlantZAfrica. Online: <https://pza.sanbi.org/streptocarpus-dunnii>.
- Vogel, S., 1954. Blütenbiologische Typen als Elemente der Sippengliederung dargestellt anhand der Flora Siidafrikas. Gustav Fischer Verlag, Jena.

- Weber, A., Bellstedt, D., Möller, M., 2021., Die Gesneriaceen Afrikas und ihre vogelblütigen Vertreter: *Streptocarpus dunnii* und *S. myoporoides*. Der Palmengarten 85, 125-138.
- Wesselingh, R.A., 2007. Pollen limitation meets resource allocation: towards a comprehensive methodology. *New Phytologist* 174, 26-37.
- Wester, P., Cairampoma, L., Haag, S., Schramme, J., Neumeyer, C., Claßen-Bockhoff, R., 2020. Bee exclusion in bird-pollinated *Salvia* flowers: the role of flower color versus flower construction. *International Journal of Plant Sciences* 181, 770-786.
- Whittaker, D.J., 2022. *The Secret Perfume of Birds: Uncovering the Science of Avian Scent*. John Hopkins University Press, Maryland.
- Wilcock, C., Neiland, R., 2002. Pollination failure in plants: why it happens and when it matters. *Trends in Plant Science* 7, 270-277.
- Yamaji, F., Ohsawa, T.A., 2016. Field Experiments of Pollination Ecology: The Case of *Lycoris sanguinea* var. *sanguinea*. *Journal of Visualized Experiments* 117, e54728.
- Zoeller, K.C., Steenhuisen, S.L., Johnson, S.D., Midgley, J.J., 2016. New evidence for mammal pollination of *Protea* species (Proteaceae) based on remote-camera analysis. *Australian Journal of Botany* 64, 1-7.

Chapter 2

**Done speculating: Camera traps and experimental evidence confirm
the long-suspected importance of sunbirds as pollinators of
Streptocarpus dunnii (Gesneriaceae)**

2.1 ABSTRACT

Although floral traits can provide clues about which pollinators visit a plant species, the interaction can only be confirmed by direct pollinator observations and experimentation. A shift from insect to bird pollination in *Streptocarpus* (Gesneriaceae) has been inferred from the derived position of *Streptocarpus dunnii* which has densely packed red, tubular flowers which conform to the bird pollination syndrome, in contrast to the light magenta to pale violet flowers of related species which are considered to be insect-pollinated. *Streptocarpus dunnii* occurs among rocks in open grassland habitat which has hindered previous attempts to observe bird pollinators of this species. I used camera traps to identify floral visitors and recorded over 300 visits by malachite sunbirds which carried large loads of pollen, at three study sites. Greater double-collared sunbirds visited less frequently, at one site, and typically robbed flowers rather than feeding legitimately. Selective exclusion experiments revealed that *S. dunnii* does not produce fruit and seeds when birds, but not insects, are excluded from flowers. Supplemental hand-pollination experiments showed that seed production in natural populations is not pollen-limited. Breeding system experiments showed that *S. dunnii* is completely self-compatible, but reliant on pollinators for reproduction. This study confirms previous suggestions that *S. dunnii* represents the outcome of a novel shift from insect to bird pollination in *Streptocarpus* and also highlights the importance of malachite sunbirds for pollination of high-altitude grassland plant species.

Keywords: sunbirds, pollination syndrome, floral traits, breeding system, camera traps, selective exclusion, pollen limitation, self-compatibility

2.2 INTRODUCTION

Animal pollination is a key process to ensure the continued reproduction of many plants (Kearns, Inouye & Waser, 1998; Whelan, Wenny & Marquis, 2008; Ollerton, Winfree & Tarrant, 2011) and is considered to play a role in plant diversification (Gervasi & Schiestl, 2017). Bird pollination contributes to the maintenance of ecosystems (Whelan, Wenny & Marquis, 2008) and is found in many regions all over the world (Stiles, 1981; Cronk & Ojeda, 2008; Pauw, 2019).

Flowers often exhibit morphological traits and possess rewards which are attractive to certain animal pollinator groups; these suites of traits are known as pollination syndrome (Faegri & van der Pijl, 1979; Johnson & Bond, 1994; Castellanos, Wilson & Thomson, 2003; Fenster *et al.*, 2004). Moreover, plant species pollinated by birds often show particular floral traits which are attractive to birds (Brown & Kodric-Brown, 1979; Fenster *et al.*, 2004). Flowers pollinated by birds are often red or orange or have a combination of colours which could also include yellow, blue or green (Johnson & Bond, 1994; Rodríguez-Gironés & Santamaría, 2004; Cronk & Ojeda, 2008). In comparison to bee-pollinated plant species, bird-pollinated plants have larger nectar volumes (Nicolson, 2002; Cronk & Ojeda, 2008) and nectar sugar concentration is relatively dilute (Baker, 1975; Pyke & Waser, 1981; Nicolson, 2002). Differences among pollinating birds can result in even smaller scale floral trait patterns (Johnson & Nicolson, 2008), such as differences in nectar composition. Nectar sugar composition normally consists of glucose, fructose and sucrose with sucrose often being found at the highest concentration in hummingbird-pollinated plants (Freeman *et al.*, 1984; Stiles & Freeman, 1993) and hexose being dominant in passerine-pollinated plants (Nicolson, 2002). However, in a study conducted on ornithophilous *Erica* species, 29 species were found to be sucrose dominant while only eight species had hexose dominant nectars (Barnes, Nicolson & van Wyk, 1995). Bird-pollinated flowers often have a long and narrow corolla tube, which can accommodate bird beaks (Castellanos, Wilson & Thomson, 2003; Cronk & Ojeda, 2008). Furthermore, bird-pollinated flowers lack scent (Knudsen *et al.*, 2004; Cronk & Ojeda, 2008). While hummingbirds tend to hover while feeding, other flowering visiting birds, such as sunbirds, use perches while feeding (Anderson, Cole & Barrett, 2005; Johnson, Kiepiel & Robertson, 2020).

As predicted, birds have been found to be effective pollinators of plants that conform to the bird pollination syndrome (e.g. Johnson, 1996; van der Niet, Cozien & Johnson, 2015; Kiepiel, Brown & Johnson, 2022). It is often less clear whether these plants depend strictly on birds for pollination as plants pollinated by birds are often visited by other animals (Whelan, Wenny & Marquis, 2008; Krauss *et al.*, 2017). When other visitors visit flowers adapted for pollination by birds, they may be able to feed on nectar without pollinating (transferring pollen between reproductive parts of flowers) (Inouye, 1980; Castellanos, Wilson & Thomson, 2003; Botes, Johnson & Cowling, 2009). These visitors are known as nectar thieves, nectar robbers or even parasites (McDade & Kinsman, 1980) and could have a negative impact on plant reproductive success because they exploit nectar resources without contributing to pollen transfer (Vaughton, 1996; Irwin, Brody & Waser, 2001). McDade and Kinsman (1980) found that the amount of nectar available in *Aphelandra golfodulcensis* and *Justicia aurea* flowers was decreased more by visitors (other birds and insects) that pierce through the side of flowers than those that actually pollinated the flowers (hermit hummingbirds). Excluding birds is a useful way to determine how much birds alone contribute to reproductive success (Ratto *et al.*, 2018). Johnson and van der Niet (2019) showed that access by birds increased fruit set, but *Satyrium neglectum* subsp. *woodii* was poorly pollinated and produced nearly no fruit when exposed to insects only. In a study by Hargreaves, Johnson and Nol (2004) on *Protea roupelliae*, the exclusion of birds led to a large significant decrease in seed set even though there were many insect visitors. These studies show that insect visitors are often ineffective as pollinators of plant species adapted for pollination by birds.

Streptocarpus forms part of the diverse family Gesneriaceae, known commonly as Cape Primroses (Chen *et al.*, 2020). The genus is characterized by high levels of floral morphological diversity which is thought to reflect a diversity of pollination systems (Hilliard & Burt, 1971; Möller & Cronk, 1997; Möller *et al.*, 2019; Chen *et al.*, 2020). The genus consists of 176 species which are mainly found in southern and central Africa, including Madagascar and the Comoro Islands, but four species which are not closely related to the African taxa occur in southeast Asia (Hilliard & Burt, 1971; Möller & Cronk, 2001; de Villiers, 2008; Nishii *et al.*, 2015). *Streptocarpus* has been found to be comprised of two clades, where *Colpogyne*, *Linnaeopsis* and the subgenus

Streptocarpus (including the species *Streptocarpus dunnii*) is placed in the second clade (Nishii *et al.*, 2015). *Streptocarpus dunnii* is closely related to the species *S. pole-evansii* and *S. denticulatus* (Hilliard & Burt, 1971; Nishii *et al.*, 2015). *Streptocarpus dunnii* and *S. pole-evansii* produces a red pigment granule, known as a naphthaquinone, namely dunnione (Hilliard & Burt, 1971). *Streptocarpus denticulatus* produces a different pigment, a chalcone quinone (Hilliard & Burt, 1971). Solid pigment granules are produced in only these three species in *Streptocarpus*, indicating their close relation (Hilliard & Burt, 1971). *Streptocarpus* flowers which are purple, pink or blue with wide openings and tube-shaped, wide corollas are associated with bee pollination (Möller *et al.*, 2019). Species such as *Streptocarpus candidus* and *Streptocarpus fanniniae* possess these traits and scent and are considered to be insect-pollinated (Jong & Burt, 1975; Harrison *et al.*, 1999; Möller *et al.*, 2019). Flowers with narrow tubes and wide “ventral and lateral” petals are associated with moth and butterfly pollination; *Streptocarpus johannis*, for example, could be butterfly-pollinated (Tsou *et al.*, 2014). *Streptocarpus myoporoides* and *Streptocarpus dunnii* are the most unusual species in the *Streptocarpus* group as they possess tubular, red corollas, which other species do not possess, and they have been predicted to be bird-pollinated (Weber, Bellstedt & Möller, 2021). However, literature on the pollination biology within the genus *Streptocarpus* is scarce. Most of the pollinators of *Streptocarpus* species are speculated based on traits aligning with pollination syndromes or a few observations only (Möller *et al.*, 2019; Weber, Bellstedt & Möller, 2021).

The morphological traits of *S. dunnii*, including its red colour and its long curved floral tubes, suggests that it is bird-pollinated (Harrison, Möller & Cronk, 1999; Hughes *et al.*, 2007), however there has not been any literature confirming this except for a few anecdotal observations (Möller pers. obs. in Hughes *et al.*, 2007; pers. obs. Bellstedt, Hughes & Möller in Möller *et al.*, 2019; Weber, Bellstedt & Möller, 2021). Bird pollination of a species cannot be assumed on the basis of floral traits, as long-tubed red flowers can also be pollinated by insects such as butterflies (Johnson & Bond, 1994). Hughes *et al.* (2007) reported the observation of a single visit by a malachite sunbird (*Nectarinia famosa* L.), at Verloren Vallei Nature Reserve in South Africa, Mpumalanga. *Streptocarpus dunnii* is found in rocky environments with large boulders, in and around rock clefts (Hughes *et al.*, 2007), which may make it particularly difficult

to see birds visiting it, as it grows close to the ground and thus may be hidden from view.

This study investigated the pollination biology of the South African plant species, *Streptocarpus dunnii*. The main objectives of this chapter were to quantify floral traits of *S. dunnii* for comparison with other bird-pollinated species, establish the breeding system and determine reliance on pollinators for reproduction, identify floral visitors and determine their contribution to reproductive success and finally, to determine if current pollination service limits reproductive success through pollen limitation.

2.3 MATERIALS AND METHODS

2.3.1 Study site and species

Streptocarpus dunnii is stemless and usually produces only a single leaf in its lifetime but populations with several leaves have been found in Barberton (Hilliard & Burt, 1971). It produces a dense inflorescence (Hughes *et al.*, 2007) but is monocarpic and flowers die after setting seed (Hilliard & Burt, 1971; Hook, Sheridan & Reid, 2018). *Streptocarpus dunnii* can commonly be found in shaded and moist rocky habitats, in open grasslands, at the base of large boulders or in the clefts of rocks (Möller & Cronk, 2001; Hughes *et al.*, 2007). The very small seeds of this species are released from spiral shaped fruit and are considered to be dispersed by wind (Hilliard & Burt, 1971). The species occurs in Drakensberg and Steenkampsberg, located within Mpumalanga (Hughes *et al.*, 2007) and some areas in Swaziland (Hilliard & Burt, 1971).

Streptocarpus dunnii flowers from November to February (Hilliard & Burt, 1971).

Fieldwork on *S. dunnii* was done in eSwatini at Malolotja Nature Reserve (26°08'28" S, 31°08'08" E) and in South Africa, at Field and Stream Farm (25°25'29" S, 30°01'02" E; Tonteldoos), Verloren Vallei Nature Reserve (25°18'14" S, 30°07'27" E; Verloren Vallei) and Mountainlands Nature Reserve (25°45'02" S, 31°06'03" E) in Mpumalanga. Field and Stream farm is found on the Tonteldoos road and is therefore referred to as Tonteldoos in this study. Fieldwork took place from 14th December 2021 to 26th January 2022.

2.3.2 Floral traits

2.3.2.1 *Measurement of flower and inflorescence traits*

Floral measurements were recorded for flowers sampled at Tonteldoos (n=26), Malolotja (n=20), Mountainlands (n=11) and Verloren Vallei (n=20). One flower was collected randomly per individual plant at each site for as many plants as possible. The following was measured for each flower to the closest 0.01 millimeters using digital callipers: flower tube length; sepal length; opening height of flower; whole flower height; opening width; whole flower width; and anther and stigma length. Tube length was measured from the base of the flower to the point at which the petals fuse. Sepal length was measured from the receptacle to the tip of the sepal. Flower opening height and width were measured as the maximum vertical and horizontal distances, respectively, across the mouth of the floral tube. Whole flower height and width were measured as the maximum vertical and horizontal distances, respectively, across the flower face including the petals. The filament was measured from the base of the filament to the furthest tip of the anther. The style was measured from the base of the style to the tip of the stigma. For every flower measured, evidence of robbing (pierced tissue at the base of the floral tube) was also noted.

The number of open flowers per inflorescence was recorded from camera trap video footage.

2.3.2.2 *Scanning electron microscopy*

Scanning electron microscopy was used to examine stages of floral development in *S. dunnii* flowers. Different developmental stages and parts of flowers of *S. dunnii* were subjected to electron microscopy, including the stigma, anther, ovary and flower bud. Samples were preserved in 70% ethanol after harvesting. For primary fixation, samples were placed in vials and covered in 3% buffered glutaraldehyde to rehydrate samples. Thereafter, samples underwent a buffer wash. The glutaraldehyde was removed and samples were covered with 0.05M sodium cacodylate buffer and left for five minutes. This procedure was repeated. The sodium cacodylate was removed for the dehydration step. Samples were covered with 10% ethanol for 10 minutes, then removed and covered with 30% ethanol for 10 minutes. This was repeated for 50% ethanol for 10 minutes, 70% ethanol for 10 minutes and 90% ethanol for 10 minutes. Finally, samples were placed in 100% ethanol for 10 minutes and this step was repeated twice. For the

last round of 100% ethanol, the samples were transferred to the critical point drying (CPD) baskets in a Petri dish, immersed in the 100% ethanol, for critical point drying. The CPD baskets were then placed in the Quorum K850 CPD machine. During CPD, ethanol is replaced with liquid CO₂. The CO₂ is heated and pressurised to its critical point, at which the liquid converts to gas without the damaging effects of surface tension on the sample. This results in a dry, intact sample. Finally, the dried samples are mounted onto scanning electron microscopy stubs, using tape on the stubs for samples to stick.

2.3.2.3 *Nectar volume and concentration*

Nectar measurements were taken to quantify volumes of available nectar and nectar produced over 24 hours, as well as nectar concentration and sugar composition for *S. dunnii*. Nectar standing crop volume was measured at Tonteldoos (n=14), Malolotja (n=18), Mountainlands (n=6) and Verloren Vallei (n=35) within an hour of midday, using 5µL microcapillaries. To measure the amount of nectar produced by flowers over a 24-hour period, inflorescences were bagged using mesh organza bags, tightened at the base of inflorescences, to prevent visitors from accessing nectar. After 24 hours, nectar volume of flowers isolated from pollinators was measured using 5µL microcapillaries (Tonteldoos, n=29; Malolotja, n=20; Verloren Vallei, n=30).

A Bellingham and Stanley 0–50% refractometer was used to measure nectar concentrations (Tonteldoos, n=46; Malolotja, n=20; Verloren Vallei, n=42).

2.3.2.4 *Flower colour analysis*

To quantify flower colour, spectral reflectance was recorded for flowers from populations at Tonteldoos (n=9), Malolotja (n=8) and Mountainlands (n=6). For each flower, spectral reflectance was recorded for the lower front floral sepal (longitudinal and cross section) as well as for the upper outer and inner surfaces of the floral tube. The spectral reflectance of a leaf from each plant was also recorded. Spectral reflectance over the UV-visible 300-700nm range was recorded using an Ocean Optics USB 2000 spectrometer with the fibre optic reflection probe held at 45° to the surface of the floral part. An Ocean Optics Mini-DT light source with a spectral range of c. 200-1100nm was used. An Ocean Optics WS-1 diffuse reflectance standard was used to calibrate

spectra. Spectra were recorded with Ocean Optics SpectraSuite software at intervals of 0.34nm. Integration time was set to 2300ms, with three scans averaged for each reading, and boxcar width set to two.

2.3.3 *Breeding system experiments*

Breeding system experiments were set up at Tonteldoos (n=7) and Verloren Vallei (n=2) to determine if *S. dunnii* is self-compatible and if it depends on pollinators for seed production. To conduct breeding system experiments, inflorescence branches with flowers in bud stage was bagged with mesh bags to exclude pollinators. When these flowers opened and flowers were in female phase with receptive stigmas, one raceme on each inflorescence was randomly assigned to each of three different treatments. One raceme was selected for manual cross-pollination with pollen from another plant, at least five meters distant, one raceme was assigned to manual self-pollination with pollen from the same plant and a third raceme was left unmanipulated to test for autonomous self-fertilization. To distinguish between the different treatments, different coloured wires were wrapped around the base of each raceme. The treated inflorescence was bagged again to prevent visitors from contaminating pollination treatments, until developed fruits were collected approximately two months after pollination treatments were applied.

The mean number of seeds produced for unmanipulated, self and cross treatments in the breeding system experiments were used to calculate indices of self-compatibility and autofertility. The index of self-compatibility (ISC) was calculated according to the formula of Becerra and Lloyd (1992). If values are close to zero, the species is self-incompatible whereas values closer to one indicate self-compatibility.

$$ISC = \frac{\text{Mean reproductive success Self}}{\text{Mean reproductive success Cross}}$$

The autofertility index (AFI) was calculated using the formula in Lloyd and Schoen (1992). Values close to zero indicate dependence on pollinators for seed production; values close to one, indicate that the species is not reliant on pollinators for reproduction.

$$AFI = \frac{\text{Mean reproductive success Auto}}{\text{Mean reproductive success Cross}}$$

2.3.4 Visitor observations and camera trapping

Direct observations were conducted to observe visitors to flowers of *S. dunnii* over five days at Verloren Vallei and over six days at the Tonteldoos study population. Additionally, twelve Bushnell NatureView HD motion trigger cameras (model 119740, Bushnell Outdoor Products) were used to record flower visitors. Cameras were set to video mode for 60 seconds with a video size of 1920x1080 on 24-hour camera mode. Sensitivity level was set to high and video sound was on. Cameras were fitted with close focussing 460mm and 600mm lenses and set up at these distances from the focal inflorescences. Cameras were set up using gorilla tripods and rocks in the surroundings were used to stabilize and conceal cameras. Cameras were set up in the mornings and SD cards changed each day when doing fieldwork at Tonteldoos and Verloren Vallei. Cameras were also set up and left for three to five weeks at Tonteldoos, Verloren Vallei (19 December 2021 to 25 January 2022), Malolotja and Mountainlands (5 January to 22 January 2022). For each interaction recorded by the cameras, the date of the interaction, time of day, gender and species of the flower visitor and the number of open flowers on an inflorescence was noted. Additionally, the number of flowers probed, proportion of flowers visited in an inflorescence out of the number of open flowers, whether visitors visibly contacted anthers and or stigmas, and the number of instances of each, whether pollen was visible on the head and or on the beak was recorded. Aspects of feeding behaviour which was noted included, direction of probing, (with or counter to floral curvature), direction of feeding movement within an inflorescence, (whether upward, downward, or horizontal) hovering or perching behaviour, and nature of the perch used, as well as any visible instances of nectar robbing by piercing the floral tube in contrast to legitimate probing of flowers through the flower opening.

2.3.5 Selective exclusion experiments

Selective exclusion experiments were used to determine the contribution of birds and of potential smaller insect visitors to fruit and seed set. Exclusion experiments were set up at Verloren Vallei (n=10) and Tonteldoos (n=8 replicates). Each replicate in the exclusion experiments consisted of three treatments: complete exclusion, for which the

entire inflorescences was covered with a plastic mesh cage (aperture size: 10x13mm) and additionally covered in bridal veil gauze, to exclude all possible visitors; a bird exclusion treatment in which the entire inflorescences was covered with a plastic mesh cage only (aperture size: 10x13mm), to exclude bird visitors but allow access by smaller, insect visitors, and a control inflorescence which was tagged but otherwise unmanipulated, allowing access for all visitors. As far as possible, inflorescences of similar size (number of flowers in bud) were assigned to the different treatments within each replicate. For each treatment inflorescence, the number of treatment flowers (buds) was recorded and any open flowers which might already have received pollinator visits, were removed. Cages were left in place through peak flowering, and fruits were harvested after approximately ten weeks.

2.3.6 Pollen limitation

Pollen-supplementation experiments were conducted at Tonteldoos (n=30) and Verloren Vallei (n=30) to determine whether fecundity in *S. dunnii* is limited by pollen receipt. To test for pollen limitation, a raceme on a random inflorescence was identified and cross-pollinated with pollen from another plant and a second raceme on the same plant was tagged as a control but otherwise unmanipulated, subjected to natural pollination. Cross pollen was applied by unfolding the anthers to expose the pollen and applying it directly to the stigma of the treatment flower. Treatment and control racemes were marked with coloured wires. Where possible, an equal or similar number of flowers (typically 3 to 5) was assigned to both treatments on each experimental plant. For each replicate (plant) the number of flowers assigned to hand-pollination and control treatments was recorded.

A pollen limitation index (PL index) was calculated according to the formula of Larson and Barrett (2000) using the mean number of seeds produced by flowers assigned to the hand supplementation and control treatments in the pollen limitation experiments, as:

$$PL\ index = 1 - \frac{Natural}{Supplemented}$$

If the value is close to zero, the plant species is not pollen-limited whereas values close to 1 indicate strong pollen limitation of fecundity. Values falling near and below 0.5,

indicate species show intermediate level of not being pollen limited and values near and above 0.5, indicate that species show an intermediate level of being pollen limited.

2.3.7 Pollen and ovule production

Pollen and ovule numbers were estimated for 13 flowers to determine the pollen-ovule ratio of *S. dunnii*. To quantify pollen grains found in the anther of each flower, unopened anthers were placed in 1ml 70% ethanol in an Eppendorf tube. The anther was opened using a dissecting needle to release pollen and the Eppendorf tube was vortexed for 60 seconds to suspend the pollen grains in solution. From each sample, a 200µl subsample was placed on a microscope slide. Melted fuchsin gel was used to stain the pollen grains. The stained pollen grains were counted at 10x magnification under a compound microscope (Zeiss AXIO fluorescence microscope). The mean number of grains across subsamples was multiplied by 5 to estimate the total pollen grains found in one flower.

Ovaries placed in 70% ethanol were dissected, dried overnight to loosen ovules, and all ovules were removed from the ovary tissue for counting. Ovules were counted using a stereo microscope (Zeiss Steri 2000-C) at 0.65x magnification. To determine the pollen-ovule ratio, the estimate of the number of pollen grains per flower was divided by the estimated number of ovules counted for 13 flowers.

2.3.8 Data analyses

All statistical analyses were conducted in SPSS version 28 (IBM). To test for differences between floral measurements, nectar volume and concentration among sites, Generalized Linear Models (GLMs) with a Gaussian distribution were used. Site was included as a fixed factor in the model. GLMs were also used to test for differences among treatments in breeding system, selective exclusion and pollen limitation experiments. Numbers of fruits produced per flower, seeds produced per ovule and seeds per flower were analysed with a binomial distribution and logit link function. Generalized Estimating Equations with an exchangeable correlation matrix were used to account for correlated responses where multiple treatments were applied to a plant. Sites were pooled for the breeding system analysis, as there were only two replicates at the Verloren Vallei site. In instances where there was a lack of variance, because no flowers

assigned to a particular treatment produced any fruit or seeds, prevented model convergence, one 0 was replaced with a 1 in that treatment (Zuur, Ieno & Smith, 2007). Significance was assessed using Wald statistics. To adjust significance for multiple comparisons, the sequential Šidák method was used. Data was back-transformed from log scales in order to plot means and standard errors. Graphs were produced using SigmaPlot 8.0. Figures with graphics were produced using CorelDRAW X4 (version 14.0.0.567).

Colour spectra were analysed using R (2021) and using the package ‘pavo’ (Maia et al., 2013). Local regression smoothing was used to optimize spectra with a 0.15 smoothing parameter.

2.4 RESULTS

2.4.1 *Floral traits*

2.4.1.1 Flower measurements

Flower number per inflorescence ranged from 12 to 88 across sites. Tube length ranged from 29 to 44mm; floral tubes were longest at Verloren Vallei and shortest at Malolotja (Table 1). This trend applied to other aspects of flower size including sepal length, opening width, whole flower width and anther length, for which Tonteldoos and Verloren Vallei had the largest floral measurements. Height and width of the flower opening ranged from 7mm to 9mm and 6mm to 7.5mm respectively (Table 1).

Table 1. Floral and inflorescence trait measurements for four studied populations of *Streptocarpus dunnii*. Values are means (\pm SE). Numbers in parentheses indicate samples size (number of flowers or plants). Different superscript letters indicate significant differences in post-hoc comparisons among sites for any trait.

Site	Tonteldoos	Malolotja	Mountainlands	Verloren Vallei	χ^2	p
Trait						
Tube length (mm)	36.28 \pm 0.49 (26) ^a	33.00 \pm 0.56 (20) ^b	33.44 \pm 0.76 (11) ^b	37.00 \pm 0.56 (20) ^a	35.93	<0.001
Sepal length (mm)	6.69 \pm 0.15 (26) ^a	5.10 \pm 0.17 (20) ^b	5.66 \pm 0.23 (11) ^b	7.23 \pm 0.17 (20) ^a	97.54	< 0.0001
Opening height (mm)	8.82 \pm 0.17 (26) ^{ab}	7.21 \pm 0.19 (20) ^c	8.19 \pm 0.26 (11) ^a	9.12 \pm 0.19 (20) ^b	60.82	<0.001
Opening width (mm)	7.56 \pm 0.17 (26) ^a	5.81 \pm 0.19 (20) ^b	6.22 \pm 0.26 (11) ^b	7.55 \pm 0.19 (20) ^a	67.56	<0.001
Flower height (mm)	17.01 \pm 0.36 (26) ^a	13.73 \pm 0.41 (20) ^b	15.58 \pm 0.55 (11) ^c	18.74 \pm 0.41 (20) ^d	79.25	<0.0001
Flower width (mm)	16.99 \pm 0.44 (26) ^a	13.56 \pm 0.51 (20) ^b	14.20 \pm 0.68 (11) ^b	18.49 \pm 0.51 (20) ^a	59.13	<0.001
Filament length (mm)	38.09 \pm 0.52 (26) ^a	33.32 \pm 0.59 (20) ^b	32.59 \pm 0.80 (11) ^b	39.67 \pm 0.59 (20) ^a	90.48	<0.001
Style length (mm)	38.45 \pm 0.89 (26) ^a	34.52 \pm 1.01 (20) ^b	35.87 \pm 1.36 (11) ^{ab}	39.36 \pm 1.01 (20) ^a	14.52	0.002
Robbing rate (%)	7.7 (26)	15 (20)	9.1 (11)	10 (20)		
24hr nectar volume (μl)	4.15 \pm 0.58 (29, 10)	4.70 \pm 0.70 (20, 6)	/	5.86 \pm 0.57 (30, 6)	4.63	0.099

Standing crop nectar volume (μl)	1.52 ± 0.57 (14)	0.44 ± 0.50 (18)	0 ± 0.87 (6)	0.84 ± 0.36 (35)	3.01	0.391
Sugar concentration (%)	19 ± 0.6 (46, 28)	19.5 ± 0.84 (20)	/	17.3 ± 0.6 (42, 18)	6.26	0.044
Open flowers per inflorescence	55.66 ± 2.10 (8) ^a	50.45 ± 1.02 (19) ^b	/	34.50 ± 1.10 (11) ^c	144.19	<0.0001

2.4.1.2 Scanning electron microscopy

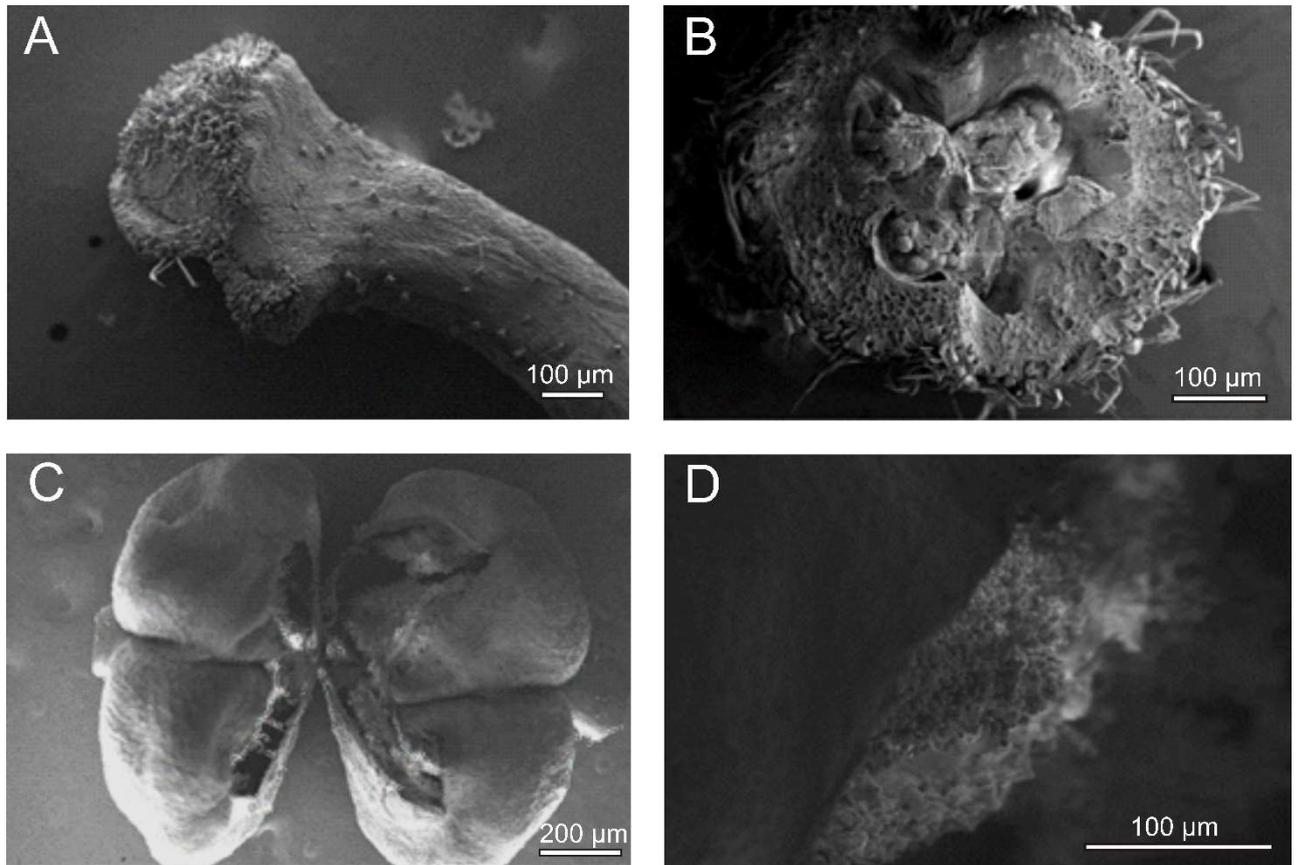


Figure 1. Scanning electron microscopy images of floral parts of *Streptocarpus dunnii*: stigma (A), a cross-section of the ovary (B), the opened anther locule (C), the interior of the anther, showing the pollen grains (D).

The surface of the stigma had finger-like protrusions, possibly aiding adherence of pollen grains (Fig. 1A). The butterfly-shaped anther of *S. dunnii*, held a large amount of pollen on the inner surfaces (Fig. 1C, D). The ovary contained a large number of ovules (mean ovules per flower 3301.54 ± 298.07) (Fig. 1B).

2.4.1.3 Nectar volume and concentration

Nectar volumes did not differ among sites for standing crop or for the volume produced over 24 hours (Table 1). Standing crop nectar volume (mean \pm SE, $0.80 \pm 0.32 \mu\text{l}$; range zero to $14 \mu\text{l}$) was significantly less than nectar volume accumulated over 24 hours ($4.94 \pm 0.31 \mu\text{l}$; range zero to $16 \mu\text{l}$; $\chi^2=88.05$, $p < 0.0001$; Fig. 2).

The mean nectar concentration across sites was $18.4 \pm 0.37\%$. There were no significant differences in nectar sugar concentration among sites (Table 1).

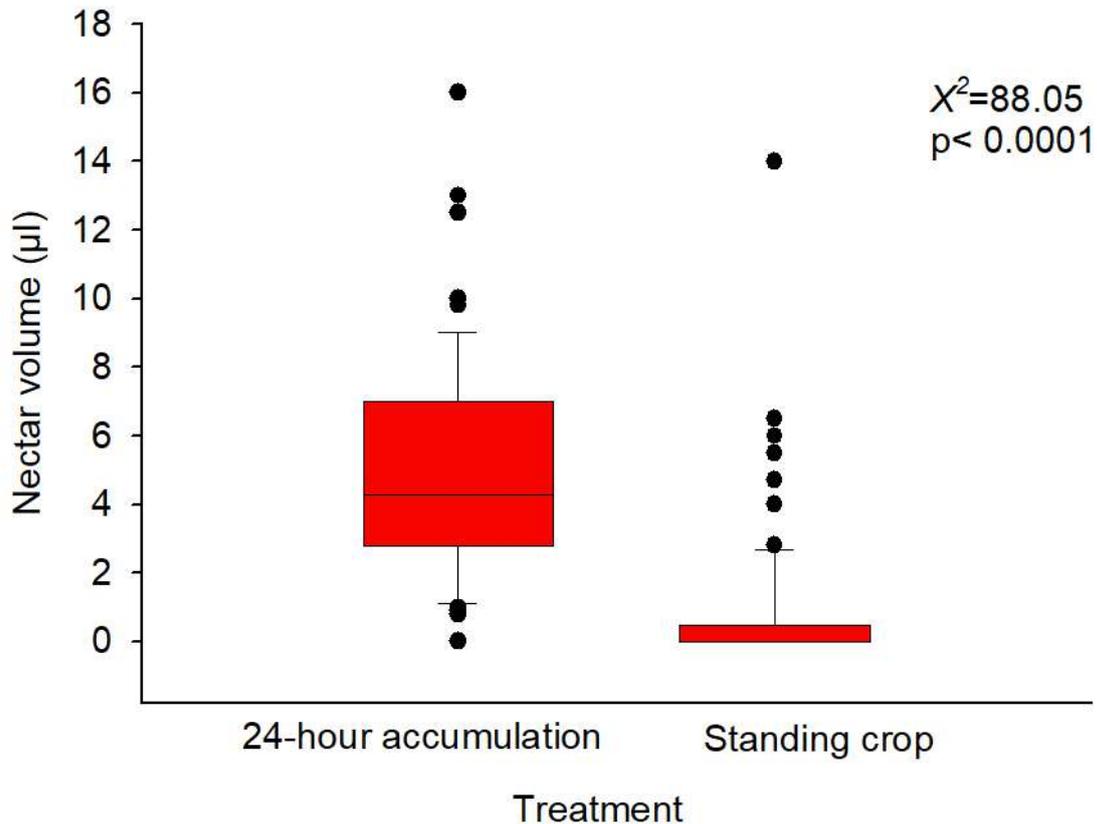


Figure 2. Nectar volume of *Streptocarpus dunnii* at Tonteldoos, Malolotja and Verloren Vallei, measured after flowers were bagged to determine nectar produced over 24 hours, and standing crop nectar, which indicates the amount typically available to foraging pollinators.

2.4.1.4 Flower colour analysis

S. dunnii showed consistent spectral reflectance across sites with an increase in reflectance from about 550nm until 650nm for flower petals (Fig. 3). There was a smaller increase in reflectance around 400nm. The floral tube reflectance peaked at about 400nm and only slightly dropped around 500 to 550nm where the petal reflectance dropped more substantially. The reflectance of the floral petal and tube thus

falls partly in the UV reflectance range (100-400nm). The spectral reflectance of the leaf peaked at about 550nm and increased again at about 670nm (Fig. 3A).

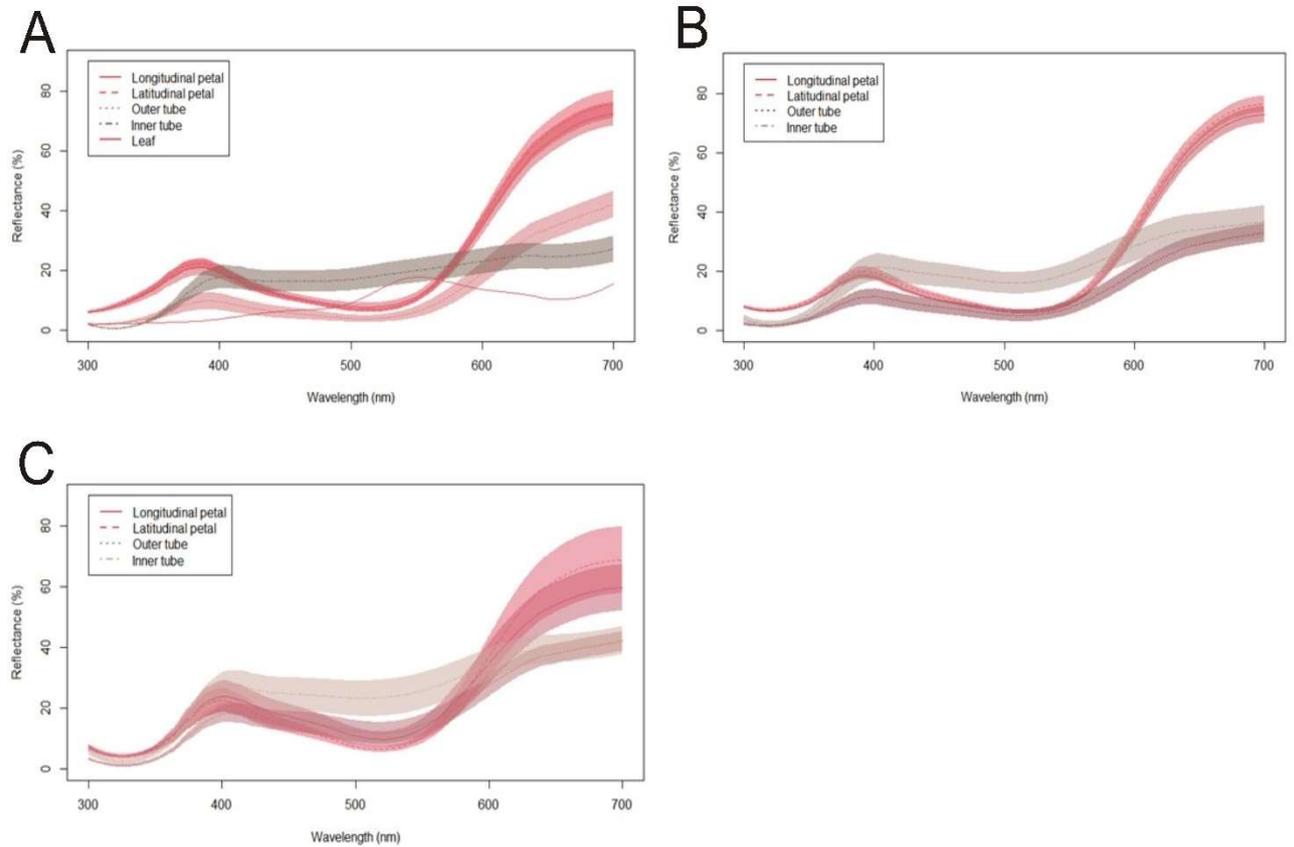


Figure 3. Spectral reflectances of flowers of *Streptocarpus dunnii* from Tonteldoos site (A), Malolotja site (B), Mountainlands site (C). Lines indicate means and shaded regions indicate 95% confidence intervals.

2.4.2 Breeding systems

There was an overall significant difference in fruit production between unmanipulated, cross and self-treatments ($\chi^2=9.01$, $p=0.011$). The cross and self-treatment produced significantly more fruits than the unmanipulated treatment (both pairwise $p < 0.0001$). Seeds per fruit was similar for cross and self-treatments ($\chi^2=0.10$, $p=0.751$). Treatments differed significantly overall in seed production per flower ($\chi^2=81.47$, $p < 0.0001$); the number of seeds produced following self and cross-pollination treatments was similar ($p=0.961$) and significantly greater for both than in the unmanipulated treatment (both $p < 0.0001$).

The index of self-compatibility calculated for *S. dunnii* seeds was 1, indicating *S. dunnii* is completely self-compatible. The autofertility index for fruits and seeds was calculated to be 0 showing *S. dunnii* is completely dependent on pollinators.

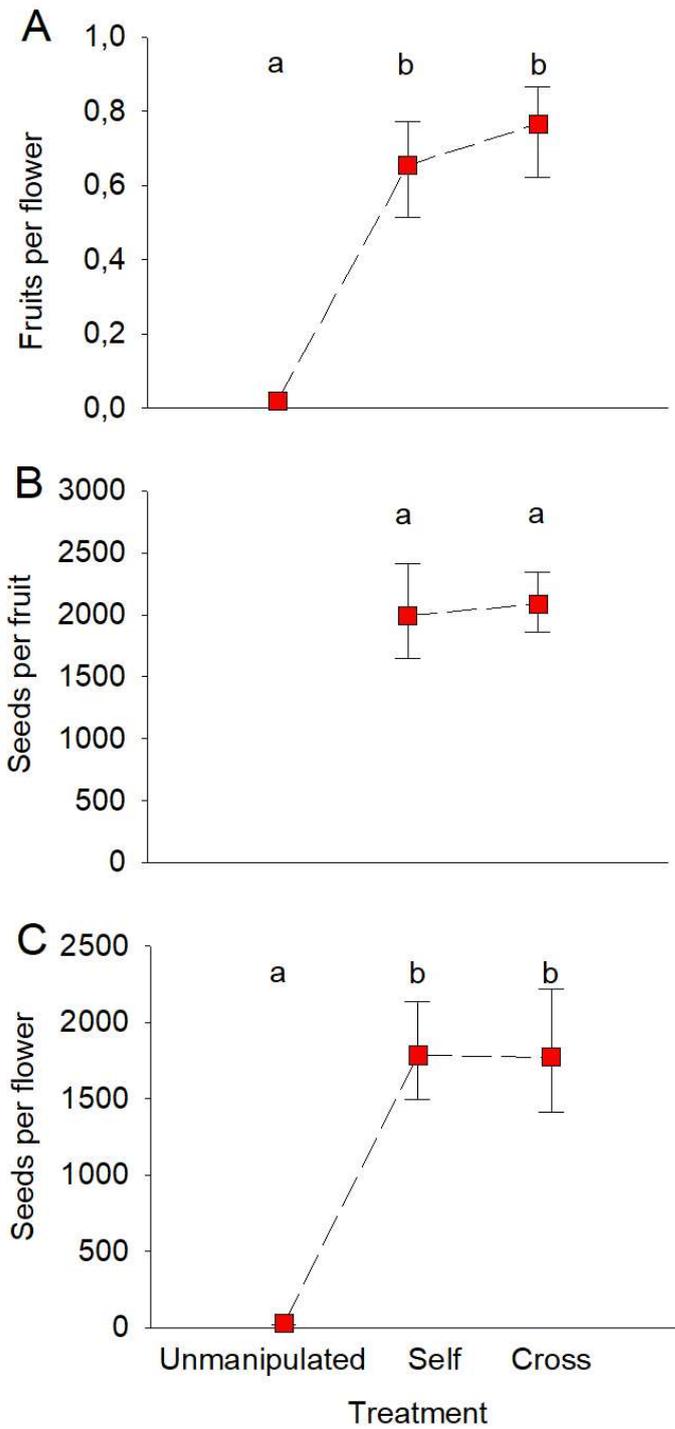


Figure 4. Reproductive success of flowers subjected to controlled hand-pollinations to assess the breeding system of *Streptocarpus dunnii*: fruits produced per flower (A), number of seeds produced per fruit (B) (no fruits or seeds were produced in the unmanipulated treatment) and the number of seeds produced per flower (C). Symbols indicate means \pm SE. Within each panel, symbols that share a letter do not differ significantly.

2.4.3 Pollinator observations

During direct observations and throughout fieldwork, sunbirds were frequently seen in the vicinity of *S. dunnii* plants, but were not observed visiting flowers, presumably because the rocky habitat prevented line of sight observations of plants from at a distance and observer proximity disturbed birds when close to *S. dunnii* plants.

Motion trigger cameras recorded a total of 337 videos of sunbirds feeding on flowers of *S. dunnii*, as well as one video of a butterfly visitor of the *Papilio* genus (Table 2). Videos of birds of the same sex which were recorded within 5 minutes of each other were considered to be one visit by the same bird. From these videos, a total of 281 records of foraging by male and female malachite sunbirds, *Nectarinia famosa*, identified this species as the most frequent visitor at all sites, with the exception of Mountainlands Nature Reserve, where no pollinators were recorded. Visits by malachite sunbirds contributed 100%, 100% and 56.69% of recorded visits at Verloren Vallei, Tonteldoos and Malalotja respectively. At Malalotja Nature reserve, greater double-collared sunbirds, *Cinnyris afer*, were also recorded visiting *S. dunnii* (42% of 156 visits by sunbirds), but only male birds functioned as legitimate pollinators, inserting their beaks into floral tubes and contacting reproductive parts to probe flowers legitimately (12 records); all recorded foraging by female greater double-collared sunbirds involved illegitimate feeding by piercing the side of floral tubes to feed on nectar without contacting floral reproductive parts (55 records). Malalotja had the largest number of recorded sunbird visits (174 videos) over 19 camera days (Table 2). At Tonteldoos site and at Verloren Vallei male malachite sunbirds greatly outnumbered females whereas at Malalotja, female malachite sunbirds dominated (Table 2).

In 81% of recorded foraging bouts by sunbirds, pollen was visible on the bird's beak, head or both (Table 3). The frequency at which birds carried visible pollen loads, as

well as pollen placement varied among bird species and gender (Table 3). The majority (ranging between 75 and 100%) of malachite sunbird visitors carried visible pollen loads on their beaks, on the upper mandible area, from the middle of the beak to the nostril (Table 3). In most (85.7%) of the recorded foraging bouts by male greater double-collared sunbirds, pollen was visible on birds' beaks, but pollen was seldom evident on bills of female birds (only visible in 16.2% of foraging bouts). Most (92.9%) of male greater double-collared sunbirds had visible pollen loads on their foreheads. At all sites, female sunbirds were more likely than males to carry pollen on their foreheads (Table 3).

Consistent with the pattern observed for visible pollen loads, female birds were more frequently observed robbing flowers. Of the 68 visits by female greater double-collared sunbirds recorded on video footage, none showed female birds inserting their beaks in floral tubes or contact with stigmas, consistent with the 100% robbing rate (Table 2, 3; Fig. 6F). Most foraging bouts included evidence of stigma contact (range approximately 75-95% of foraging bouts across sites), with the exception of male malachite sunbirds at Malolotja, for which only one of two visits involved stigma contact (Table 2; Fig. 6D). Malachite sunbirds were not observed to rob flowers, with a single exception of one foraging bout by a female bird at Verloren Vallei. Male malachite sunbirds were observed to both rob flowers and feed legitimately within a single foraging bout but never to exclusively rob flowers in a foraging bout (Fig. 6E).

Birds typically probed 20 to 50% of open flowers in each foraging bout. The mean (\pm SE) number of flowers probed per plant per foraging bout by malachite sunbirds ranged from 8 ± 3.89 at Tonteldoos to 17.22 ± 0.74 at Verloren Vallei. Birds primarily made use of the leaf of the inflorescence as a perch but also used other rocks, a branch, sturdy weeds in close proximity, as well as flowers of the inflorescence itself or the ground (Fig. 6). Hovering behaviour was observed only rarely, to reach uppermost flowers in the inflorescence when there was no other perch available (Fig. 6B).

All birds that visited flowers probed with the floral curve at all sites, often feeding from below, facing the flowers (Fig. 6). No consistent pattern in feeding movement within an inflorescence was observed for sunbirds.

Bird visitation occurred throughout the day from 4am to 6pm (Fig. 5). The highest visitation rates occurred in the early to mid-morning hours from 5am to about 10am. Visitation decreased over midday hours and increases again in the afternoon, at most sites (Figure 5).

Table 2. Number of visitors recorded on *Streptocarpus dunnii* on camera trap footage at three study populations.

Site	Tonteldoos	Malolotja	Verloren Vallei	Total
N foraging bouts recorded	34	157	146	337
Calendar days	11	19	12	42
Number of camera days	11	19	13	43
Number of focal plants	8	19	11	38
Sunbirds	34	156	146	336
Malachite ♂	30	2	127	159
♀	4	87	19	110
Greater double-collared ♂	0	12	0	12
♀	0	55	0	55
Butterflies (Papilio)	0	1	0	1

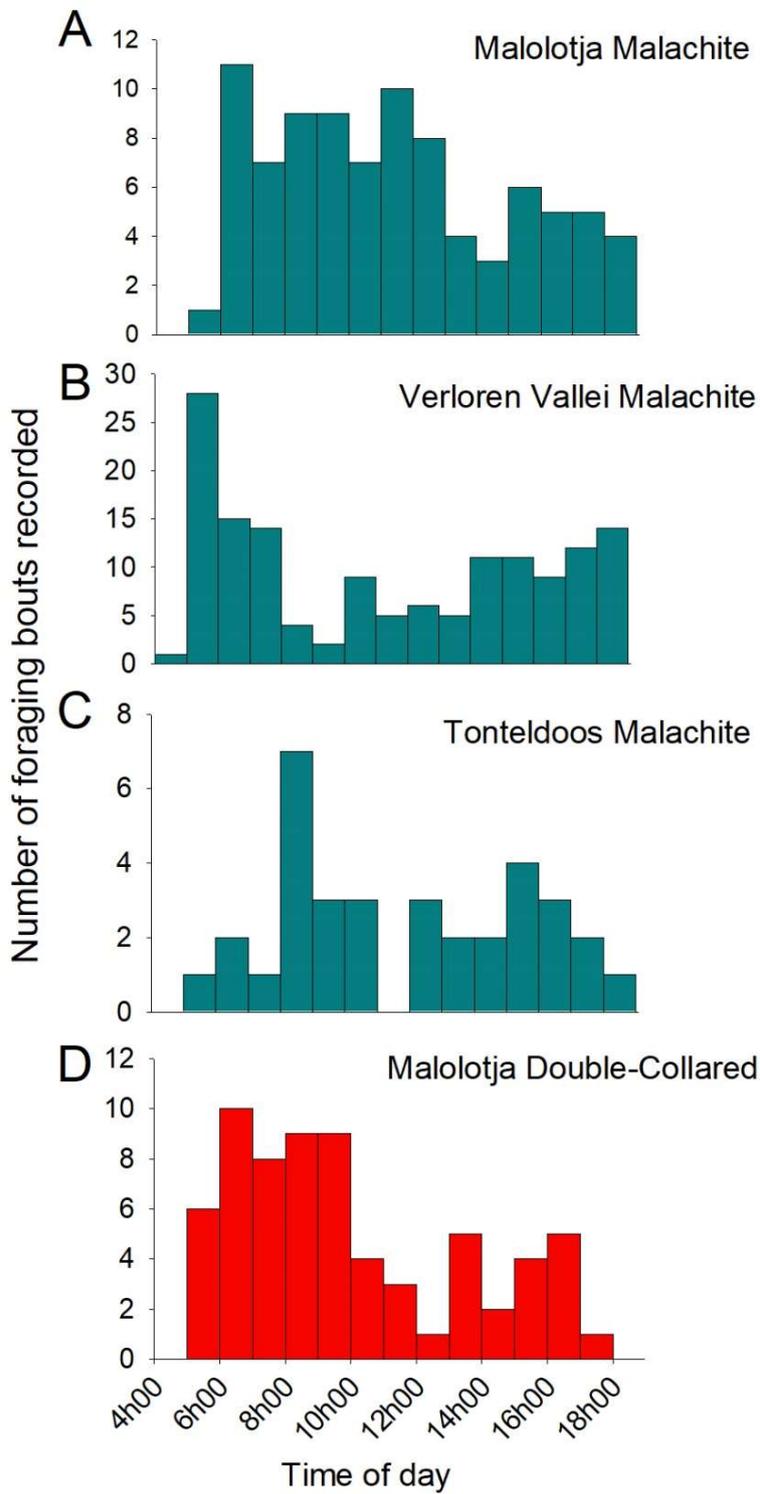


Figure 5. Diel patterns of sunbird foraging behaviour for malachite and greater double-collared sunbirds, recorded in three populations of *Streptocarpus dunnii*. Malachite sunbirds at Malolotja (A), Verloren Vallei (B), and Tonteldoos (C) and greater double-collared sunbirds at Malolotja (D). Note differences in y-axis scaling.

Table 3. Details of sunbird foraging behaviour from camera trap footage. Percentages for visible pollen loads indicate the percentage of foraging bouts in which pollen was visible on the beak or the head.

Species	Malachite						Greater double-collared		χ^2	p
Site	Tonteldoos		Malolotja		Verloren Vallei		Malolotja			
	♂ (30)	♀ (4)	♂ (2)	♀ (87)	♂ (127)	♀ (19)	♂ (12)	♀ (55)		
Visible pollen on beak (%)	96.7	75	100	96.6	92.9	94.7	83.3	10.9	369.98	<0.0001
Visible pollen on head (%)	56.7	54.1	0	16.1	55.1	89.5	91.7	0	20.54	<0.001
Number of flowers probed	17.17 ± 1.47	8 ± 3.89	10 ± 4	14.83 ± 1.05	17.49 ± 0.74	11.11 ± 1.71	12.33 ± 1.78	0	132.30	<0.0001
Percentage of open flowers probed (%)	32.12	19.8	21.6	31.2	50.4	34.8	36.5	0	146.73	<0.0001
Stigma contact (%)	96.7	75	50	96.6	87.4	89.5	100	0	336.07	<0.0001
Robbing (%)	0	0	0	0	0	5.3	41.7	100	1232.92	<0.0001
Hovering (%)	0	0	0	1.1	13.4	0	0	0	4.81	0.028
Perch on leaf (%)	73.5	50	100	52.9	52.6	52.6	41.7	63.6		

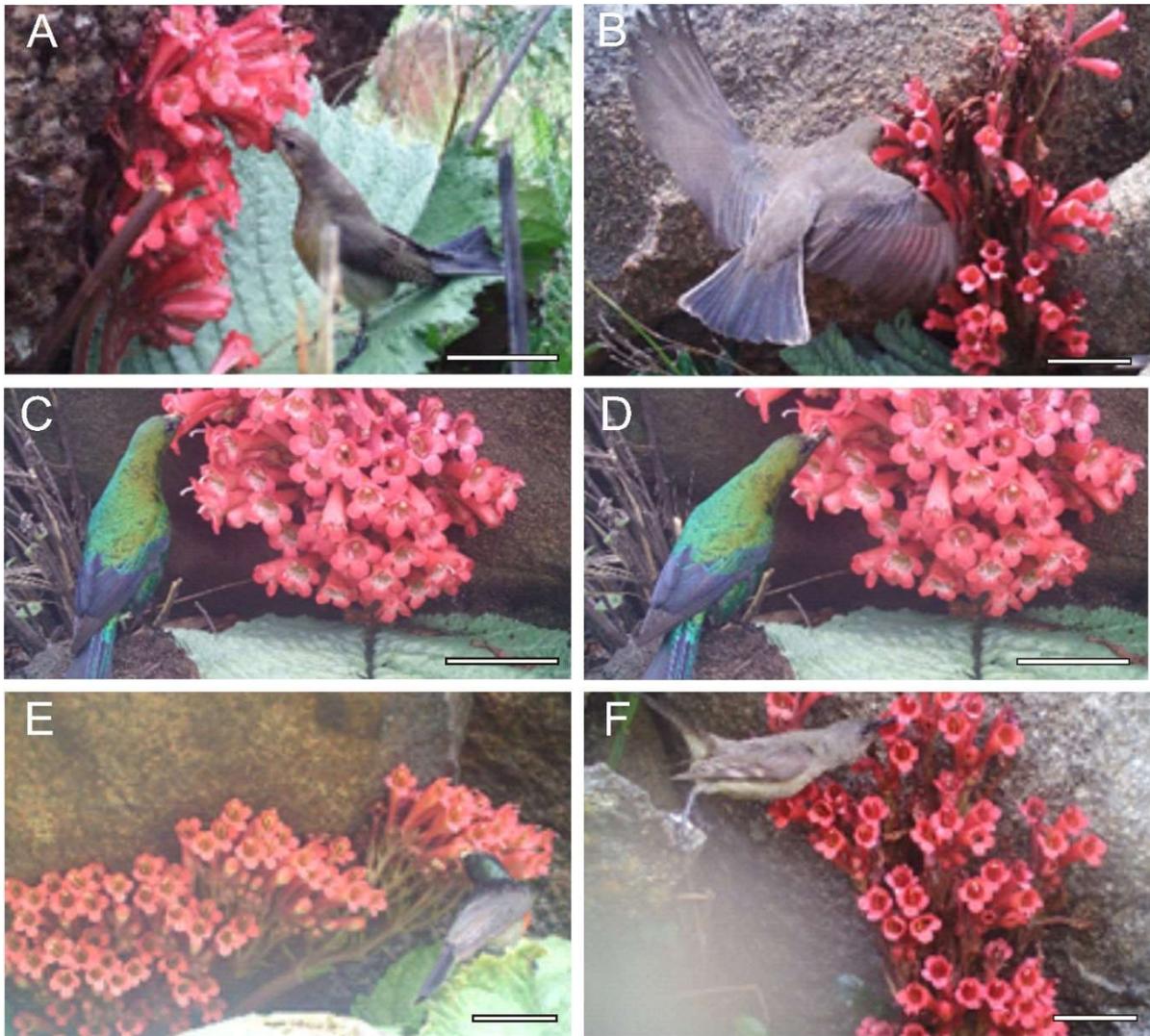


Figure 6. Still images from camera trap footage illustrating details of sunbird behaviour during foraging on inflorescences of *Streptocarpus dunnii*. Female malachite sunbird perching on the plant leaf while visiting flowers (A), and hovering while feeding (B); male malachite sunbird utilising vegetation as a perch. Note visible pollen where the bill and head meet and proximity of sunbird head and flower stigma (C); the same male malachite sunbird, illustrating contact of pollen on head with floral stigma (D); male greater double-collared sunbird feeding on flowers, note pollen on bill (E); female double-collared sunbird using a rock to perch; note absence of visible pollen load (F). Scale bars: 4cm.

2.4.4 Pollen and ovule production

The mean (\pm SE) pollen number per flower was 15695.77 ± 1477.09 and mean (\pm SE) ovule per flower was 3301.54 ± 298.07 . The pollen-ovule ratio was 5.70 ± 0.95 .

2.4.5 Selective exclusion experiments

None of the racemes from which all visitors were excluded produced any fruits (Fig. 7). Only one of the racemes in the bird exclusion treatments (caged) at Verloren Vallei produced fruits, possibly because a bird could reach through the cage to the flowers after the inflorescence grew closer to the cage than anticipated as flowers opened after experimental set-up. There was a significant difference in fruit production among treatments ($\chi^2 = 59.81$, $p < 0.0001$) but no significant difference between sites ($\chi^2 = 0.55$, $p = 0.457$; Fig. 7A). Racemes open to all visitors produced significantly more fruits than those excluding all and only bird visitors at both sites ($p < 0.0001$).

There was a significant difference between sites ($\chi^2 = 1482.71$, $p < 0.0001$) and among treatments ($\chi^2 = 5991.63$, $p < 0.0001$) for seed production per fruit (Fig. 7B). Racemes from which all visitors were excluded and those excluding birds only both produced significantly less seeds than racemes open to all visitors at Tonteldoos (both $p < 0.0001$). All treatments differed significantly at Verloren Vallei ($p < 0.05$), with seeds per fruit in the bird exclusion treatment intermediate to that in the control and all-exclusion treatments, as a result of the single raceme from which birds were excluded which produced fruits and seeds.

There was a significant difference in seed production per flower among treatments ($\chi^2 = 192.87$, $p < 0.0001$) and between sites ($\chi^2 = 6.83$, $p = 0.009$; Fig. 7C). The racemes exposed to all visitors produced significantly more seeds than the racemes excluding all visitors and those excluding birds at the Tonteldoos site ($p < 0.0001$) and at the Verloren Vallei site ($p = 0.034$).

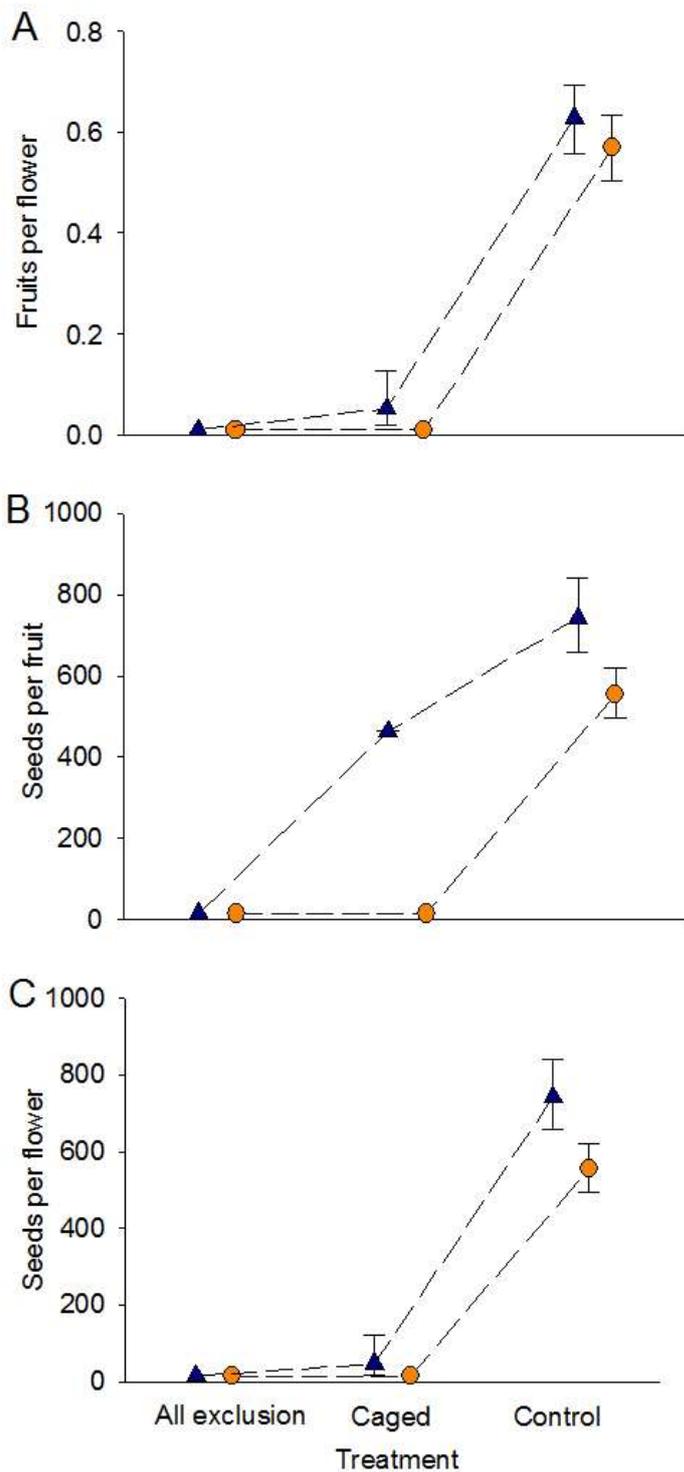


Figure 7. Effects of selective exclusion experiments on the reproductive success of *Streptocarpus dunnii* at Verloren Vallei (blue triangles) and Tonteldoos (orange circles): Fruits produced per flower (A), the seeds produced per fruit (B) and seeds produced per flower (C). Symbols indicate means \pm SE.

2.4.6 Pollen-supplementation experiments

There was an overall significant difference in fruit production between treatments ($\chi^2 = 9.56$, $p = 0.002$; Fig. 8A) but not between sites ($\chi^2 = 0.01$, $p = 0.925$). There was no significant difference between treatments at Tonteldoos ($p = 0.702$) but significantly more fruits were produced on the pollen supplemented racemes at the Verloren Vallei site ($p < 0.0001$).

No significant effect of treatment was found for seeds produced per fruit ($\chi^2 = 0.21$, $p = 0.649$) although plants at Verloren Vallei produced more seeds per fruit overall than did those at Tonteldoos ($\chi^2 = 8.40$, $p = 0.004$).

There was no overall significant difference in seeds produced per flower between the pollen-supplemented and unmanipulated racemes ($\chi^2 = 0.94$, $p = 0.332$) but significantly more seeds were produced at Verloren Vallei than at Tonteldoos ($\chi^2 = 11.84$, $p = 0.001$).

The pollen limitation index for fruit set at the Tonteldoos site was 0.13 and 0.24 at Verloren Vallei. The pollen limitation index for seed set per flower at the Tonteldoos site was 0.01 and 0.02 at Verloren Vallei. The pollen limitation index for seed set per fruit at Tonteldoos was 0.07 and 0.02 at Verloren Vallei. Overall, these low indices showed that fecundity of *S. dunnii* is not pollen-limited.

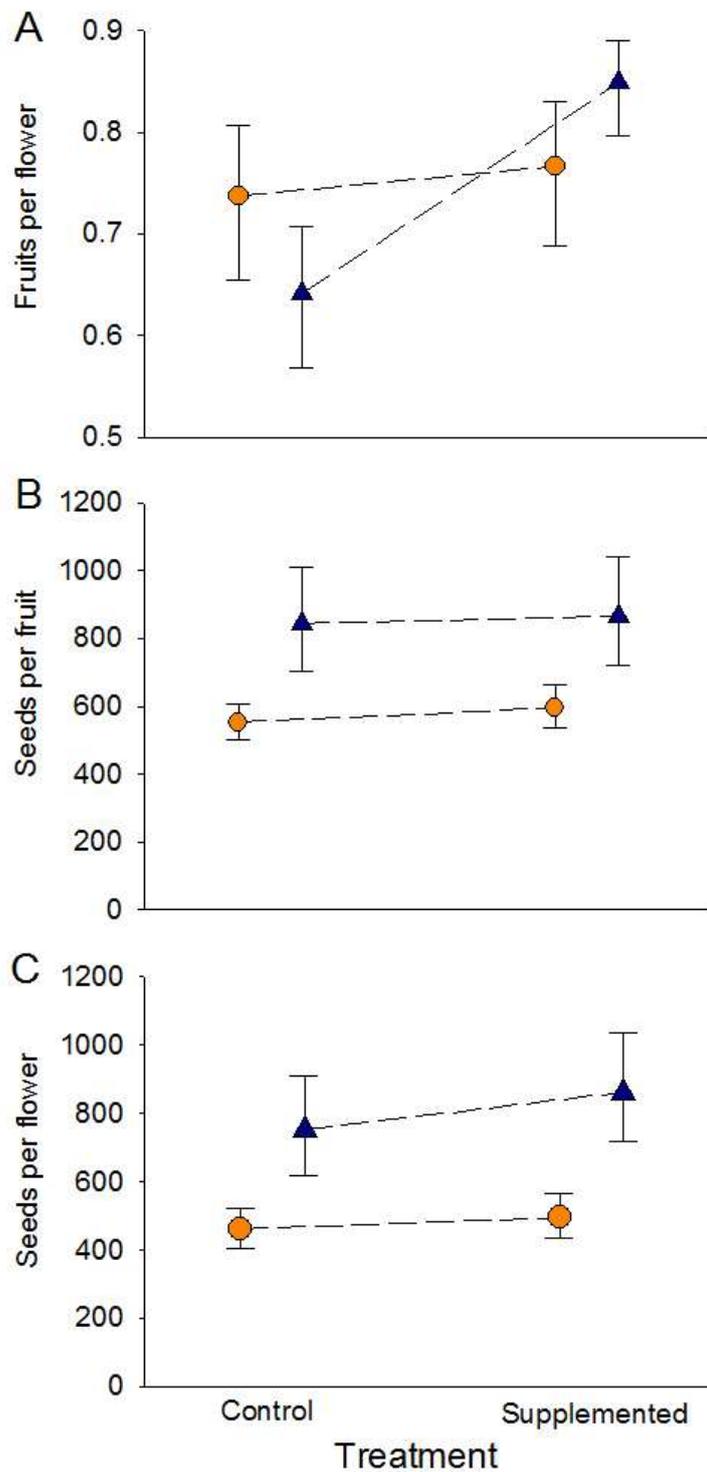


Figure 8. The fruit and seeds produced from naturally-pollinated flowers and hand-pollinated flowers with cross-pollen in pollen limitation experiments conducted on *Streptocarpus dunnii* at Verloren Vallei (blue triangles) and Tonteldoos (orange circles): proportion fruits produced per flower (A), seeds produced per fruit (B) and seeds produced per flower (C). Symbols indicate means \pm SE.

2.5 DISCUSSION

In this study, camera traps enabled confirmation of a long-held hypothesis that the red, unscented tubular flowers of *S. dunnii*, exceptional in the genus, are indicative of pollination by sunbirds. Although I failed to directly observe any visits by birds to flowers, almost 300 foraging bouts by malachite and greater double-collared sunbirds were recorded by motion trigger cameras at three study populations in eSwatini and South Africa. Details of bird behaviour and interactions with flower, gleaned from camera trap footage, suggest that malachite sunbirds, in particular, male birds, are the primary pollinators of *S. dunnii*. Most bird visitors carried visible pollen on their heads and or bills, but double-collared female sunbirds often visited without evidence of visible pollen loads and were more often observed feeding by robbing without contacting reproductive parts of flowers. Morphological matching between flowers and bird dimensions is closer for male malachite sunbirds than for other sunbird visitors, consistent with their role as primary pollinators. Flower colour, morphology and nectar traits largely align with those associated with bird pollination. A peak in the UV region, atypical for ornithophily but common in insect-pollinated species, possibly suggests that pollination by birds in *Streptocarpus* is derived from ancestral insect pollination, although this requires confirmation with molecular methods. Breeding system experiments confirmed that *S. dunnii* is self-compatible but completely dependent on pollinators for reproduction, and selective exclusion experiments confirmed that sunbird visitation is critical for seed production. Pollen supplementation showed that seed production is not limited by pollinator activity, consistent with the high levels of pollinator activity recorded in *S. dunnii* study populations as well as with the five-fold difference between nectar produced in 24 hours and available as standing crop. Videos showed that birds typically probed 20 to 50% of open flowers on an inflorescence, likely contributing to the low observed levels of pollen limitation but also potentially resulting in high levels of geitonogamy. Success of camera traps in this study highlights their utility for both confirming hard to observe interactions, as well as for deriving details of interactions that are otherwise impossible to observe.

The floral traits of *S. dunnii* investigated were found to be similar to those of other bird-pollinated species. The floral tube lengths ranged from a mean of 33 to 37mm across sites, consistent with bird-pollinated flowers having long tubes to accommodate bird

bills (Cronk & Ojeda, 2008; Castellanos, Wilson & Thomson, 2003). The nectar volume accumulated over 24-hours in flowers of *S. dunnii* (approximately 4-6 μ l) was lower than the typical volume in flowers pollinated by specialised passerine nectarivores (10-30 μ l; Johnson and Nicolson, 2008) but higher than typically found in bee and fly-pollinated flowers (Wolff, 2006). Nectar levels may not be as high as found in other bird-pollinated flowers if large numbers of flowers with less nectar cumulatively provide similar volumes to those provided by plants with fewer flowers but more nectar per flower, and *S. dunnii* inflorescences were densely packed with an average of 34 open flowers per inflorescence to 55 open flowers per inflorescence across sites, which would provide a large total nectar volume per plant for birds. Significant differences between nectar accumulated over 24 hours and available as standing crop indicated that bird activity likely significantly decreases available nectar (mean standing crop nectar was less than 1 μ l compared to the 24-hour production volume c. 5 μ l). Nectar concentration in *S. dunnii* fell within the 15-25% range expected for species pollinated by specialised passerine nectarivores, (Johnson & Nicolson, 2008). The sugar concentration is also less than the 40% concentration found in bee-pollinated flowers globally (Pamminger *et al.*, 2019), supporting the observation that bird-pollinated flowers have dilute nectar concentrations (Pyke & Waser, 1981).

The reflectance spectra of *S. dunnii* flowers found in this study is similar to that recorded for other bird-pollinated flowers (e.g. Johnson and Bond, 1994; Meléndez-Ackerman, Campbell and Waser, 1997; Shrestha *et al.*, 2013). *Streptocarpus dunnii* produces dunnione in flowers and this could be why there is a peak in the 400nm region. The production of dunnione may aid in the attraction of sunbirds but it also has alternative functions, such as protecting against microbial infections (Céspedes *et al.*, 2014). Sunbirds have an ultraviolet sensitive colour vision system (Ödeen and Håstad, 2010). *Streptocarpus dunnii* colour spectral analysis showed that flowers reflect in the UV range, as well as in the red region. Ödeen and Håstad (2010) found that the wavelength of maximum absorbance of some sunbird species was 371nm and *S. dunnii* flowers also reflect in this range confirming that *S. dunnii* floral reflectance lies in the visible range for sunbirds, which likely facilitates detection of flowers by foraging sunbirds.

Controlled hand-pollination experiments showed that *S. dunnii* is self-compatible and produces fruit and seed similar to that produced in the cross-pollination treatment following self-pollination. The self-compatibility index for seed production was 1, confirming that *S. dunnii* is completely self-compatible. Details of sunbird foraging behaviour from camera trap footage suggest that self-compatibility likely plays an important role in the reproductive success of *S. dunnii*: as birds probe flowers, pollen locules dehisce and release visible clouds of thousands of pollen grains, resulting in large pollen deposits on the sunbird head and bill. Birds also often visit many flowers at a single inflorescence. The large amount of pollen deposited on sunbirds and the many visits to flowers of the same inflorescence make self-pollination inevitable. This suggests that that pollinator mediated self-pollination and subsequent self-fertilisation may represent a significant component of reproductive success for *S. dunnii*.

Streptocarpus teitensis, found in Kenya, was also found to be self-compatible but reliant on insects for pollination (Otieno, Joshi & Rutschmann, 2021), so self-compatibility is not unique in the genus. Otieno, Joshi and Rutschmann (2021) suggest that the capacity for self-pollination in *S. teitensis* could protect this species from the negative impact of pollinator declines. In contrast, *S. dunnii* lacks capacity for autonomous self-pollination and would be vulnerable to pollinator decline. The absence of reproductive success in exclusion experiments further confirm the critical importance of sunbirds for reproductive success in *S. dunnii*.

Camera traps proved extremely useful in this study enabling collection of an abundance and variety of data of exceptional quality on pollinator interactions with *S. dunnii* from footage despite never observing a single interaction directly. Most importantly, I was able to confirm identification of the pollinator species involved, which has previously been extremely challenging due to the habitat and growth habit of plants making it almost impossible to observe birds on low growing plants amongst rocks. The most frequent visitors captured on camera footage were malachite sunbirds and double-collared sunbirds. Additionally, many critical details of the pollination process could be seen in footage: for example, the process of pollen release from the flower and deposition onto the forehead of the bird visitor during nectar feeding was clearly visible in video footage, as contact of visible pollen deposits on birds' heads and bills with floral stigmas, as birds fed on flowers. The behaviour of sunbirds while feeding, which

could not normally be seen when doing direct observations, could also be observed using camera footage. The single leaf produced by *S. dunnii* was shown to be a commonly used perch for sunbirds, as predicted by Cronk and Ojeda (2008). Despite previous reports of hovering by malachite sunbirds (Geerts & Pauw, 2009b; Wester, 2013), hovering was only rarely recorded in interactions with *S. dunnii*: the highest incidence of hovering was for 14% of total visits at Verloren Vallei, where hovering was almost exclusively associated with birds feeding from the uppermost flowers on an inflorescence, in instances when no alternative perch was available.

Details of pollen placement and corresponding differences in the frequency at which different sunbird species legitimately visit or rob flowers observed in this study showed the importance of trait matching between bird bill and *S. dunnii* flower size for the effective pollination of flowers and could also explain the observation of greater double-collared sunbirds feeding on *S. dunnii* at Malalotja but not at other sites. Bird lists of all four study sites indicate that both malachite sunbirds and greater double-collared sunbirds are found in all these areas (SABAP2). It is therefore puzzling that double-collared sunbirds were only observed visiting *S. dunnii* at Malalotja Nature Reserve. The floral tube length, sepal length, opening width, whole flower width and anthers of *S. dunnii* was significantly shorter for plants at Malalotja and Mountainlands than for those at Tonteldoos and Verloren Vallei. Malachite sunbird bill lengths range from 29 to 34mm (Rebelo, 1987; Downs, 2004) which matches the floral tube measurements of *S. dunnii*, ranging from 33 to 37mm on average across sites, while greater double-collared sunbird bill lengths range from 24 to 29mm (Rebelo, 1987). This size match likely plays a role in the effective pollination of *S. dunnii*, as the beak of malachite sunbirds more frequently contact the anthers and stigmas of flowers, as seen in video footage where 75-90% of visits by females and 50-92% of visits by male malachite sunbirds included contact with stigmas. Geerts and Pauw (2009a) identified a guild of flowers in the Cape Floral Region that appear to be hyperspecialized for pollination by malachite sunbirds in particular and, based on floral traits, especially tube length, *S. dunnii* could be considered to belong to this guild. Geerts and Pauw (2009a) found that while malachite sunbirds were legitimate pollinators of several Cape plant species, all with floral tube lengths exceeding 30mm, other bird species such as *Cinnyris chalybea* (Southern double-collared sunbird), *Anthobaphes violacea* (Orange-breasted sunbird)

and *Zosterops virens* (Cape white-eye), with shorter bill lengths, typically only robbed these longer tubed flowers (Geerts & Pauw, 2009a), a pattern which is possibly also observed in this study for interactions of malachite and greater double-collared sunbirds with *S. dunnii*. The long floral tube lengths of *S. dunnii* flowers mean it may be more difficult for greater double-collared sunbirds to reach to the base for nectar, promoting illegitimate feeding behaviour frequently observed for female greater double-collared sunbirds. Geerts and Pauw (2009a) also found that greater double-collared sunbirds could only reach the nectar of long-tubed flowers when piercing a hole in the flower base, whereas malachite sunbirds, for which long-tubed flowers were morphologically better adapted, more often insert their bills into flowers. However, nectar could be more easily accessible for greater double-collared sunbirds at the base of the smaller-sized flowers at Malolotja, with their shorter bill lengths. The higher percentage of pollen on the male greater double-collared sunbird head compared to the malachite sunbirds, supports the idea that they have shorter beaks and thus they have to insert their heads deeper into flowers to reach nectar, allowing for the anthers to touch their heads more instead of their bills as much as the malachite sunbird. The shorter floral tube lengths at Malolotja and Mountainlands could be an early indication of evolutionary adaptation of *S. dunnii* to shorter-billed bird visitors, specifically the male greater double-collared sunbird. If plants adapt their floral tube lengths to the shorter bills, this may encourage female greater double-collared sunbirds to insert their bills in the corolla tube instead of robbing but further investigations would have to be done over time to consider this as a possibility.

The territorial behaviour of malachite sunbirds, which lead to the exclusion of other bird visitors in Cape plants as found by Geerts and Pauw (2009a), may be another explanation for the absence of greater double-collared sunbirds in sites other than Malolotja. Malachite sunbirds are known to be territorial birds, especially at dense inflorescences (Skead, 1967). Geerts and Pauw (2009a) also found that malachite sunbirds defend their territories against shorter-billed visitors, which can decrease levels of robbing by shorter-billed visitors. These results suggest that there may be a possibility that malachite sunbirds could eventually exclude greater double-collared sunbirds from visiting *S. dunnii* at Malolotja, resulting in more legitimate visits and an overall increase in reproductive output. Another possible reason for double-collared

sunbirds visiting Malolotja is that there may not be many other plant species for greater double-collared sunbirds to visit in the area. The greater double-collared population could also be more abundant and thus require more resources at Malolotja. According to Cruden (1977), the more efficient pollen transfer is, the lower the pollen-ovule ratio of a plant species will be. The pollen-ovule ratio for *S. dunnii* was 5.70 ± 0.95 , which is much lower than that of other xenogamous species (Cruden, 1977). This aligns with the reproductive success found in *S. dunnii* due to its high pollen transfer efficiency.

Streptocarpus dunnii can be described as facultatively xenogamous because it is able to self-pollinate but is adapted for cross pollination (Cruden & Lyon, 1989). The efficient pollen transfer by malachite sunbirds found here also provides additional support for the finding of Hughes et al. (2007), who observed low genetic variation in *S. dunnii* due to the open habitat it is found in and the vagility of the malachite sunbird. Malachite sunbirds are not restricted to the rocky niche of *S. dunnii* because other nectar-providing plant species are found between habitats, facilitating pollen transfer between populations (Hughes et al., 2007).

Exclusion experiments showed that fruits and seeds were produced almost exclusively in *S. dunnii* plants to which sunbird visitors had access, whereas insects did not contribute to reproductive success. Combined with results of pollen limitation experiments, which showed that reproductive success in the species is not limited by pollen receipt, these results suggest that for *S. dunnii*, sunbirds provide high levels of pollinator service, facilitating optimal seed production within the limits of resources available for reproduction. The absence of reproductive success when birds were excluded from *S. dunnii* is a result similar to that found in bird-pollinated plants in other studies (Payne, Symes & Witkowski, 2016; Geerts & Pauw, 2009b; Johnson, McQuillan & Kirkpatrick, 2010; Brown, Downs & Johnson, 2010), although insects have been shown to contribute to reproductive success in some species with ornithophilous flowers (Mayfield, Waser & Price, 2001; Patrick, Johnson & Duffy, 2018; Kunitake et al., 2004). Caution is required when interpreting results of exclusion experiments such as these, because large butterflies are also excluded by cages that exclude birds (e.g., Butler, Cozien & Johnson, 2022). However, only one butterfly was recorded visiting *S. dunnii* in over 300 recorded foraging bouts, suggesting that the contribution of butterflies to pollination in *S. dunnii* is negligible at best. Importantly, the camera traps

used in this study have been shown to be effective for recording Lepidopteran visitors (Johnson *et al.*, 2019; Castañeda-Zárate, Johnson & van der Niet, 2021), confirming that the absence of recorded butterfly visits is a reliable indicator of absence of butterfly visits. Thus, high levels of pollination and reproductive success can be attributed to visitation primarily by malachite sunbirds.

2.6 CONCLUSION

Results of this study confirm that malachite sunbirds are the primary pollinators of *S. dunnii*. Camera trap footage was essential in this study for identifying flower visitors and documenting details of interactions with flowers. Sunbirds appear extremely effective in pollinating *S. dunnii*, as seen in its high fruit and seed output when exposed to sunbirds. Differences in frequency of visible pollen loads and in legitimate versus robbing behaviour of malachite and double-collared sunbirds suggest that *S. dunnii* may be hyperspecialized for pollination by malachite sunbirds in particular.

2.7. REFERENCES

- Anderson B, Cole WW, Barrett SC. 2005. Specialized bird perch aids cross-pollination. *Nature* 435: 41-42.
- Baker HG. 1975. Sugar concentrations in nectars from hummingbird flowers. *Biotropica* 37-41.
- Barnes K, Nicolson SW, van Wyk BE. 1995. Nectar sugar composition in *Erica*. *Biochemical Systematics and Ecology* 23: 419-423.
- Becerra JX, Lloyd DG. 1992. Competition-dependent abscission of self-pollinated flowers of *Phormium tenax* (Agavaceae): a second action of self-incompatibility at the whole flower level? *Evolution* 46: 458-469.
- Botes C, Johnson SD, Cowling RM. 2009. The birds and the bees: using selective exclusion to identify effective pollinators of African tree aloes. *International Journal of Plant Sciences* 170: 151-156.
- Brown JH, Kodric-Brown A. 1979. Convergence, competition, and mimicry in a temperate community of hummingbird-pollinated flowers. *Ecology* 60: 1022-1035.
- Brown M, Downs CT, Johnson SD. 2010. Pollination of the red-hot poker *Kniphofia laxiflora* (Asphodelaceae) by sunbirds. *South African Journal of Botany* 76: 460-464.
- Butler HC, Cozien RJ, Johnson SD. 2022. For the birds? Contrasting pollination and breeding systems of the paintbrush lilies *Scadoxus puniceus* and *S. membranaceus* (Amaryllidaceae). *Plant Systematics and Evolution* 308: 1-11.
- Castañeda-Zárate M, Johnson SD, van der Niet T. 2021. Food reward chemistry explains a novel pollinator shift and vestigialization of long floral spurs in an orchid. *Current Biology* 31: 238-246.
- Castellanos MC, Wilson P, Thomson JD. 2003. Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. *Evolution* 57: 2742-2752.
- Céspedes CL, Salazar JR, Ariza-Castolo A, Yamaguchi L, Ávila JG, Aqueveque P, Kubo I, Alarcón J. 2014. Biopesticides from plants: *Calceolaria integrifolia* sl. *Environmental Research* 132: 391-406.

- Chen YY, Nishii K, Kidner C, Hackett CA, Möller M. 2020. QTL dissection of floral traits in *Streptocarpus* (Gesneriaceae). *Euphytica* 216: 1-20.
- Cronk Q, Ojeda I. 2008. Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany* 59: 715-727.
- Cruden RW, Lyon DL. 2019. Facultative xenogamy: examination of a mixed mating system. In: Bock JH, Linhart YB, eds. *The Evolutionary Ecology of Plants*. CRC Press, 171-207.
- Cruden RW. 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* 31: 32-46.
- de Villiers MJ. 2008. Phylogenetic and population genetic studies in the genus *Streptocarpus* Lindl. (Gesneriaceae DC.). Unpublished D. Phil. Thesis, Stellenbosch University.
- Downs CT. 2004. Some preliminary results of studies on the bill and tongue morphology of Gurney's Sugarbird and some southern African sunbirds. *Ostrich-Journal of African Ornithology* 75: 169-175.
- Faegri K, van der Pijl L. 1979. *The principles of pollination ecology*. Oxford: Pergamon Press.
- Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* 35: 375-403.
- Freeman CE, Reid WH, Becvar JE, Scogin R. 1984. Similarity and apparent convergence in the nectar-sugar composition of some hummingbird-pollinated flowers. *Botanical Gazette* 145: 132-135.
- Geerts S, Pauw A. 2009a. Hyper-specialization for long-billed bird pollination in a guild of South African plants: the Malachite Sunbird pollination syndrome. *South African Journal of Botany* 75: 699-706.
- Geerts S, Pauw A. 2009b. African sunbirds hover to pollinate an invasive hummingbird-pollinated plant. *Oikos* 118: 573-579.
- Gervasi DD, Schiestl FP. 2017. Real-time divergent evolution in plants driven by pollinators. *Nature Communications* 8: 1-8.

- Hargreaves AL, Johnson SD, Nol E. 2004. Do floral syndromes predict specialization in plant pollination systems? An experimental test in an “ornithophilous” African Protea. *Oecologia* 140: 295-301.
- Harrison CJ, Möller M, Cronk QC. 1999. Evolution and development of floral diversity in *Streptocarpus* and *Saintpaulia*. *Annals of Botany* 84: 49-60.
- Hilliard OM, Burt BL. 1971. *Streptocarpus*: An African Plant Study. Pietermaritzburg: University of Natal Press.
- Hook I, Sheridan H, Reid C. 2018. Trichomes and naphthoquinones protect *Streptocarpus dunnii* Hook. f. against environmental stresses. *South African Journal of Botany* 119: 193-202.
- Hughes M, Möller M, Edwards TJ, Bellstedt DU, de Villiers M. 2007. The impact of pollination syndrome and habitat on gene flow: a comparative study of two *Streptocarpus* (Gesneriaceae) species. *American Journal of Botany* 94: 1688-1695.
- Inouye DW. 1980. The terminology of floral larceny. *Ecology* 61: 1251-1253.
- Irwin RE, Brody AK, Waser NM. 2001. The impact of floral larceny on individuals, populations, and communities. *Oecologia* 129: 161-168.
- Johnson KA, McQuillan PB, Kirkpatrick JB. 2010. Bird pollination of the climbing heath *Prionotes cerinthoides* (Ericaceae). *International Journal of Plant Sciences* 171: 147-157.
- Johnson SD, Balducci MG, Bijl A, Castañeda-Zárate M, Cozien RJ, Ortmann CR, van der Niet T. 2020. From dusk till dawn: camera traps reveal the diel patterns of flower feeding by hawkmoths. *Ecological Entomology* 45: 751-755.
- Johnson SD, Bond WJ. 1994. Red flowers and butterfly pollination in the fynbos of South Africa. In: Arianoutsou M, Groves RH, eds. *Plant-animal interactions in Mediterranean-type ecosystems*. Dordrecht: Springer, 137-148.
- Johnson SD, Kiepiel I, Robertson AW. 2020. Functional consequences of flower curvature, orientation and perch position for nectar feeding by sunbirds. *Biological Journal of the Linnean Society* 131: 822-834.
- Johnson SD, Nicolson SW. 2008. Evolutionary associations between nectar properties and specificity in bird pollination systems. *Biology Letters* 4: 49-52.

- Johnson SD, van der Niet T. 2019. Bird pollination in an African *Satyrium* (Orchidaceae) confirmed by camera traps and selective exclusion experiments. *Plant Systematics and Evolution* 305: 477-484.
- Johnson SD. 1996. Bird pollination in South African species of *Satyrium* (Orchidaceae). *Plant Systematics and Evolution* 203: 91-98.
- Jong K, Burt BL. 1975. The evolution of morphological novelty exemplified in the growth patterns of some Gesneriaceae. *New Phytologist* 75: 297-311.
- Kearns CA, Inouye DW, Waser NM. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics* 83: 112.
- Kiepiel I, Brown M, Johnson SD. 2022. A generalized bird pollination system in *Schotia brachypetala* (Fabaceae). *Plant Biology* 24: 806-814.
- Knudsen JT, Tollsten L, Groth I, Bergström G, Raguso RA. 2004. Trends in floral scent chemistry in pollination syndromes: floral scent composition in hummingbird-pollinated taxa. *Botanical journal of the Linnean Society* 146: 191-199.
- Krauss SL, Phillips RD, Karron JD, Johnson SD, Roberts DG, Hopper SD. 2017. Novel consequences of bird pollination for plant mating. *Trends in Plant Science* 22: 395-410.
- Kunitake YK, Hasegawa M, Miyashita T, Higuchi H. 2004. Role of a seasonally specialist bird *Zosterops japonica* on pollen transfer and reproductive success of *Camellia japonica* in a temperate area. *Plant Species Biology* 19: 197-201.
- Larson BM, Barrett SC. 2000. A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society* 69: 503-520.
- Lloyd DG, Schoen DJ. 1992. Self-and cross-fertilization in plants. I. Functional dimensions. *International Journal of Plant Sciences* 153: 358-369.
- Maia R, Eliason CM, Bitton PP, Doucet SM, Shawkey MD. 2013. pavo: an R package for the analysis, visualization and organization of spectral data. *Methods in Ecology and Evolution* 4: 906-913.
- Mayfield MM, Waser NM, Price MV. 2001. Exploring the 'most effective pollinator principle' with complex flowers: bumblebees and *Ipomopsis aggregata*. *Annals of Botany* 88: 591-596.

- McCarren S, Midgley J, Coetzee A. 2021. Sending private messages: Floral ultraviolet signals are correlated with pollination syndromes in *Erica*. *Journal of Pollination Ecology* 30: 289-298.
- McDade LA, Kinsman S. 1980. The impact of floral parasitism in two neotropical hummingbird-pollinated plant species. *Evolution* 944-958.
- Meléndez-Ackerman E, Campbell DR, Waser NM. 1997. Hummingbird behavior and mechanisms of selection on flower color in *Ipomopsis*. *Ecology* 78: 2532-2541.
- Möller M, Atkins H, Barber S, Purvis D. 2019. The living collection at the Royal Botanic Garden Edinburgh illustrates the floral diversity in *Streptocarpus* (Gesneriaceae). *Sibbaldia: The International Journal of Botanic Garden Horticulture* 155-177.
- Möller M, Cronk QC. 1997. Origin and relationships of *Saintpaulia* (Gesneriaceae) based on ribosomal DNA internal transcribed spacer (ITS) sequences. *American Journal of Botany* 84: 956-965.
- Möller M, Cronk QC. 2001. Phylogenetic studies in *Streptocarpus* (Gesneriaceae): reconstruction of biogeographic history and distribution patterns. *Systematics and Geography of Plants* 71: 545-555.
- Nicolson SW. 2002. Pollination by passerine birds: why are the nectars so dilute? *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 131: 645-652.
- Nishii, K., Hughes, M., Briggs, M., Haston, E., Christie, F., DeVilliers, M.J., Hanekom, T., Roos, W.G., Bellstedt, D.U., Möller, M., 2015. *Streptocarpus* redefined to include all Afro-Malagasy Gesneriaceae: Molecular phylogenies prove congruent with geographical distribution and basic chromosome numbers and uncover remarkable morphological homoplasies. *Taxon* 64, 1243-1274.
- Ödeen A, Håstad O. 2010. Pollinating birds differ in spectral sensitivity. *Journal of Comparative Physiology A* 196: 91-96.
- Ollerton J, Winfree R, Tarrant S. 2011. How many flowering plants are pollinated by animals? *Oikos* 120: 321-326.
- Otieno M, Joshi N, Rutschmann B. 2021. Flower visitors of *Streptocarpus teitensis*: implications for conservation of a critically endangered African violet species in Kenya. *PeerJ* 9: e10473.

- Pamminger T, Becker R, Himmelreich S, Schneider CW, Bergtold M. 2019. The nectar report: quantitative review of nectar sugar concentrations offered by bee visited flowers in agricultural and non-agricultural landscapes. *PeerJ* 7: e6329.
- Patrick KL, Johnson SD, Duffy KJ. 2018. High levels of fecundity in small and isolated populations of a self-compatible *Aloe* pollinated by opportunistic birds and bees. *Plant Biology* 20: 780-788.
- Pauw A. 2019. A bird's-eye view of pollination: Biotic interactions as drivers of adaptation and community change. *Annual Review of Ecology, Evolution, and Systematics* 50: 477-502.
- Payne SL, Symes CT, Witkowski ET. 2016. Of feathers and fur: differential pollinator roles of birds and small mammals in the grassland succulent *Aloe peglerae*. *Austral Ecology* 41: 952-963.
- Pyke GH, Waser NM. 1981. The production of dilute nectars by hummingbird and honeyeater flowers. *Biotropica* 260-270.
- R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ratto F, Simmons BI, Spake R, Zamora-Gutierrez V, MacDonald MA, Merriman JC, Tremlett CJ, Poppy GM, Peh KSH, Dicks LV. 2018. Global importance of vertebrate pollinators for plant reproductive success: a meta-analysis. *Frontiers in Ecology and the Environment* 16: 82-90.
- Rebello AG. 1987. Bird Pollination in the Cape Flora. In: Rebello AG, ed. *A Preliminary Synthesis of Pollination Biology in the Cape Flora*. Pretoria: CSIR, 83-108.
- Rodríguez-Gironés MA, Santamaría L. 2004. Why are so many bird flowers red? *PLoS Biology* 2: e350.
- Shrestha M, Dyer AG, Boyd-Gerny S, Wong BB, Burd M. 2013. Shades of red: bird-pollinated flowers target the specific colour discrimination abilities of avian vision. *New Phytologist* 198: 301-310.
- Skead CJ. 1967. *Sunbirds of Southern Africa*. Cape Town: Balkema.
- Southern African Bird Atlas Project 2 (SABAP2). Online: <https://sabap2.birdmap.africa/>.
- Stiles FG, Freeman CE. 1993. Patterns in floral nectar characteristics of some bird-visited plant species from Costa Rica. *Biotropica* 191-205.

- Stiles FG. 1981. Geographical aspects of bird-flower coevolution, with particular reference to Central America. *Annals of the Missouri Botanical Garden* 323-351.
- Tsou Y, Hsu HC, Wang CC, Kuo YF, Wang CN, Möller M. 2014. Quantification of floral shape variation in the F2 hybrids of two *Streptocarpus* species with contrasting pollination syndrome. The 7th International Symposium on Machinery and Mechatronics for Agriculture and Biosystems Engineering (ISMAB) May 21-23: 191-196.
- van der Niet T, Cozien RJ, Johnson SD. 2015. Experimental evidence for specialized bird pollination in the endangered South African orchid *Satyrium rhodanthum* and analysis of associated floral traits. *Botanical Journal of the Linnean Society* 177: 141-150.
- Vaughton G. 1996. Pollination disruption by European honeybees in the Australian bird-pollinated shrub *Grevillea barklyana* (Proteaceae). *Plant Systematics and Evolution* 200: 89-100.
- Weber A, Bellstedt D, Möller M. 2021. Die Gesneriaceen Afrikas und ihre vogelblütigen Vertreter: *Streptocarpus dunnii* und *S. myoporoides*. *Der Palmengarten* 85: 125-138.
- Wester P. 2013. Sunbirds hover at flowers of *Salvia* and *Lycium*. *Ostrich* 84, 27-32.
- Whelan CJ, Wenny DG, Marquis RJ. 2008. Ecosystem services provided by birds. *Annals of the New York Academy of Sciences* 1134: 25-60.
- Wolff D. 2006. Nectar sugar composition and volumes of 47 species of *Gentianales* from a southern Ecuadorian montane forest. *Annals of Botany* 97: 767-777.
- Zuur A, Ieno EN, Smith GM. 2007. Analyzing ecological data. New York: Springer Science & Business Media.

CHAPTER 3

Evidence for sunbird pollination in the cliff-dwelling South

African endemic tree *Alberta magna*

3.1 ABSTRACT

The field of pollination biology has grown in recent decades but many plant-pollinator interactions are yet to be studied. Pollination by birds in southern Africa is thought to be important in several plant families but in many cases is inferred based on ornithophilous floral traits, unconfirmed by formal pollination studies. *Alberta magna* (Rubiaceae) is one such species for which bird-pollination has been predicted based on floral traits, but for which data on pollination biology are lacking, possibly due to its restricted distribution and an unusual cliff-edge habitat, which makes the trees difficult to access for study. I measured floral traits of *A. magna* and confirmed that red flower colour, tubular morphology and copious and dilute nectar all align with the ornithophilous pollination syndrome. Direct observations and motion-activated camera traps recorded over 100 visits to flowers of *A. magna*, primarily by southern double-collared (*Cinnyris chalybeus*; 69%) and olive sunbirds (*Cyanomitra olivacea*; 26%). Selective exclusion of flower visitors indicated that *A. magna* is dependent on birds for reproductive success: exclusion of bird visitors decreased seed production three-fold relative to naturally pollinated flowers, whereas exclusion of insects did not affect fecundity. *Alberta magna* was found to produce large numbers of seedless fruits, however supplemental hand-pollination experiments revealed that fecundity is not pollen limited. Fruit set following controlled hand pollinations with self and cross pollen was inadequate to establish self-compatibility. Overall, only 14% of naturally pollinated flowers and 12% of manually cross-pollinated flowers developed seeds, and further research is needed to determine the underlying cause of the low reproductive success of *A. magna*. This study confirmed that *A. magna* is pollinated almost exclusively by *Cinnyris chalybeus* and *Cyanomitra olivacea* and confirmed the value of camera traps for documenting pollination interactions in study systems in which accessibility limits human observations.

Keywords: sunbirds, pollination syndrome, pollen limitation, breeding system, camera traps, selective exclusion

3.2 INTRODUCTION

Pollination is crucial for the reproductive success of plants (Katumo et al., 2022). Pollination is an example of a mutualism where visitors of plants disperse pollen while plants provide nectar for visitors (Mitchell et al., 2009). Visitors can include butterflies, bees, bats, flies, moths, rodents and birds. At least 10 000 plant species are reported to be pollinated by nectarivorous birds (Johnson, 2022) and consequently birds play a crucial role in providing this service in the ecosystem (Abrahameczyk, 2019; Pauw, 2019). Because of their ability to travel extensive distances, birds are known to carry pollen for great distances facilitating gene flow (Cronk and Ojeda, 2008; Pauw, 2019). Pollen is transferred onto different parts of the bird's body depending on the floral structure of the plant species, but the head is the area pollen is most transferred to (Johnson, 1996; Geerts and Pauw, 2009). The effectiveness of pollen transfer is partially dependent on how well a pollinator morphologically matches the flower they are visiting (Rico-Guevara et al., 2021).

Pollination syndromes are combinations of floral traits and rewards associated with attraction of particular animal pollinators (Fenster et al., 2004). Many studies have found bird pollinated species which conform to the ornithophilous syndrome (Johnson, 1996; Ollerton et al., 2008; van der Niet et al., 2015; Kiepiel et al., 2022). Flowers conforming to this syndrome are unscented, often vivid red or orange in colour, have long and tubular corollas and nectar is dilute and copious (van der Pijl, 1961; Johnson, 1996; Nicolson, 2002; Knudsen et al., 2004). However, the validity of pollination syndromes has been questioned (Dellinger, 2020) as studies have found that floral traits do not always fit according to syndromes (Waser et al., 1996; Ollerton et al., 2009). In one study, only one third of plant species' primary pollinators, as identified according to visitation frequencies, were accurately predicted by syndromes based on floral traits (Ollerton et al., 2009). These exceptions to pollination syndromes emphasize the importance of rigorous field studies to verify syndrome-based predictions of pollination systems (Johnson and Steiner, 2000; van der Niet, 2021).

African nectar-feeding birds are characterized by Fleming and Muchhala (2008) as being large, non-hovering specialist birds. Nectarivorous birds which are dominant in Africa include specialist nectar feeding sunbirds and sugarbirds as well as opportunistic nectar feeders including white eyes, weavers and bulbuls (Botes et al., 2007; Fleming and Muchhala, 2008; Kiepiel et al., 2022). Adaptation to different pollinators, including birds, has contributed to the abundant floral diversity found in southern Africa (Goldblatt et al. 1999;

Johnson, 2010). Within southern Africa bird pollination is well known from the fynbos, but also occurs in the summer rainfall grasslands and forest habitats and occurs in a range of plant families including Proteaceae, Ericaceae, Orchidaceae and Iridaceae (Johnson, 2010).

The plant family Rubiaceae, including the tribe Alvertae (Bremer and Eriksson, 2009), consists of three clades: the southeast African *Alberta magna*, the Madagascan *Nematostylis* and *Razafimandimbisonia* (Kainulainen et al., 2009). *Alberta magna* is morphologically unique and can be described as taxonomically isolated as no other species forms part of its clade (Puff et al., 1984; Kainulainen et al., 2009). Traits which separate *A. magna* from *Nematostylis* and *Razafimandimbisonia* species include that it has a panicle-shaped cyme, awl-shaped stigma and the calyx lobe expands after anthesis (Kainulainen et al., 2009). The flowers of *A. magna* have been argued to be bird-pollinated while that of *Razafimandimbisonia* and *Nematostylis* is presumed to be butterfly-pollinated (Puff et al., 1984). *Alberta magna* produces distinct, bright red fruits and flowers that has earned it the common name, the Natal flame bush (van Staden et al., 1990). Floral traits of *A. magna*, in particular, the bright red colour and elongated floral tubes are consistent with ornithophily (Vogel, 1954). Anecdotal observations suggest both opportunistic generalist (*Zosterops*, Vogel, 1954) and specialist nectar-feeding bird species (*Nectarinia* sp., Puff 1984) visit flowers of *A. magna*. Sunbirds of the *Nectarinia* genus (species unidentified) were observed feeding on *A. magna* nectar on the Natal South Coast (Puff et al., 1984). Vogel (1954) observed Cape White-eyes (*Zosterops virens*) visiting cultivated *A. magna* trees in Kirstenbosch Botanical Gardens. However, some species such as *Razafimandimbisonia minor*, on which butterflies have been observed to feed, are described as being an intermediate form between bird and butterfly flowers because their corolla shapes are similar to that of *A. magna*, the floral tubes gradually widen and there is no landing place for butterflies (Puff et al., 1984). This overlap in floral traits with other closely related, florally similar, butterfly-pollinated plant species, suggests that *A. magna* could also be butterfly-pollinated or have a mixed pollination system including birds and butterflies as visitors (e.g. Manning and Goldblatt, 2005; Butler et al., 2022). A thorough observational and experimental study is therefore required to confirm the pollination system of *A. magna*.

This study investigates the pollination biology of *Alberta magna*. Specifically, I objectively quantify floral traits of *A. magna*, establish the breeding system and determine reliance on pollinators for reproduction, identify floral visitors and determine their contribution to

reproductive success and finally, determine if current pollination service limits reproductive success through pollen limitation.

3.3 METHODS AND MATERIALS

3.3.1 *Study site and species*

Alberta magna is a flowering evergreen small to medium-sized tree (Ben-Jaacov et al., 1991) native to South Africa (Ben-Jaacov et al., 1991). *Alberta magna* has a restricted distribution (Drewes et al., 1998) occurring in KwaZulu-Natal and the east of the Eastern Cape Province (Puff et al., 1984). Under natural conditions, the species is restricted to a highly specific habitat where grasslands and evergreen forest meet on the edge of cliffs (Puff et al., 1984). In lower coastal areas, the species can be found in wooded ravines (van Staden et al., 1990).

Three natural populations, including two in the midlands and a third on the south coast of KwaZulu-Natal, South Africa were the main focus of this study: Two populations were located within the greater Karkloof Conservancy: the population at Karkloof Canopy Tours (29°19'22.03"S, 30°15'45.65"E) consisted of approximately 20 trees and the population at Mbona Private Nature Reserve (29°17'31"S, 30°20'54" E) consisted of three trees. Both populations in the Karkloof Conservancy were located at cliff edges where Midlands Mistbelt Grassland meets patches of Eastern Mistbelt Forest. The study population on the south coast at Umtamvuna Nature Reserve (31°01'11.1"S, 30°10'16.6"E), consisted of approximately 15 trees on the edge of a steep gully. Visitors were also recorded and observed on flowers from two cultivated trees at the Pietermaritzburg Botanical Gardens (29°36'28.5"S, 30°20'52.0"E) and one cultivated tree at the Pietermaritzburg Hospice (-29°36'19.1"S, 30°19'56.4"E).

3.3.2 *Floral traits*

3.3.2.1 *Measurement of flower and inflorescence traits*

Morphological measurements were recorded for one flower from every flowering tree at each of the study sites. For each flower, digital callipers were used to measure flower tube length; sepal length; opening height of flower; whole flower height; opening width; whole flower width; anther and stigma length. Tube length was measured from the base to the point which petals fuse. Sepal length was measured from the receptacle to the tip of the sepal. Opening height and width were measured as the maximum vertical and horizontal lengths respectively,

across the mouth of the floral tube. Whole flower height and width was measured as the maximum vertical and horizontal lengths respectively, across the face of the flower, including the petals. Filaments were measured from the base to the furthest tip of the anther. The style was measured from the base to the tip of the stigma. For each measured flower, whether or not there was evidence of floral larceny (slit or piercing at the base of the floral tube) was also recorded. The number of flowers on each focal inflorescence used for camera traps was also recorded.

3.3.2.2 *Scanning electron microscopy*

Young flower buds, mature buds, cross-sections of fruits, as well as anthers and stigmas of flowers of *Alberta magna* were viewed using scanning electron microscopy to examine floral development. For primary fixation, floral parts and fruits were covered in glutaraldehyde in glass jars with lids and refrigerated at 4°C for one week. Thereafter, samples underwent a buffer wash three times for five minutes in 0.05M phosphate buffer. Samples were then dehydrated by being submerged in 10%, 30%, 50%, 70% and 90% ethanol respectively for 10 minutes each. After being submerged in 100% ethanol three times, samples were placed in the critical point dryer (CPD) baskets and submerged in a Petri dish with ethanol for the second and third times. The samples in the CPD baskets were then transferred to the Quorum R850 CPD under 100% ethanol. Throughout CPD the ethanol was replaced with liquid carbon dioxide. The carbon dioxide is then heated and pressurised to a critical point where liquid becomes gas, resulting in dried and intact samples. Samples were then viewed and photographed under the Zeiss EVO LS 15 Variable Pressure Scanning Electron Microscope (VP SEM).

3.3.2.3 *Nectar volume, concentration and composition*

To quantify floral nectar available to visitors (standing crop) as well as the amount of nectar produced over 24 hours, nectar was measured at Karkloof, Mbona (sites combined; n=4 and 7 respectively) and Umtamvuna (n=6 and 8 respectively). Nectar standing crop volume was measured from flowers collected around midmorning, when pollinators are active. Nectar was also collected from flowers from which pollinators were excluded for 24 hours prior by enclosing inflorescences in mesh pollinator exclusion bags. For both, nectar volume was measured using 5µl microcapillary tubes. Nectar concentration was also recorded, using a Bellingham and Stanley 0–50% refractometer (Karkloof and Mbona, n=13; Umtamvuna, n=7).

To determine nectar composition, nectar of five flowers, from Karkloof and Mbona, was transferred onto filter paper. Nectar sugar composition was analysed by high performance liquid chromatography using a Shimadzu (LC-20AT) high performance liquid chromatograph. Refractive index (RID-10A) was used for detection with a Phenomenex column (Rezex RCM-Monosaccharide, 200 × 780mm 8µm) and isocratic separation was achieved using high-purity water as the mobile phase.

3.3.2.4 *Flower colour analysis*

To determine the spectral reflectance of *A. magna* flowers, flowers from Karkloof (n=8), Mbona (n=1) and Hospice (n=1) were sampled. Spectral reflectance was recorded over the UV-visible 300-700nm range, for the outer petal, inner petal, the outer floral tube and the inner floral tube using an Ocean Optics USB 2000 spectrometer and a fibre optic reflection probe at 45° to the surface of the object, in an optic holder. An Ocean Optics Mini-DT light source with a spectral range of c. 200-1100nm was used. An Ocean Optics WS-1 diffuse reflectance standard was used to calibrate spectra, which was captured with Ocean Optics SpectraSuite software at intervals of 0.34nm.

3.3.3 *Breeding system experiments*

To determine the breeding system of *A. magna*, controlled hand pollination experiments were conducted in Karkloof (n=10) and Mbona (n=2). To prevent contamination with non-treatment pollen, inflorescences were bagged in the bud stage prior to pollination treatments and bagged again after flowers were manipulated. The breeding system experiments consisted of three treatments which were applied within an inflorescence: to assess self-compatibility, flowers assigned to a self-pollination treatment were hand-pollinated with pollen from the same tree and flowers assigned to a cross-pollinated control treatment were hand-pollinated with pollen from a different individual tree from a minimum of 3m away. To assess potential for autonomous self-fertilisation, flowers were unmanipulated but tagged and bagged as described above. Treated flowers were marked with coloured wires. The index of self-compatibility (ISC) for the species was calculated by dividing the mean fruit or seeds produced from the self-treatment by the cross-treatment (Becerra and Lloyd, 1992). A value close to 0 indicates self-incompatibility while a value close to 1 indicates self-compatibility. The index of autofertility (IAF), to assess capacity for reproduction independent of pollinators, was calculated by dividing the mean fruit or seeds produced from the unmanipulated-treatment by the cross-treatment (Lloyd and Schoen, 1992). A value close to 0

indicates that the species is dependent on pollinators for reproductive success and a value close to 1 indicates that the species can reproduce independently of pollinators.

3.3.4 Pollinator observations

To determine the visitors of *A. magna*, visitors were recorded using direct observations and camera traps at Karkloof, Mbona, Umtamvuna and the Botanical Gardens site. Two to five camera traps were set up at each site over a total period of 70 days from June 2021 to March 2022. Direct observations by one to three observers during the same period added approximately 40 cumulative observer hours of direct observations. *Alberta magna* trees were observed from a distance of not closer than 5 meters. Binoculars were used to assist identification of birds in direct observations. Bushnell NatureView HD cameras (model 119740) were positioned in trees to record visits to flowers. Cameras were set to record video for 60 seconds at high sensitivity with a resolution of 1920x1080. Cameras were initially set to record during day and night hours (24-hour mode) but switched to day mode after no visits were recorded at night for several weeks and to avoid excessive wind trigger during night hours reducing memory available for daytime recording. Close-focussing lenses with focal distances of 600mm or 460mm lens were used and cameras were set up at these respective distances, from the focal inflorescence. Cameras were positioned facing inflorescences, attached to branches or poles using Gorilla TriPods (Z-02) and secured with cable ties.

Camera trap video footage was analysed to identify the visitor species and to record details of each foraging bout, including the number of open flowers on an inflorescence, number of probes, number of individual flowers visited, frequency of visible stigma and/or anther contact. For all visits by birds, the following was noted: instances of visible pollen on the head or beak, the direction from which flowers were probed (visiting from the top, side or bottom of flowers), whether birds probed flowers from a perch or while hovering, and whether feeding movement occurred upwards or downwards within each inflorescence. Finally, all visible instances of robbing in which birds fed on nectar by piercing the floral tube at the base, but without contacting reproductive parts of the flower, were also recorded. Videos of the same visitor species and sex on the same inflorescence, recorded within 5 minutes of each other were considered as a single foraging bout.

3.3.5 Selective exclusion experiments

To quantify the contributions of different flower visitors to reproductive success in *A. magna*, selective exclusion experiments were set up in Karkloof (n=13) and Mbona (n=7). Selective

exclusion treatments were set up on inflorescences in the bud phase to ensure no pollination had taken place prior to setting up the experiment. Each replicate in the exclusion experiment consisted of three treatments 1) complete exclusion, for which an inflorescence was covered with a plastic wire mesh cage (aperture size: 10x13mm) and covered with bridal veil fabric to exclude all possible visitors, 2) bird-exclusion set-ups for which inflorescences were covered with plastic wire mesh cages only, excluding bird visitors but allowing for smaller insect visitors, 3) a control set-up where an inflorescence was not covered with anything, allowing for all visitors to visit. One to two inflorescences per tree were assigned to each treatment, with a total of 20 replicates. Each treatment was tagged, and the number of flowers in each treatment inflorescence was recorded.

3.3.6 Pollen limitation

To determine whether reproductive success in *A. magna* is limited by pollen receipt under natural conditions, pollen supplementation experiments were conducted at Karkloof (n=11) and Mbona (n=6). Two paired treatments were applied on each of the 17 inflorescences on seven trees. For the pollen supplementation treatment, naturally pollinated flowers were hand-pollinated with supplemental cross-pollen from another tree a minimum of 3m away. Naturally pollinated flowers on the same inflorescence were tagged but otherwise left unmanipulated, to serve as controls. The pollen limitation index for the species was calculated as 1 minus the mean number of fruits or seeds produced in the control treatment divided by that in supplemented treatment (Larson and Barrett, 2000). A value close to 0 indicated that pollen receipt does not limit reproductive success, whereas values close to one indicates pollen-limitation.

3.3.7 Pollen and ovule production

To determine the pollen-ovule ratio of *A. magna*, the number of pollen grains and ovules was assessed for 13 flowers. To quantify pollen grains per flower, the fused anthers and the secondary pollen presenter region of the style were dissected out of unopened flowers and placed in 1ml 70% ethanol in a 2ml Eppendorf tube. The Eppendorf tube was then vortexed 1 minute to release and suspend pollen grains in solution. From the suspended solution, 200µl was subsampled and placed on a microscope slide with melted fuchsin gel to stain the pollen grains and covered with a cover slip. The pollen grains were then counted at 10x magnification under a compound microscope (Zeiss AXIO fluorescence microscope). The mean of the thirteen subsamples were multiplied by 10 to get an estimate of the total pollen

grains found in one flower. The ovaries of thirteen flowers were cut open and counted using a stereo microscope (Zeiss Steri 2000-C) at 0.65x magnification. To determine the pollen-ovule ratio, the mean of the number of pollen grains per flower was divided by the mean number of ovules counted for the thirteen flowers.

3.3.8 Data analyses

Floral traits were compared among sites using generalized linear models in SPSS version 28 (IBM). Floral measurements, nectar volume and concentration, were modelled in generalized linear models with a Gaussian distribution. Breeding system, selective exclusion and pollen limitation experiments were analysed using Generalized Estimating Equations with an exchangeable correlation matrix to account for replication of treatments within trees. The number of fruits per flower, seeds per fruit and seeds per flower produced were modelled using Generalized Linear Models (GLM), with a binomial distribution and logit link function. Sites were pooled for the analysis of experimental data, as there were too few replicates (two) at the Mbona site to allow comparison among sites. Significance was assessed using generalized score statistics. The sequential Šidák method was used to adjust significance for multiple pairwise contrasts.

Data was back transformed from the logit scale to plot means and standard errors. Graphs were produced using SigmaPlot 8.0. Figures with graphics were created using CorelDRAW X4 (version 14.0.0.567).

Colour spectra were analysed using R (2021) and using the package ‘pavo’ (Maia et al., 2013). Local regression smoothing was used to optimize spectra with a 0.15 smoothing parameter.

3.4 RESULTS

3.4.1 Floral traits

3.4.1.1 Flower measurements

The number of open flowers per inflorescence ranged from 2 to 25 across sites and did not vary among sites (Table 1). No significant differences among sites were detected for any of the floral or nectar traits measured in Karkloof and Umtamvuna. Mean tube length was similar at both sites at approximately 24mm; whole flower height was measured at

approximately 6mm. The percentage of flowers that showed evidence of robbing was 13% and 25% at Karkloof and Umtamvuna respectively.

Table 1. Floral and inflorescence traits of *Alberta magna* at Karkloof and Umtamvuna Nature Reserve. Values in brackets indicate number of flowers except for the open flowers per inflorescence, where values in brackets indicate number of inflorescences.

Measurement	Karkloof	Umtamvuna	χ^2	p
Open flowers per inflorescence	10.09 ± 0.89 (47)	6.25 ± 3.04 (4)	1.47	0.226
Tube length (mm)	23.16 ± 0.51 (15)	24.63 ± 0.98 (4)	1.76	0.185
Sepal length (mm)	4.03 ± 0.16 (15)	4.45 ± 0.30 (4)	1.53	0.216
Opening height (mm)	3.67 ± 0.18 (15)	3.75 ± 0.35 (4)	0.05	0.819
Opening width (mm)	3.66 ± 0.25 (15)	3.30 ± 0.48 (4)	0.45	0.503
Whole flower height (mm)	5.95 ± 0.25 (15)	5.95 ± 0.49 (4)	0.00	0.995
Whole flower width (mm)	6.38 ± 0.35 (15)	6.03 ± 0.68 (4)	0.21	0.644
Anther (mm)	19.08 ± 0.75 (15)	18.63 ± 1.46 (4)	0.08	0.782
Stigma (mm)	33.45 ± 0.65 (15)	36.15 ± 1.27 (4)	3.59	0.058
Flowers robbed (%)	13.33 (15)	25 (4)		
24hr nectar volume (µl)	5.69 ± 1.62 (7)	8.25 ± 1.51 (8)	1.34	0.246
Standing crop nectar volume (µl)	1.74 ± 0.43 (4)	1.39 ± 0.35 (6)	0.39	0.535
Sugar concentration (%)	15.73 ± 0.76 (13)	15.86 ± 1.03 (7)	0.01	0.921

3.4.1.2 Scanning electron microscopy

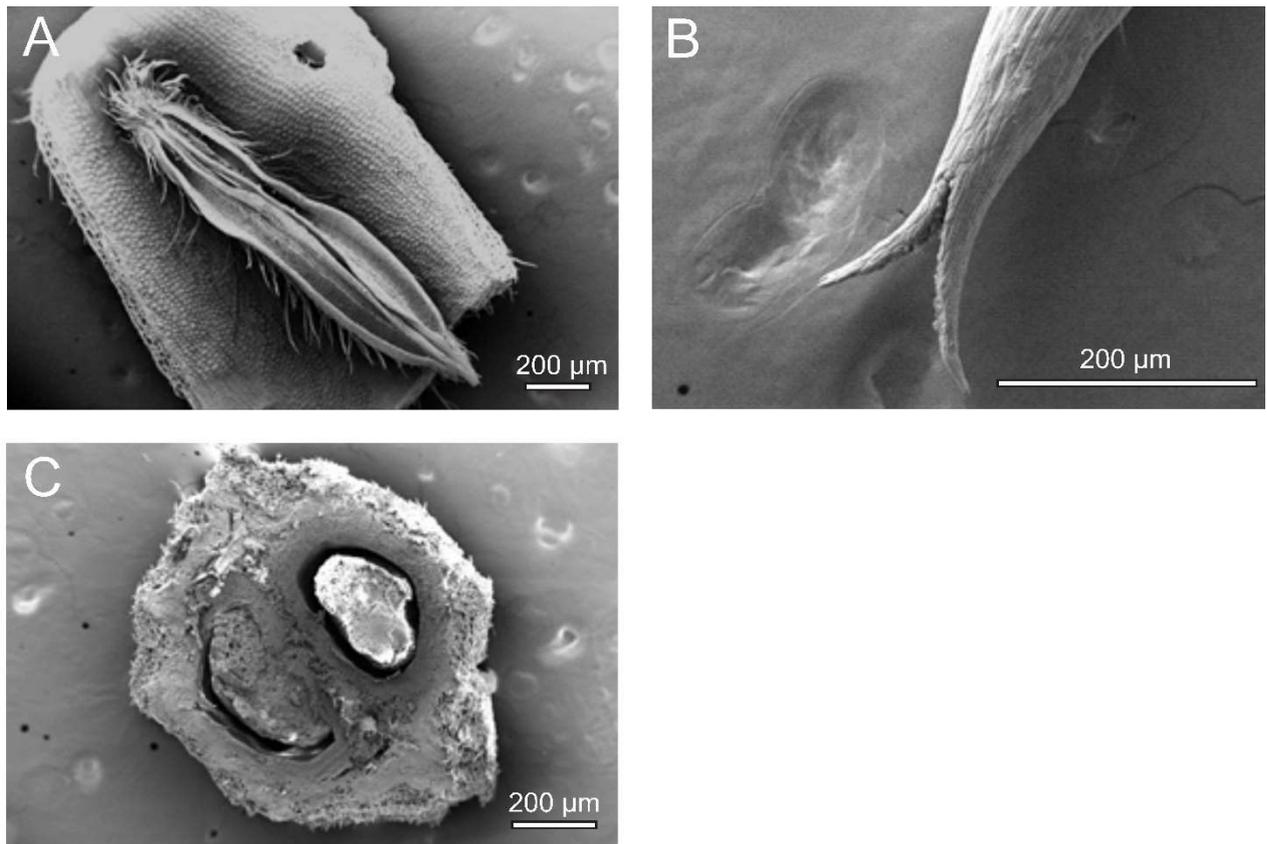


Figure 2. Scanning electron microscopy images of reproductive parts of *Alberta magna* flowers: anther (A), stigma (B), cross-section of ovary (C).

The anthers of *A. magna* are found fused within the floral tube (Fig. 1A), where pollen is produced but secondary pollen is also presented on the style, just below the stigma (Fig. 1B). *Alberta magna* produces two ovules (Fig. 1C).

3.4.1.3 Nectar volume, concentration and composition

The mean nectar volume did not differ between sites (24 hour bagged nectar: $\chi^2=1.34$, $p=0.246$; standing crop nectar: $\chi^2=0.39$, $p=0.535$; Table 1) but was significantly greater for flowers bagged for 24 hours ($7.05 \pm 0.90\mu\text{l}$) than available as standing crop ($1.53 \pm 1.12\mu\text{l}$; $\chi^2=14.94$, $p<0.001$; Fig. 2). Sugar concentration was similar across sites ($15.8\% \pm 0.61$). Nectar of *A. magna* was composed of $43.4 \pm 1.26\%$ glucose, $48.5 \pm 1.24\%$ fructose and $8.2 \pm 0.90\%$ sucrose sugars (mean \pm SE, $n=5$).

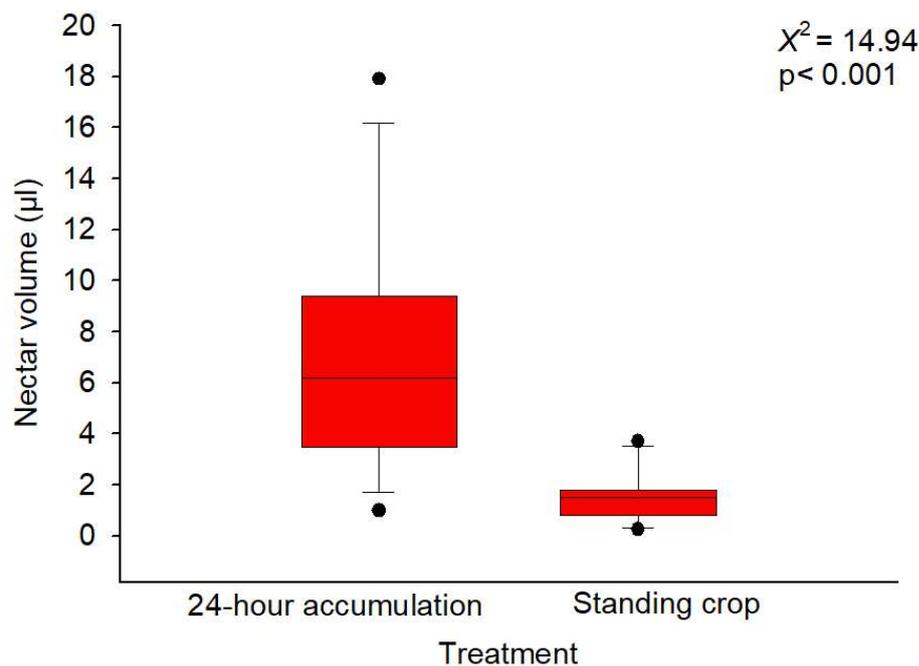


Figure 2. Nectar volumes in flowers of *Alberta magna* measured after flowers were bagged to exclude pollinators and quantify nectar production over 24 hours, and available to foraging pollinators as standing crop.

3.4.1.4 Flower colour analysis

The spectral reflectance of *A. magna* floral petals and tubes showed a sharp increase at 550nm and high, uniform reflectance in the red region from 550nm to 650nm (Fig. 3). There was no peak in reflectance in the ultraviolet range (300-400nm).

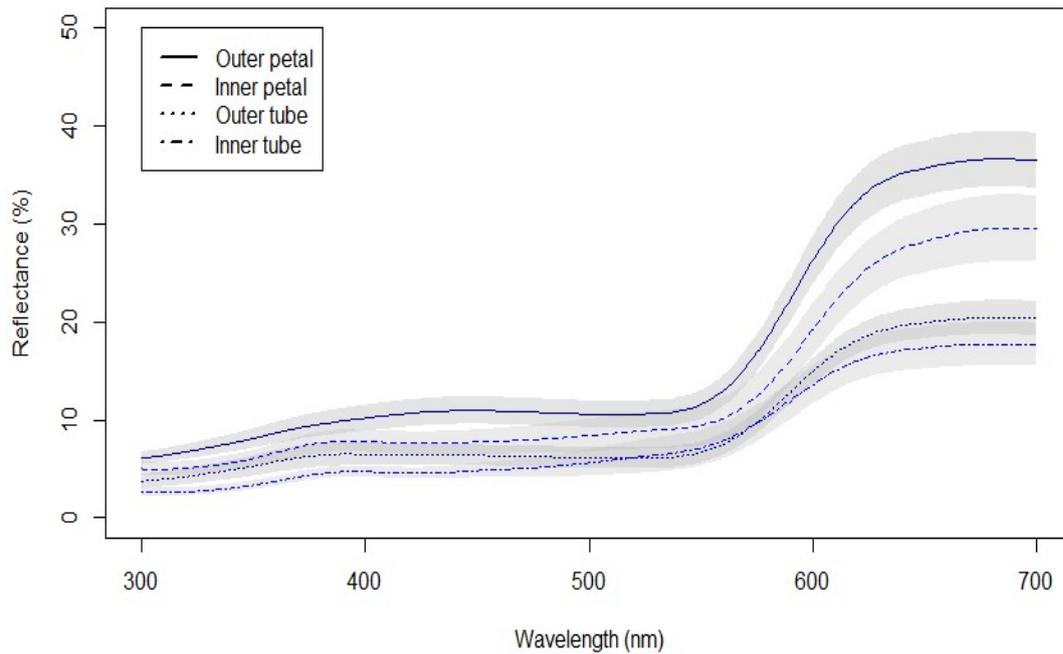


Figure 3. Spectral reflectance of *Alberta magna* flowers. Lines show mean and 95% confidence intervals (CI) for flowers from trees at Karkloof, Mbona and Pietermaritzburg hospice.

3.4.2 Breeding systems

Pollination treatment did not affect reproductive success in the breeding system experiment (fruits produced per flower $\chi^2=2.61$, $p=0.271$, Fig. 4A; seeds produced per fruit $\chi^2=1.74$, $p=0.418$, Fig. 4B; seeds produced per flower $\chi^2=1.96$, $p=0.376$, Fig. 4C).

Self-compatibility and autofertility indices varied substantially depending on the reproductive output measurement used: The self-compatibility index for fruits produced per flower was 0.88, for seeds produced per fruit, 0.91, and for seeds produced per flower, 0.27. The autofertility index for fruits produced per flower was 0.93, for seeds produced per fruit 0.91 and seeds per flower 0.36.

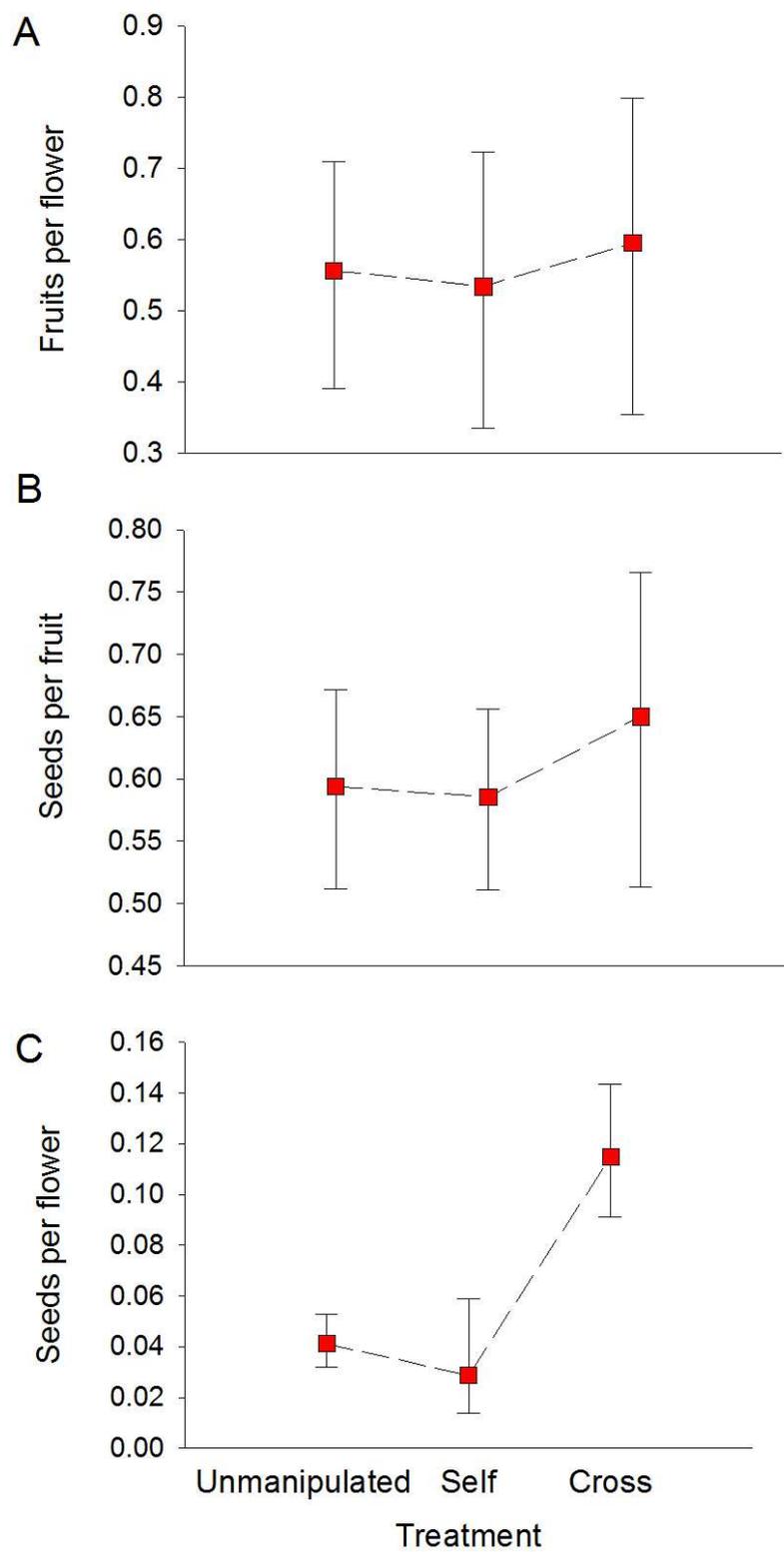


Figure 4. Reproductive success of flowers of *Alberta magna* subjected to different controlled pollination treatments: fruits produced per flower (A), number of seeds produced per fruit (B) and seeds produced per flower (C). Symbols indicate means \pm SE.

3.4.3 Pollinator observations

During over 40 hours of direct field observations, 90 foraging bouts by sunbird visitors were observed. Over 70 camera-days, a total of 106 visits by sunbirds and three visits by butterflies were recorded. The most common flower visitor in camera footage, was the southern double-collared sunbird (*Cinnyris chalybeus*; 69% of the recorded foraging bouts), which was the dominant visitor at Karkloof and Mbona (Table 2). Recorded visits by southern double-collared sunbirds were predominately by male individuals (84.93% of total 73 recorded visits). The olive sunbird (*Cyanomitra olivacea*) was a common visitor at Mbona (43.14% of total 51 recorded visits) and in the Pietermaritzburg Botanical Gardens (75% of total 8 recorded visits). Amethyst sunbirds (*Chalcomitra amethystina*) were captured less frequently on camera traps than other sunbirds (only four recorded visits). Collared sunbirds were seen on *A. magna* only at Umtamvuna occasionally through direct observations and only captured once on camera traps.

Camera footage showed that birds move their beak with the curve of the flower when feeding. Feeding orientation was flexible: birds fed from the top, side or base of the inflorescence depending on the availability of a suitable perch. Foraging movements within an inflorescence were unpredictable, although foraging more often involved horizontal than vertical movement within an inflorescence (Table 3). In more than half of recorded foraging bouts, collared and olive sunbirds utilised a branch of the inflorescence itself as a perch (Table 3). Camera trap footage across the more than 100 foraging bouts recorded did not include any cases of hovering while feeding for any of the four sunbird species. Robbing behaviour was also not evident in any of the recorded foraging bouts.

Overall, 36.54% of birds carried visible pollen loads, and close to 100% of visits included contact with stigmas (Table 3). Pollen was more often visible on birds' heads than on their beaks ($40.91 \pm 5.24\%$ of birds with visible pollen on the head; $11.24 \pm 3.35\%$ with pollen visible on the beak, across all sunbird visitors; Table 3). The frequency at which pollen was visible on birds' heads and beaks varied among bird species: visible pollen loads on birds' heads were observed for in two-thirds of foraging bouts at Mbona and all bouts recorded at the Pietermaritzburg gardens for olive sunbirds, and in two thirds of foraging bouts by amethyst sunbirds, but only for 18% and 33% of foraging bouts by southern double-collared sunbirds at Karkloof and Mbona respectively (Table 3). Olive sunbirds carried pollen on their beaks more often than other sunbirds (15% at Mbona and 50% at PMB gardens). Pollen was

only rarely seen on the beaks of southern double-collared sunbirds (5% and 11% at Karkloof and Mbona respectively) while none of the three amethyst sunbirds observed carried visible pollen on their beaks.

Birds were found to probe at least 50% of open flowers on an inflorescence, with the exception of olive sunbirds at the gardens (12%). Amethyst sunbirds probed the largest number of flowers per inflorescence (22 ± 6.43), followed by olive sunbirds (approximately 14 probes) and southern double-collared sunbirds probing significantly less than the other species (approximately 9 probes).

The diel patterns of foraging activity differed among sunbird species (Fig. 5). Southern double-collared sunbirds visitors were observed throughout the day, from 7am until 7pm (Fig. 5A). Olive sunbird visits also occurred throughout the day but foraging commenced earlier: visits were recorded from 4am until 6pm with a peak in visitation between 1 and 2pm (Fig. 5B). Amethyst sunbirds visited primarily in the morning, between 9am and 11am (Fig. 5C).

Table 2. Details of visitation to flowers of *Alberta magna*, recorded on camera traps.

Site	Karkloof	Mbona	PMB Gardens	Umtamvuna	Total
Number of videos with visitors	48	51	8	2	109
Days	48	16	4	2	70
Number of focal plants	9	12	1	2	24
Sunbirds					106
Southern double-collared sunbird ♂	37	23	1	1	73
♀	8	3	0	0	
Amethyst sunbird ♂	0	2	0	0	4
♀	0	1	1	0	
Olive sunbird	0	22	6	0	28
Collared sunbird	0	0	0	1	1

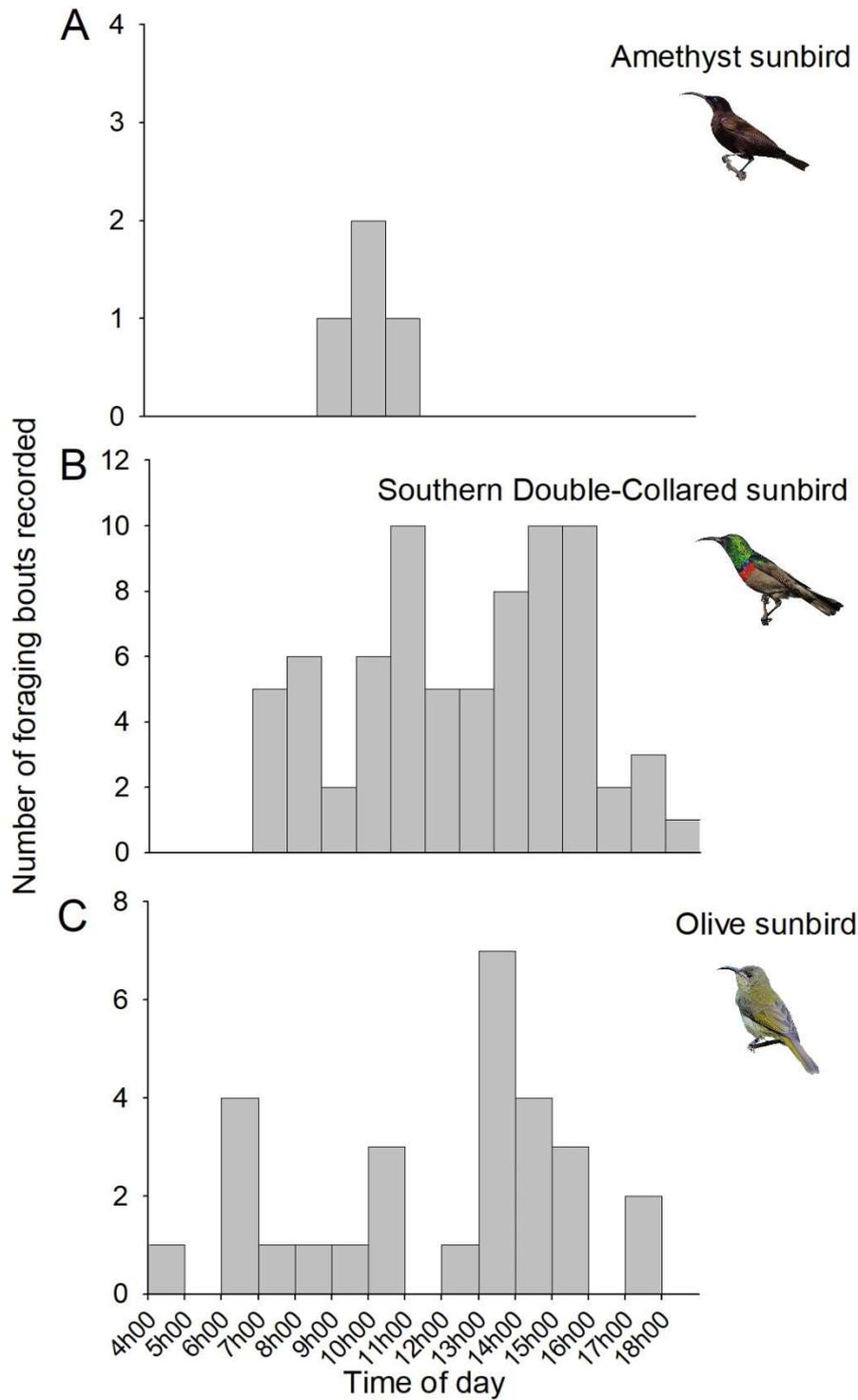


Figure 5. Diel patterns of sunbird pollinator activity, based on camera trap footage of three species of sunbird foraging on flowers of *Alberta magna*: Amethyst sunbird (A), southern double-collared sunbird (B) and olive sunbird (C). Note differences in y-axes.

Table 3. Details of sunbird foraging behaviour and pollen placement obtained from camera trap footage. Numbers in parentheses indicate numbers of recorded foraging bouts for each sunbird species at each site. Sites that share letters do not differ significantly.

Species	Southern double-collared		Amethyst		Olive		χ^2	p		
Site	Karkloof (45)	Mbona (26)	Mbona (3)	Mbona (22)	PMB Gardens (6)					
Visible pollen loads	17.78	26.92	100	59.09	100					
on beak (% of birds)	5	11.1	a	0	a	15	50	a	5.33	0.070
on head (% of birds)	17.5	33.3	a	66.7	ab	68.4	100	b	29.46	<0.001
Number of probes	9.53 ± 0.86	8.12 ± 1.67	a	22 ± 6.43	b	15.09 ± 1.87	13 ± 2.78	b	19.03	<0.001
% open flowers probed	52.0	50	a	73.3	a	60.8	12.3	a	2.18	0.337
Stigma contact (% of probed flowers)	100	70.8	a	100	a	90.9	100	a	0.55	0.759
Perch on branch of inflorescence (%)	88.9	53.9		33.3		54.5	83.3			



Figure 6. Images from camera trap footage illustrating interactions of sunbirds with flowers of *Alberta magna*. Olive sunbirds feeding on flowers from a perched position above (A) and below the inflorescence (B); stigma contact with heads of male (C) and female (D) amethyst sunbirds feeding on flowers; stigma contact on the heads of male (E) and female (F) southern double-collared sunbirds feeding on flowers. Scale bars: 2.5cm.

3.4.4 Pollen-ovule ratios

The mean (\pm SE) pollen count per flower was 1853.08 ± 394.56 . There were two ovules in all ovaries. The mean (\pm SE) pollen-ovule ratio was calculated to be 926.54 ± 197.28 .

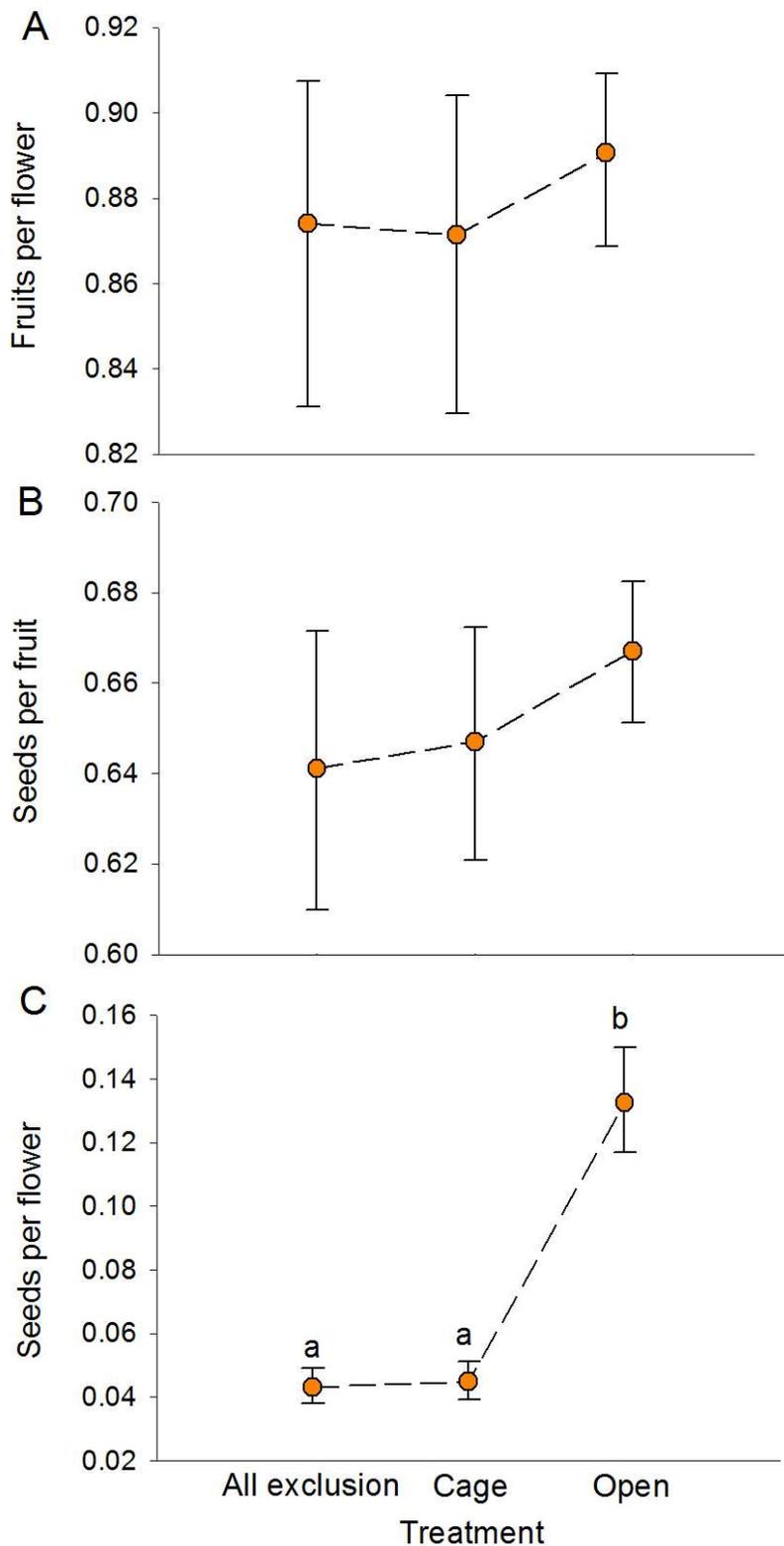


Figure 7. Effects of selective exclusion of different flower visitors on reproductive success of *Alberta magna*, based on three measures of reproductive success: fruits produced per flower (A), seeds produced per fruit (B) and seeds produced per flower (C). Symbols indicate mean \pm SE. Symbols that share a letter do not differ significantly.

3.4.5 *Selective exclusion experiments*

Analysis of reproductive success in selective exclusion experiments found no significant differences among treatments for fruits produced per flower ($\chi^2=0.75$, $p=0.688$; Fig. 7A) or for seeds produced per fruit ($\chi^2=1.07$, $p=0.586$; Fig. 7B). The number of seeds produced per flower was similar for racemes from which birds were excluded and from which all visitors excluded, but significantly greater for naturally-pollinated racemes ($\chi^2=70.78$, $p<0.001$; Fig. 7C).

3.4.6 *Pollen-supplementation experiments*

Pollen supplementation experiments found no significant differences between pollen-supplemented and control treatments for fruit produced per flower ($\chi^2=1.41$, $p=0.235$; Fig. 8A) or seeds produced per fruit ($\chi^2=1.90$, $p=0.168$; Fig. 8B). The pollen limitation indices calculated based on fruits per flower (0.00) and seeds per fruit (0.08) also indicated an absence of pollen limitation. The index of pollen limitation calculated based on seeds per flower suggested intermediate pollen limitation (IPL = 0.64), although no difference in the number of seeds produced per flower was found between pollen-supplemented and control flowers ($\chi^2=3.56$, $p=0.059$; Fig. 8C).

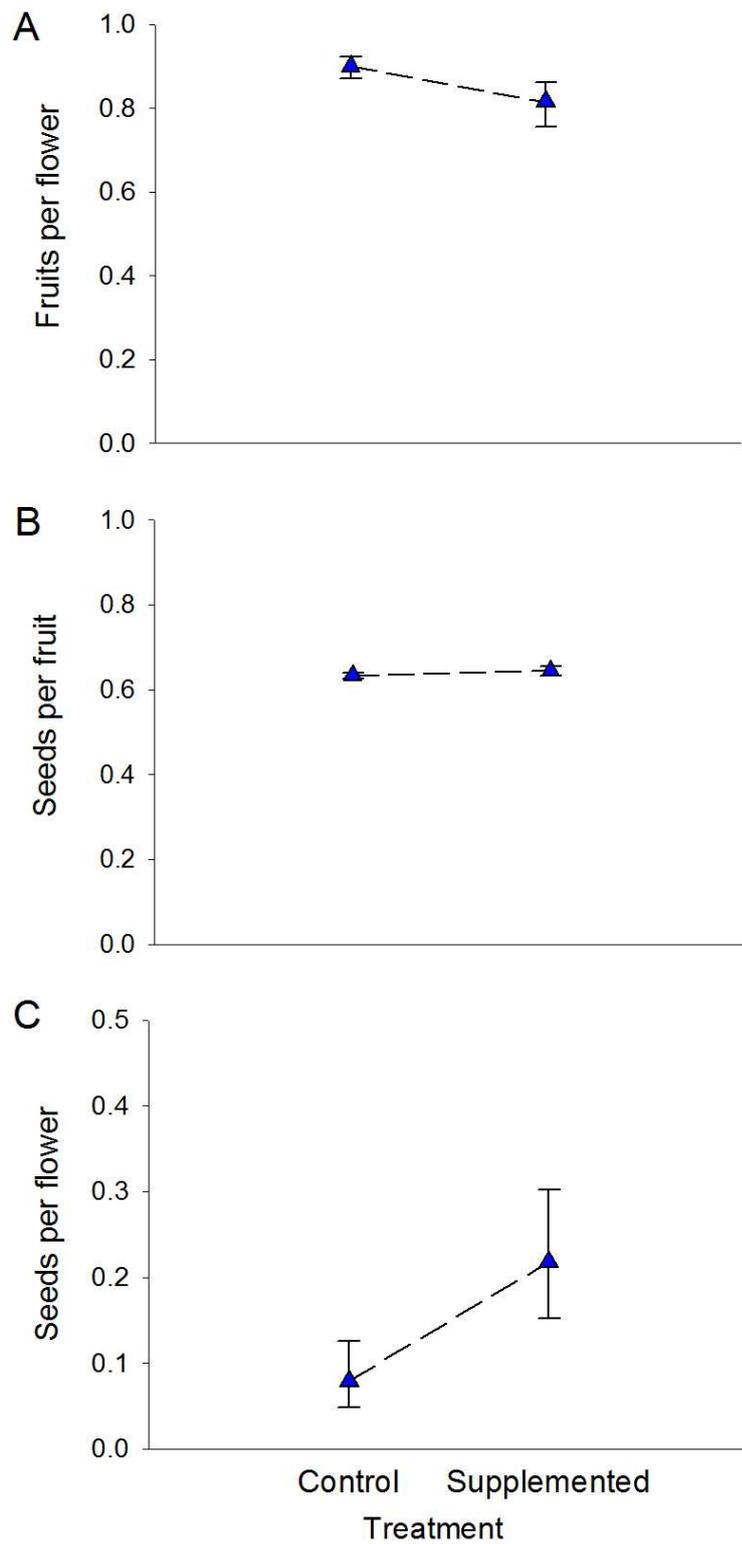


Figure 8. Reproductive success of *Alberta magna* naturally pollinated flowers and flowers subjected to pollen-supplementation treatment: proportion of fruits produced per flower (A), seeds produced per fruit (B) and seeds produced per flower (C). Symbols indicate means \pm SE.

3.5 DISCUSSION

In this study I was able to thoroughly document the pollination biology of *A. magna* through direct observations, camera trap recordings, and hand pollination and selective exclusion experiments, to verify the trait-based prediction of bird-pollination for this species. Objective quantification of floral traits of *A. magna* confirmed typical traits of an ornithophilous pollination syndrome which included copious nectar, low nectar concentrations, long floral tube lengths and colour reflectance typical for bird-pollinated plants. Controlled hand-pollination experiments suggested that *A. magna* is not self-compatible and showed that the species produces a low seed set even when hand-pollinated. Southern double-collared and olive sunbirds were the most common visitors to *A. magna*, whereas visits by amethyst sunbirds were recorded only occasionally. The exclusion of birds led to a decline in reproductive success while the exclusion of insects had a negligible effect. Supplemental pollination with cross-pollen revealed that *A. magna* is not pollen-limited, despite very low levels of fecundity in naturally pollinated flowers.

In this study camera trap footage enabled the identification of bird visitors of *A. magna* to the species level and the gathering of information which could not be obtained from direct observations. I was able to identify double-collared sunbirds and olive sunbirds as the main visitors of *A. magna* from camera footage. Several aspects of the feeding behaviour of sunbirds, including details of pollen placement on the body, contact with reproductive structures, perching behaviour and frequency of probing which cannot normally be picked up when doing direct observations, were obtained from camera footage in this study. While other plant species may provide a perch in the form of a branch, stem or leaf (Cronk and Ojeda, 2008), the inflorescence branch of *A. magna* provides adequate support for sunbirds to utilise it as a perch while feeding.

Morphological matching of *A. magna* flowers and bird bill dimensions size may play a role in effective pollination: pollen placement on birds' heads typically occurred when birds fully inserted their bills into flowers. The bill length of the olive sunbird may represent a closer match with *A. magna* floral tube and stigma lengths since pollen was seen on heads of olive sunbirds more often than on heads of southern double-collared sunbirds. Pollen placement on the head rather than the bill may be beneficial as pollen grains carried in the feathers could remain in feathers longer during bird flight and not blow off as easily as from the bill (Rocca and Sazima, 2013). There is a close match between floral tube (23.47 ± 0.47 mm across sites)

and bill lengths of southern double-collared sunbirds which range from 20 to 24mm (Downs, 2004). Pollen was observed on the heads of southern double-collared sunbirds three times more than on their bills, possibly also reflecting the close match between bill and floral dimensions. Visits by southern double-collared sunbirds also included visible stigma contact for 70.8% and 100% of flowers probed, at Mbona and Karkloof respectively. During feeding, sunbirds' heads make contact with the longer (34.02 ± 0.63 mm across sites) stigma and pick up secondary pollen or transfer pollen onto the stigma. The secondary presentation of pollen on the longer style likely also facilitates additional deposition of pollen on feathers of pollinating birds' heads. Olive sunbirds bill lengths range from 25 to 29mm (Downs, 2004), slightly longer than that of southern double-collared sunbirds and longer than flowers of *A. magna*. Some of the bill of the olive sunbird would be outside of the floral tube but this would still allow for the head to contact the secondary pollen presenter on the exerted part of the style, as well as the stigma, since style length of *A. magna* averages 33.5 to 36.2mm at Karkloof and Umtamvuna respectively (Table 1). There were more cases of olive sunbirds with pollen on their heads (70.37%) than bills (22.22%) and this percentage was also higher than that found for southern double-collared sunbirds. Stigma contact was observed for 90.9% and 100% of flowers probed at the respective sites. There were only a total of three visits from amethyst sunbirds even though their bill lengths are similar to that of olive sunbirds (25-30mm; Rebelo, 1987). The size matching of bird bills and floral tubes is a commonly recognised phenomenon, where larger billed birds visit larger flowers and birds with small bills visit smaller flowers (Biddick and Burns, 2018; Rico-Guevara et al., 2021). This could be an active process whereby birds look specifically for flowers with matching traits or it could be passive whereby birds feed randomly on flowers that are accessible for their bill size (Biddick and Burns, 2018). Suggested reasons for the coevolution of bird bill and flower length matching is that it deters unwanted or less effective visitors and provides a mutual benefit to both bird (nectar is easily obtainable) and plant (efficient pollination) (Rico-Guevara et al., 2021).

Visitation by sunbirds to flowers of *A. magna* recorded in this study showed that sunbirds did not visit flowers notably more in the morning, as found in other studies where there is a peak in mid-morning visits (Butler et al., 2022) or later in the morning (Sun et al., 2017; Huang et al., 2018; Chen et al., 2019). Sunbird visits to *A. magna* were instead spread out throughout the day and were common until 4pm.

Exclusion experiments revealed that seed production per fruit were similar across treatments, however open racemes produced approximately threefold more seeds per flower than those excluding birds and those excluding all visitors. Thus, the exclusion of sunbirds significantly decreased reproductive success. The significant effect of sunbird-exclusion on reproductive success implicates olive sunbirds and southern double-collared sunbirds as pollinators of *A. magna* and confirms that seed production would decline in the absence of these sunbirds. The similarity in seed production for racemes subjected to bird-exclusion and exclusion of all visitors, demonstrates that insects do not contribute to reproductive success in *A. magna*.

Visitor observations and exclusion experiments in this study together clearly implicate sunbirds as pollinators of *A. magna*. Butterflies were only captured on camera trap footage on a total of three out of the 106 bird videos captured but pollen could not be observed on butterflies because they are too small on footage. Studies conducted on other plant species, such as those by Butler et al. (2022) and McElveen and Meyer (2020) were able to use camera traps to detect and record butterfly visitation while Johnson et al. (2019) and Steen (2012) was able to detect and record hawkmoth visits. Thus, the lack of footage found in this study is likely due to the absence of butterflies and not because of the inability of cameras to detect butterflies. The confirmation of bird pollination in this species, for which all close relatives are butterfly pollinated, is indicative a possible shift from butterfly to bird pollination in the group. Confirmation of the pollination systems for the Madagascan species and application of molecular methods could confirm this hypothesized pollinator shift.

Alberta magna floral traits including spectral reflectance and morphology were generally consistent with those associated with bird pollination, although nectar characteristics were surprising for a species pollinated by sunbirds. The spectral reflectance of *A. magna* was similar to that of other bird-pollinated plant species peaking in the range of about 550 to 650nm (Johnson and Bond, 1994; Meléndez-Ackerman et al., 1997; Shrestha et al., 2013). However, there was no peak in the UV range and sunbirds are known to have an ultraviolet sensitive colour vision system (Ödeen and Håstad, 2010). Thus, the lack of UV reflectance in *A. magna* could mean that flowers are not as easily detectable to sunbirds as other UV reflecting flowers. However, it has been found that sunbirds do not necessarily prefer UV reflecting flowers, but rewards found within the flowers instead (Meléndez-Ackerman et al., 1997; McCarren et al., 2021) and thus, the lack of UV reflectance is not inconsistent with pollination by sunbirds.

Nectar volume for flowers bagged to quantify nectar production over 24 hours was 6 and 8 μ l respectively at the two study sites where this was sampled, below the average volume determined for sunbirds (10-30 μ l; Johnson and Nicolson, 2008). This recorded accumulated nectar volume, is higher than for insect-pollinated flowers (Cronk and Ojeda, 2008), but low compared to other flowers pollinated by generalist birds (40–100 μ l; Johnson and Nicolson, 2008). Standing crop nectar was on average less than 2 μ l per flower in both study sites. This is significantly lower than accumulated nectar volume, suggesting that flower visitors consume a substantial amount of nectar. The nectar sugar concentration of approximately 15% is consistent with the dilute nectar typical of many bird-pollinated plants. *Alberta magna* nectar sugar concentration is low but within the range observed for bird-pollinated plants (15-20%; Johnson and Nicolson, 2008) and is lower than the 36% concentration typically found in bee-pollinated flowers (Pyke and Waser, 1981). Various explanations have been proposed to explain the low nectar concentration found in passerine-pollinated plants: birds require high water volumes (there is more water in dilute nectar), it is easier for nectar uptake (less viscous) or the low concentration may act as a deterrent to bee visitors (Baker, 1975; Bolten and Feinsinger, 1978). Glucose and fructose levels were found to be 43% and 49%, while sucrose was only 8%, which is comparable to many other species with low sucrose levels such as *Greyia sutherlandii* and *Chasmanthe aethiopica* (Goldblatt et al., 1999), which are pollinated by passerines (Nicolson, 2002). However, the low sucrose level contrasts with the conclusion of Johnson and Nicolson (2008), who found that plants pollinated by specialist passerines, such as sunbirds, usually have high sucrose levels (40-60%). This result also differs from the majority of ornithophilous *Erica* species which were found to be sucrose dominant (Barnes et al., 1995). The low sucrose level found for *A. magna* is more typical for generalist bird-pollinated plant species, which are in the range of 0-5% (Johnson and Nicolson, 2008) than for species pollinated by specialist nectar feeding sunbirds. Although hexose-dominant nectars are more commonly associated with pollination by opportunistic nectar feeding birds (which lack the sucrase enzyme required to digest sucrose), sunbirds are flexible and can feed on both hexose and sucrose dominated nectars. Thus, the sugar composition of *A. magna* is atypical but not inconsistent with pollination by sunbirds.

Fruit set per flower was not a good indicator of reproductive success in *A. magna*. For exclusion experiments, fruit set ranged around 80% for all three treatments, but there were no seeds in most of these fruits. For this reason, two metrics for seed production was used, seeds produced per fruit and seeds produced per flower. Approximately 60% of fruits produced two

seeds across breeding system treatments. Seeds produced per flower shows how few seeds are produced in all treatments, where only 13% of flowers produced seeds in the open treatment. The use of different measures of reproductive output was thus useful to compare reproductive success at different scales.

Controlled hand pollinations to determine the breeding system of *A. magna* indicate that the species is weakly self-compatible and may produce very low levels of seed set by means of autonomous self-pollination. Seed production from flowers hand pollinated with self-pollen, and for unmanipulated flowers, was approximately a third of that from flowers hand-pollinated with cross-pollen and indices of autofertility (0.36) and self-compatibility (0.27) based on seed production per flower indicated some potential for self-compatibility and weak antifertility to contribute to reproductive success. However, seed production per flower from both manual cross-pollination and in naturally pollinated flowers (14% and 12% respectively), and only weak evidence for pollen limitation, suggest that improved receipt of cross pollen will not alleviate natural limited reproductive success.

Despite the significant contribution of sunbird pollinators to seed production in *A. magna*, seed production under natural pollination conditions output is still surprisingly low and this extremely low seed output may contribute to the rarity of the species. Limited access to required resources may play a role in the low seed output of *A. magna* (Lee, 1988). Germination rates of naturally produced seeds are also low, which has proved a challenge in horticulture (Van Staden, 1990). Longevity of trees that do germinate may mitigate effects of low seed production and germination rates. *Alberta magna* may have previously experienced inconsistent pollinator visits and thus developed overproduction of ovules as a method to adapt to unpredictable visitation (Burd, 1995). Long-lived plants often have strong self-incompatibility systems since the chance of geitonogamy is higher in plants with more flowers (Gibbs and Bianchi, 1999). Other potential explanations for low fruit set, including seed abortion, should be investigated.

The usefulness of camera traps in this study is incontrovertible, but challenges associated with using camera traps were also experienced. Motion detecting cameras detect any movement, and in this study, windy conditions led to high levels of false triggering in which videos were recorded, but no flower visitors were recorded. Wind trigger often resulted in inefficient utilisation of available memory storage as cards were filled by continuous wind footage in a few hours and no longer available to record visitors, shortly after set-up. Setting

up cameras in *A. magna* trees which were very tall also proved a challenge, sometimes requiring a pole to attach a camera to if a tree could not be climbed. Most flowers in the trees at the Umtamvuna site were impossible to reach and thus cameras could only be placed in a small proportion of the flowering trees due to accessibility constraints. Characteristic of plant structure, including susceptibility to wind trigger, and accessibility of plants and flowers, affect the utility of camera traps in a study system, but in the case of *A. magna* these challenges could largely be overcome by deploying cameras over many months of an extended flowering season.

3.6 CONCLUSION

This study confirmed that *A. magna* floral traits conform to an ornithophilous pollination syndrome and are indicative of reliance of the species on sunbirds for pollination. Three species of sunbirds were observed to visit flowers, carry visible pollen loads and contact stigmas as they fed on flowers, confirming their effectiveness as pollinators. Exclusion experiments revealed that sunbirds are important for reproductive success of *A. magna*: sunbird access increased overall reproductive output threefold (from 4% to 12%). Despite the low levels of natural seed set (only c. 14% of naturally pollinated flowers develop into fruits with seeds), pollen supplementation experiments showed that *A. magna* is not strongly pollen limited. Data presented in this study thus confirm the critical role of sunbirds in pollination of *A. magna*, but also identify the need to investigate other, non-pollinator causes of low natural fecundity in this rare species.

3.7 REFERENCES

- Abrahamczyk, S., 2019. Comparison of the ecology and evolution of plants with a generalist bird pollination system between continents and islands worldwide. *Biological Reviews* 94, 1658-1671.
- Baker, H.G., 1975. Sugar concentrations in nectars from hummingbird flowers. *Biotropica* 37-41.
- Barnes, K., Nicolson, S.W., van Wyk, B.E., 1995. Nectar sugar composition in *Erica*. *Biochemical Systematics and Ecology* 23, 419-423.
- Becerra, J.X., Lloyd, D.G., 1992. Competition-dependent abscission of self-pollinated flowers of *Phormium tenax* (Agavaceae): a second action of self-incompatibility at the whole flower level? *Evolution* 46, 458-469.
- Ben-Jaacov, J., Ackerman, A., Tal, E., Jacobs, G., 1991. Vegetative propagation of *Alberta magna* by tissue culture and grafting. *HortScience* 26, 74-75.
- Biddick, M., Burns, K.C., 2018., Phenotypic trait matching predicts the topology of an insular plant–bird pollination network. *Integrative Zoology* 13, 339-347.
- Bolten, A.B., Feinsinger, P., 1978. Why do hummingbird flowers secrete dilute nectar? *Biotropica* 10, 307-309.
- Botes, C., Johnson, S. D., Cowling, R.M., 2008. Coexistence of succulent tree Aloes: partitioning of bird pollinators by floral traits and flowering phenology. *Oikos* 117, 875-882.
- Bremer, B., Eriksson, T., 2009. Time tree of Rubiaceae: phylogeny and dating the family, subfamilies, and tribes. *International Journal of Plant Sciences* 170, 766-793.
- Brody, A.K., Mitchell, R.J., 1997. Effects of experimental manipulation of inflorescence size on pollination and pre-dispersal seed predation in the hummingbird-pollinated plant *Ipomopsis aggregata*. *Oecologia* 110, 86-93.
- Burd, M., 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *The Botanical Review* 60, 83-139.
- Burd, M., 1995. Ovule packaging in stochastic pollination and fertilization environments. *Evolution* 49, 100-109.
- Butler, H.C., Cozien, R.J., Johnson, S.D., 2022. For the birds? Contrasting pollination and breeding systems of the paintbrush lilies *Scadoxus puniceus* and *S. membranaceus* (Amaryllidaceae). *Plant Systematics and Evolution* 308, 1-11.

- Chen, K., Lu, J., Wang, C., 2019. Effective pollination of *Aeschynanthus acuminatus* (Gesneriaceae) by generalist passerines, in sunbird-absent East Asia. *Scientific reports* 9, 1-12.
- Chmel, K., Ewome, F.L., Gómez, G.U., Klomberg, Y., Mertens, J.E., Tropek, R., Janeček, Š., 2021. Bird pollination syndrome is the plant's adaptation to ornithophily, but nectarivorous birds are not so selective. *Oikos* 130, 1411-1424.
- Cronk, Q., Ojeda, I., 2008. Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany* 59, 715-727.
- Dellinger, A.S., 2020. Pollination syndromes in the 21st century: where do we stand and where may we go? *New Phytologist* 228, 1193-1213.
- Downs, C.T., 2004., Some preliminary results of studies on the bill and tongue morphology of Gurney's Sugarbird and some southern African sunbirds. *Ostrich-Journal of African Ornithology* 75, 169-175.
- Drewes, S.E., Horn, M.M., Connolly, J.D., Bredenkamp, B., 1998. Enolic iridolactone and other iridoids from *Alberta magna*. *Phytochemistry* 47, 991-996.
- Fenster, C.B., Armbruster, W.S., Wilson, P., Dudash, M.R., Thomson, J.D., 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* 375-403.
- Fleming, T.H., Muchhala, N., 2008. Nectar-feeding bird and bat niches in two worlds: pantropical comparisons of vertebrate pollination systems. *Journal of Biogeography* 35, 764-780.
- Geerts, S., Pauw, A., 2009. Hyper-specialization for long-billed bird pollination in a guild of South African plants: the Malachite Sunbird pollination syndrome. *South African Journal of Botany* 75, 699-706.
- Gibbs, P.E., Bianchi, M.B., 1999. Does late-acting self-incompatibility (LSI) show family clustering? Two more species of Bignoniaceae with LSI: *Dolichandra cynanchoides* and *Tabebuia nodosa*. *Annals of Botany* 84, 449-457.
- Goldblatt, P., Manning, J.C., Bernhardt, P., 1999. Evidence of bird pollination in Iridaceae of southern Africa. *Adansonia* 21, 25-40.
- Huang, Z.H., Luo, W.H., Huang, S.X., Huang, S.Q., 2018. Sunbirds serve as major pollinators for various populations of *Firmiana kwangsiensis*, a tree endemic to South China. *Journal of Systematics and Evolution* 56, 243-249.
- Johnson, S.D., 1996. Bird pollination in South African species of *Satyrium* (Orchidaceae). *Plant Systematics and Evolution* 203, 91-98.

- Johnson, S.D., 2010. The pollination niche and its role in the diversification and maintenance of the southern African flora. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365, 499-516.
- Johnson, S.D., 2022. Bird pollination. *Current Biology* 32, R1059-R1060.
- Johnson, S.D., Balducci, M.G., Bijl, A., Castañeda-Zárate, M., Cozien, R.J., Ortmann, C.R., van der Niet, T., 2020. From dusk till dawn: camera traps reveal the diel patterns of flower feeding by hawkmoths. *Ecological Entomology* 45, 751-755.
- Johnson, S.D., Bond, W.J., 1994. Red flowers and butterfly pollination in the fynbos of South Africa, in: Arianoutsou, M., Groves, R.H. (Eds.), *Plant-animal interactions in Mediterranean-type ecosystems*. Springer, Dordrecht, pp. 137-148.
- Johnson, S.D., Nicolson, S.W., 2008. Evolutionary associations between nectar properties and specificity in bird pollination systems. *Biology Letters* 4, 49-52.
- Johnson, S.D., Steiner, K.E., 2000. Generalization versus specialization in plant pollination systems. *Trends in Ecology & Evolution* 15, 140-143.
- Kainulainen, K., Mouly, A., Khodabandeh, A., Bremer, B., 2009. Molecular phylogenetic analysis of the tribe Alberteae (Rubiaceae), with description of a new genus, *Razafimandimbisonia*. *Taxon* 58, 757-768.
- Katumo, D.M., Liang, H., Ochola, A.C., Lv, M., Wang, Q.F., Yang, C.F., 2022. Pollinator diversity benefits natural and agricultural ecosystems, environmental health, and human welfare. *Plant Diversity* 44, 429-435.
- Kiepiel, I., Brown, M., Johnson, S.D., 2022. A generalized bird pollination system in *Schotia brachypetala* (Fabaceae). *Plant Biology* 24, 806-814.
- Knight, T.M., Steets, J.A., Vamosi, J.C., Mazer, S.J., Burd, M., Campbell, D.R., Dudash, M.R., Johnston, M.O., Mitchell, R.J., Ashman, T., 2005. Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution, and Systematics* 467-497.
- Knudsen, J.T., Tollsten, L., Groth, I., Bergström, G., Raguso, R.A., 2004. Trends in floral scent chemistry in pollination syndromes: floral scent composition in hummingbird-pollinated taxa. *Botanical Journal of the Linnean Society* 146, 191-199.
- Larson, B.M., Barrett, S.C., 2000. A comparative analysis of pollen limitation in flowering plants. *Biological journal of the Linnean Society* 69, 503-520.
- Lee, T.D., 1988. Patterns of fruit and seed production, in: Doust, J.L., Doust, L.L. (Eds.), *Plant reproductive ecology: patterns and strategies*. Oxford University Press, New York, pp. 179-202.

- Lloyd, D.G., Schoen, D.J., 1992. Self-and cross-fertilization in plants. I. Functional dimensions. *International Journal of Plant Sciences* 153, 358-369.
- Maia, R., Eliason, C.M., Bitton, P.P., Doucet, S.M., Shawkey, M.D., 2013. pavo: an R package for the analysis, visualization and organization of spectral data. *Methods in Ecology and Evolution* 4, 906–913.
- Manning, J. C., Goldblatt, P., 2005. Radiation of pollination systems in the Cape genus *Tritoniopsis* (Iridaceae: Crocoideae) and the development of bimodal pollination strategies. *International Journal of Plant Sciences* 166, 459-474
- McCarren, S., Midgley, J., Coetzee, A., 2021. Sending private messages: Floral ultraviolet signals are correlated with pollination syndromes in *Erica*. *Journal of Pollination Ecology* 30, 289-298.
- McElveen, D., Meyer, R.T., 2020. An effective and affordable camera trap for monitoring flower-visiting butterflies in sandhills: with implications for the frosted elfin (*Callophrys irus*). *Journal of Pollination Ecology* 26, 12-15.
- Meléndez-Ackerman, E., Campbell, D.R., Waser, N.M., 1997. Hummingbird behavior and mechanisms of selection on flower color in *Ipomopsis*. *Ecology* 78, 2532-2541.
- Mitchell, R.J., Flanagan, R.J., Brown, B.J., Waser, N.M., Karron, J.D., 2009. New frontiers in competition for pollination. *Annals of Botany* 103, 1403-1413.
- Nicolson, S.W., 2002. Pollination by passerine birds: why are the nectars so dilute? *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 131, 645-652.
- Ödeen, A., Håstad, O., 2010. Pollinating birds differ in spectral sensitivity. *Journal of Comparative Physiology A* 196, 91-96.
- Ollerton, J., Alarcón, R., Waser, N.M., Price, M.V., Watts, S., Cranmer, L., Hingston, A., Peter, C.I., Rotenberry, J., 2009. A global test of the pollination syndrome hypothesis. *Annals of Botany* 103, 1471-1480.
- Ollerton, J., Cranmer, L., Stelzer, R., Sullivan, S., Chittka, L., 2008. Bird pollination of Canary Island endemic plants. *Nature Precedings* 1-18.
- Pauw, A., 2019. A bird's-eye view of pollination: Biotic interactions as drivers of adaptation and community change. *Annual Review of Ecology, Evolution, and Systematics* 50, 477-502.
- Puff, C., Robbrecht, E., Randrianasolo, V., 1984. Observations on the SE African-Madagascan genus *Alberta* and its ally *Nematostylis* (Rubiaceae, Alberteae), with a

- survey of the species and a discussion of the taxonomic position. *Bulletin du Jardin botanique national de Belgique/Bulletin van de Nationale Plantentuin van België* 54, 293-366.
- Pyke, G.H., Waser, N.M., 1981. The production of dilute nectars by hummingbird and honeyeater flowers. *Biotropica* 260-270.
- R Core Team., 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rebelo, A.G., 1987. Bird Pollination in the Cape Flora, in: Rebelo, A.G. (Ed.), *A Preliminary Synthesis of Pollination Biology in the Cape Flora*. CSIR, Pretoria, pp. 83–108.
- Rico-Guevara, A., Hurme, K.J., Elting, R., Russell, A.L., 2021. Bene “fit” assessment in pollination coevolution: mechanistic perspectives on hummingbird bill–flower matching. *Integrative and Comparative Biology* 61, 681-695.
- Rocca, M.A., Sazima, M., 2013. Quantity versus quality: identifying the most effective pollinators of the hummingbird-pollinated *Vriesea rodigasiana* (Bromeliaceae). *Plant Systematics and Evolution* 299, 97-105.
- Shrestha, M., Dyer, A.G., Boyd-Gerny, S., Wong, B.B., Burd, M., 2013. Shades of red: bird-pollinated flowers target the specific colour discrimination abilities of avian vision. *New Phytologist* 198, 301-310.
- Steen, R., 2012. Pollination of *Platanthera chlorantha* (Orchidaceae): new video registration of a hawkmoth (Sphingidae). *Nordic Journal of Botany* 30, 623-626.
- Sun, S.G., Huang, Z.H., Chen, Z.B., Huang, S.Q., 2017. Nectar properties and the role of sunbirds as pollinators of the golden-flowered tea (*Camellia petelotii*). *American Journal of Botany* 104, 468-476.
- Symes, C.T., Nicolson, S.W., 2008. Production of copious dilute nectar in the bird-pollinated African succulent *Aloe marlothii* (Asphodelaceae). *South African Journal of Botany* 74, 598-605.
- van der Niet, T., 2021. Paucity of natural history data impedes phylogenetic analyses of pollinator-driven evolution. *New Phytologist* 229, 1201-1205.
- van der Niet, T., Cozien, R.J., Johnson, S.D., 2015. Experimental evidence for specialized bird pollination in the endangered South African orchid *Satyrium rhodanthum* and analysis of associated floral traits. *Botanical Journal of the Linnean Society* 177, 141-150.
- van der Pijl, L., 1961. Ecological aspects of flower evolution. II. Zoophilous flower classes. *Evolution* 44-59.

- van Staden, J., Kelly, K., Stormanns, C., 1990. Dormancy and germination of *Alberta magna* seeds. South African Journal of Botany 56, 542-545
- Vogel, S., 1954. Blütenbiologische Typen als Elemente der Sippen-gliederung dargestellt anhand der Flora Siidafrikas. Gustav Fischer Verlag, Jena.
- Waser, N.M., Chittka, L., Price, M.V., Williams, N.M., Ollerton, J., 1996. Generalization in pollination systems, and why it matters. Ecology 77, 1043-1060.
- Wilcock, C., Neiland, R., 2002. Pollination failure in plants: why it happens and when it matters. Trends in Plant Science 7, 270-277.

Chapter 4

4.1 DISCUSSION

Southern Africa hosts a diversity of plant species which are pollinated by specialist birds, including sunbirds and sugarbirds (Johnson and Steiner, 2000; Johnson and Steiner, 2003; Johnson, 2004; Fleming and Muchhala, 2008). This study on *Alberta magna* and *Streptocarpus dunnii* confirmed sunbird pollination in two species which were hypothesized, based on floral traits to also be sunbird-pollinated, but for which observations had been challenging. The aims of this study were to determine if floral traits align with ornithophilous pollination syndrome; to determine self-compatibility and dependency on pollinators; to determine the primary pollinator species and finally, to determine if reproductive success was limited by pollen. Through the use of observations and experiments, I was able to determine the role of sunbirds in the pollination of *A. magna* and *S. dunnii*. This study highlighted that plant species with similar floral traits that share an animal pollinator group can differ significantly in their pollination systems.

Implications from the documentation of bird pollination in the two study species for evolution of bird pollination, as well as for conservation of the study species, are discussed below. Some methodological challenges associated with the use of camera traps in this study are described and ideas for future research, to address both unresolved issues and questions arising from this study, are proposed.

Pollination systems of *Streptocarpus dunnii* and *Alberta magna*: similarities and differences

Although this study confirmed that both study species are, as predicted from floral traits, reliant on sunbirds for pollination, the sunbird species involved differed. Objective quantification of floral traits for both study species also confirmed general alignment with ornithophily but found some exceptions for colour and nectar traits that did not strictly conform to the typical bird-pollination syndrome.

Streptocarpus dunnii was found to be visited by two species of sunbird: malachite sunbirds were the dominant visitor recorded at two study sites in Mpumalanga, and greater double-collared sunbirds also visited at the study site in eSwatini. However, malachite sunbirds are likely the primary pollinators in the light of both morphological trait matching and bird behaviour observed on flowers: trait matching between bill length and floral tube length is

closer for malachite sunbirds than for greater double-collared sunbirds, which have shorter beaks, and the majority of visits by double-collared sunbirds at Malolotja consisted of robbing flowers whereas as most visits by malachite sunbirds involved contact with floral reproductive parts. Legitimate visits by malachite sunbirds and predominant robbing by greater double-collared sunbirds suggest hyperspecialization for pollination by malachite sunbirds in *S. dunnii*.

Both *Streptocarpus dunnii* and *Alberta magna* have highly specialised pollination systems, dependant on just one or two species of sunbird for pollination, but the sunbird species on which the respective species rely for pollination, differ: *S. dunnii* was pollinated primarily by malachite sunbirds with a potential, secondary contribution by greater double-collared sunbirds. *Alberta magna* was visited by southern double-collared and olive sunbirds, with a few visits by amethyst sunbirds. The primary pollinator of *A. magna*, based on pollen loads and visitation frequency, is the southern double-collared sunbird. The different sunbird pollinators of the two studied plant species may reflect differences in ecological niches of the plants which correspond to habitat preferences of their respective sunbird pollinators.

Streptocarpus dunnii, is a species of open, high elevation grassland whereas *A. magna* is found along cliffs on forest edges. Although malachite sunbirds occur in diverse habitats including fynbos, they prefer open habitats, and are frequent in high elevation grasslands such as that where *S. dunnii* occurs (Skead, 1967). In contrast, the most common visitors to *A. magna*, olive sunbirds and southern double-collared sunbirds, are known to prefer woodland and Afromontane Forest habitats (Skead, 1967).

For both *S. dunnii* and *A. magna*, copious and dilute nectar, long floral tube lengths and colour spectra were consistent with expectations for traits associated with bird-pollination. In both study species, however, exceptions were also observed. Floral spectral reflectance of *S. dunnii* included the typical peak in the red region, but also a peak in the UV region, which may indicate a vestigial trait from an insect-pollinated ancestor. This difference is present because *S. dunnii* contains the pigment, dunnione, while the colour expression of *A. magna* is likely the result of anthocyanin colour expression. Nectar traits of *A. magna* were largely consistent with sunbird pollination, with the exception of the relatively low sucrose content of the nectar. However, nectar traits do not always reflect pollination systems (e.g. Manning and Goldblatt, 2007) and sunbirds are physiologically capable of feeding on hexose-dominated nectar. In both species, nectar volumes per flower were relatively low for sunbird-pollinated species, however, this may be compensated for by large floral displays in both *S. dunnii* and

A. magna which results in provision of a nectar resource that is nevertheless attractive for birds.

Evolutionary implications of evidence for bird-pollination in *S. dunnii* and *A. magna*

The bird pollination systems of *S. dunnii* and *A. magna* documented in this study, both represent a novel or previously unconfirmed pollination system in the respective plant lineages, providing insight into potential evolutionary pathways by which bird-pollination may evolve. In both systems, the shift to bird-pollination and adaptation to a novel pollinator is associated with colonization or occupation of a novel region or habitat. Confirmation of the direction of the pollinator shift in both cases requires molecular analyses, but some speculations can be made based on commonalities associated with the evolution of bird-pollination systems in the two study species.

In *Streptocarpus*, pollination by birds is thought to have arisen twice, independently (Weber et al., 2021): Bird-pollination is also hypothesized (but unconfirmed) for one other *Streptocarpus* species, with similarly unusual floral traits for the genus. *Streptocarpus myoporoides*, which, in common with *S. dunnii*, also has a large, robust and dense inflorescence of scarlet flowers (Weber et al., 2021). Closely related *Streptocarpus* species are pollinated by insects including bees and long-tongued flies, and flowers of insect-pollinated *Streptocarpus* species differ in colour, size and in inflorescence size from *S. dunnii*. Flowers of insect-pollinated species are white, mauve, often with nectar guides, and usually have few or even a single flower in some species (Weber et al., 2021). The exceptional inflorescence size of *S. dunnii*, with tens of flowers, may thus reflect selection to develop a large display for attraction of birds, to provide nectar quantities associated with bird pollination, or both. Insect-pollinated *Streptocarpus* species typically occupy forest habitats, whereas the bird-pollinated *S. dunnii* occurs in rocky, high elevation grassland habitats. *Streptocarpus myoporoides* is restricted to Mozambique where it occurs on steep wet, cliff faces in Afromontane Forest (Weber et al., 2021), a habitat which is possibly more similar to that of insect-pollinated *Streptocarpus* than the grassland habitat of *S. dunnii*.

Relatives of *A. magna* are thought to be pollinated by butterflies, and most occur in humid, forest habitats (Puff et al., 1984). *Alberta magna* is unique within the group both because it is the only species that occurs in South Africa, in contrast to the remainder which are found in Madagascar, and for being the only species pollinated by birds in a group pollinated by butterflies. Shifts between bird and butterfly-pollination are known from other southern

African plant groups including *Clivia* (Kiepiel and Johnson, 2014), *Scadoxus* (Butler et al., 2022) and *Tritoniopsis* (Manning and Goldblatt, 2007). Butterfly-pollination is thought to be commonly derived from bird-pollination in the fynbos biome, evolving as plants adapted to butterflies that initially exploited red, sunbird-pollinated flowers and were subsequently co-opted as pollinators by flowers (Johnson and Bond, 1994). In such cases, the direction of the shift is thought to be from bird to butterfly pollination (Johnson and Bond, 1994), whereas adaptation for bird pollination may represent shift from butterfly-pollination to bird-pollination.

Thus, evolution of bird-pollination in both *S. dunnii* and *A. magna* appears to be associated with occupation of a new habitat or geographic region, or both, and adaptation to birds may have occurred as the previous insect or butterfly pollinators were absent or less frequent as in the former habitat or region.

Conservation implications of pollination and breeding systems of *Streptocarpus dunnii* and *Alberta magna*:

In this study, exclusion experiments confirmed that sunbirds are critical for reproductive success in both *S. dunnii* and *A. magna*. Controlled hand pollinations, which determined the breeding system of the study species, showed that both are dependent on sunbirds for pollination. Self-compatibility differed between the species: *S. dunnii* is completely self-compatible whereas *A. magna* is characterised by very low levels of self-compatibility and antifertility: self-pollinated flowers and flowers from which pollinators were excluded showed low levels of seed set (c. 3% of flowers set seed), whereas approximately 12 to 14 % of naturally pollinated flowers of *A. magna* set seed.

The self-compatibility of *S. dunnii* likely results in high levels of self-pollination and self-mating, as sunbirds typically visited multiple flowers in the dense inflorescence in a single foraging bout, making self-pollination inevitable. Quantification of the contribution of self-pollination to reproductive success requires molecular methods (e.g. Duffy et al., 2020). Hughes et al. (2007) observed a high level of variation at the population level in *S. dunnii* and that it does not suffer from inbreeding depression. The outcrossing in the species may be present because of the delay in development of the style and stigma maturation, allowing for temporal and spatial separation of male and female phases (Hughes et al., 2007).

The breeding system of *A. magna* was less unambiguous, but results suggest it is self-incompatible. Birds were also observed to visit multiple flowers in an inflorescence in a single foraging bout on *A. magna*, but the low level of self-compatibility overserved for this species likely limits the contribution of selfing to reproductive success.

Both *S. dunnii* and *A. magna* showed a significant reduction in seed output when birds were excluded from flowers. Birds are thus important pollinators to both species but did contribute much more to seed output per flower in *S. dunnii* (increase from 5% to 80% seed set) than in *A. magna* (increase from 4% to 12% of flowers setting seed). Results of exclusion experiments in this study add to the existing literature of decreased reproductive output when bird pollinators are excluded from bird-pollinated plants (Ratto et al., 2018; Amorim et al., 2022; Butler et al., 2022). This emphasizes the importance of birds as pollinators and insects which did not contribute to reproductive output, indicating that insects cannot fulfil the role of birds as pollinators in these systems.

Pollen limitation is commonly found in plant species (Burd, 1994; Knight et al., 2005) but neither species in this study showed evidence of pollen limitation of reproductive success. For *Streptocarpus dunnii*, supplemental hand pollinations showed that reproductive output is not affected by pollen limitation; current high levels of pollinator activity are associated with optimal levels of pollination. Pollen supplementation experiments in *A. magna* were less conclusive: no difference was found between reproductive success of naturally pollinated and pollen-supplemented flowers, but the calculated pollen limitation index of 0.64 is indicative of intermediate pollen limitation. Of greater concern for conservation of *A. magna*, is the low overall reproductive success. Naturally pollinated flowers set approximately 15% seed, and pollen addition does not increase seed set beyond 20%. Thus, alternative explanations for the low seed set in *A. magna* require investigation.

Populations of both study species occur in conservation areas: *S. dunnii* occurs in Nature Reserves in both South Africa (Mountainlands Nature Reserve, Verloren Vallei Nature Reserve) and in eSwatini (Malolotja Nature Reserve). *Alberta magna* occurs in at least two sites in the Karkloof Conservancy and in the Umtamvuna Nature Reserve. Information from this study can therefore inform conservation strategies in these managed areas, to ensure that the sunbird pollinator species, on which both study species depend, are considered in any conservation management plan.

Methodological issues identified in this study and suggestions for future research

Camera traps were shown to obtain information which could not be gathered through conventional human observations, however, this study showed that camera traps are more or less effective depending on the environment they are used in. It was challenging to observe birds visiting *S. dunnii* through direct observations in the rocky habitat it is found in but camera traps captured over 300 bird visits to the species. *Alberta magna* had a severely restricted distribution and was found in a highly specialised habitat where it is difficult to reach inflorescences, but some could be reached and cameras recorded over 100 bird visits. The habitat *S. dunnii* is found in made it very difficult to do direct observations but was ideal for camera traps. The rock clefts allowed for cameras to be concealed from potential theft or interference, and cameras could be placed easily in a stable position between rocks. The rocks also blocked any possible wind, reducing the chances of the memory card being filled by wind-triggered footage. These ideal conditions allowed for a high success rate and low rate of false triggering by wind and enabled many videos to be gathered in a much shorter time than required for the study of *A. magna*. The cliff-edge habitat of *A. magna* made the positioning of camera traps challenging as trees and inflorescences were hard to access and reach. The windy conditions resulted in memory cards reaching full capacity within a single afternoon. The positioning of tall trees on slopes made it difficult to get to most trees and most branches were out of reach, even when climbing trees. Although there were more challenges when gathering footage for *A. magna*, the potential to accumulate videos over the extended flowering period of this species, eventually enabled accumulation of adequate detail to characterise pollination interactions.

This study successfully combined camera trapping and pollinator exclusion to identify bird pollinators and quantify their importance for pollination. Cameras have been useful in other studies involving large vertebrate pollinators (e.g. Cozien et al., 2019; Amorim et al., 2022; van der Niet et al. 2022) as well as for some smaller invertebrate pollinators (e.g. Castañeda-Zárate et al., 2021; Butler, 2022). However, as well as the challenges associated with plant form and habitats being more or less suitable for camera traps, this study also highlights the need to formally test camera sensitivity especially for smaller flower visitors, to extend the use of camera traps to verify other pollination systems. In particular, it will be invaluable to test camera efficacy for butterflies, long-proboscid flies and bees, using known or continuously recorded visitation rates by different species and cameras at different focal distances (see Ortmann and Johnson, 2021) to confirm utility of cameras for these visitors. Testing motion trigger camera sensitivity for other, especially smaller visitors, could extend

use of cameras to confirm the numerous cases of hypothesized but unverified pollination systems for many other species in the southern African flora (e.g. Vogel, 1954; Johnson and Wester, 2017).

4.2 CONCLUSIONS

Application of camera traps in this study highlights their utility for confirming pollination in systems in which rarity and habitats have posed challenges for more traditional direct methods. Pollination systems documented for two rare species provide inspiration for evolutionary studies of shift direction and evolution of bird pollination and can help inform conservation of both study species. Insight from this study also suggests that for the conservation of *Alberta magna*, investigation of causes of low natural seed set as detected in this study, may be required. It would also be useful to extend studies on breeding system of this species to confirm whether it is self-incompatible, and to provide clarity on why the species is restricted in its distribution and how to conserve it.

Pollination scientists should take advantage of advancing technologies which can be used in the field to obtain data, such as camera traps, particularly for pollination systems which have been difficult to study due to rarity or complex habitats. Specific testing and evaluation of camera trapping methods to enable extension of similar methods to those used in this study, to other pollination systems, holds promise to develop research into elusive and hard to study plant-pollinator interactions.

4.3 REFERENCES

- Amorim, M.D., Maruyama, P.K., Baronio, G.J., Azevedo, C.S., Rech, A.R., 2022. Hummingbird contribution to plant reproduction in the rupestrian grasslands is not defined by pollination syndrome. *Oecologia* 199, 1-12.
- Burd, M., 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *The Botanical Review* 60, 83-139.
- Butler, H.C., Cozien, R.J., Johnson, S.D., 2022. For the birds? Contrasting pollination and breeding systems of the paintbrush lilies *Scadoxus puniceus* and *S. membranaceus* (Amaryllidaceae). *Plant Systematics and Evolution* 308, 1-11.
- Castañeda-Zárate, M., Johnson, S.D., van der Niet, T., 2021. Food reward chemistry explains a novel pollinator shift and vestigialization of long floral spurs in an orchid. *Current Biology* 31, 238-246.
- Cozien R.J., van der Niet, T., Johnson S.D., Steenhuisen S.L., 2019. Saurian surprise: lizards pollinate South Africa's enigmatic hidden flower. *Ecology* 100, 261-263.
- Duffy, K.J., Patrick, K.L., Johnson, S.D., 2020. Outcrossing rates in a rare "ornithophilous" aloe are correlated with bee visitation. *Plant Systematics and Evolution* 306, 23.
- Fleming, T.H., Muchhala, N., 2008. Nectar-feeding bird and bat niches in two worlds: pantropical comparisons of vertebrate pollination systems. *Journal of Biogeography* 35, 764-780.
- Hughes, M., Möller, M., Edwards, T.J., Bellstedt, D.U., de Villiers, M., 2007. The impact of pollination syndrome and habitat on gene flow: a comparative study of two *Streptocarpus* (Gesneriaceae) species. *American Journal of Botany* 94, 1688-1695.
- Johnson, S.D., 2004. An overview of plant-pollinator relationships in southern Africa. *International Journal of Tropical Insect Science* 24, 45-54.
- Johnson, S.D., Bond, W.J., 1994. Red flowers and butterfly pollination in the fynbos of South Africa, in: Arianoutsou, M., Groves, R.H. (Eds.), *Plant-animal interactions in Mediterranean-type ecosystems*. Springer, Dordrecht, pp. 137-148.
- Johnson, S.D., Steiner, K.E., 2000. Generalization versus specialization in plant pollination systems. *Trends in Ecology & Evolution* 15, 140-143.
- Johnson, S.D., Steiner, K.E., 2003. Specialized pollination systems in southern Africa. *South African Journal of Science* 99, 345-348.
- Kiepiel, I., Johnson, S.D., 2014. Shift from bird to butterfly pollination in *Clivia* (Amaryllidaceae). *American Journal of Botany* 101, 190-200.

- Knight, T.M., Steets, J.A., Vamosi, J.C., Mazer, S.J., Burd, M., Campbell, D.R., Dudash, M.R., Johnston, M.O., Mitchell, R.J., Ashman, T., 2005. Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution, and Systematics* 467-497.
- Manning, J. C., Goldblatt, P., 2005. Radiation of pollination systems in the Cape genus *Tritoniopsis* (Iridaceae: Crocoideae) and the development of bimodal pollination strategies. *International Journal of Plant Sciences* 166, 459-474.
- Ortmann, C.R., Johnson, S.D., 2021. How reliable are motion-triggered camera traps for detecting small mammals and birds in ecological studies? *Journal of Zoology* 313, 202-207.
- Puff, C., Robbrecht, E., Randrianasolo, V., 1984. Observations on the SE African-Madagascan genus *Alberta* and its ally *Nematostylis* (Rubiaceae, Alberteae), with a survey of the species and a discussion of the taxonomic position. *Bulletin du Jardin botanique national de Belgique/Bulletin van de Nationale Plantentuin van België* 54, 293-366.
- Ratto, F., Simmons, B.I., Spake, R., Zamora-Gutierrez, V., MacDonald, M.A., Merriman, J.C., Tremlett, C.J., Poppy, G.M., Peh, K.S.H., Dicks, L.V., 2018. Global importance of vertebrate pollinators for plant reproductive success: a meta-analysis. *Frontiers in Ecology and the Environment* 16, 82-90.
- Skead, C.J., 1967. *Sunbirds of Southern Africa*. Balkema, Cape Town.
- van der Niet, T., 2021. Paucity of natural history data impedes phylogenetic analyses of pollinator-driven evolution. *New Phytologist* 229, 1201-1205.
- van der Niet, T., Cozien, R.J., Castañeda-Zárate, M., Johnson, S.D. 2022. Long-term camera trapping needed to identify sunbird species that pollinate the endangered South African orchid *Satyrium rhodanthum*. *African Journal of Ecology* 60, 1278-1205.
- Weber, A., Bellstedt, D., Möller, M., 2021. Die Gesneriaceen Afrikas und ihre vogelblütigen Vertreter: *Streptocarpus dunnii* und *S. myoporoides*. *Der Palmengarten* 85, 125-138.