Crypsis in Non-flying Mammal Pollinated Proteaceae:

Novel Adaptations and Evidence of Nectarivorous Bird Avoidance.

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THESIS

Submitted in fulfilment of the requirements for a **Master of Science** degree by full dissertation

July 2018

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Key Phrases:

Proteas, Crypsis, Dead Leaf Retention, Therophily, Nectar Robbing.

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Connolly, A. and Midgley, J.J. Crypsis in Non-Flying-Mammal Pollinated Protea Species: A strategy for nectarivorous bird avoidance. Unpublished, to be submitted to *The South African Journal of Botany*.

Connolly, A. and Midgley, J.J. Test of hypotheses for dead leaf retention in *Protea*; flammability, fertilisation, and crypsis. Unpublished, to be submitted to *The South African Journal of Botany.*

ACKNOWLEDGMENTS:

I would like to extend my sincerest thanks to Jeremy Midgley, my supervisor, for guidance and encouragement throughout this process and for having unwavering faith in my abilities. I would also like to express my gratitude for this opportunity which has been made possible by the Biological Sciences Department and the University of Cape Town. Lastly, I could not have reached this point without my family and I am incredibly humbled by their support throughout my academic career. To my mother who supported me financially and emotionally, I only wish you were here to see me finally finish it!

ABSTRACT

A defining feature of the non-flying mammal pollinated (NMP) syndrome is inflorescence crypsis whereby flowers are close to the ground and somewhat hidden within the canopy. A number of species in the Cape Proteaceae are NMP, two of which were chosen as focal species for this study: *Protea amplexicaulis* and *Protea humiflora*. This study investigated the two previously suggested hypotheses for crypsis: hidden flowers are more difficult for nectarivorous birds to access, or hidden flowers provide greater cover for small mammal pollinators from aerial predators. Using remote triggered cameras, *P. amplexicaulis* and *P. humiflora* inflorescences were observed over the 2017 flowering period, noting visitation by birds and small mammals and assessing the legitimacy of birds as pollinators. In the literature, bird visitation to exposed inflorescences is suggested to be rare, but this study showed that it is considerable. Observations of camera footage suggest that birds are in fact illegitimate pollinators and thus nectar rob. Bird visitation to exposed inflorescences was more than tenfold that of hidden inflorescences, suggesting that crypsis is likely a strategy to avoid nectar robbing by birds. Both *P. amplexicaulis* and *P. humiflora* have been observed to retain dead leaves, which may contribute to their cryptic nature. Alternative hypotheses for dead leaf retention in Proteaceae – that it may increase flammability or result in a below canopy spike in nutrients post fire (selfish fertilization) – were assessed and rejected. Sampling of eight local *Protea* species showed that dead leaf retention is not a consequence of prolonged live leaf retention, with *P. amplexicaulis* retaining dead leaves for up to 6 years. The removal of dead leaves in 30 *P. amplexicaulis* individuals resulted in a significant decrease in the number of inflorescences hidden from aerial view, thus suggesting that dead leaf retention may be a strategy to enhance crypsis and thus forms part of the NMP syndrome. This research expands on the knowledge of the NMP syndrome; providing evidence in support of an anti- nectar robbing crypsis function, discovering a novel crypsis

adaptation regarding dead leaf retention, and casting doubt on the Restricted Distributions hypothesis for the evolution of the syndrome.

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INTRODUCTION

1.1 Non-flying Mammal Pollination in Proteaceae

Non- flying mammal pollination (NMP) was first suggested in the 1930's by Porsch (1934). It remained controversial for decades as evidence was anecdotal due to the often cryptic nature of NMP flowers and the shyness of small mammals. The basis for NMP was also questioned by many in the pollination biology community due to the limited mobility of non-flying mammals in comparison to other biotic pollen vectors such as birds (eg. Faegri & van der Pijl 1979). Evidence in support of NMP begun to accumulate as researchers invented novel methods circumventing the issues inherent with observing small mammals on cryptic flowers. These methods included live-trapping in the vicinity of the plants, assessing fur and faecal pollen loads, and the use of florescent powders (See Wiens *et al.* 1983, Carthew & Slater 1991, Goldingay *et al.* 1991, Hackett & Goldingay 2001, Biccard & Midgley 2009). Recently, with the development of remote-triggered cameras, observational evidence of NMP has become possible (see Lombardi *et al.* 2013, Melidonis & Peter 2015, Zoeller *et al.* 2016).

Plants pollinated primarily by non-flying mammals tend to exhibit convergent suites of floral traits leading to the definition of an NMP syndrome. The NMP syndrome in Cape Proteas was thoroughly explored by Rebelo and Breytenbach (1987) and includes the following. NMP Proteas bear flowers close to the ground (geoflorous growth) with often cryptic inflorescences which are reddish-brown in colour. They produce copious amounts of concentrated nectar and emit a yeasty scent (Wiens & Rourke 1978, Johnson *et al.* 2001). Florets have an effective 10mm distance between stigma and nectar pools (Wiens *et al.* 1983) ensuring pollen transfer onto the rostrum and whiskers of small mammals which increases the chances of effective pollen transfer as pollen sticks to the small hairs of the small mammal.

NMP Proteas flower in early winter to late spring thus providing food for small mammals during months of resource scarcity (Fleming & Nicholson 2002).

A number of *Banksia* species in Australia have been confirmed as NMP (Cunningham 1991, Goldingay *et al.* 1991, Hackett & Goldingay 2001), most commonly pollinated by marsupials. In the Cape floristic region, around 35 species have been identified as NMP based solely on their floristic traits, while a number have been confirmed as NMP with experimental and observational evidence (Wiens et al 1983, Rourke & Wiens 1977, Fleming & Nicholson 2002, Biccard & Midgley 2009, Melidonis & Peter 2015, Zoeller *et al.* 2016). NMP Proteas in the Cape tend to be pollinated primarily by a number of small mammal species. The evolution of NMP in Proteaceae has been suggested as a shift from bird pollination due to the restricted populations in which NMP Proteas tend to reside. This 'restricted population hypothesis' initially put forth by Wiens and colleagues (1983) suggests that as *Protea* species moved into localized habitats, forming smaller, restricted populations, the frequency of bird visitation would have decreased as birds prefer to forage on larger populations.

1.2 Cryptic Inflorescences as a Pollination Adaptation

A defining feature of the NMP syndrome is the visual concealment of inflorescences, termed crypsis. The geoflorous growth and generally dense shrubbery of NMP Proteas contribute to the cryptic nature of flowers. Hypotheses as to the functionality of crypsis were suggested by Wiens and colleagues (1983) but have yet to be further investigated.

The first potential explanation for crypsis is that it provides safety to small mammal pollinators from nocturnal raptors. It is well established in the literature that small mammals prefer to forage in sheltered environments (Sih 1980; Longland & Price 1991; Bowers & Dooley 1993; Manson & Stiles 1998; Muñoz *et al.* 2009) and has been shown to affect seed selection (Perea *et al.* 2011), seed fate (Sivy *et al.* 2011), and overall foraging behaviour

(Brown *et al.* 1988; Kotler *et al.* 2002). Allowing small mammals to forage within the safety of the shrub would likely increase the time spent pollinating at a given inflorescence, and encourage visitation to multiple inflorescences within an individual protea. This hypothesis has been criticised as nocturnal raptors in the Fynbos are few (Rebelo and Breytenbach 1987).

Alternatively, it was suggested that crypsis is a strategy to reduce visitation by nectarivorous birds. As birds are attracted to flowers visually (Raven 1972), crypsis should preclude birds from visitation, especially given that sunbirds and sugarbirds are not known to frequent the ground or explore dense shrubbery unless enticed. Birds are likely illegitimate pollinators, or at least highly inefficient pollen vectors, for NMP Proteas due to morphological mismatches. The stigma-nectar length of NMP flowers (typically 10 mm) results in pollen attaching to the slippery bill where it will easily wipe off rather than sticking in the facial feathers. Bird pollinated plants tend to have tube lengths which correlate with bill length of the main avian pollinator as to ensure pollen attaches to the head feathers rather than the bill (Geerts & Pauw 2009). Additionally, the small, inwardly curved styles of NMP Proteas (Wiens *et al.* 1983) would inhibit effective pollen deposition as the bird beak is highly unlikely to come into contact with the style. Thus, birds visiting NMP Proteas would likely be nectar thieves. Nectar robbing can negatively affect the reproductive success of a plant through a loss of pollen without effective deposition, the reduction of nectar rewards, or by damaging the inflorescence itself (Traveset *et al.* 1998; Irwin *et al.* 2001; Burkle *et al.* 2007; Irwin *et al.* 2010). Visitation by birds to inflorescences thus might negatively affect reproductive success of the NMP Protea, most likely by reducing nectar rewards for legitimate pollinators, reducing attraction via scent as nectar contains most of the scent of the *Protea* (Steenhuisen *et al.* 2010), or by removing pollen loads without effectively depositing it.

1.3 Dead Leaf Retention in Proteaceae

This thesis originated from a discussion about dead leaf retention as observed in *Protea amplexicaulis*. Dead leaf retention has yet to be investigated in proteas, although hypotheses have been suggested (He *et al.* 2011, Midgley & Bond 2011). With rodent behaviour in mind given that *P. amplexicaulis* is thought to be NMP, I suggested a possible link between small mammal pollination and foliage density. Upon further research, specifically that of crypsis in NMPs and the hypotheses for this suggested by Wiens et al (1983), it became apparent that this link was in fact theoretically feasible. If crypsis is an adaptation to NMP in its own right, then it would be likely for some crypsis-enhancing strategies to have evolved beyond simply geoflorous growth.

While investigating the phylogeny of the Australian *Banksia* and its fire-related traits, He and colleagues (2011) noted dead leaf retention in 25 species of the *Banksia* genus and suggested that this trait proliferated in the Miocene approximately 25.9 million years ago. It has also been observed in a Cape Protea, with *P. amplexicaulis* described as retaining dead leaves for up to 6 years (Midgley & Bond 2011). The adaptive significance of this trait in Proteaceae has remained uncertain, however due to the similarities between the Cape Floristic Region (CFR) and Australian Proteaceae species and habitats, the selective pressure was likely the same across the family.

Dead leaf retention, termed marcescence, is observable in multiple plant species from a number of genera in variable habitats and is thought to provide a variety of functional advantages such as insulation and protection against desiccation (Harris et al 2004, Smith 1979, Otto & Nilsson 1981). While marcescence in Proteaceae is likely to have a functional explanation, leaf abscission time is a fundamental ecological plant characteristic and correlated with multiple physiological and morphological traits (Reich *et al.* 1992), as well as habitat (Escudero & del Arco 1987). Thus, marcescence could simply be a result of the plants overall leaf longevity strategy.

Two hypotheses were put forward regarding the adaptive significance of marcescence in Proteaceae by He and colleagues (2011) seeking to explain the trait in Australian *Banksia*. Fire is an ancient and major driver in Australian ecosystems, with many plant species thought to have developed certain traits, such as pyriscent serotiny, as fire-adaptations (He *et al.* 2011; Keeley *et al.* 2011). Plants in the CFR are also thought to be fire-adapted, with many fynbos species dependent on fire for seed germination and flowering (De Lange & Boucher 1990; Brown *et al.* 2003; Lamont & Downes 2011). Flammability is one such trait which is thought to have evolved in fire prone systems, with increased individual flammability causing a localized spike in fire intensity. According to the *kill thy neighbour* hypothesis (Bond & Midgley 1995), this will benefit the individual by killing the neighbouring plants and thereby providing a space of low competition in which its seedlings can establish. The low water content of dead leaves would undoubtedly impact local fire intensity (Midgley & Bond 2011), and this is the basis of the flammability hypothesis put forward for marcescence (He *et al.* 2011). However, this hypothesis assumes that neighbouring plants are mostly fire-avoiders rather than fire-adapted reseeders or resprouters which would senesce in low-intensity fires anyway. He *et al.* (2011) also suggest that incineration of dead leaves would result in a highly localized release of nutrients into the soil below the canopy of the mother plant, and thus create a fertilized micro-climate in which seedlings can establish and thrive. If this is correct, one would expect more seedlings to survive beneath the canopy of a burnt mother plant and that they would exhibit increased growth due to the additional nutrients. However, the selfish-fertilization hypothesis assumes extremely limited movement of nutrients and no seed dispersal post fire, which is unlikely in protea systems (Smith 1970; Grier 1975; Slingsby & Bond 1983; Bond 1988; Auld & Denham 1999; He *et al.* 2004).

Alternatively, I suggest that marcescence in Proteaceae forms part of the NMP syndrome, functioning as extra foliage in order to increase the degree of crypsis of inflorescences. If crypsis is an important factor in breeding success, either by means of reducing nectar robbing or increasing time spent by small mammals within the shrub, then it follows that additional traits may develop in order to enhance this effect. The retention of dead leaves would provide extra foliage with which to conceal flowers with and make accessing internal inflorescences difficult for birds. The additional foliage would also contribute to the shelter provided by the shrub thus benefiting both small mammals and the plant through shelter enrichment. This would indicate a non-floristic-specific trait may form a part of the NMP syndrome but having evolved in only a handful of species.

1.4 Thesis Statement and Study Objectives

Pollination by small mammals comprises a suite of plant adaptations. These mostly floristic traits converge into a syndrome. Crypsis is a morphological trait associated with the NMP syndrome in which the concealment of flowers likely increases pollination efficiency - either by reducing illegitimate pollen removal, discouraging nectar thieves, or encouraging more time spent pollinating - and thus contributes to breeding success. In Proteas, where seed set is already low (Wiens *et al.* 1983), this boost in pollination efficiency could be a significant fitness advantage . Thus, additional morphological traits may be associated with NMP as crypsis-enhancing strategies, one such trait I suggest is dead leaf retention. The broad objective of this study is to explore crypsis as it related to the NMP syndrome and investigate whether additional traits may form a part of this phenomenon.

This study aims to firstly explore the pollination systems of two NMP classified species, *Protea amplexicaulis* R.Br and *Protea humiflora* Andrews, and gather the first observational evidence of these species' primary pollinators. Secondly, I will thoroughly explore crypsis, the adaptive significance of hiding inflorescences, and the implications thereof. This will be the first study which delves into the phenomenon of floral crypsis in NMP Proteas and is likely to produce novel insights. Finally, I will explore dead leaf retention in Cape Proteas, the viability of previously put forth hypotheses for its functional significance, as well as my own hypothesis relating dead leaf retention to crypsis and the NMP syndrome. More specifically, the following hypotheses will be tested:

1) Protea amplexicaulis and P. humiflora are pollinated primarily by small mammals. This is tested by means of camera trapping to observe visitor frequency to inflorescences.
2) Non-flying mammal pollinated Proteas hide flowers to avoid nectar robbing birds. Through use of camera traps we explore whether the degree of crypsis affects visitation to inflorescences by birds and small mammals.
3) Dead leaf retention in NMP Proteas represents a crypsis adaptation by providing more foliage to hide inflorescences. We assess how dead leaf retention affects crypsis of inflorescences by removing dead leaves and noting the change in exposure of inflorescences.

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2.

INVESTIGATING THE NON-FLYING MAMMAL POLLINATION SYNDROME IN TWO CAPE PROTEAS.

Abstract

Non-flying mammal pollinated species are often inferred as such based solely on their floristic traits fitting into a defined ‘syndrome’. Many Proteaceae have thus been predicted as primarily pollinated by small, ground dwelling mammals such as rodents in the Cape Floristic Region and sugar gliders in Australia. The majority of evidence gathered on the pollination systems of Cape proteas has been inferential, such as analysis of faecal and fur pollen loads of live-trapped rodents, as observation of nocturnal, shy small mammals was considered impossible. With the development of motion and heat triggered cameras, pollinator species and behaviours are now directly observable. This study investigates the pollination systems of *Protea amplexicaulis* and *Protea humiflora* by means of camera trapping over a 16-day period during peak flowering in Jonaskop, South Africa. Six small mammal species were observed foraging on inflorescences throughout the study period, with their fur and whiskers contacting pollen presenters and likely resulting in effective pollen transfer. This study concludes that both *P. amplexicaulis* and *P. humiflora* are primarily pollinated by small mammal species, although surprisingly extensive nectarivorous bird visitation was also noted.

Introduction

Plants which are pollinated by specific pollinator functional groups tend to exhibit convergent suites of floral traits or ‘pollination syndromes’ (Johnson & Steiner 2000, de Merxem *et al.* 2009; Ollerton *et al*. 2009). While these syndromes often correctly predict the primary pollinators of a plant, this needs to be tested via observation and pollination experiments. Many floral traits represent adaptations to multiple pollinators or even minor pollinators (Spears 1983, Waser *et al.* 1996, Johnson & Steiner 2000, de Merxmen *et al.* 2009), and as such the ‘syndrome’ may not accurately or comprehensively represent the pollination ecology of the plant.

The non-flying mammal pollination (NMP) syndrome represents a very distinct suite of floral traits which favour small, ground-dwelling mammals. These floral traits include inflorescences which are close to the ground (geoflorous) and often hidden from view (cryptic), copious and viscous nectar, a yeasty scent, and dull red-brown bracts (Rourke & Wiens 1977; Wiens *et al.* 1983; Rebelo & Breytenbach 1987). A number of Australian and South African Proteaceae and other families haven been identified as NMP based on their morphology alone as observational evidence is hard to come by due to the often nocturnal and shy nature of small mammals such as rodents (Wester *et al.* 2009; Bridges & Noss 2011). Technological advances, namely the development of heat and movement triggered cameras, has allowed for novel insights into these mammal pollination systems (Bridges and Noss 2011). The ability to directly observe visitors to inflorescences as well as their behaviour and visit frequency has recently allowed for five Cape proteas to be confirmed as NMP (see Melidonis & Peter 2015, Zoeller *et al.* 2016).

Protea amplexicaulis R. Br. and *Protea humiflora* Andrews. are classic examples of NMP Proteas in the Cape, featuring heavily in the literature (Rourke & Wiens 1977; Wiens *et* *al.* 1983; Rebelo & Breytenbach 1987). *Protea amplexicaulis* is a low sprawling shrub which is endemic to the Cape Floristic Region and found mostly on north-facing sandstone slopes, often forming extensive, dense stands, or scattered in moister, southern-facing plains (Rourke 1980). Commonly found in small, relatively isolated populations, *P. humiflora* is an erect to sprawling shrub generally restricted to hot, dry northern-facing slopes (Rourke 1980). The floral traits of *P. amplexicaulis* and *P. humiflora* are consistent with the general NMP syndrome as described above (Rebelo & Breytenbach 1987). A number of observations, including live-trapping of rodents near suspected NMP proteas and assessing fur and faecal pollen loads, were conducted (see Rourke & Wiens 1977; Wiens *et al.* 1983; Fleming & Nicholson 2002) which implied that a handful of small mammals, mostly rodents, are the primary pollinators of *P. amplexicaulis* and *P. humiflora*. The small mammals most commonly associated with these two proteas were the Cape striped field mouse (*Rhabdomys pumilio pumilio),* the spiny mouse (*Acomys subspinosus*), and the Namaqua rock rat (*Micaelamys namaquensis*) (Rourke & Wiens 1977; Wiens *et al.* 1983). Investigation into the pollination biology of *P. amplexicaulis* and *P. humiflora* remains incomplete as previous studies did not have access to remote-triggered cameras and thus could not observe pollination directly or observe pollinator behaviour and visitation rates.

This study aims to gather observational evidence, through the use of remote triggered cameras, of the pollinators of *P. amplexicaulis* and *P. humiflora*. We expect to observe a number of small mammal visitors, namely the species described in Wiens *et al.* (1983), Fleming and Nicholson (2002), and Zoeller *et al.* (2016). Steenhuisen *et al*. (2015) also found evidence of visitation by small carnivores such as the Cape Grey mongoose (*Galerella pulverulenta*) and nectarivorous birds, although this occurred rarely. Furthermore, notes were taken regarding visitor behaviour, the duration of visits, and whether or not the visitors were likely to have picked up or deposited pollen.

Methods

Fifteen P. amplexicaulis and 5 *P. humiflora* individuals were chosen for observation on the north facing slopes of Jonaskop, in the Riviersonderend mountain range in the Western Cape, South Africa (-33.58107° S; 19.30219° E, approximately 1000m above sea level), near to the study sites of Wiens *et al.* (1983). *Protea amplexicaulis* is found scattered throughout the mountains, occurring sparsely on rocky outcrops and forming relatively dense stands in open, flat areas, while *P. humiflora* occurs in one or two isolated locations. The *P. humiflora* individuals selected were situated mid-way up the summit, in a small population near the old quarry. *Protea amplexicaulis* is found in locally dominant stands within the area known locally as 'Jonasplaats'. The selected *P. amplexicaulis* individuals were grouped within different areas or habitat types within Jonasplaats: four were positioned on and around a small hill, four in a dominant stand in a low-lying flat area, four in a small isolated group on a rocky outcrop, and three were scattered throughout a *Protea repens* stand.

Remote-triggered cameras were employed to observe inflorescences during peak flowering. Only inflorescences in which the bracts had fully opened but only the outer ring of florets had dehisced anthers were chosen. To allow for a better view and ensure that the sensors would be triggered by small mammal presence, inflorescences had to be exposed by removing interfering branches and leaves. Bushnell camera traps (Trophy Cam HD Max-Colour LCD, 119577C and Bushnell Trophy Cam 119466, Kansas City, MO,U.S.A.) were focused on either a specific inflorescence or on a cluster of inflorescences. Cameras were mounted on tripods, stabilised by rocks, about 1-1.5 m away as per the prescribed focal distance. Throughout the study period, batteries and SD cards were checked and replaced as necessary. Cameras were set to a 'normal' sensitivity level and recorded video footage of 30 seconds when triggered during both day and night. Masking tape was placed over the infrared LEDs to prevent over exposure at night. A visit was defined as an event where the visitor was observed actively foraging on an inflorescence. Back-to-back visits by the same species were only counted as new if they occurred more than five minutes after the previous visit.

The observation period lasted a total of 16 days, *P. amplexicaulis* being observed in early August 2017 and *P. humiflora* in early September as to coincide with peak flowering. Due to issues with three of the cameras, ultimately 26 *P. amplexicaulis* inflorescences were observed on a total of 13 individuals and four *P. humiflora* inflorescences on four individuals.

Observations were used to identify small mammal visitor species and describe their behaviour. Video footage was assessed for visitor type and species, notable behaviour, and time spent visiting.

Results

Protea amplexicaulis had a larger range of small mammal visitors, comprising 6 species five of which were rodents, while *P. humiflora* was visited by only two species, both rodents (Table1). The 26 *P. amplexicaulis* inflorescences were visited by small mammals a total of 201 times (mean 7.7 visits per inflorescence) over the 16-day observational period. *Protea humiflora* inflorescences were visited an average of 9 times (n=4) with a total of 36 visits over the 16 days.

All visits occurred at night except for those by the Cape striped field mouse (*Rhabdomys pumilio*) which wasactive during the day. The majority of visits involved probing of the inflorescence, with 22 occurrences of the visitor only sniffing the inflorescence, and 25 occurrences of bract chewing specific to the Cape striped field mouse. *Rhabdomys pumilio* was the only destructive small mammal visitor, often chewing on bracts, with 5 occurrences of inflorescences having been completely destroyed. Pollen transfer was deemed feasible for all events involving probing or chewing of bracts as multiple parts of the visitor’s bodies came into contact with florets, notably the hair and whiskers on the snout when lapping nectar form the base of the florets. Additionally, small mammals commonly climbed onto the inflorescence, with 75% of visitors to *P. humiflora* positioning their feet on mature florets.

Table 1: List of small mammal species having visited *P. amplexicaulis* and *P. humiflora* inflorescences during a 16-day observational period in Jonaskop.

Protea Species	Small Mammal Species	Common Name	Total Visits
P. amplexicaulis	*Micaelamys namaquensis*	Namaqua Rock Rat	73
	Rhabdomys pumilio	Cape Striped Field Mouse	65
	Elephantulus edwardii	Cape Elephant Shrew	40
	Acomys subspinosus	Spiny Mouse	14
	Myomyscus verreauxii	Verreaux’s Mouse	5
	Otomys irroratus	South African Vlei Rat	4
P. humiflora	*Acomys subspinosus*	Spiny Mouse	20
	Micaelamys namaquensis	Namaqua Rock Rat	16

The Cape striped field mouse was found visiting *P. amplexicaulis* individuals in all four locations in Jonaskop (Figure 1) and visited inflorescences previously visited by all other small mammal species (Figure 2). The Namaqua rock rat (*Micaelamys namaquensis*) was observed on *P. amplexicaulis* individuals mostly at the small, rocky hill (‘Koppie’) location, in conjunction with the Cape elephant shrew (*Elephantulus edwardii*) which was restricted to this area (Figure 1). The spiny mouse (*Acomys subspinosus*) and the Namaqua rock rat were never observed visiting the same inflorescence of *P. amplexicaulis* (Figure 2) although they do not overlap significantly in habitat (Figure 1). These two rodent species overlap in the *P. humiflora* stand as well, but were observed visiting the same flowers (Figure 3). Interactions between small mammal species are negative overall, with high numbers of visitation to an inflorescence by one species correlating with low or no visits by another species, as seen by the majoritively negative trends in Figure 3. The relationship between *M. namaquensis* and *E. edwardii* shows a somewhat positive trend in visits to inflorescences.

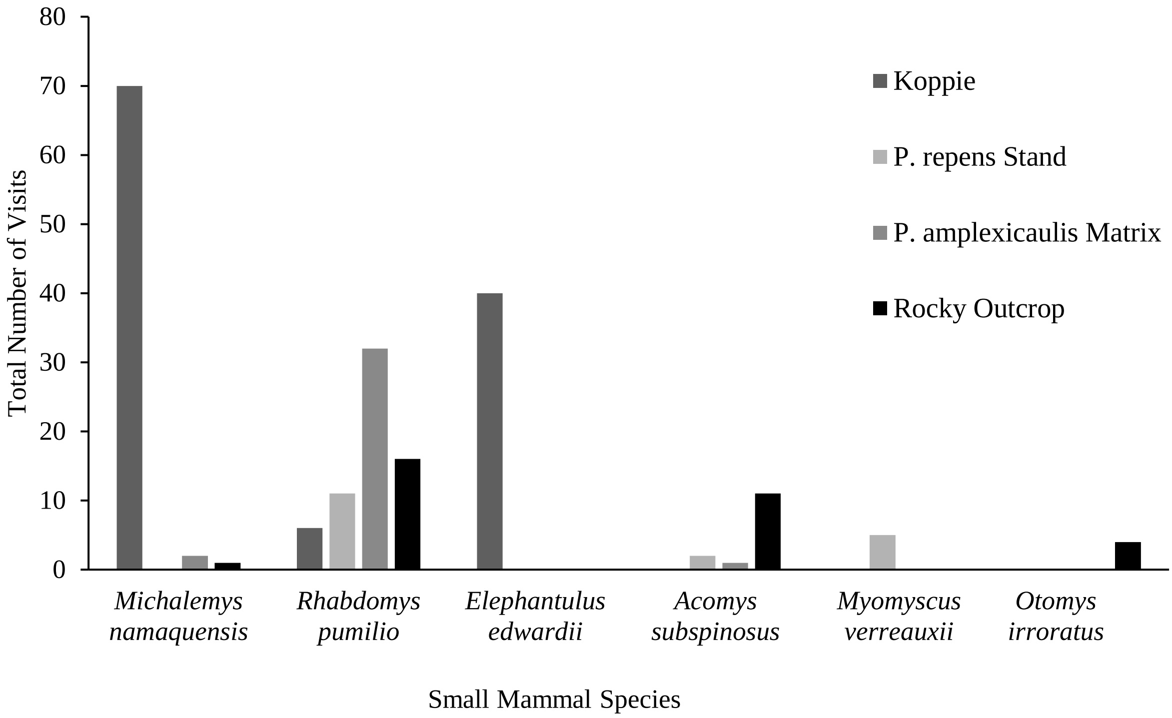

Figure 1: The total number of inflorescence visits by the 6 observed small mammal species, separated into the 4 different locations and habitat types in which the *P. amplexicaulis* (n=26) individuals were situated within Jonaskop.

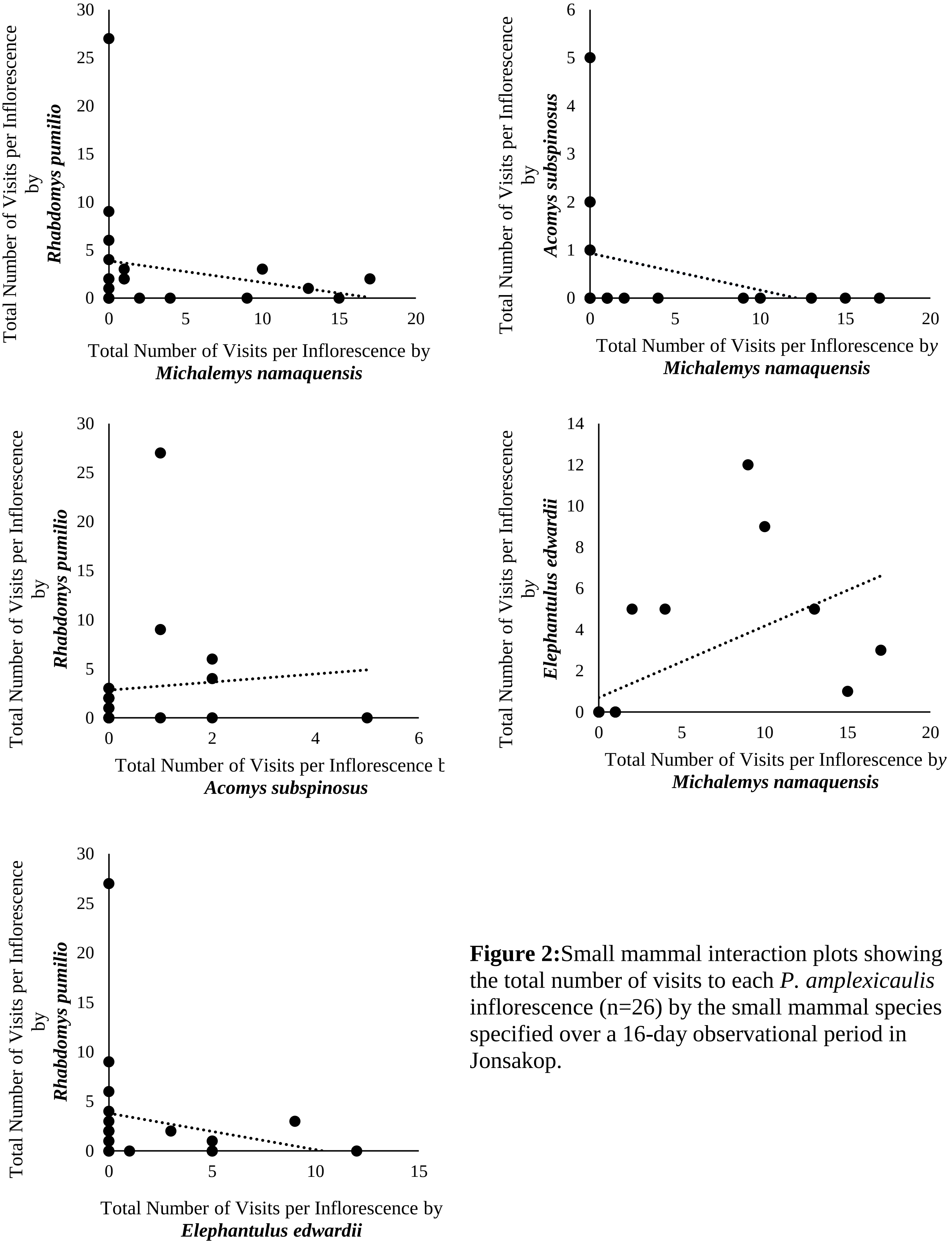

Figure 2: Small mammal interaction plots showing the total number of visits to each *P. amplexicaulis* inflorescence (n=26) by the small mammal species specified over a 16-day observational period in Jonsakop.

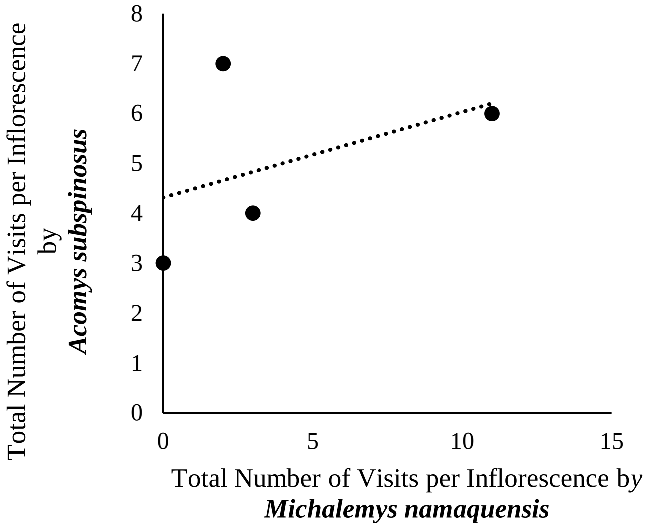

Figure 3: Small mammal interaction plot showing the total number of visits to each *P. humiflora* inflorescence (n=4) by *Micaelamys namaquensis* and *Acomys subspinosus* over a 16-day observational period in Jonsakop.

A number of potential nectar competitors were also observed visiting *P. amplexicaulis* and *P. humiflora* inflorescences. Nectarivorous bird visitation was substantial comprising 35% of all *P. amplexicaulis* visits and 84.5 % of *P. humiflora* visits. In addition to this, the Cape grey mongoose, *Galerella pulverulenta,* was observed on two different camera traps in Jonaskop, totalling five visits to *P. amplexicaulis* inflorescences over the 16 days, and recorded as being destructive to the flowers on three out of the five visits (Figure 4).

Figure 4: Footage of a Cape Grey Mongoose foraging on *P. amplexicaulis* inflorescences in Jonaskop, South Africa.

Discussion

These results have supported previous work on *P. amplexicaulis* and *P. humiflora* (by Rourke & Wiens 1977; Wiens *et al.* 1983; Fleming & Nicholson 2002) in that it has confirmed that they are primarily pollinated by small mammals, mostly rodents. This study also highlights the importance of observational research in pollination biology as it can reveal potential minor pollinators or nectar thieves such as the carnivores and birds seen visiting inflorescences in this study.

Small mammal species were the most common visitors (60%) to *P. amplexicaulis* and consistently made contact with the reproductive structures of the flowers, gathering pollen on their whiskers, fur, and feet. The high number of bird visits to *P. humiflora* is likely a high estimate as flowers were exposed and thus more attractive to birds. Furthermore, birds are most likely illegitimate pollinators, thus nectar thieves, due to morphological incompatibility – average bill length far exceeds stigma-nectar length of flowers (See chapter 3). Despite the comparatively low number of small mammal visits (15.5% of total visits), *P. humiflora* is likely primarily pollinated by small mammals as their behaviour facilitates successful transfer of pollen that sticks to their fur. The slightly higher small mammal visitation rates per inflorescence to *P. humiflora* than to *P. amplexicaulis* further suggests that it is NMP as *P. amplexicaulis* certainly is.

It is important to note that the visitation rates found in this study may not accurately reflect the normal visitation to these inflorescences, as flowers had to be exposed in order to observe them. Small mammals have a preference for dense shrubbery (Sih 1980; Longland & Price 1991; Bowers & Dooley 1993; Manson & Stiles 1998; Muñoz *et al.* 2009) and thus cryptic inflorescences may have increased small mammal visitation rates. Nevertheless, that small mammals frequently visit exposed flowers suggests that crypsis is not necessary for small mammal visitation. Even the diurnal species, *R. pumilio*, frequently visits exposed inflorescences in this study (Table 1) and in other studies (Melidonis & Peter 2015, Zoeller *et al.* 2016). Presumably, crypsis is of less value to nocturnal small mammals - which are the majority of visitor species - given the low numbers of nocturnal raptors in the habitat. I suggest that floral crypsis may have other functions, besides that of hiding small mammal visitors.

Spikes in visitation by small mammal species with location, as shown in figure 3, is due mostly to their habitat preferences. For example, the Cape elephant shrew resides in semi-isolated rocky habitats with many boulders and crevices (Smit *et al.* 2007) such as the rocky hill at which it was observed. The lack of cross-over in inflorescence selection between *Micaelamys namaquensis* and *Acomys subspinosus* for *P. amplexicaulis* (Figure 2) may indicate resource portioning, although they were found to visit the same *P. humiflora* flowers (Figure 3). The interactions between small mammal species visitation rates are negative overall, which was to be expected as there is no obvious benefit of foraging on an inflorescence frequented by another species. Also, there is no evidence to suggest nocturnal temporal subdivision of resources among small mammal species (Zoeller *et al.* 2016). Interestingly, *M. namaquensis* and *E. edwardii* seem to interact positively, often visiting the same inflorescences. This may be due to *E. edwardii's* crepuscular habit.

In terms of small mammal pollinator species, these results are consistent with previous research. *Protea humiflora* pollen loads were found on five small mammal species by Wiens *et al*. (1983), these species were the same as those found visiting *P. amplexicaulis* in this study some 25 years later. Only two of these species were observed visiting the *P. humiflora* flowers in this study. This is likely due to the isolated and singular location of the four *P.humiflora* individuals This study is the first to observe *Otomys irroratus* probing inflorescences , with Malidonis and Peter (2015) finding pollen in scat and on snouts and Zoeller *et al.* (2016) having recorded its presence but never observing probing or foraging behaviour. While a number of *O. irroratus* individuals were seen in the vicinity of *P. amplexicaulis*, only four events of inflorescence probing were recorded, indicating that this is likely a minor small mammal pollinator species. The array of small mammal pollinators observed suggests that the NMP syndrome in these Proteas promotes visitation by multiple species, ranging from the small Verreaux's Mouse to the large vlei rat.

This chapter furthermore highlights the benefits of employing motion or heat triggered cameras in pollination research. It has allowed for observation without impacting natural behaviours and provided valuable insight into the secrets of nocturnal rodent pollinators. It is worth noting that even the use of camera technology may not comprehensively identify all pollinators (Bridges & Noss 2011), and that the potential contribution of insects to pollen transfer is overlooked due to camera sensitivity levels being set for vertebrate observations.

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3.

CRYPSIS IN NON-FLYING-MAMMAL POLLINATED PROTEA SPECIES: A STRATEGY FOR NECTARIVOROUS BIRD AVOIDANCE.

Abstract

Non-flying mammal pollinated Proteas (NMPPs) typically have cryptic inflorescences. Two hypotheses have been suggested for this; hidden flowers are more difficult for nectarivorous birds to access or hidden flowers provide greater cover for small mammal pollinators from aerial predators. Using camera traps we quantified bird visitation on exposed flowers of two NMPPs *P. amplexicaulis* and *P humiflora* and noted it is considerable. We also noted that while birds often remove pollen, they are unlikely to deposit it. This suggests that birds are illegitimate pollinators and thus nectar thieves worth deterring. Comparison of bird visits to *P. humiflora* inflorescences showed that exposed inflorescences are visited more than tenfold that of hidden inflorescences. No signs of small mammal predation were detected. We conclude that crypsis is likely a strategy for nectarivorous bird avoidance. Furthermore, the high prevalence of birds visiting exposed NMPPs suggests that the NMP syndrome did not evolve due to a lack of suitable bird pollinators.

Introduction

The inflorescences of non-flying mammal pollinated Proteas (NMPPs) tend to be cryptic in that they are hidden from external view by shrubbery (Rourke & Wiens 1977). The NMP syndrome also includes cauliflorous or axillary inflorescences which are close to the ground, dull coloured, and musty in odour (Wiens *et al.* 1983). This cryptic syndrome results in inflorescences which are accessible primarily by animals scrambling among internal branches and detected through smell, thus suggesting it is a syndrome evolved in favour of small mammal pollination. Wiens *et al.* (1983) suggested that crypsis in NMPPs favours small mammals by providing protection from aerial predators within their dense canopy . Alternatively, it has been suggested that hiding inflorescences is a vital strategy to minimize nectar robbing by birds, given the high nectar loads of NMPPs (Wiens *et al.* 1983; Rebelo & Breytenbach 1987).

Visitation to inflorescences by illegitimate pollinators (nectar robbing) can negatively affect the reproductive success of a plant through a loss of pollen without effective deposition, the reduction of nectar rewards, or by damaging the inflorescence itself (Traveset *et al.* 1998; Irwin *et al.* 2001; Burkle *et al.* 2007; Irwin *et al.* 2010). While NMPPs typically have a nectar-to-stigma distance of 10 mm (Wiens *et al.* 1983), Cape nectarivorous birds have significantly longer beaks with 18-23 mm in the Southern Double Collared sunbird, *Cinnyris chalybea,*; 20-23 mm in the Orange breasted sunbird, *Anthobaphes violacea,*; and 29-35 mm in the Malachite sunbird, *Nectarinia famosa,* (Geets & Pauw 2009). In bird pollinated sugarbushes, the stigma protrudes “about one beak’s length above the nectar source” (Rebelo 1987), thus pollen is deposited mostly on the crown and throat rather than on the smooth beak where it is likely to brush off before being deposited. The short nectar-to-stigma distance in NMPPs will thus likely result in ineffective pollen deposition on visiting birds. As nectar contains most of the scent of the *Protea* (Steenhuisen *et al.* 2010), this may further affect small mammal visitors which rely mostly on odour for detection (Wiens *et al.*1983*).* The reduction of nectar rewards, scent attractants, and the visitation by birds, will likely negatively impact visitation rate and duration of small mammals.

The extent to which birds visit NMPP inflorescences and the consequences of these visits has yet to be explored. Visitation by birds is thought to be rare given that the inflorescences are visually obscured and produce a more viscous nectar than typical bird pollinated flowers. For example, in three NMPPs the sugar content ranged between 33-37%, while three bird pollinated proteas ranged between 18-24.4% (Wiens *et al.* 1983). However, the higher sugar content of NMPP nectar may also result in increased return visits by nectarivorous birds as they learn where to find an easy, valuable meal. Ad hoc observations, such as those made by Wiens and colleagues (1983), further suggest that bird visitation to NMPPs is rare. Understanding whether birds visit NMPPs is crucial for evaluation of present explanations for the evolution of the NMP syndrome. Wiens *et al* (1983) argue that the distribution patterns of NMPPs would preclude them from being visited by birds; they are typically small obscure plants occurring in small populations in unusual habitats (such as in arid fynbos). Thus NMPPs would not provide sufficient reliable resources for nectarivorous birds, and an alternative pollination syndrome would have evolved. If it is shown that birds are significant visitors, then this suggests the restricted distributions and populations hypothesis (RDPH) is probably incorrect.

The aerial predation hypothesis for crypsis in NMPPs relies on the expectation that small mammals, especially rodents, will likely spend more time visiting in a dense, protected canopy than an open one. The preference for shelter-enriched microhabitats by rodents has been repeatedly observed (Sih 1980; Longland & Price 1991; Bowers & Dooley 1993; Manson & Stiles 1998; Muñoz *et al.* 2009) and shown to cause differential seed selection (Perea *et al.* 2011), seed fate (Sivy *et al.* 2011) and overall foraging behaviour (Brown *et al.* 1988; Kotler *et al.* 2002). An increase in perceived shelter may well result in longer visitation durations, thus supporting the anti-predation hypothesis. However, as mentioned by Rebello and Breytenbach (1987), nocturnal avian predators are rather limited in the fynbos systems.

Due to the cryptic nature of the inflorescences, previous studies on NMPPs have yet to compare the frequency of small mammal visits versus that of birds. Through the use of remotely-triggered cameras (similarly used by Zoeller *et al* 2016), I observed the prevalence of avian visitors to exposed inflorescences and the effects of this on small mammal pollinators during peak flowering of two NMPPs, *Protea amplexicaulis* and *Protea humiflora*. Furthermore, I compared bird visitation rates between exposed and hidden inflorescences, as well as the seed set of paired hidden and exposed inflorescences. Where possible I also noted whether visitors contacted the stigma or pollen presenters, and any incidences of predation on small mammal visitors.

Methods

Sampling Site & Species

Protea amplexicaulis and *P. humiflora* were chosen as study species as they are the focus species of most previous NMPP studies and easily located. Both possess traits typical of NMPPs, with sprawling shrubbery hiding dark coloured, bowl-shaped flowers. Fifteen *P. amplexicaulis* and five *P. humiflora* individuals were chosen for observation on the north facing slopes of Jonaskop, in the Riviersonderend mountain range in the Western Cape, South Africa (33.58107 S; 19.30219 E, approximately 1000m above sea level), near to the study sites of Wiens *et al.* (1983). *Protea amplexicaulis* is found scattered throughout the mountains, occurring sparsely on rocky outcrops and forming relatively dense stands in open, flat areas, while *P. humiflora* occurs in one or two isolated locations. The *P. humiflora* individuals selected were situated mid-way up the summit, in a small population near the old quarry, while the *P. amplexicaulis* individuals were scattered around the area known locally as ‘Jonasplaats’.

Inflorescence Selection

Inflorescences were chosen carefully as to standardise the developmental stage of observed inflorescences. Only inflorescences in which the bracts had fully opened and at most the outer ring of florets had dehisced anthers were considered (Figure 1) so as to ensure that the observation period fell over peak flowering. As this study aims to assess visitation to non-cryptic flowers, only relatively exposed inflorescences were chosen. As naturally exposed flowers occur rarely in the cryptic NMPPs, a handful of inflorescences had to be manually exposed by cutting covering branches. Ultimately, 26 *P. amplexicaulis* inflorescences were observed on a total of 13 individuals and four *P. humiflora* inflorescences on four individuals.

Figure 1: A *Protea humiflora* inflorescence just prior to peak flowering in Jonaskop, South Africa.

Camera Trapping

Remote-triggered cameras are becoming a popular means to observe plant-pollinator interactions and have proven to be highly successful in both monitoring visitation as well as allowing for small details to be observed (Zoeller *et al.* 2016). To observe bird and small mammal visitation to our two study species, Bushnell camera traps (Trophy Cam HD Max-Colour LCD, 119577C and Trophy Cam 119466, Kansas City, MO,U.S.A) were focused on either a specific inflorescence or on a cluster of inflorescences. Cameras were mounted on tripods, stabilised by rocks, about 1-1.5 m away as per the prescribed focal distance. Throughout the study period, batteries and SD cards were checked and replaced as necessary. Two of the high definition cameras (Trophy Cam HD Max-Colour LCD, 119577C) were fitted with lenses having a focal distance of 25 cm, which provided more detailed footage.

Cameras were set to a ‘normal’ sensitivity level and recorded video footage of 30 seconds when triggered during both day and night. Masking tape was placed over the infrared LEDs to prevent over exposure at night. The observation lasted a total of 16 days, *P. amplexicaulis* being observed in early August and *P. humiflora* in early September as to coincide with peak flowering. In addition to observing exposed *P. humiflora* inflorescences, cameras were positioned to observe the shrub as a whole while noting the positions of inflorescences and their degree of crypsis (Exposed, partial crypsis, and hidden). Cameras were triggered by bird visitors as they flew into the shrub, and we took note of whether they visited the known positions of non-visible inflorescences.

Seed set

To assess the effect of crypsis on pollination success, seed set for inflorescences of different degrees of crypsis were measured. Fourteen *P. amplexicaulis* individuals were chosen prior to peak flowering in August 2017, with one fully exposed inflorescence and one hidden inflorescence marked on each shrub. The marked inflorescences were all from this current year’s flowers and had begun to open. In late November 2017, once enough time had passed for seeds to set, the cones were. Cones were then assessed for viable seeds by identifying whether the seeds were swollen and solid (viable endosperm- containing seeds) or just fibrous (sterile seeds). For cones which produced any viable seeds, the number of seeds set relative to the number of floret grooves in the inflorescence (hence the maximum number of possible seeds) were counted.

Data Analysis

A visit was defined by any presence of a bird or small mammal in which the inflorescence was found and touched by the visitor. Visits to inflorescences were only counted as new if they occurred more than five minutes after the previous visit. Visits having occurred during the day on day 1 were disregarded so as to not bias the study towards day-time visitors such as birds. This had no effect on observed trends or relationships, it essentially just reduced the overall number of bird visits. Footage was analysed for: visitor species, activity (such as probe or sniff), visit duration, whether the visitor visibly picked up pollen and on what; whether the visitor touched the floret with its feet; and whether the visitor could feasibly deposit pollen. Simple statistics regarding the visitation rates and behaviour of birds and small mammals were compiled. To assess the effect of bird visits on small mammal visitation in *P. amplexicaulis*, we compared the mean number of small mammal visits to inflorescences which had been visited by a bird within the study period (n =14), and inflorescences which had not (n =12) by means of a Mann-Whitney-Wilcoxon test for independent, non-normal samples (Hollander & Wolfe 1973). Additionally, we conducted a Wilcoxin signed-rank test for paired samples (Hollander & Wolfe 1973) on small mammal visitation before a bird had visited the inflorescence and after. The pattern of visitation over time was plotted for birds and small mammal visits to both *P. amplexicaulis* and *P.humiflora,* using only inflorescences which had been visited by both birds and small mammals within the 16 days (n =14 and n =4 respectively). Differences in visit duration by small mammals were assessed by means of a Mann-Whitney-Wilcoxon test for independent, non-normal samples (Hollander & Wolfe 1973) on the proportion of visits falling within the three recorded duration categories (visits lasted <5; 5-30; or >30 seconds) for inflorescences which had been visited by birds and those that hadn't. While *P. humiflora* observations were valuable in terms of behaviour and visit frequency, they could not be assessed for the effect of birds on small mammals due to the small sampling size (n = 4). We compared the mean number of bird visits per inflorescence for the three levels of crypsis via multiple Mann-Whitney-Wilcoxon tests. All formal statistical testing was computed by R statistical software (R Core team, 2016).

Results

Avian Visitation

Birds were found to visit both *P. amplexicaulis* and *P. humiflora* extensively over the 16 day observation period (Figure 2). Small mammal visits per inflorescence are similar between *P. amplexicaulis* and *P. humiflora* (7.6 and 9 respectively, roughly 0.5 visits per day per inflorescence over 16 days). Small mammals were the dominant visitors to *P. amplexicaulis* inflorescences (202 total visits) but a significant number of bird visits were observed as well (118 total visits). Birds visited *P. humiflora* inflorescences at a much higher rate with 202 birds observed overall in comparison to only 36 small mammal visits.

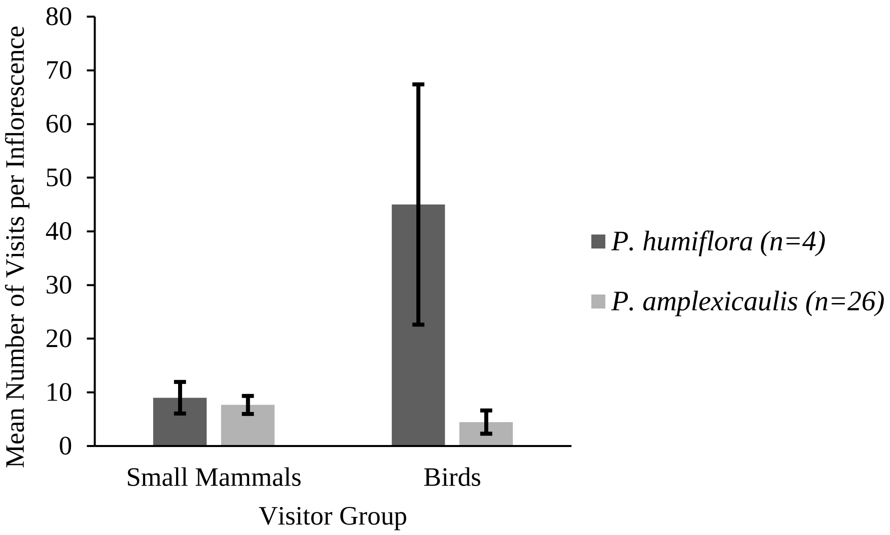

Figure 2: Mean number of small mammal and bird visits per *P. humiflora* and *P. amplexicaulis* inflorescence over a 16 day period. Error bars represent standard error.

Figure 3: Bird visitors to NMPP inflorescences often grasped the inflorescence resulting in pollen transfer to the feet. This can be seen above with a male Orange Breasted Sunbird on a *P. humiflora* inflorescence.

Pollen was removed by birds in 64% of bird visits where it was possible to tell in terms of footage quality and bird positioning. The high quality videos of *P. humiflora* allowed us to observe that out of the 47 incidences of pollen removal, 9 of these were on the beak and 38 resulted in pollen transfer to the feet (See Figure 3). There were no observations of feasible pollen deposition by birds considering the position of the stigma. The most common bird visitor was the Orange-breasted Sunbird (*Anthobaphes violacea*), with males accounting for 51% of total bird observations and females accounting for 41%. The remaining 8% of bird visits were made up of unidentifiable species (due to footage quality) as well as the Southern Double-collared Sunbird, *Cinnyris chalybeus,* (Observed twice), the Malachite Sunbird, *Nectarinia famosa*, and the Cape Grassbird, *Sphenoeacus afer* (each observed once).

Effect of Birds on Small Mammal Visitation

The difference in small mammal visitation to *P. amplexicaulis* inflorescence where birds had been observed visiting and when they were completely absent from observations of the inflorescence was not significant ($W = 62.5$, $n_1 = 14$, $n_2 = 12$ $p = 0.277$) (Figure 4). Similarly, when looking exclusively at *P. amplexicaulis* inflorescence which were visited by both birds and rodents during the 16 day observation period, there was no difference in average small mammal visitation numbers before a bird had been observed visiting and after ($W = 32.5$, $n = 14$, $p = 0.6364$) (Figure 4). Although the averaged pattern shows that the presence of birds does not significantly affect small mammal visitation rates, looking instead at the individual *P. amplexicaulis* inflorescences visited by both birds and small mammals does indeed show a pattern. Inflorescences were visited dominantly by either birds or small mammals, with high numbers of one coinciding with low numbers of the other (Figure 6).

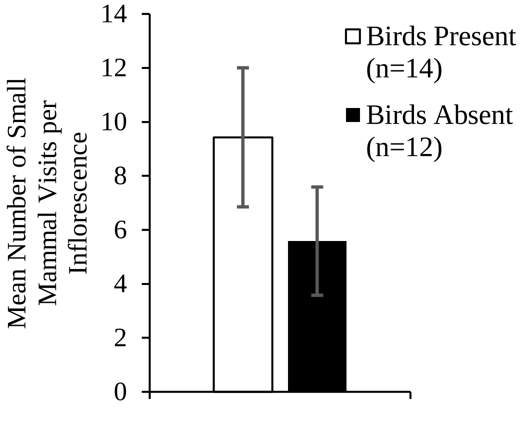

Figure 4: Mean number of small mammal visits over a 16 day period per inflorescence of *P. amplexicaulis* where birds had been observed visiting the flower (Birds Present) and where birds had not (Birds Absent). Error bars represent standard error.

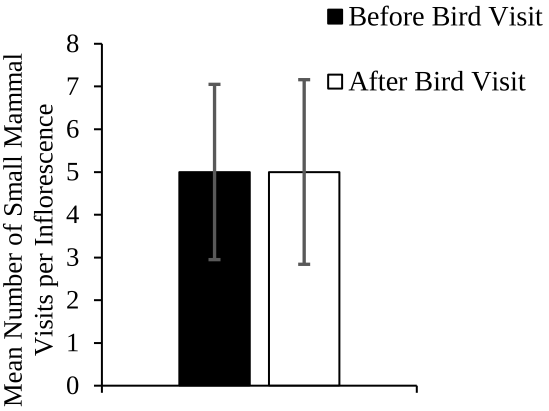

Figure 5: Mean number of small mammal visits over a 16 day period per inflorescence of *P. amplexicaulis* before birds had been observed visiting (Before Bird Visit) and after (After Bird Visit). Error bars represent standard error

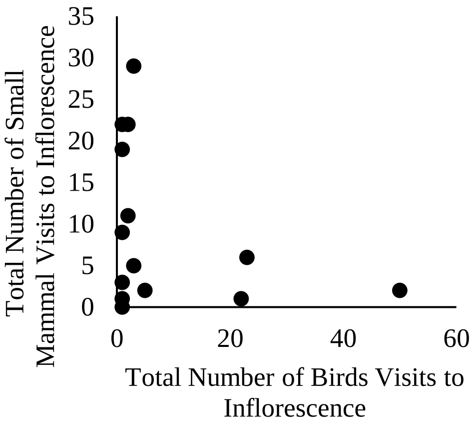

Figure 6: Total number of small mammal visits over a 16 day period to *P. amplexicaulis* inflorescence (n = 14) in relation to the total number of bird visits.

To investigate how bird and small mammal visitation changes with floret maturity, hence time since initial floret opening, I plotted the mean visitation rates over the 16 day time period (Figure 7). Small mammal visits to *P. amplexicaulis* seem to be random over time and show no connection to bird visits (Figure 7 A). Bird visits to *P. amplexicaulis* slowly climb up, reaching a maximum of 1.5 visits to inflorescences per day and then dropping off sharply. This pattern is not reflected by the *P. humiflora* observations, in which bird visitation reaches a peak early on (day three) and then slowly tapers off. Small mammal visitation in *P. humiflora* is low and drops off by day 9 (Figure 7B) which is in stark contrast to small mammal visits in *P. amplexicaulis* which occur throughout the 16 days.

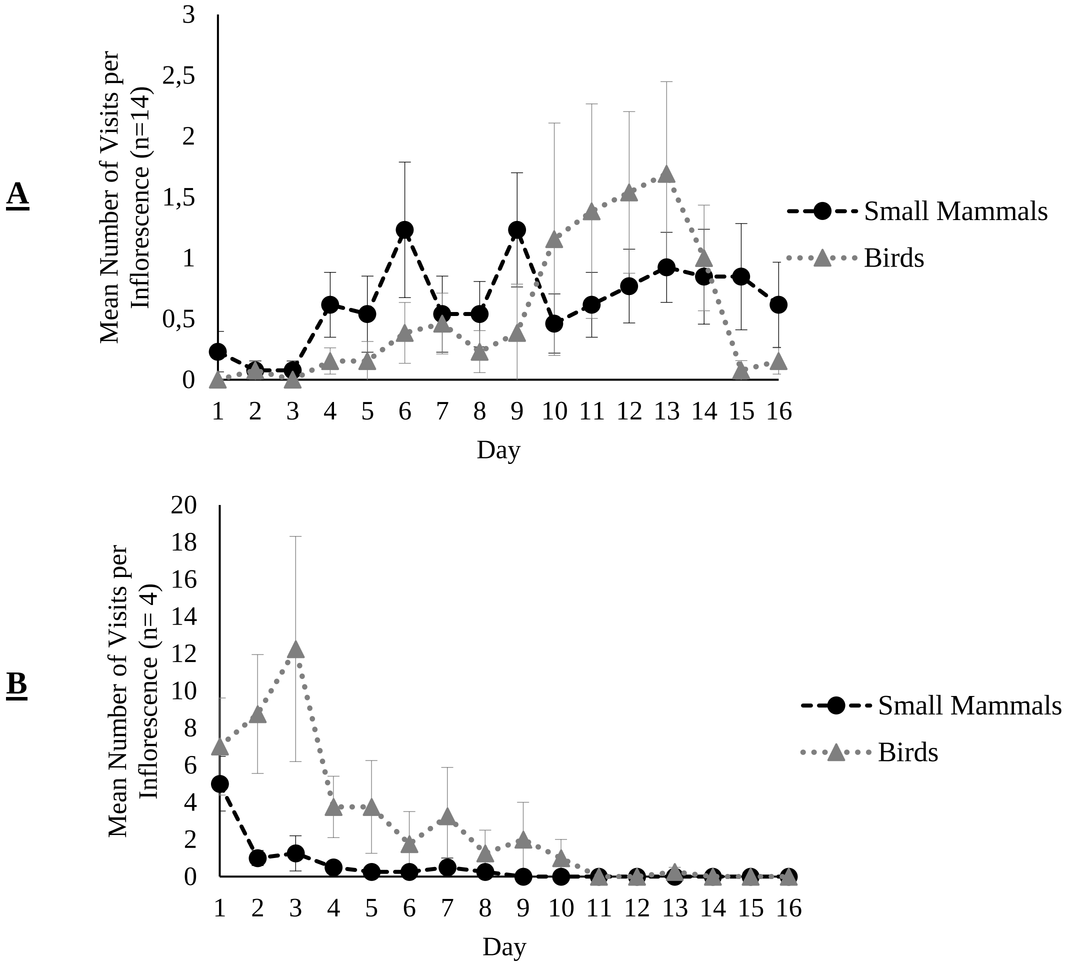

Figure 7:_Mean number of small mammal and bird visits per *P. amplexicaulis* (**A**) and *P. humiflora* (**B**) inflorescence across the 16 day observation period (broken up per 24hours). Error bars represent standard error.

The duration of small mammal visits to *P. amplexicaulis* showed no clear pattern when compared to the number of birds having previously visited the inflorescence. However, it was noted that small mammal visits lasting longer than 30 seconds did not occur once an inflorescence had been visited by over 15 birds. When comparing *P. amplexicaulis* inflorescences which were visited by birds and those which were not, the proportion of small mammal visits that fell into duration categories of less than five seconds; between five and 30 seconds; and more than 30 seconds, were not significantly different (W = 48 , n1 = 14, n2 = 12 p = 0.491 ; W = 55, n1 = 14, n2 = 12 p = 0.840 ; W = 55.5, , n1 = 14, n2 = 12 p = 0.864, respectively) (Figure 8).

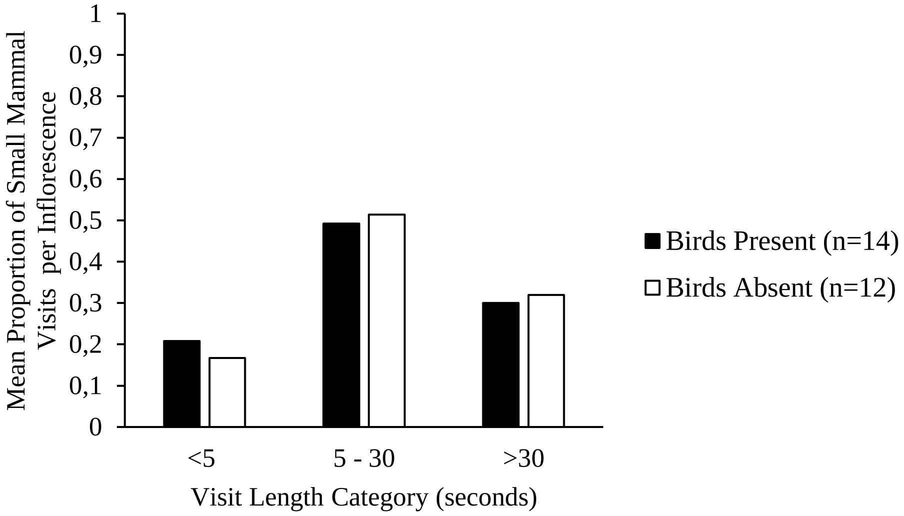

Figure 8: The proportion of small mammal visits per *P. amplexicaulis* inflorescence which had visit durations of less than 5 seconds, between 5 and 30 seconds, and more than 30 where birds had been observed visiting the flower (Birds Present) and where birds had not (Birds Absent).

Avian Visitation and Crypsis

The degree of crypsis of an inflorescence was found to significantly affect its visitation by birds (Figure 9). Exposed flowers had a mean visitation rate of more than tenfold that of hidden flowers (mean= 50.25 ± 22.383 mean visits to exposed inflorescence;mean= 3.87 ±1.287 mean visits to hidden flowers) which was a significant difference (W = 28 , n_1 = 8, n_2 = 4 p = 0.046). Partially exposed inflorescences were visited an average of 20 times over the

16 day observational period, an insignificant change from exposed and hidden inflorescent visitation rates (W = 10 , $n_1 = 4$, $n_2 = 4$, p = 0.685; W = 25.5 , $n_1 = 8$, $n_2 = 4$ p = 0.119, respectively).

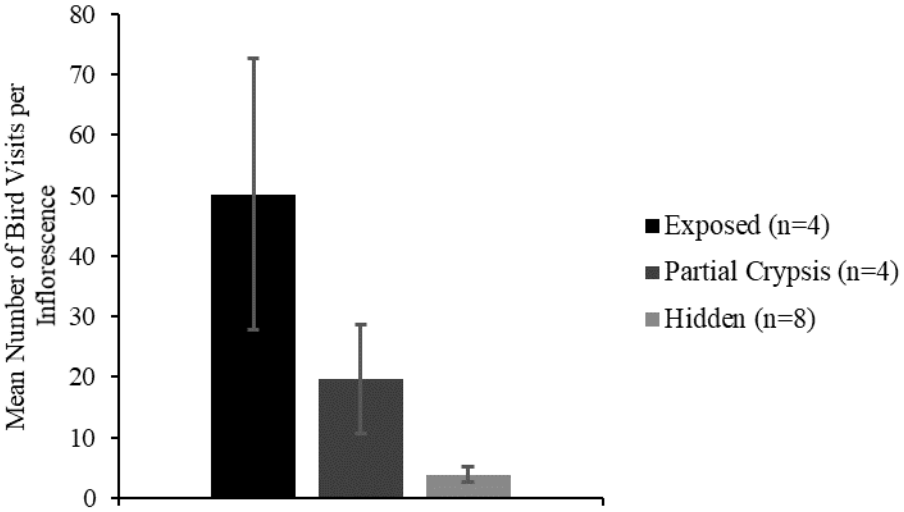

Figure 9: The mean number of bird visits per *P. humiflora* inflorescence which had crypsis levels of either Exposed, Partial, or Hidden over the 16 day observational period. Error bars represent standard error.

Whether the seed set for exposed flowers differs to that of hidden flowers was inconclusive as only five out of 28 collected cones had set seed, the other 23 of which resulted in a seed count of zero. The hidden cones which set seed had a mean seed set of 9.4 ± 4.55 % (n = 3) while the exposed cones had a mean seed set of 5.7 ± 3.18 % (n = 2). A paired comparison between two of the cones which came from the same individual (one hidden, one exposed) showed that the hidden inflorescence had nearly double the seed set of its paired exposed inflorescence (18.4% hidden; 9.6 % exposed).

There were no observations of aerial predation on small mammals over the course of the 16 days for either *P. amplexicaulis* or *P. humiflora*. However, a single boomslang, *Dispholidus typus*, was seen hunting within one of the *P. amplexicaulis* shrubs.

Discussion

The restricted distributions and populations hypothesis (Wiens *et al.* 1983) for Non-flying Mammal Pollination (NMP) relies on bird visitation to NMP Proteas being restricted due to the isolated nature of NMP populations. Our study has clearly shown that nectarivorous birds visit NMPProteas, sometimes even more frequently than small mammals visit as seen in the *P. humiflora* study. This is the first observation of its kind, with avian visitation to NMPPs previously thought of as rare. For example, Zoeller *et al* (2016) noted bird visits comprised 5.5% of vertebrate visits in *P. scabra* and 2.3% of visits in *P. decurrens* but 0% in *P. subulifolia* and *P. cordata*. To film the former two species an effort was made to expose the inflorescences and therefore these are probably maximum values for bird visits. In stark contrast, we found that *P. humiflora*, which is found in few, isolated habitats such as our sample stand in Jonaskop had a mean visit rate of 45 birds per inflorescence over 16 days, compromising 84% of total vertebrate visits while bird visits comprised 35% of all visits to *P. amplexicaulis*. The species studied by Zoeller *et al.* (2016) had much smaller inflorescences than our study species, which may explain such a large difference in bird visitation numbers as bird-pollinated proteas tend to have much larger inflorescences. The evolution of the non-flying mammal pollination syndrome needs to be re-evaluated given these novel observations which undermine the RDPH.

In addition to showing that nectarivorous birds visit NMPPs, we have shown that they are likely illegitimate pollinators. Birds pick up pollen more often than not, but as expected due to the NMPP nectar-stigma distance of 10 mm, the pollen brushes onto their smooth beak and feet. While there have been observations of successful pollination by bird feet (Johnson & Brown 2003), the frequency of birds standing on mature florets was very low (38 out of 202 total bird visits to *P. humiflora*). When observing small mammal visits, it is clear that the small hairs on the rostrum would easily pick up and correctly deposit pollen. Thus, birds are probably illegitimate pollinators of NMPPs, not only robbing the inflorescence of nectar but also removing pollen loads without legitimate deposition.

As the primary method of locating inflorescences is smell in small mammals and the majority of scent is in nectar, we expected that small mammal visits would decrease as nectar loads are diminished, thus nectar thieves would have a negative effect on pollinator visits. However, the lack of overall change in small mammal visitation with bird visitation and the random distribution of visits over the entire flowering period, suggest that sequential floret opening maintains nectar loads and scent to a sufficient degree. This is not to say that birds do not affect small mammal visits at all, as *P. amplexicaulis* inflorescences were either visited by mostly birds or by mostly small mammals (Figure 6). The cause of this effect is still unclear, as bird presence in itself is unlikely to have a direct effect due to the majority of small mammal visitors being nocturnal.

A high degree of inflorescence crypsis clearly protects against avian nectar thieves. We have shown that not only are exposed flowers visited by birds at a higher than expected rate, but that this visitation rate is tenfold that of hidden inflorescences. Thus we have supported the hypothesis that crypsis provides protection from nectar thieves. Whether or not crypsis, or indeed visitation by illegitimate pollinators, affects the reproductive success of the NMPPs can only be confirmed by looking at seed set. Unfortunately, either due to drought or the notoriously low seed-set in Cape Proteaceae (Wiens *et al.* 1983), our seed set experiment has too few replicates (23 out of 28 cones did not set seed). However, it is interesting to note that seed set of one of the hidden cones (18%) was almost double that of its paired exposed cone and fell above the 6-15% range reported in Wiens *et al.* (1983). In terms of the aerial predation hypothesis, we did not observe a single occurrence of predation on small mammals. This was also not previously found in Zoeller *et al.* (2016) (Jeremy Midgley per comms). Given that these were exposed inflorescences where small mammals would be exposed to predation, if aerial predation was occurring it would have been observed on our cameras. Furthermore, we observed substantial visitation by small mammals to terminally borne and exposed inflorescences which suggest that they do not necessarily keep to the safety of the shrubbery.

In conclusion, the potential for excessive nectar robbing by birds on non-cryptic flowers may explain a wide range of cryptic strategies in NMPPs. We suggest that while nectar thieves do not seem to affect small mammal pollinator visitation, they may decrease the pollination efficiency of NMPPs by reducing pollen loads without legitimate deposition.

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4.

TEST OF HYPOTHESES FOR DEAD LEAF RETENTION IN *PROTEA*; FLAMMABILITY, FERTILISATION, AND CRYPSIS.

Abstract

Firstly, we show that dead leaf retention occurs in three of the surveyed seven species of Cape *Protea* and that it is not a consequence of prolonged live leaf retention. *Protea amplexicaulis* retains dead leaves for up to six years and was used as a focal species to test two previously suggested hypotheses for dead leaf retention. Although dead leaves were found to contain high levels of calcium, they do not appear to positively affect below canopy seedling growth. Below canopy seedlings were neither more numerous nor larger than those in the open. The selfish-fertilization hypothesis was thus rejected. I also reject the flammability hypothesis because *P. amplexicaulis* is a short plant that is unlikely to affect the post-fire survival of its much taller near neighbours and in any event both it and its most common near neighbours are fire sensitive reseeders. This study suggests that dead leaf retention is a further trait that facilitates crypsis of inflorescences of non-flying mammal pollinated proteas and found that dead leaf removal significantly decreased the proportion of flowers hidden from view.

Introduction

Dead-leaf retention (marcescence) occurs in many species and is thought to provide a variety of functional advantages. These include protection against freezing (Harris *et al.* 2004, Smith 1979), to promote nutrient cycling (Otto and Nilsson 1981 - but see Escudero & del Arco 1987), and to inhibit growth of below canopy competing plants and seedlings

(Nilsson 1983). Marcescence has recently been observed in Proteaceae in 25 species of the *Banksia* genus in Australia (He *et al.* 2011). He *et al.* (2011) provided no data on length of live or dead leaf retention, nor dead leaf nutrient contents. They merely argued that nutrients released by burned dead leaves would favour seedlings that grew below parent plants canopies in the post-fire environment (here termed the selfish fertilisation hypothesis). Given the nutrient poor environment in which these species occur, retaining dead leaves rather than dropping them and allowing them to decay below parent plants likely incurs some nutrient costs to adult plants. The extent of costs would depend on the number of leaves in each age class, how long live leaves are retained, the degree of nutrient reabsorbtion from released leaves, and how rapidly released dead leaves decay. Thus a short live leaf longevity and rapid leaf litter decay would greatly increase the costs of dead leaf retention. However, live leaf longevity in nutrient poor systems can reach several years and decay rates of released leaves can also reach several years. Midgley and Enright (2000) showed that Cape Proteaceae keep their leaves for between 1 and 8 years with a mean of about 3 years. Mitchell and Coley (1987) showed that released *Protea repens* leaves only lost about 20% of their dry mass over 3 years. Given that most of the leaves on a mature individual plant will be less than 3 years old, and that decay rates are long, dead leaf retention in a typical Cape proteoid will make minor impact on individual plant nutrient cycling. More critically, the selfish fertilisation hypothesis assumes an absence of both seed dispersal and post-fire nutrient dispersal of ashed leaves from parent plants. *Banksia* species have winged seeds and thus will likely be dispersed by both wind and water, although many of those with dead leaves may be short, dispersal needs only to be > 1 m per fire, which is likely (Merwin et al 2012). Furthermore, post-fire nutrients are often poorly soluble, such as CaO, and easily blown by wind. In summary, if marcescence is correlated with an extended live leaf retention and some seed and nutrient dispersal, this would undermine the selfish fertilisation hypothesis.

Another possibility is that because dead leaf retention will strongly influence flammability, it may open space up for post-fire recruitment via the *kill thy neighbour* hypothesis (Bond & Midgley 1995). Dead leaf retention and associated local increase in flammability may influence mortality of neighbouring plants and thereby provides more space for seedlings of flammable parents to establish. To test this hypothesis, information on near neighbour interactions is needed as well as on their fire sensitivity. This hypothesis also relies on limited seed dispersal; seeds of flammable mutants must remain under parent and under adjacent near neighbour canopies.

Midgley and Bond (2011) noted dead leaf retention in *Protea amplexicaulis*. It is a low sprawling shrub with serotinous cones and is non-flying mammal pollinated (NMP) (Rourke & Wiens 1977; Wiens *et al.* 1983). As with most NMP Proteas, its inflorescences are cryptic, being axillary and geoflorous, and hidden amongst the shrubbery. Wiens *et al.* (1983) suggested that crypsis may have evolved to protect rodent pollinators from avian predators. It has also been suggested that crypsis is a strategy to decrease nectar robbing by illegitimate bird pollinators (Wiens *et al.* 1983; Rebelo & Breytenbach 1987), See Chapter 3. Crypsis of flowers may also provide protection from predation by larger mammals, such as the Chacma Baboon (*Papio ursinus*). Botha and Pauw (2017) found that baboons preyed upon a significant number of inflorescences when moving through an area, finding 12-29% of *Protea nerifolia* inflorescences were destroyed by baboons in three different sites.

The dense shrubbery created by retaining dead leaves assists in hiding flowers and hence forms a part of the cryptic nature of *P. amplexicaulis* inflorescences. Thus we propose that marcescence in NMP Proteaceae may have evolved in response to the cryptic inflorescence advantage. By means of defoliation experiments, we investigate whether dead leaves do in fact contribute to overall crypsis of inflorescences as well as the effect of marcescence on perceived below-canopy shelter.

Methods

Sampling Sites

Two sampling sites were chosen in which *P. amplexicaulis* is known to occur in dense stands, along with many other South African *Protea* species. The first was on the south-west facing slopes of Bainskloof pass, Western Cape, South Africa (33.62818 S; 19.09990 E, approximately 586m above sea level). The majority of sampling was completed on the steep slope to the west of the R301. The east side of the road at the same location provides a post-fire environment as a large area of the Bainskloof vegetation burnt in a natural fire in late April 2015. The second site of research was on the north facing slopes of Jonaskop (33.58107 S; 19.30219 E, approximately 1000m above sea level). Mature *P. amplexicaulis* plants are found scattered on Jonasplaats, which occurs along the east side of the access road. Sampling took place at both localities during *P. amplexicaulis* late-winter flowering in August 2016.

To determine dead and live leaf retention strategies of *P. amplexicaulis,* it and co-occurring *Protea* species were analysed. The seven other *Protea* species (*P. humiflora, P. laurifolia, P. lorifolia, P. nana, P. nitida, P. repens,* and *P. subulifolia)* were assessed with regards to the maximum age of retained leaves both dead and alive. A randomly selected branch from each of 10 individuals from each species was assessed per site, except for those only found at one site (*P. humiflora, P. lorifolia,* and *P. subulifolia* only found in Jonaskop) for which only 10 individuals were assessed in total. Leaf age can be inferred, with an accuracy of one year, from the annual stem growth of the plant, which is marked by node scarring or by branching events in Cape Proteaceae. Annual branch growth in Proteaceous species is marked by small, circular swellings (nodes), which can be counted from the outermost branch to infer age of the node and hence the age of the leaves along the branch (Lamont 1985, Wills 2003). Only nodes with more than three fully expanded leaves attached to the relevant stem were included in leaf longevity counts. The maximum age of dead and alive leaves were averaged per species. The live-leaf ages of *P. amplexicaulis, P. humiflora,* and *P. subulifolia* were then compared to the overall live-leaf longevity of the other five species using a Mann-Whitney Wilcoxon test for independent, non-normal samples. This and all other formal statistical analysis was conducted in R statistical software (R Core team, 2016).

Determining the potential costs of marcescence to a plants' nutrients cycle requires quantifying the amount of dead leaves retained by any given *P. amplexicaulis* individual. Thus, we counted the number of branches retaining dead leaves (with at least three leaves) on 15 randomly chosen *P. amplexicaulis* individuals in Jonaskop. The age of the individuals was standardised by aging five *P. repens* in the immediate area (five vegetation stands assessed overall), assuming that the *P. repens* individuals will be the same age as *P. amplexicaulis* individuals in the same stand as post-fire seedlings would have established concurrently. All stands were estimated to be around 15 years old. We collected a randomly chosen branch with dead leaves from 10 randomly chosen *P. amplexicaulis* individuals. A section on the branch was identified as one year's growth via means of node scarring as described previously. The dead leaves situated within this section were removed, dried, and ground. An elemental analysis was completed in a Spectroscout energy-dispersive X-ray Fluorescence analyser, calibrated according to certified standards. The amount (g) was then multiplied by the mean number of dead branches to calculate the amount of nutrients retained each year per individual.

Flammability Hypothesis

Dead leaf retention strongly influences flammability; *P. amplexicaulis* was rated highly flammable in Burger and Bond (2015). To investigate whether the *kill thy neighbour* flammability hypothesis (Bond & Midgley 1995) is applicable to *P. amplexicaulis* individuals, we assumed that inter-specific near neighbours needed to frequently occur relatively closely and that these neighbours should be resprouters. Reseeders die in even the mildest of fires and are therefore unaffected by a localised spike in fire intensity. The nearest woody neighbours within a 2-meter radius of 30 *P. amplexicaulis* plants were recorded per site. Neighbouring plants more distant than this are unlikely to be affected by *P. amplexicaulis* flammability. Other *P. amplexicaulis* plants were recorded as nearest neighbours if applicable.

Selfish-fertilization Hypothesis

The selfish-fertilization hypothesis (He *et al.* 2011) predicts *P. amplexicaulis* seedlings will do better beneath the canopy of burnt mother plants, than in the open. Sampling took place at the Bainskloof site, in an area which burnt 16 months prior. *P. amplexicaulis* seedlings occurring within a $1m^2$ transect around the centre of a burnt individual's canopy were measured for height using callipers. Measurements were then taken for seedlings falling within a $1m^2$ transect 1.5 meters horizontally to the right of the parental transect. If another burnt *P. amplexicaulis* individual was found to the right, then the plot was moved to the left of the original burnt individual. This was repeated for a total of 36 paired transects. Using a Wilcoxin signed-rank test for paired samples (Hollander & Wolfe 1973), the number of seedlings found beneath mother-plant canopies and within the matrix was compared. The difference in the average height of seedlings between parental and matrix samples was assessed in the same way, however the sample size was drastically reduced (n=12) as many paired transects did not have seedlings present in both transects. To investigate the potential amount of nutrients released by a *P. amplexicaluis* individual when burnt, we multiplied the average amount per years' worth of dead leaves by the average retention time of dead leaves in *P. amplexicaulis.*

Crypsis Hypothesis

The contribution of dead leaves to overall crypsis in *P. amplexicaulis* individuals was assessed in two forms. Firstly, in line with the small mammal protection hypothesis for crypsis, we investigated how dead leaves contribute to the degree of shelter beneath the canopy. We used below canopy light exposure as a proxy for degree of shelter. At the Jonaskop site, 20 randomly selected *P. amplexicaulis* shrubs were sampled. Using a GoPro HERO4 camera, an image from the base of the plant facing the sky, was taken and analysed using *Image.J* (Schneider *et al.* 2012) in order to estimate the percentage of sky visible. With the camera kept in the same place, the shrub was then stripped of all dead leaves, and another image was taken and analysed. Before the repeat photo was taken, the branches were allowed to fall back into their natural positions as they were often moved around during defoliation. Outputs from the Image.J analyses were used to investigate the difference between below-canopy light with and without dead leaves, using a students paired t-test.

To determine how dead leaf retention may conceal flowers, the locations of inflorescences produced in 2016 were determined in 30 *P. amplexicaulis* plants in the Jonaskop site. The locations were divided into 3 crypsis levels: completely exposed with the centre of the flower clearly visible from above, partially covered, and hidden. This categorisation was then redone once all dead leaves had been pruned from the plant. The flower locations of the 30 individuals before and after dead leaf removal were compared by means of a Chi-Squared test with three categories.

Protea amplexicaulis was observed to be heavily predated upon by Chacma baboons (*Papio ursinus)*; fresh flower heads that were ripped off bushes littered the Bainskloof sites. The rate of predation by baboons on *P. amplexicaulis* individuals was quantified by counting the number of ripped-off fresh flower heads laying within several 3m^2 plot in dense *P. amplexicaulis* areas. The total number of remaining this current year's flowers was also quantified for all *P. amplexicaulis* individuals whose canopy lay within the plot. Ten plots were randomly chosen and sampled. Within the same site, 30 individuals displaying evidence of baboon predation were assessed with regards to flower location (as previously described) to see whether inflorescences of a certain crypsis level are more likely to be picked. This was analysed by a Chi-Squared test with three categories.

Results

Of the eight *Protea* species which were assessed for their leaf longevity strategy, *P. amplexicaulis*, *P. humiflora*, and *P. subulifolia* were found to retain dead leaves. These species retained live leaves for a similar period of about two to three years, with *P. amplexicaulis* retaining live leaves for a mean of 2.7 years, *P. humiflora* for 2.9 years, and *P. subulifolia* for 2 years. Mean dead leaf retentions were 3.5, 2.8, and 2.8 years respectively. The live leaf retention strategies of the three marcescent species were not found to be statistically different to the average of the other five species ($W = 451.5$, $n_1 = 35$, $n_2 = 90$ and $p = 0.51$ respectively). *P.laurifolia*'s leaf longevity strategy stands out from the other 6 species, with live leaves retained for up to 8 years (Figure 1).

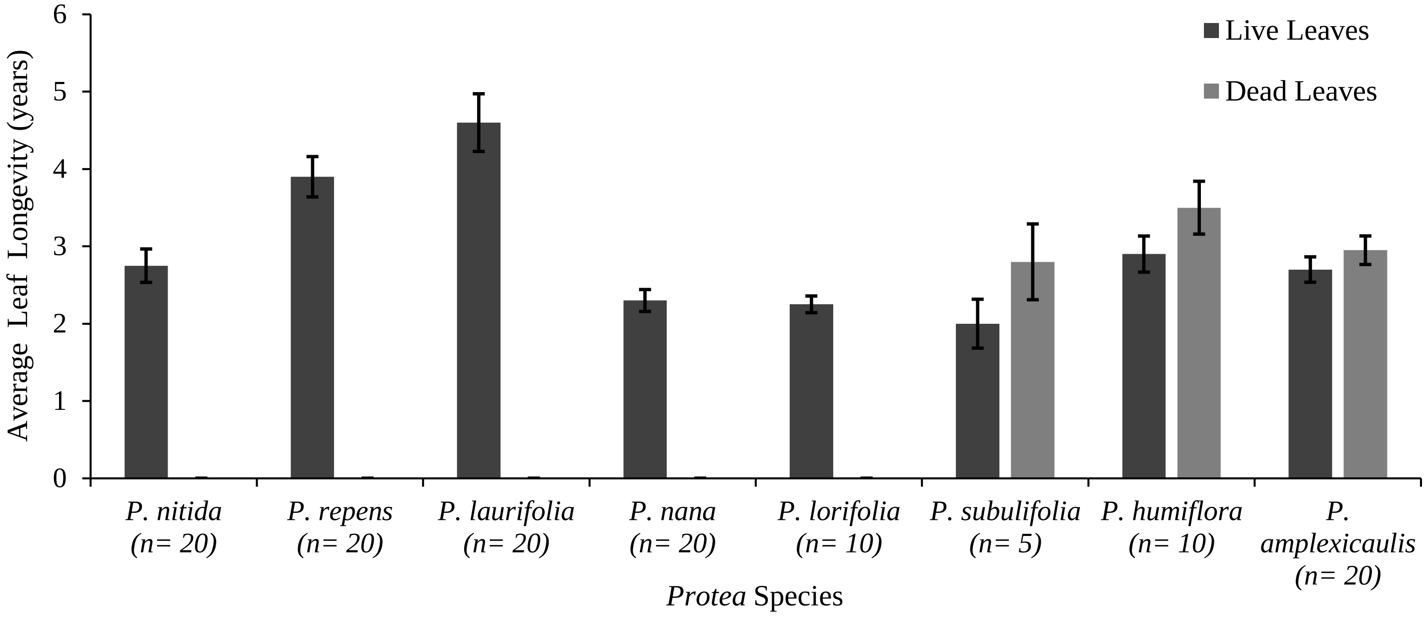

Figure 1: Leaf retention strategies of eight *Protea* species sampled at the Bainskloof and Jonaskop sites. Bars represent the maximum age in years of dead and alive leaves, averaged over *n* individuals per species. Error bars represent standard error.

To calculate the potential nutrient cycling cost of marcescence, we assessed the nutrient content of dead leaves and observed the usual leaf decay rate in the area. The mean number of branches retaining dead leaves on any given *P. amplexicaulis* individual is 57.4 ±10.35 (n = 15), with an average 3 years' worth of dead leaves per branch (Figure 1). Nutrient analysis concluded that the dead leaves store significant amounts of calcium (Ca), among other elements such as magnesium (Mg), potassium (K), and phosphorus (P). By retaining dead leaves rather than dropping them, the *P. amplexicaulis* individual retains 3.542 ±0.204 g Ca; 0.063 ± 0.026 g Mg; 0.149 ±0.014 g K; and 0.049 ±0.011 g P per year (n =10). The decomposition rate of leaf litter from non-marcescent proteas was found to be relatively slow, with the litter layer beneath a *P. repens* individual reaching depths of over 10 cm (Figure 2).

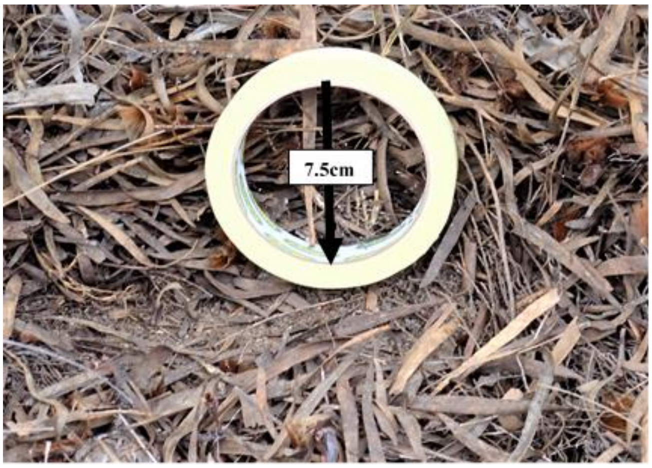

Figure 2: A photo documenting the leaf litter layer found beneath a *Protea repens* individual in Jonaskop with a roll of tape (7.5cm inner diameter) for scale.

Flammability Hypothesis

Protea repens was by far the most common woody species found within two meters of *P. amplexicaulis* individuals at both sites, with the second most common being another *P. amplexicaulis* plant (Figure 3). Both of these species are reseeders and are sensitive to mortality by fire. There were two occurrences of unidentified woody neighbours, and three occurrences of no woody neighbour within a two-meter radius of *P. amplexicaulis* individuals at the Jonaskop site (Figure 3).

Selfish-fertilization Hypothesis

No significant differences were found between the number of seedlings established beneath the canopy of a burnt individual (42 total seedlings) and within the vegetation matrix 1.5m away (46 total seedlings) ($V = 128$, $n = 36$, $p = 0.771$). The average height of the seedlings per transect were also found to be statistically similar ($V = 41$, $n = 12$, $p = 0.910$), with an overall mean height of 50.2 ± 17.6 mm ($n = 42$) in the transects below burnt *P. amplexicaulis* individuals and 50.5 ± 18.8 mm ($n = 46$) in the matrix (Figure 4). Based on our nutrient analysis, an individual *P. amplexicaulis* retains 9.918 ± 0.176 g Ca in their dead leaves at any point in time, such as just prior to being burnt.

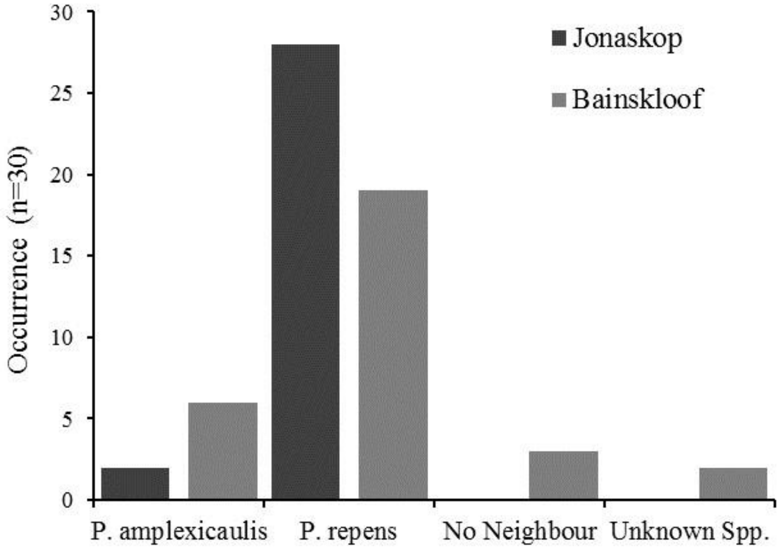

Figure 3: The nearest woody plant species within a 2m radius of *P. amplexicaulis* individuals sampled at the Bains kloof and Jonaskop sites (n= 30 *P.amplexicaulis* individuals per site).

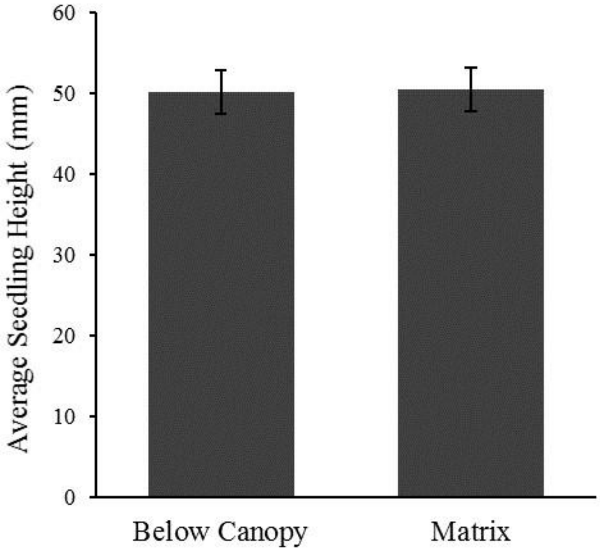

Figure 4: The height (mm) of established *P. amplexicaulis* seedlings averaged over 36 paired 1m^2 transects below burnt *P. amplexicaulis* individuals and 1.5m away in the matrix, as sampled at the Bainskloof post-fire site in August 2016. Error bars represent standard error.

Crypsis Hypothesis

The removal of dead leaves from *P. amplexicaulis* individuals resulted in a significant 12.8 ± 7.17 % mean increase in below canopy light exposure ($t_{(19, 1)} = -8.02$, $p < 0.001$) (Figure 5) and a significantly different distribution with regards to flower exposure ($\chi^2_{(2)} = 156.59$, $p < 0.001$). The percentage of flowers completely hidden within the canopy decreased from 68% to 38%, while the number of fully exposed flowers increased by 13% and partially covered increased by 17% (Figure 6).

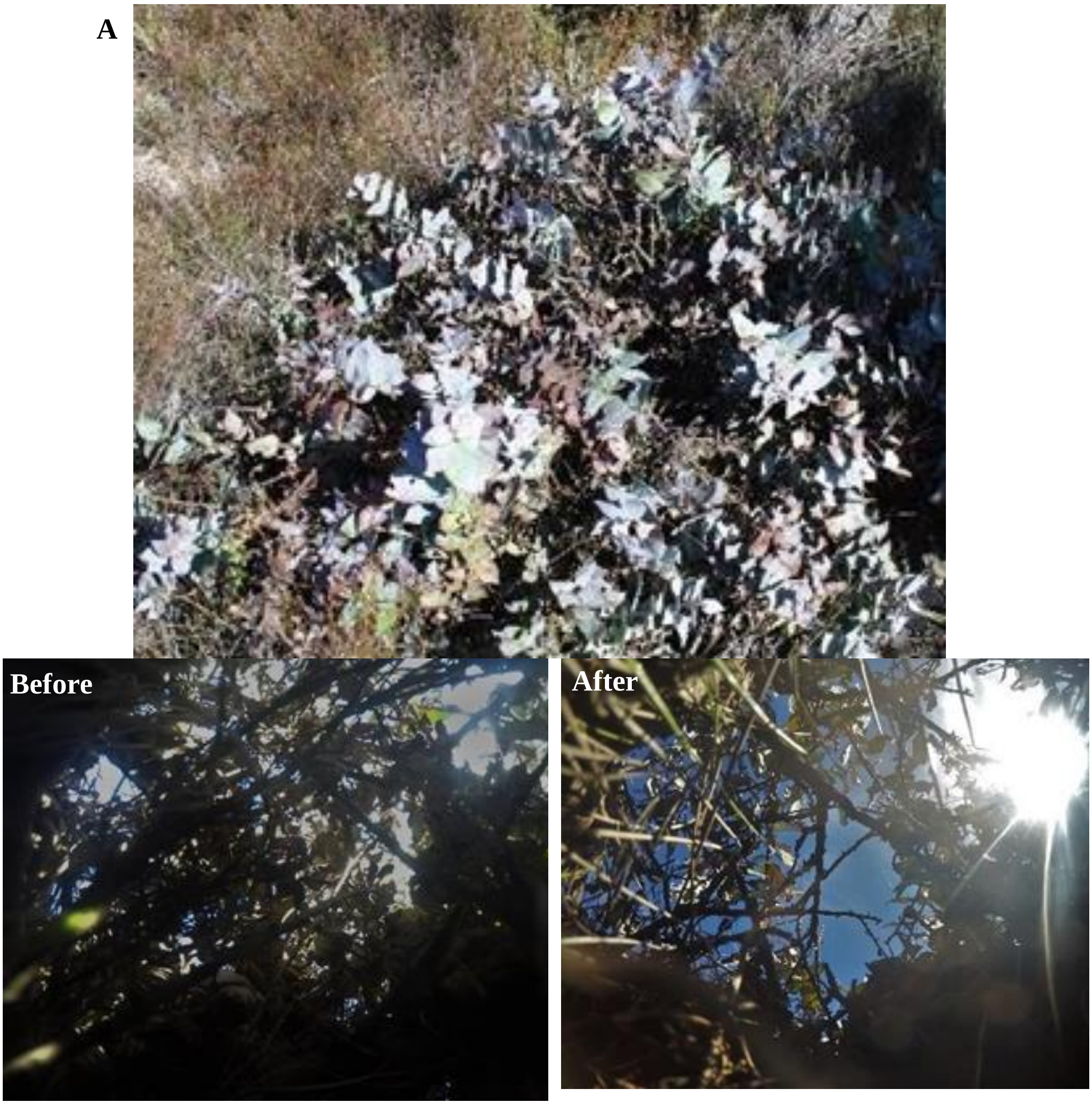

Figure 5: One of the *P. amplexicaulis* individuals sampled at the Jonaskop site (**A**) and the below canopy sky visibility before (left) and after (right) the removal of dead leaves.

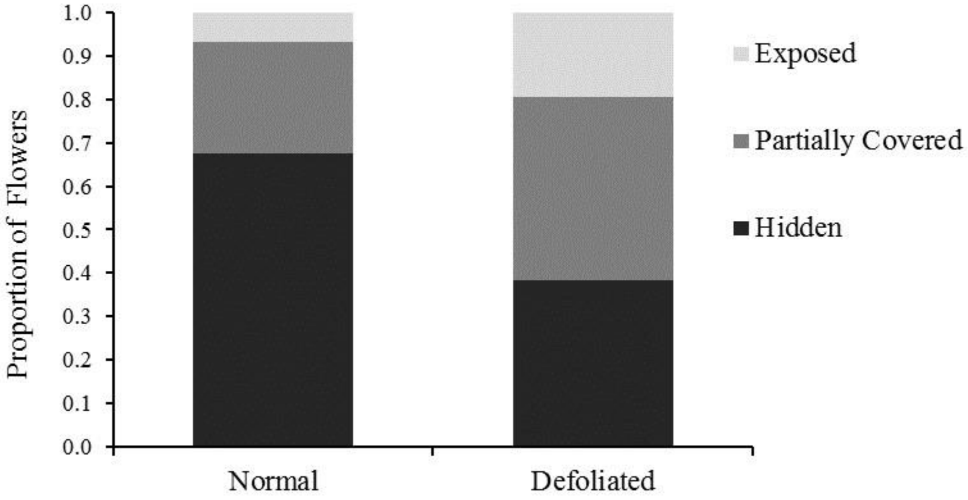

Figure 6: The effect of dead leaf removal ('Defoliated') on the distribution of flowers within *P. amplexicaulis* shrubs, with regards to visual exposure based on 30 individuals at the Jonaskop site (n= 327 flowers).

There was evidence for substantial predation on *P. amplexicaulis* flowers by baboons at the Bainskloof with almost half of 2016 flowering output removed (mean = 47.5 ± 2.01 %; n = 763). To assess whether baboon predation was affected by flower location, the exposure distributions were compared between predated upon individuals in Bainskloof and untouched individuals in the Jonaskop site (n_1 = 349 flowers/ 30 individuals; n_2 = 347 flowers/ 32 individuals, respectively). There was no significant difference between the flower location distribution of plants subjected to high levels of baboon predation and those subjected to no predation ($\chi^2_{(2)}$ = 2.86, p = 0.239). Thus, crypsis did not deter baboons; they were able to locate hidden flowers.

Discussion

Marcescence in Cape *Protea* species appears to be relatively infrequent (three out of 8 species) and is decoupled from the plant's live leaf longevity. Dead leaves contain some

Calcium, but because leaf decay rates are low the amount of calcium may not be biologically significant in terms of nutrient cycling. Also, given that soil calcium does not limit tree growth in fynbos (Bond 2010), it is unlikely to have a major impact on seedling growth. That seedlings frequently established in the open suggests dispersal occurs and therefore the selfish fertilisation hypothesis is unlikely. Even the seedlings recorded as growing under canopy cannot be assumed to be off-spring from that plant due to the likeliness of seed dispersal. In any event below canopy plants were not larger than seedlings in the matrix. Thus, we reject the selfish seedling hypothesis.

Protea amplexicaulis often had *P. repens* as it's near neighbour and 22% of the time it was its own near neighbour. Both the selfish fertilisation hypothesis and the kill thy neighbour hypothesis rely on limited seed dispersal. However, Bond (1988) as well as Manders (1986) have shown that hairy protea seeds are able to disperse locally by tumbling along the ground. Secondly as both *P. repens* and *P. amplexicaulis* are reseeders, and not fire-avoiders, there is no real advantage of increased flammability because these species would die in a relatively low-intensity fire. Also, as *P. repens* is significantly taller (frequently > 2 m) than *P. amplexicaulis* (< 0.5 m tall), it is unlikely that the latter would affect fire mortality of the former. Finally, as nutrient-rich ash is often dispersed by wind and water post-fire (Smith 1970, Grier 1975), it is unlikely that dead leaf retention results in below canopy fertilisation.

Marcescence as a strategy for reduced flower robbing by illegitimate pollinators is the most likely explanation given the results of this study. Although defoliation resulted in a significant 12% decrease in below-canopy shelter, this is likely not biologically significant as small mammals are unlikely to notice such a minor change in shelter. Due to the dense, shrub-like canopy of *P. amplexicaulis*, illegitimate, visually searching pollinators such as birds would have limited access to flower heads, and as such only be able to steal nectar from fully exposed, and to a lesser extent partially exposed, flowers. The significant shift in flower exposure once dead leaves had been removed from *P. amplexicaulis* individuals (Figure 6) highlights the potential role of marcescence in reducing the prevalence of nectar robbing by birds. Both before and after defoliation, most flowers were fully hidden within the canopy. While this is commonly observed in many rodent pollinated species and likely attributable to the need for flowers to be close to the ground and accessible to rodents (Rebelo & Breytenbach 1987), the 30% decrease in the number of flowers potentially exposed to birds was due primarily to the cover provided by dead leaves. Thus, the evolution of marcescence may be due to an enhanced crypsis strategy specific to NMP Proteas. Two other lines of evidence support this hypothesis. Firstly, we note that the other two species which were observed to be marcescent (*P. humiflora* and *P. subulifolia*) are also small mammal pollinated. Secondly, many of the marcescent *Banksia* species observed to retain dead leaves by He *et al.* (2011), possess traits synonymous with non-flying mammal pollination (Cunningham 1991; Goldingay *et al.* 1991; George 1984) and are potentially visited by illegitimate bird pollinators.

While marcescence may provide protection from bird visitors, it does not reduce the rate of predation by baboons. This is due to the nature of their foraging behaviour, in which the baboon searches through the bush rather than being attracted by sight or smell. While baboon predation may be more harmful than nectar thieving birds – due to the destructive process of ripping off the entire flower- it is unlikely that this event is common enough to require a specific defence.

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5.

SYNTHESIS AND CONCLUSIONS

5.1 Synthesis

This study set out to explore non-flying mammal pollination (NMP) in Cape Proteas, specifically the adaptive significance of crypsis and potential morphological traits which enhance this. Through the use of camera technology, this study found evidence in support of *Protea amplexicaulis* and *Protea humiflora* being primarily pollinated by small mammals, with the main pollinator species similar to those in the literature (Rourke & Wiens 1977; Wiens *et al.* 1983).. Additional observations of small carnivore visitation similar to Steenhuisen *et al.* (2015) were also made, with the Cape Grey Mongoose observed foraging on flowers on five occasions. Bird visitation to exposed flowers was unexpectedly high given it was previously considered rare for NMP Proteas (Wiens et al 1983), making up 35% of all *P. amplexicaulis* visits and 84.5% of total *P. humiflora* visits.

The adaptive significance of concealing flowers in NMP plants had not been investigated prior to this study, although hypotheses were suggested by Wiens *et al.* (1983). Our results showed not only that bird visitation to exposed NMP inflorescences was high, but that they are unlikely effective pollinators and thus represent nectar thieves. The effect of nectar robbing by birds on subsequent small mammal visitation or behaviour was found to be limited. This may be expected as the majority of small mammal pollinators are nocturnal and thus temporally separated from nectarivorous birds.However the reduction in nectar, and thereby scent (Steenhuisen *et al.* 2010), was expected to reduce attractiveness of an inflorescences to small mammals as well as the associated nectar reward. Given that birds were often seen to pick up pollen (64% of visits) but deemed unlikely to legitimately deposit pollen, I suggest that the main cost of nectar robbing in this case is a reduction in pollen loads without effective pollination which impacts the overall reproductive success of the plant (see Irwin *et al.* 2010). Given the already low seed set in NMP Cape Proteas (Wiens *et al.*1983), this nectar robbing by birds is worth avoiding. Crypsis is shown to be an effective strategy against nectarivorous birds as I found that hidden flowers were visited 92% less that exposed ones. This effect is likely due to the foraging behaviour of birds relying mostly on visual cues (Raven 1972).

Novel investigation into leaf longevity and dead leaf retention in Cape Proteas was conducted in Chapter 4. Live leaf longevity differs amongst species, ranging from two to four years for most Proteas but with *Protea laurifolia* retaining live leaves for up to 8 years. Three species; *P. amplexicaulis, P. humiflora,* and *P subulifolia*, were found to retain dead leaves, averaging around three years retention time. All three of these species are confirmed NMP (Rourke & Wiens 1977, Wiens *et al.* 1983, Zoeller *et al.* 2016). Upon rejection of previous hypotheses of He *et al.* (2011), a novel hypothesis for marcescence in Proteaceae was suggested and supported by the results of this study. Dead leaf retention was found to increase the proportion of completely hidden inflorescences by 30% in a defoliation experiment on *P. amplexicaulis* individuals. I thus suggest that dead leaf retention in Proteaceae developed as a means to reduce nectar robbing by nectarivorous birds.

5.2 Conclusions

In conclusion, visitation by illegitimate bird pollinators to exposed flowers is substantial and likely negatively impacts gene flow in NMP Proteas. Hiding flowers greatly reduces visitation by birds and thus crypsis is a vital adaptation and a defining morphological trait in NMP Proteas. Enhancing the overall cryptic nature of inflorescences within a plant, dead leaf retention can be seen as an adaptation to NMP and contributes to the defence against nectar robbing birds. Noting the high number of avian nectar thieves found in this study and that this was found to be an extremely rare event in Zoeller et al (2016), I suggest that NMP species with larger inflorescences, such as *P. amplexicaulis*, are more susceptible to nectar robbing as hiding them from site is practically more difficult. The three marcescent proteas identified in this study have larger inflorescences (*P. amplexicaulis* 60-80 mm, *P. humiflora* 60-80 mm, *P. subulifolia* 45-60 mm in diameter) than those non-marcescent species studied by Zoeller *et al.* (2016) (*P. scabra* 30-50 mm, *P. cordata* 40-50 mm, *P. decurrens* 30-50 mm in diameter) (Rourke 1980). Thus, I suggest that marcescence developed in NMP species with more conspicuous inflorescences as an additional defence against avian nectar thieves.

5.3 Implications

Observations of small mammal pollination in this study contribute to a growing knowledge on NMP syndromes and NMP in Cape Proteas, and provides further example of the utility of remote-triggered cameras in pollination biology. This study has made novel insight into nectar robbing and suggests morphological traits associated with defence against nectar thieves. The observation of substantial bird visitors to NMP *Protea* inflorescences casts doubt on the current explanation for the evolution of the NMP syndrome. While the pollination system of the basal *Protea* species has yet to be described, current phylogenetic reconstructions suggest bird pollination as the ancestral condition (Valente *et al.* 2010). Wiens et al (1983) suggest that the shift from bird to small mammal pollination occurred as a consequence of highly specialized plant species becoming ecologically restricted due to changes in habitat. The 'restricted populations hypothesis' goes on to suggest that as species became more restricted, attractiveness to birds decreased as birds prefer to forage on larger stands (Turner *et al.* 2011), and thus a shift to NMP occurred. However, our results showing high levels of bird visitation to exposed inflorescences undercuts the basis of this evolutionary hypothesis. It is especially notable that the *P. humiflora* individuals observed were in a highly localized, restricted stand and that bird visitation was more frequent than that to *P. amplexicaulis* individuals situated in larger, mixed species stands. The restricted distributions hypothesis for the evolution of the NMP syndrome must thus be re-evaluated.

5.4 Issues and Future Research

Although advances in camera technology have allowed for novel observation into small mammal pollination (Wester *et al.* 2009, Melidonis & Peter 2015, Zoeller *et al.* 2016, Lombardi *et al.* 2017), it is still challenging to observe inflorescences in their naturally cryptic state.. It is likely that small mammal visitation numbers would be higher on cryptic inflorescences and that their behaviour, namely visit duration and foraging tendencies, may be affected by the relative exposure. Furthermore, some small mammal species may be excluded from observations as they are shyer and remain in well sheltered areas.

As the trigger sensitivity for cameras is set to pick up vertebrates, visitation by insects and their role in pollination may be overlooked by this study. Furthermore, pollinator species assemblages as well as events such as nectar robbing by birds or baboons may fluctuate substantially within populations and due to environmental factors, such as resource limitation and drought.

The direct effect of nectar robbing in NMP Proteas on breeding success must still be investigated. Unfortunately, due to low proportion of seeds set and the majoritively male functioning of Cape Proteas, the comparison of seed set for hidden versus exposed *P. amplexicaulis* inflorescences was inconclusive. A number of experiements could be conducted to further investigate the effect of bird visitation on breeding success of the plant. Bagging of inflorescences during the day versus night or building cages to exclude visitors may help reveal the pollination effectiveness of birds and small mammals. Defoliating entire plants – thereby exposing all inflorescences – and assessing the average seed set for these plants versus for non-defoliated plants would provide the necessary data to comprehensively investigate the effect of crypsis.

The effect of nectar robbing on small mammal pollination is inherently difficult to investigate in-field and may be better investigated through choice trials with captive rodents.

Phylogeny of NMP and marcescence should be constructed in order to further investigate whether dead leaf retention represents a pollination adaptation whereby it increases the level of crypsis for NMP inflorescences.

5.5 The Importance of a Multi-Disciplined Approach

I believe that in part, this thesis highlights the importance of thinking holistically when hypothesizing and reaching beyond ones specialisation. A pollination biologist would likely not give the leaves of a plant a second thought, never mind mention a phenomenon such as marcescence in their description of the plants' syndrome. Similarly, a fire ecologist would observe dead leaves and immediately associate it with flammability. The novel connection between dead leaf retention and pollination syndromes put forth in this study was made possible by a collaboration of knowledge on fire ecology, pollination biology, animal behaviour, and plant-animal interactions.

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