The natural history, sensory ecology and biogeography of the seed dispersal of large fruits in Fynbos

by

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Plagiarism Statement

The conception, planning, data collection and analyses, and writing of this study were entirely my own, except where explicitly mentioned otherwise.

I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publications in my thesis, and where co-authorships are involved, my co-authors have agreed that I may include the publications. This includes:

- **Chapther 2**: **White JDM**, Bronner GN, Midgley JJ. 2017. Camera-trapping and seed-labelling reveals widespread granivory and scatter-hoarding of nuts by rodents in Fynbos. *African Zoology* 52 (1): 31-41.
- **Chapter 3**: **White JDM**, Midgley JJ. 2017. Dispersal of semi-fleshy fruits to rocky crevices by a rock-restricted rodent. *South African Journal of Science* 113: 1-5.
- **Appendix 1**: Midgley JJ, **White JDM**, Johnson SD, Bronner GN. 2015. Faecal mimicry by seeds ensures dispersal by dung beetles. *Nature Plants* 1 (10): 15141.
- **Appendix 2**: Midgley JJ, **White JDM**. 2016. Two dung beetle species that disperse mimetic seeds both feed on eland dung. *South African Journal of Science* 112 (7/8): 1-3.

Chapters 2 and 3, and Appendix 1 and 2 which are adapted from publications and co-authored with either one of my supervisors, Jeremy Midgley and Gary Bronner. Their contributions were through discussions, fieldwork and manuscript editing. Steve Johnson did the scent analysis in Appendix 1. Michael Cramer assisted me with the code and discussion around the boosted regression tree analysis in Chapter 6.

Signed by candidate

Joseph Douglas Mandla White

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Abstract

Seed dispersal is a crucial life-history stage for the regeneration of all reseeding plants. In the Fynbos Biome of the Greater Cape Floristic Region (GCFR) of South Africa, at least 100 plant species have seed traits that suggest they are scatter-hoarded by small mammals. The aims of this thesis were to investigate the dispersal biology of large-fruited Fynbos plants by: 1) determining the spatial and taxonomic extent of scatter-hoarding through seed trials, investigating both dry, nut-like and semi-fleshy fruits; 2) trait-based analyses investigating selective drivers of seed colour polymorphisms in flat-winged, serotinous *Leucadendron* and 3) modeling the relative influence of environmental, biological and disturbance drivers in predicting the distributions of serotinous and scatter-hoarded *Leucadendron*. Seed predation and scatter-hoarding by small mammals was widespread, yet locally patchy. Four new fynbos species with large, nut-like fruits were confirmed to be scatter-hoarded, by either *Acomys subspinosus* or *Gerbilliscus paeba*. Many species with small, nut-like fruits, with no clear dispersal or defense strategies, suffered intense seed predation by the non-hoarding small mammals, *Rhabdomys pumilio* and *Micaelamys namaquensis*. The large, dry, nut-like fruits of *Ceratocaryum argenteum* have a globally unique, alternative strategy for effective dispersal. The nuts emit a strong scent that mimics the scent of herbivore dung and exploits the olfactory sensory perception of dung beetles, which rolled and buried the seeds. The semi-fleshy fruits of the rock-restricted *Heeria argentea* were dispersed by the mutualistic *M. namaquensis*, which consumed only the pericarp, allowing for germination, as well as moving fruits to fire-protected, rocky outcrops. Many flat-winged *Leucadendron* seeds, with polymorphic brown or black seed coats, displayed background matching with post-fire soils. This plant defense likely reduces predation by visually cued avian granivores. Finally, modeling results suggest that the distributions of both serotinous and scatter-hoarded plants are strongly negatively and positively influenced by more intense seasonal drought and longer fire return interval in the GCFR, respectively. Overall, this thesis advances our understanding of large-fruited Fynbos plants, providing unique insights into their natural history, ecology, evolution, conservation and biogeography.

Chapter 1

Introduction

1.1 Seed dispersal

Seed dispersal is a fundamental stage in the life cycle of adult plants, as it connects the end of their reproductive cycle with the establishment of their offspring (Wang & Smith, 2002). Plants and their seeds display many morphological structures uniquely associated with different seed dispersal methods (Wenny, 2001). These dispersal-related structures require a cost from the plant, suggesting there is a benefit to dispersal of their seeds (Howe & Smallwood, 1982; Wenny, 2001). The main benefits of local seed dispersal to plants that have been proposed are: (a) escape from disproportionate seed and seedling mortality caused by distance- or density-dependent factors near conspecifics (Janzen, 1970; Connell, 1971); (b) colonization of rare, unpredictable or ephemeral disturbances; and (c) directed dispersal to fixed microhabitats which are suitable for establishment and growth (Howe & Smallwood, 1982).

These advantages are not mutually exclusive and determining the relative role of each for different plants can help inform us on the importance of different dispersal agents. Furthermore, seeds dispersed by animals can face potentially very different seed fate pathways (Theimer, 2005). To better understand how plants survive this stage of their life cycle, it is necessary to understand the natural history of these seed dispersal relationships. One of these pathways is where the animal may act as both seed predator and/or disperser by consuming and damaging a significant proportion of seeds they handle, known as synzoochory (Theimer, 2005; Gómez, Schupp & Jordano, 2018). For example, scatter-hoarding animals may temporarily cache seeds for later consumption, and occasionally fail to recover some of these caches, thereby acting inadvertently as seed dispersers (Vander Wall, 1990).

Understanding this type of basic natural history is particularly important for the mega-diverse Core Cape Subregion (CCR), previously known as the Cape Floristic Region, but now forming part of the Greater Cape Floristic Region (GCFR), in the southwestern Cape of South Africa (Manning & Goldblatt, 2012) (Figure 1.1). Within a small area (±90 760 km^2) there is a mega-diversity of vascular plant species (± 9383), with hyper-endemism (68%), 1719 Fynbos endemic threatened species and 20 listed plant extinctions for the province (SANBI, 2017). In this small region, with its disproportionate diversity, conservation management and the many critically endangered plant species are under ongoing pressure. The vast majority of Fynbos plants have assumed or unknown dispersal mechanisms. Identifying and understanding mutualistic seed disperser interactions with seeds is crucial to understanding the bottlenecks in the life cycle of plants (Wang & Smith, 2002), especially for species with low recruitment rates, such as the critically endangered *Widdringtonia cedarbergensis* (White et al., 2016).

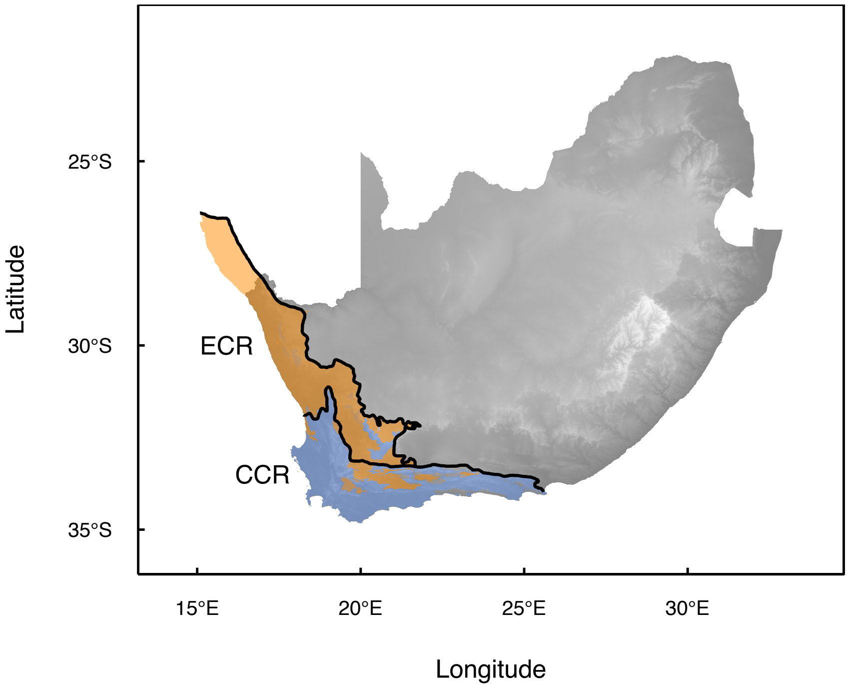


Figure 1.1 Map of the Greater Cape Floristic Region and its components, the Cape Core Subregion (CCR) and the Extra Cape Subregion (ECR) within southwestern South Africa and extending into Namibia. Both the CCR and ECR are mostly congruent with the Fynbos (blue) and Succulent Karoo (orange) Biomes, respectively, though both have a mix of each biome to a small extent.

There are several published studies on the various mechanisms and pathways of seed predation and dispersal in the Cape flora (Myburg & Rust 1975; Bond & Slingsby 1983; Bond 1984; Bond & Slingsby 1984; Bond & Breytenbach 1985; Manders 1986; Brits 1987; Bond 1988; Fraser 1990; Holmes 1990; Botha & le Maitre 1992; Christian 2001; Midgley et al. 2002; Midgley & Anderson 2005; Steenhuisen & Johnson 2012; Rusch et al. 2013a; Rusch et al. 2013b; Rusch et al. 2014; Botha & Pauw 2017; van Blerk et al. 2017; Weighill et al. 2017). However, due to the exceptional plant species richness, many families, genera and species dispersal mechanisms remain assumed or unknown.

1.2 Seed dispersal in the Fynbos Biome

The CCR is dominated by fire-prone and fire-adapted fynbos shrublands, where post-fire regeneration of non-sprouting shrubs is dependent on either canopy- or soil-stored seeds (le Maitre & Midgley, 1992). There are two major threats to seeds in this region: 1) seed predation either pre- or post-dispersal by insects, ants, small mammals or birds (Myburg & Rust, 1975; Fraser, 1990) or 2) fire damage (Ferrandis, Herranz & Martınez-Sánchez, 1999; Newton, Bond & Farrant, 2006). Post-fire seed predation will be negligible for soil-stored seeds, while serotinous, wind-dispersed seeds are still at risk of predation, likely from immigrating bird granivores or declining small mammal granivore populations (Chalmandrier et al., 2013). Recruitment of fynbos seedlings is rare during inter-fire intervals under closed canopies; some recruitment does occur in open vegetation and in gaps, but most of these seedlings will suffer mortality before maturing (Kraaij & van Wilgen, 2014). Another reason for post-fire recruitment pulses is that many fynbos plants have fire- and/or smoke-induced germination cues, where seedlings can take advantage of the more favourable post-fire conditions of increased light, a flush of nutrients and decreased competition (le Maitre & Midgley, 1992). Most Fynbos plants produce small, dry fruits that have no clear adaptations for dispersal and are presumed passively dispersed (Manning & Goldblatt, 2012). These small fruits would generally be insignificant to small mammal or bird seed predators. Plants that are serotinous (300+ species in the Cape flora) lack persistent soil-seed reserves and store their seeds in closed woody cones in the canopy, which are only released after several years or when the parent senesces, usually due to an environmental cue, such as fire disturbances (Bond, 1984; Lamont et al., 1991; Lamont & Enright, 2000). Globally, serotinous seeds typically have seed appendages such as wings, plumes and parachutes, likely for wind dispersal, with the major exception being the Myrtaceae (Lamont et al., 1991). In fynbos, there is a relative absence of fleshy fruits that are attractive to birds, despite being relatively common in adjacent vegetation types, such as Afromontane forest or Strandveld (le Maitre & Midgley, 1992). Therefore seed dispersal by birds and other mechanisms, such as attachment to animals, appear to be proportionately insignificant or understudied in the GCFR (Johnson, 1992).

Alternatively, seeds can persist by being stored in the soil during inter-fire intervals, where the seeds will be more protected from seed predators and fire than if they remained on the soil surface (Fraser, 1990). There are two known biotic dispersal mechanisms that allow seeds to efficiently enter the soil bank – myrmecochory and scatter-hoarding. Myrmecochorous plants are very common in fynbos (1300+ species) and produce seeds with a fleshy, nutrient-rich elaiosome that is attractive to ants (Johnson, 1992). Ants collect these seeds and carry them to underground burrows where the elaiosome is consumed, while the seed is abandoned and remains intact in the soil (Bond & Slingsby, 1983). Alternatively, seeds that are scatter-hoarded typically have a thick, hard seed coat and lack an elaiosome (Midgley et al., 2002) (Figure 1.2). Ants do not interact with scatter-hoarded seeds due to their lack of an elaiosome and large size (Midgley & Anderson, 2005). A number of fynbos species produce seeds that fall into a putative scatter-hoarding dispersal syndrome and have the generalized characteristics that include a seed coat that is more than 2 mm thick and is larger than 5 mm in length (Midgley & Anderson, 2005). Throughout this thesis, dry, nut-like fruits with hard seed coats are called nuts (or generally referred to as seeds), while semi-fleshy, typically recalcitrant fruits are called fruits. Adaptations for dispersal have varied evolutionary origins, meaning that dispersal syndromes are useful only as general tool and their classification cannot substitute empirical studies of the dispersal mechanism (Howe & Smallwood, 1982).

Figure 1.2 Seeds representing three of the major seed dispersal modes in the Fynbos Biome of South Africa, including: a) the dry, hard nut-like fruits (*Leucadendron pubescens*), b) flat-winged seeds (*Leucadendron laureolum*), and c) elaiosome-covered seeds (*Leucadendron sericeum*).

1.3 Nuts in the Fynbos Biome

Scatter-hoarding has long been the focus of research in other ecosystems/habitat types for both ecological and evolutionary studies (Brodin, 2010). However, it has only recently been shown to exist in fynbos (Midgley et al., 2002). Midgley et al. (2002) found evidence that a biotic disperser was burying *Leucadendron sessile* (Proteaceae) nuts, and showed that *Acomys subspinosus* (Muridae) was both a common rodent at the site and the only rodent that buried nuts in laboratory conditions. Midgley et al. (2002) concluded that they had found the first evidence for scatter-hoarding in the Fynbos Biome.

Midgley and Anderson (2005) expanded on this original study when they found three new nut-fruited species were dispersed and buried. They showed evidence that *Leucadendron concavum*, *Willdenowia incurvata* (Restionaceae) and *Ceratocaryum argenteum* (Restionaceae) nuts were buried by biotic dispersers at four new sites. Furthermore, they also found laboratory evidence for burial behaviour by *Gerbilliscus paeba* (Muridae). Using the definition of nuts above, they suggested that there are likely 60-100 plants, in the Restionaceae and

Proteaceae plant families alone, with seed characteristics that fit the putative scatter-hoarding dispersal syndrome.

The recent availability of camera traps to study small, nocturnal or cryptic animal behaviours presented a major opportunity to improve our understanding of the plant-animal interactions in the GCFR. Broadening the scope of Midgley & Anderson (2005), I primarily asked: a) how and where are Cape plants with large, nut-like or semi-fleshy fruits dispersed?; b) to what extent do Cape plants have seed defenses to reduce seed predation by small mammals and birds?; and c) why are there more or fewer scatter-hoarded and serotinous Cape plants in certain environments?

1.4 Thesis Overview

The main objective of this thesis was to explore the ecology, evolution and biogeography of scatter-hoarded fynbos plants. The main hypothesis is that large, dry and semi-fleshy, fynbos fruits are scatter-hoarded by small mammals. This required the scope of a variety of methods and taxa that are detailed in the following chapters.

In Chapter 2, I explored the taxonomic and spatiotemporal extent of scatter-hoarding, investigating the dry nuts of seven endemic fynbos plants species: *Leucadendron pubescens*, *Leucadendron loranthifolium* and *Leucadendron sessile* (Proteaceae), *Cliffortia cuneata* and *Cliffortia phillipsii* (Rosaceae), *Widdringtonia cedarbergensis* (Cupressaceae), and *Ceratocaryum argenteum* (Restionaceae). To identify small mammal responses to nuts I used seed removal and tracking experiments with camera trap observations at eight sites spread throughout the CCR and seasons.

In Chapter 3, I investigated the seed dispersal and germination of semi-fleshy, recalcitrant fruits of the rock restricted trees *Heeria argentea* (Anacardiaceae) and *Hartogiella schinoides* (Celastraceae). This included the use of seed removal and tracking experiments with camera trap observations to test Marloth's

(1925) hypothesis that *Procavia capensis* (rock hyrax) both predates and disperses *H. argentea* fruits. Additionally, to understand the potential advantages of rock outcrops to a rock restricted plant, I investigated *H. argentea* responses to a recent fire in relation to plant size and distance within or from a rock outcrop.

In Chapter 4, I examined the seed dispersal and germination of *Hyaenanche globosa* (Picrodendraceae), a small, rock restricted, locally endemic tree, reported to have highly toxic seeds. I investigated aspects of the seed biology in relation to its germination and the possibility of directed toxicity to prevent seed predation by a common seed predator.

In Chapter 5, using seed removal data, field observations and spectral data, I investigated whether seed crypsis (background matching) in serotinous Proteaceae seeds can reduce seed predation by visually cued avian granivores. Seeds are released *en masse* post-fire on heterogenous patches of ash-sand and charcoal-litter mixed substrates, providing a context for visually cued selective agents on seed colour.

In Chapter 6, I explored the mechanisms behind bottom up, environmental and disturbance, drivers of the biogeography of serotinous and scatter-hoarded *Leucadendron* (Proteaceae) and how these drivers relate to seed traits and dispersal interactions. Additionally, I investigated the potential dispersal mechanisms of *Leucadendron elimense* and *L. laxum*, two species with small, dry, nut-like fruits in order to classify this small seed type for modeling purposes.

Chapter 7, the last chapter, synthesises all of the research presented thus far, placing it in context with previous research and then providing possible future avenues of research that may follow from this thesis. Additionally, I incorporate the findings of unpublished research on *Widdringtonia cedarbergensis* and two publications I co-authored on coleopterochory, from Appendix 1 and 2, where I re-examined the dispersal of *Ceratocaryum argenteum*, which produces large nuts with a strongly scented outer tuberculate layer.

Chapter 2

Camera-trapping and seed-labelling reveals widespread granivory and scatter-hoarding of nuts by rodents in the Fynbos Biome.

2.1 Abstract

Many plant–animal interactions can be challenging to directly observe, due to species being small, cryptic and/or nocturnal. Previous research on seed predation and dispersal by rodents in the Fynbos Biome of South Africa has relied on indirect evidence, as methods for directly monitoring rodent–seed interactions were not available. The aims of the study were to determine which resident small mammals scatter-hoard nuts and the geographic, seasonal and taxonomic extent of scatter-hoarding in the Fynbos Biome. We used camera traps focused on seed stations at eight sites in the Fynbos Biome to determine the responses of small mammals to tagged nut-like fruits (nuts) of seven endemic plant species belonging to the Proteaceae (n = 3), Rosaceae (n = 2), Restionaceae (n = 1) and Cupressaceae (n = 1), as well as commercial sunflower seeds. We found *Acomys subspinosus* and *Gerbilliscus paeba* scatter-hoarded nuts, which they typically carried and buried individually. *Rhabdomys pumilio* and *Micaelamys namaquensis* only consumed nuts. *Leucadendron pubescens* and *L. loranthifolium* are added to the list of known plant species that are scatter-hoarded by rodents. Nuts of *Cliffortia cuneata* and *C. phillipsii*, and the critically endangered *Widdringtonia cedarbergensis*, were consumed but not dispersed by small mammals, whereas nuts of *Ceratocaryum argenteum* were neither consumed nor scatter-hoarded by rodents (within its native range). *Gerbilliscus paeba* and *A. subspinosus* scatter-hoarded nuts aseasonally, outside of seed-fall periods. Scatter-hoarding was widespread throughout the Fynbos Biome, although it was highly localised across and within sampled sites. The absence of scatter-hoarding rodents at sites with rodent-dispersed plants remains an important aspect for future investigation.

2.2 Introduction

Seed predators and scatter-hoarding animals may play a major role in the fate of seeds, both as consumers and dispersers, in many different habitats (Janzen, 1971; Vander Wall, 1990; Wang & Smith, 2002). The Fynbos Biome of South Africa is a global Mediterranean-climate ecosystem hotspot of extraordinary botanical diversity and endemism (Rouget et al., 2014; Cowling et al., 2015). Despite the exceptional floral diversity of the Fynbos Biome, there is a general paucity of literature on plant–animal interactions (le Maitre & Midgley, 1992; Anderson et al., 2014). Small mammals are thought to have a major impact on fynbos plant species and communities due to seed predation (Bond, 1984; Bond & Breytenbach, 1985; Botha, 1989; van Hensbergen et al., 1992; Christian, 2001), particularly in the Proteaceae, and have generally been seen as antagonists to plants. More recently, research suggests that two species of small mammals are dispersers of fynbos plant seeds (Midgley et al., 2002; Midgley & Anderson, 2005; Rusch, Midgley & Anderson, 2014).

Identifying interactions of small mammal species with seeds is challenging, as most are nocturnal (Carleton & Happold, 2013) making them difficult to observe. Previous research on seed fates in the Fynbos Biome was based on indirect circumstantial evidence garnered from (often very limited) small mammal trapping during or after seed removal trials, with no direct observation or confirmation of which species were actually consuming or scatter-hoarding nuts. In more recent studies, laboratory arena trials and/or small mammal trapping adjacent to seed trial sites has typically been used to identify which small mammals may have been seed predators and/or scatter-hoarders (Midgley et al., 2002; Midgley & Anderson, 2005; Rusch, Midgley & Anderson, 2013a). Such captive studies are problematic as they may not reflect animals' behaviours in the wild or account for differences in the trapability/detectability of species.

Recently, camera traps have been used successfully to study the behaviour of nocturnal and diurnal rodent pollinators in the Fynbos Biome (Hobbhahn & Johnson, 2013; Melidonis & Peter, 2015; Steenhuisen et al., 2015; Zoeller et al., 2016) and granivorous rodents globally (Roth & Vander Wall, 2005; Jansen et al., 2012; Warzecha & Parker, 2014; Moore & Vander Wall, 2015). Camera trapping is a non-invasive and efficient method to study trap-shy and nocturnal species in all weather conditions, and allows observations of small mammal activity and behaviour without any disruptive effects (Seufert, Linden & Fischer, 2010). Furthermore, camera traps are easy to set up and can function independently for as long as battery-life and digital storage space lasts, making them ideal for the study of small and cryptic species.

Midgley and Anderson (2005) suggested that *Acomys subspinosus* (Waterhouse, 1838) and *Gerbilliscus paeba* (A. Smith, 1836; previously assigned to the genus *Gerbillurus* but now included in *Gerbilliscus*; see Knight et al., (2013) and Monadjem et al. (2015)) were the only two scatter-hoarding small mammal species in fynbos, and that they scatter-hoard the nuts of *Leucadendron sessile* (Proteaceae) and *Willdenowia incurvata* (Restionaceae), respectively. They also found burial of *Ceratocaryum argenteum* (Restionaceae) nuts, although they did not have the appropriate data to speculate on the burial agent (Midgley & Anderson, 2005). Recently, Midgley et al. (2015) and Midgley and White (2016) found that *C. argenteum* exploits the sensory perception of dung beetles by mimicking antelope faeces, thus ensuring effective seed dispersal. Midgley and Anderson (2005) hypothesised that there are probably between 60 and 100 plant species with seed traits that suggest they are rodent dispersed in the Proteaceae and Restionaceae families alone. However, this hypothesis remains untested as there have hitherto been no direct observations of resident small mammals scatter-hoarding behaviour in the wild.

This study presents camera-trapping evidence documenting responses of resident small mammals to provisioned nuts of seven endemic fynbos plants. The primary aim was to ascertain unequivocally which species scatter-hoard nuts, their scatter-hoarding behaviours and the geographical and seasonal extent of scatter-hoarding in the Fynbos Biome. Such evidence is vital to improving understanding of the important roles small mammals are purported to play as seed predators, dispersers and hoarders in fynbos. Secondary aims were to investigate the responses of other resident small mammals to Fynbos nuts and to determine whether any additional plant families and species with nut-like fruits are dispersed and buried by small mammals.

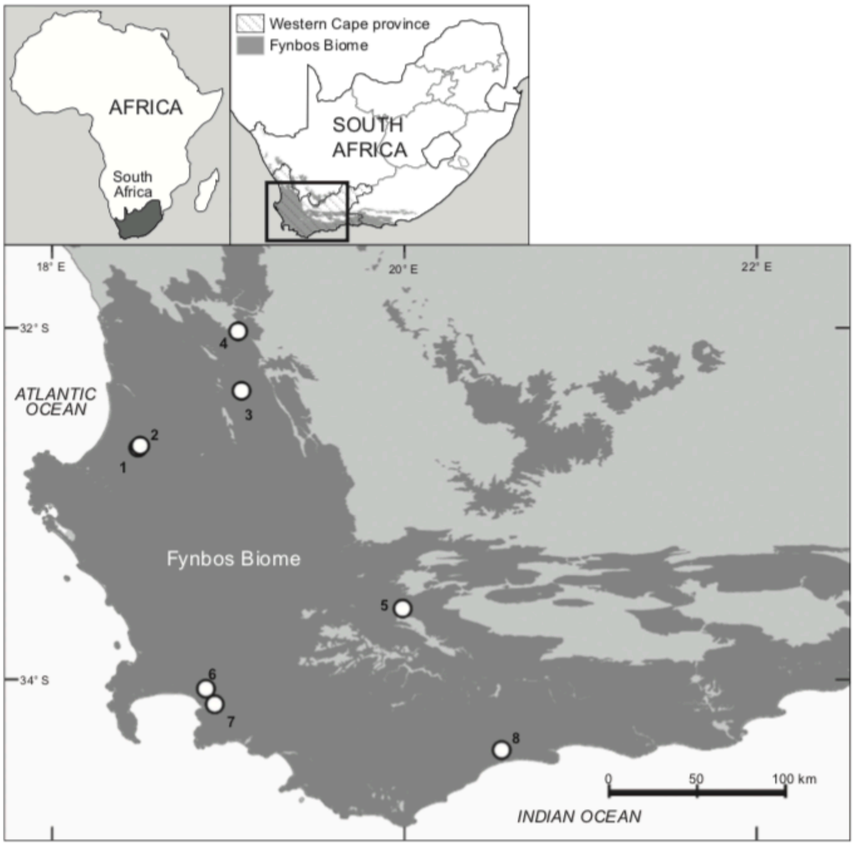


Figure 2.1 Location of the field sites used within the Fynbos Biome, South Africa. 1 = Aurora, 2 = Aurora Mountain, 3 = Middelberg, Cederberg Wilderness Area, 4 = Wolfdrif, 5 = Drie Kuilen Nature Reserve, 6 = Helderberg Nature Reserve, 7 = Sir Lowry's Pass (see Midgley et al. (2002)) and 8 = De Hoop Nature Reserve (see Midgley and Anderson (2005)).

2.3 Methods

Field sites

The study was conducted between 2013 and 2015 at sites spread across the expanse of the Fynbos Biome in the south-western Cape, South Africa. The Fynbos Biome is comprised of fire-prone shrublands, restricted to the low nutrient soils of the south-western Cape (Figure 2.1). It experiences a Mediterranean-type climate, with hot and dry austral summers (December-February) and mild, wet winters (June-August) (Mucina & Rutherford, 2006). We sampled eight field sites spread across the south-western Cape (Table 2.1), including two sites used in previous studies (Sir Lowry's Pass – Midgley et al. (2002); Rusch et al., (2013a); De Hoop Nature Reserve – Midgley and Anderson (2005)) and six new sites. Small mammal diversity varies topographically in the Cape mountains (Bond, Ferguson & Forsyth, 1980), therefore field sites included a range of topographies, including highland, mid-slope and lowland vegetation habitats. We selected sites on the basis of the presence of relatively extensive stands of indigenous nut-fruited plants. Field sites also included a mix of vegetation subtypes of the Fynbos Biome, as recognised by Mucina and Rutherford (2006). This included sand fynbos sites at Aurora, De Hoop Nature Reserve and Wolfdrif (lowland). Secondly, shale fynbos sites at Helderberg Nature Reserve and Sir Lowry's Pass (mid-slope). Lastly, we used sandstone fynbos sites at Aurora Mountain, Middelberg and Drie Kuilen Nature Reserve (highland) (Figure 2.1).

Fynbos nuts

To investigate small mammal responses to nuts of indigenous fynbos plants, we provisioned nut-like fruits (mean mass with husk in grams ± SD, *n* = 20) of the following plants at different field sites (see Table 2.2): *Leucadendron sessile* (0.20 ± 0.02), *L. loranthifolium* (0.22 ± 0.03), *L. pubescens* (0.27 ± 0.05) (Proteaceae), *Cliffortia cuneata* (0.05 ± 0.01), *C. phillipsii* (0.08 ± 0.01) (Rosaceae), *Ceratocaryum argenteum* (0.71 ± 0.09) (Restionaceae) and *Widdringtonia cedarbergensis* (0.09 ± 0.04) (Cupressaceae). As many of these nuts have thick seed coats with presumably high processing costs, we also provisioned seed stations with commercial sunflower seeds (0.05 ± 0.01) that offer a smaller reward with a very low processing cost. Furthermore, using commercial sunflower seeds provided the possibility to find a generic, non-invasive nut for use in future studies on seed interactions. Where possible, we provisioned nuts that are indigenous to the site at seed stations (see Tables 2.1 and 2.2). At Wolfdrif and De Hoop Nature Reserve, where indigenous nuts for harvesting were not available at the time of study, we used a combination of *L. sessile* and *C. argenteum* nuts and sunflower seeds, whereas at Drie Kuilen Nature Reserve we used *L. sessile* nuts.

Camera traps

We used one Ltl Acorn 6210M (Shenzen LTL Acorn Electronics Co., Shenzen, Guangdong, China) motion-activated camera trap to monitor the responses of small mammals to indigenous nuts at each seed station. To minimise disruption to nocturnal small mammal behaviour, the cameras emit 940 nm infrared 'black' flashes (Rovero et al., 2013). We focused cameras on tripods from at least 1 m away and set to record a 60-second-long video (maximum length), preceded by one to three photographs per activation event and no interval set between photograph/video sets. An 'observation' was considered as an animal having a novel encounter with a nut (ignored, inspected but not removed, eaten, or removed). Observations in which the animal consumed or removed the nut from a seed station were classed as interactions. Thus a single observation could be

spread over several videos or photographs: for example, if a small mammal consumed a nut *in situ* for 60+ s. Alternatively, a 60-second-long video sometimes contained several interactions: for example, if a small mammal consumed more than one nut *in situ* within 60 s. Once all provisioned nuts were consumed and/or removed from a site, we removed seed stations and no further observations determined. Using the above definitions, we recorded a total of 696 observations over 27 d at eight field sites.

Table 2.1 Summary of habitat and sample sizes at each respective site used in the study

Site	GPS Location	Altitude (m)	Fynbos type	Nut-fruited plants present at site	Date	No. of stations	Nut species	No. of nuts per station
1) Aurora	32° 40.163'S; 18° 29.995'E	216	Sand	*L. loranthifolium,* *L. pubescens*	16 Oct 2014	12	*L. sessile*	10
					3 Aug 2014	33	*L. sessile*	10
					30 Jan 2015	10	*L. loranthifolium*	10
					30 Jan 2015	10	*L. pubescens*	10
2) Aurora Mountain	32° 40.871'S; 18° 29.328'E	548	Sandstone	*L. loranthifolium*	30 Jan 2015	18	*L. loranthifolium*	10
3) Middelberg	32° 21.455'S; 19° 4.506'E	1165	Sandstone	*W. cedarbergensis*	10 Apr 2014	14	*W. cedarbergensis*	20
4) Wolfdrif	32° 1.155'S; 19° 3.374'E	288	Sand	*L. pubescens*	22 May 2014	49	*L. sessile* *C. argenteum* Sunflower	5 of each at same station
5) Driekuilen NR	34° 24.046'S; 20° 33.118'E	1298	Sandstone	*L. barkerae*	2 Oct 2014	10	*L. sessile*	10
6) Helderberg NR	34° 3.127'S; 18° 52.401'E	245	Shale	*C. cuneata,* *C. phillipsii*	25 Nov 2014	10	*C. cuneata*	10
						10	*C. phillipsii*	10
					12 Dec 2014	10	*C. cuneata*	10
						10	*C. phillipsii*	10
7) Sir Lowry's Pass	34° 8.428'S; 18° 55.407'E	228	Shale	*L. sessile*	1 Apr 2014	10	*L. sessile*	10
8) De Hoop NR	34° 24.046'S; 20° 33.118'E	142	Sand	*C. argenteum*	3 May 2013	11	*L. sessile* *C. argenteum* Sunflower	3 of each at same station

Small mammal responses

We typically provisioned seed stations with 10 nuts per station, with three exceptions. Firstly, at Middelberg, each station was provisioned with 20 *W. cedarbergensis* nuts. This was due to the inaccessibility of the sites, where only seven camera traps could be carried to high altitudes.

Secondly, Wolfdrif stations were supplied with five nuts of each of *L. sessile*, *C. argenteum* and sunflower at every station, and lastly at De Hoop Nature Reserve seed stations had the same three nut types but with only three of each. The number of seed stations per site varied according to availability of time and equipment, but always had a minimum of 10 stations (number of seed stations per site can be seen in Table 2.2). To determine the fate of seeds removed from seed stations, we attached 10 cm lengths of ultraviolet-reflecting thread onto nuts with fast-setting non-toxic glue (see Midgley et al. (2012)). To minimise repeated measurements of the same small mammal individuals, we placed seed stations 30–50 m apart. We monitored seed fates over a minimum of 3 d and up to 5 d at each site. We located removed seeds after sunset or before sunrise by walking in a spiral outwards for up to 10 m away from seed stations while searching for threads with an ultraviolet-emitting LED torch. When seed husk fragments were found attached to strings, the fate was considered as consumed. The fate of located seeds was classified as: consumed *in situ*, removed and consumed (together summarised as consumed in Table 2.2), or removed and buried (buried in Table 2.2) and the distance moved was recorded. Based on camera trap observations, we calculated the percentage of nuts that small mammals discovered at each site. Discovered nuts are here defined as the number of nuts first encountered by a small mammal at a seed station. Of the number of nuts discovered, we then calculated the percentage of nuts removed and remaining. The percentage of nuts removed was then split into the percentage of nuts consumed, buried or unrecovered (i.e. either the string was not located or there was no nut attached to the located string).

Observations from the camera traps allowed identifications of small mammal species and documentation of their basic behavioural responses to nuts. Small mammals were identified primarily on relative size, body to tail length ratio and species distribution data. The number of observations per seed station per day was calculated by dividing the total number of observations of each small mammal species at a site, first by the number of total seed stations presented and then by the number of days the cameras were recording for in the field. Camera trap observations, when accompanied by data on the fate of relocated nuts, allowed us to categorise the responses of resident small mammals to nuts as either ignored, inspected, consumed, scatter-hoarded or larder-hoarded. At no stage did small mammal species overlap spatially or temporally at seed stations, removing the possibility of erroneously classifying behaviours to different species. However, we were unable to discriminate between individuals of the same species.

Scatter-hoarding in the Fynbos Biome

When we located caches, the depth buried and number of nuts per cache was recorded. Burial depth is related to fire survival (Moore & Vander Wall, 2015) and nuts per cache reflects behaviour of the small mammal scatter-hoarders. The presence of more than one nut per scatter-hoarded cache (Rusch, Midgley & Anderson, 2013b) may have significant implications in the context of intra- and interspecific cache pilferage and consequently soil seed-bank dynamics. We determined the numbers of nuts removed per visit to seed stations from observations on camera traps.

Data analysis

All statistical analyses were performed in R (R Core Team, 2018a). To determine our primary and secondary aims, we tested for differences in seed consumption and burial (defined as the proportion of seeds consumed or buried of the total seeds discovered at each station) by fitting generalised linear mixed-effects models (GLMMs). The proportion of seeds consumed or buried from each station was the response variable (success/failure) and sites and stations were included as random effect factors. The glmmPQL function from the MASS package was applied for the regression. We tested for differences in seed consumption and burial between all small mammals that interacted with seeds. Only *G. paeba* was considered for tests of seasonal behaviour differences and seed interactions, as other species did not have the necessary sample sizes. Seed removal distances and fates were compared using generalised linear models. The package 'multcomp' was used to test for pairwise comparisons between factors at the 95% confidence level.

2.4 Results

Small mammal responses

All GLMM fixed effects were found to be significant predictors of consumption and burial of nuts (see Supplementary Tables 2.1 and 2.2). *Acomys subspinosus*, *G. paeba*, *Micaelamys namaquensis* (A. Smith, 1834) and *Rhabdomys pumilio* (Sparrman, 1784) were the only four small mammals observed interacting with nuts across all eight surveyed sites (Figure 2.2, Table 2.2). Cape rock elephant shrews *Elephantulus edwardii* (A. Smith, 1839), which are predominantly insectivorous (Perrin & Rathbun, 2013), and vlei rats *Otomys irroratus* (Brants, 1827), which are folivorous (Taylor, 2013), were both observed at four sites (Figure 2.2, Table 2.2) but ignored the provisioned nuts.

Acomys subspinosus (Figure 2.2a) interacted with nuts of *L. loranthifolium* at only the Aurora Mountain site. However, interactions were relatively rare (0.83 observations per seed station per day) and a small proportion (22%) of nuts were removed (Table 2.2). Of the removed nuts, 20% were buried and 10% consumed (Figure 2.3a and b, see Supplementary Table 2.1 for further information). Buried nuts were moved 2.03 ± 0.24 m (mean ± SE) from the seed stations (Figure 2.4) and were buried 2.06 ± 0.40 cm deep in the soil (Figure 2.5). Of the nuts that were removed, 70% of nuts were unrecovered (Table 2.2). It was only observed to visit field stations at night.

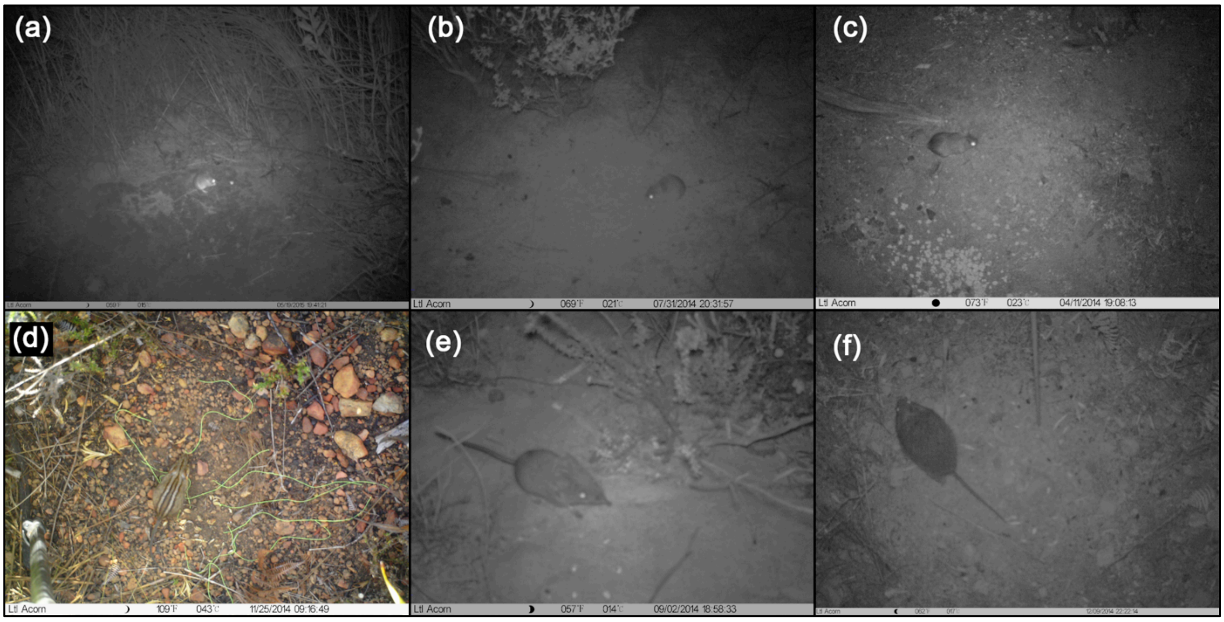


Figure 2.2 Photographs taken by remotely activated cameras of resident small mammals (a) *Acomys subspinosus* at Aurora Mountain, (b) *Gerbilliscus paeba* at Wolfdrif, (c) *Micaelamys namaquensis* at Middelberg, and (d) *Rhabdomys pumilio* at De Hoop Nature Reserve, and common small mammals that showed no interest in nuts (e) *Elephantulus edwardii* at Drie Kuilen Nature Reserve and (f) *Otomys irroratus* at Helderberg Nature Reserve.

Gerbilliscus paeba (Figure 2.2b) was observed only at night at two of the sand fynbos sites (Aurora and Wolfdrif) where it was the dominant small mammal visitor (2.16–2.55 and 1.83 observations per seed station per day, respectively) (Table 2.2). At Aurora, it was found to regularly bury local indigenous *L. loranthifolium* (21.1%), *L. pubescens* (17.8%) and non-native *L. sessile* (24.2%) nuts during three separate seed trials (August, October 2014 and January 2015; Figure 2.3f, Supplementary Table 2.1). Nuts of sunflowers (8.3%) and *L. sessile* (4.3%) were scatter-hoarded at Wolfdrif in May 2014 (Figure 2.3f). Seed husks were rarely found at both sand fynbos sites whether in situ and/or after seed removal, as reflected by the low percentage of consumed seeds (Figure 2.3e). *Gerbilliscus paeba* moved nuts 4.93 ± 0.29 m (Figure 2.4) and buried them 1.94 ± 0.10 cm deep (Figure 2.5). At Wolfdrif and Aurora *L. sessile* and sunflower seed husks were occasionally found in burrows (>15 cm deep) after removal by *G. paeba* (*n* = 23 at 18 burrows in total at both sites), but no intact nuts were ever found in burrows. *Gerbilliscus paeba* seed removal was difficult to locate and consequently had high percentages of unrecovered nuts (range: 35.1–82.2%) (Table 2.2).

Micaelamys namaquensis (Figure 2.2c) was a nocturnal visitor to seed stations at four sites in rocky, shale or sandstone fynbos. It was most frequently observed at Aurora Mountain and Middelberg, with fewer observations at Drie Kuilen Nature Reserve and Helderberg Nature Reserve (1.17, 1.95, 0.6 and 0.43–0.53 observations per seed station per day, respectively) (Table 2.2). *Micaelamys namaquensis* generally had moderate rates of seed discovery (*L. sessile* 20%, *L. loranthifolium* 16.7%, *W. cedarbergensis* 57.1%, *C. cuneata* 40% and *C. phillipsii* 20%). However, all nuts that were located after removal by *M. namaquensis* were consumed, with no burials (Figure 2.3a and b, Supplementary Table 2.1) and moved short distances (0.84 ± 0.07 m) (Figure 2.4). The percentage of unrecovered nuts was typically low for *M. namaquensis* (range: 6.7–30%) (Table 2.2).

Rhabdomys pumilio (Figure 2.2d) was the only diurnal granivore observed at six sites across the Fynbos Biome (Table 2.2), in a variety of habitats including lowland sand, mid-slope shale and highland sandstone fynbos sites (Table 2.2). It was the only small mammal observed to interact with nuts at De Hoop Nature Reserve and Sir Lowry's Pass (1.01 and 1.2 observations per seed station per day, respectively), where all discovered *L. sessile* nuts (81.8% and 50%, respectively) were consumed (Table 2.2).

Table 2.2 Summary of seed fates (percentage discovered by small mammal, percentage removed and of this the percentage consumed, buried and unrecovered) and main small mammal responses to Fynbos nuts at eight sites in the Fynbos Biome. (See following page)

Small Mammal Species	Observations /Seed pile/Day	Plant Species	Number of nuts presented at site (number of seed stations)	% Discovered	Of % Discovered		Of % Removed			Main Small Mammal Response
					% Remaining	% Removed	% Consumed	% Buried	% Unrecovered	
Acomys subspinosus	0.83	_L. loranthifolium (2)_	180 (18)	22.2	0	100	10	20	70	Consumed; Scatter-hoarded
Micaelamys namaquensis	1.17	_L. loranthifolium (2)_	180 (18)	16.7	26.7	73.3	66.7	0	6.7	Consumed
	1.95	_W. cedarbergensis (3)_	280 (14)	57.1	24.4	75.6	63.8	0	11.9	Consumed
	0.60	_L. sessile* (5)_	100 (10)	20	0	100	70	0	30	Consumed
	0.53	_C. phillipsii (6)_	200 (20)	20	0	100	75	0	25	Consumed
	0.43	_C. cuneata (6)_	200 (20)	40	15	85	66.3	0	18.8	Consumed
Gerbilliscus paeba	2.16	_L. sessile* (1)_	450 (45)	95.6	17	83	0.5	24.2	58.4	Consumed; Scatter-hoarded
	2.30	_L. pubescens (1)_	100 (10)	90	0	100	0	17.8	82.2	Consumed; Scatter-hoarded
	2.55	_L. loranthifolium (1)_	100 (10)	90	0	100	0	21.1	78.9	Consumed; Scatter-hoarded
	1.83	_L. sessile*_ (4)	245 (49)	75.5	31.4	68.6	8.1	4.3	56.2	Consumed; Scatter- and Larder-hoarded
		Sunflower** (4)	245 (49)	75.5	11.4	88.6	35.7	8.1	44.9	Consumed; Scatter- and Larder-hoarded
		C. argenteum* (4)	245 (49)	75.5	61.6	38.4	0	3.2	35.1	Inspected; Ignored; Scatter-hoarded
Rhabdomys pumilio	0.39	_L. loranthifolium (2)_	180 (18)	5.6	0	100	80	0	20	Consumed
	0.60	_L. sessile* (5)_	100 (10)	10	0	100	60	0	40	Consumed
	1.53	_C. phillipsii (6)_	200 (20)	55	0	100	100	0	0	Consumed
	0.53	_C. cuneata (6)_	200 (20)	40	32.5	67.5	67.5	0	0	Consumed
	1.20	_L. sessile (7)_	100 (10)	50	0	100	100	0	0	Consumed
	0.13	_L. sessile*_ (4)	245 (49)	5.4	100	0	0	0	0	Ignored
		Sunflower** (4)	245 (49)	5.4	80	20	20	0	0	Consumed
		C. argenteum* (4)	245 (49)	5.4	100	0	0	0	0	Ignored
	1.01	_L. sessile*_ (8)	33 (11)	81.8	40.7	59.3	59.3	0	0	Consumed
		Sunflower** (8)	33 (11)	81.8	0	100	100	0	0	Consumed
		C. argenteum (8)	33 (11)	81.8	100	0	0	0	0	Inspected; Ignored
Elephantulus edwardii	0.33	_L. loranthifolium (2)_	180 (18)	16.7	100	0	0	0	0	Ignored
	1.40	_L. sessile* (5)_	100 (10)	30	100	0	0	0	0	Ignored
	0.20	_L. sessile (7)_	100 (10)	10	100	0	0	0	0	Ignored
	0.02	_L. sessile*_ (4)	390 (78)	3.8	100	0	0	0	0	Ignored
Otomys irroratus	0.39	_L. loranthifolium (2)_	180 (18)	16.7	100	0	0	0	0	Ignored
	0.02	_L. sessile* (5)_	100 (10)	10	100	0	0	0	0	Ignored
	0.16	_C. phillipsii (6)_	200 (20)	15	90	10	10	0	0	Ignored; Consumed
	0.07	_C. cuneata (6)_	200 (20)	10	100	0	0	0	0	Ignored
	0.03	_L. sessile (7)_	100 (10)	10	100	0	0	0	0	Ignored
Mus minutoides	0.11	_L. loranthifolium (2)_	180 (18)	10	100	0	0	0	0	Ignored
Procavia capensis	0.29	_W. cedarbergensis (3)_	280 (14)	60	100	0	0	0	0	Ignored

*Indigenous to Cape fynbos but not to the site, **Not indigenous to Cape fynbos

1) Aurora, (2) Aurora Mountain, (3) Middelberg CWA, (4) Wolfdrif, (5) Driekuilen NR, (6) Helderberg NR, (7) Sir Lowry's Pass, (8) De Hoop NR

It also occurred at Helderberg Nature Reserve (0.53–1.53 observations per seed station per day, respectively) where 14% of *C. cuneata* were consumed. It predated heavily on *C. phillipsii*, consuming 55% of all experimental nuts. At Aurora Mountain, Wolfdrif and Drie Kuilen Nature Reserve (0.39, 0.13 and 0.6, observations per seed station per day), *R. pumilio* was less frequently observed and had a marginal influence on seed removal at these sites. *Rhabdomys pumilio* moved nuts short distances (0.77 ± 0.08 m) (Figure 2.4) and was never associated with buried nuts (Figure 2.3b, Supplementary Table 2.1).

Seed removal distances

Gerbilliscus paeba removed nuts significantly further than both *R. pumilio* and *M. namaquensis* (Figure 2.4). Nuts that *G. paeba* buried were moved significantly further than nuts it consumed. *Acomys subspinosus* consumed and buried nuts short distances from seed stations with no significant difference in distances between *R. pumilio* and *M. namaquensis* removal and nuts consumed by *G. paeba*, but data were limited for this species. Both *R. pumilio* and *M. namaquensis* typically moved nuts short distances to previously used eating sites under cover before consuming them.

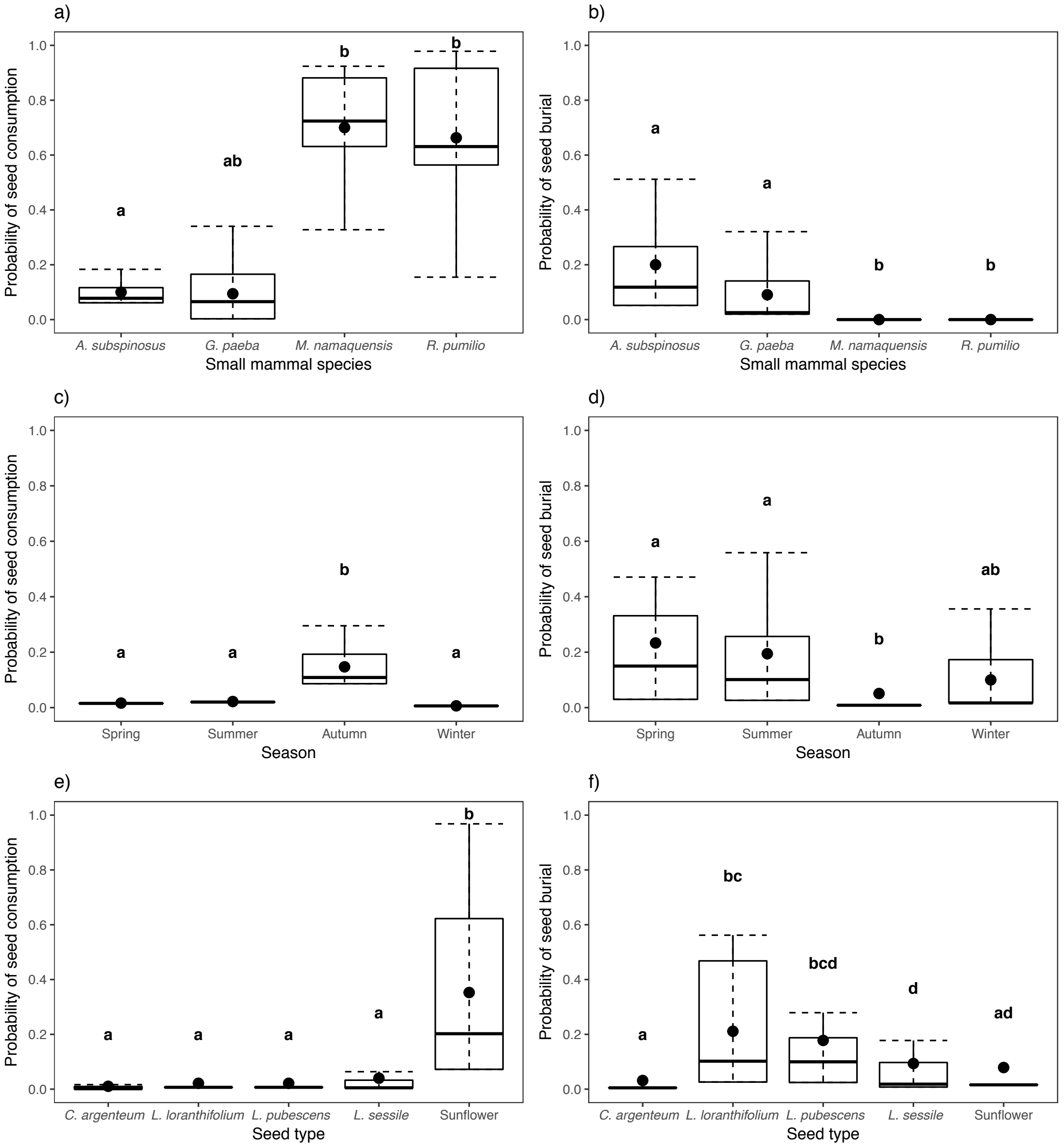


Figure 2.3 Predicted box-plots showing the probability of seed consumption and burial for all small mammal species (a and b) and for *Gerbilliscus paeba* by season (c and d) and seed type (e and f). Box-plots indicate the median, lower and upper quartiles and 1.5 × interquartile range. Means are shown as black dots. Different letters indicate a significant difference within factors of the modelled probability of seed consumption or burial ($p < 0.05$).

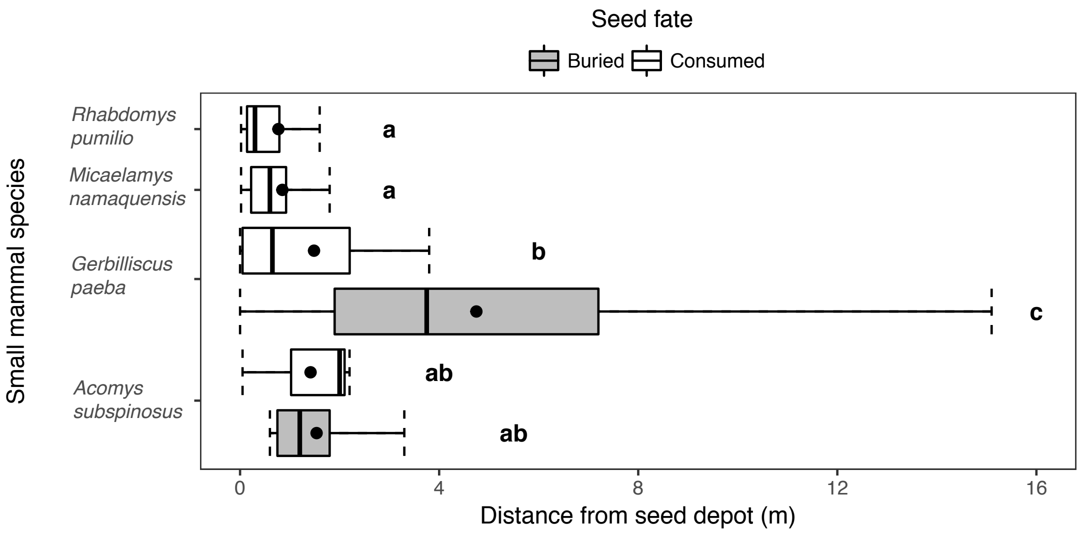


Figure 2.4 Box-plots of the distance (m) nuts were removed by small mammals from seed stations at all eight field sites. Removal distances are split for species that both consumed and buried nuts. Box-plots indicate the median, lower and upper quartiles and 1.5 × interquartile range. Means are shown as black dots. Different letters indicate a significant difference in the distance of removal between factors ($p < 0.05$).

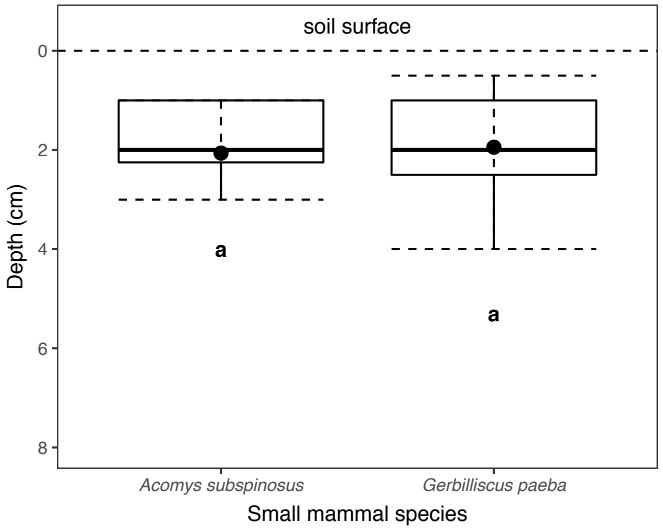


Figure 2.5 Box-plots of the depth (cm) of *Acomys subspinosus* and *Gerbilliscus paeba* caches at all sites. Box-plots indicate the median, lower and upper quartiles and 1.5 × interquartile range. Means are shown as black dots. Different letters indicate a significant difference between factors ($p < 0.05$).

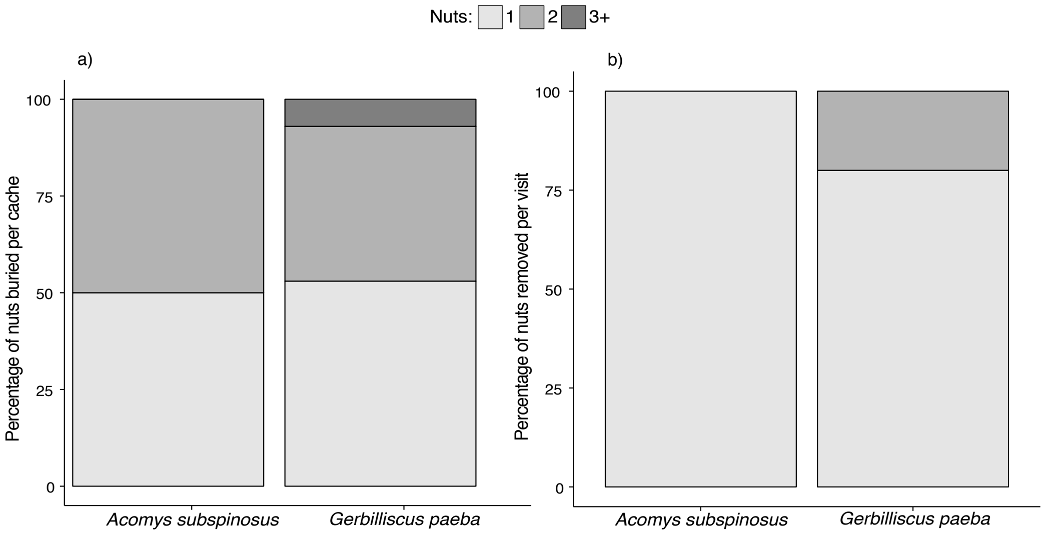


Figure 2.6 Percentage of (a) nuts buried per cache, and (b) nuts removed per visit to seed stations by *Acomys subspinosus* and *Gerbilliscus paeba* across all sites.

Scatter-hoarding in the Fynbos Biome

Averaged over all sites, *G. paeba* buried 53% of nuts removed from seed stations individually in caches, 40% in pairs and occasionally three or more nuts per cache (7%; see Figure 2.6a), whereas *A. subspinosus* was observed to bury nuts either singly or in pairs in equal proportions (Figure 2.6b). *Gerbilliscus paeba* typically removed one nut per visit (80%), but occasionally would remove two (20%), whereas *A. subspinosus* typically removed only one nut per visit (Figure 2.6b). The ratio of two-seeds-removed-per-visit:two-seeded-caches was 1:2 for *G. paeba*, indicating that some caches containing two nuts were made by repeated visits to the seed station(s). In some cases, the threads of two-seeded caches were tangled, which suggests these nuts were removed and buried simultaneously. At Aurora (August), *G. paeba* buried 18 two-seeded caches with tangled strings, indicating the two nuts were removed and buried together. These tangled string caches accounted for 62% of the observed two-seeded caches made by *G. paeba. Gerbilliscus paeba* cache burial depth (mean and 95% confidence interval) was 1.68 cm and 0.60–4.78 cm, whereas that for *A. subspinosus* was 1.80 cm and 0.44–7.26 cm (Figure 2.5).

2.5 Discussion

Camera traps

This study, and Weighill et al. (2017) (for *G. paeba* at Jakkalsfontein Private Nature Reserve), provides the first direct field evidence that *A. subspinosus* (this study) and *G. paeba* are indeed scatter-hoarders of Fynbos nuts at numerous sites (see Supplementary Video 2.1). We found three new sites where scatter-hoarding occurs (Aurora and Wolfdrif – *G. paeba*; Aurora Mountain – *A. subspinosus*) (Figure 2.1, Table 2.2), thereby confirming that scatter-hoarding is geographically widespread in fynbos, as suggested by Midgley and Anderson (2005). Although previous techniques (e.g. Midgley et al. (2002; Midgley and Anderson (2005); Rusch et al. (2013a)) did not provide irrefutable evidence that *G. paeba* and *A. subspinosus* are scatter-hoarders, our research provides conclusive evidence that they are. We demonstrated that two additional plant species (*Leucadendron loranthifolium* and *L. pubescens*) appear to be primarily dispersed by rodents. Both species are common throughout the arid western parts of the Fynbos Biome, suggesting that rodent dispersal is also widespread in the biome. *Leucadendron loranthifolium* represents a particularly interesting case of rodent dispersal, as it is the first plant species in the Fynbos Biome observed to be scatter-hoarded by two Cape scatter-hoarders in two different habitat types (sand and sandstone fynbos).

Seed predation in the Fynbos Biome

Rhabdomys pumilio and *M. namaquensis* are ubiquitous seed predators of Fynbos nuts (Table 2.2), confirming previous suggestions that *R. pumilio* is a major rodent seed predator and competitor of scatter-hoarding rodents (Rusch, Midgley & Anderson, 2014; Weighill, Huysamer & Anderson, 2017). As important granivores in fire-prone Mediterranean-like ecosystems, these ubiquitous rodents may reduce the reproductive success of plants via reduced recruitment and the depletion of soil seed banks essential for the recovery of plant communities after episodic fires (Bond & Breytenbach, 1985; Fraser, 1990;

Johnson, 1992; Rusch, Midgley & Anderson, 2014; Moore & Vander Wall, 2015) Neither species showed any indication of hoarding behaviour, suggesting that *A. subspinosus* and *G. paeba* are the only scatter-hoarding small mammals among the six species studied here. At field sites where *A. subspinosus* and *G. paeba* were not observed, seed predation by *R. pumilio* and *M. namaquensis* resulted in greatly reduced seed stocks with no dispersal benefits. This was the case for nuts of *W. cedarbergensis*, *C. cuneata*, *C. phillipsii* and *L. sessile*. These fynbos species appeared to be highly vulnerable to seed predation by small mammals. Interestingly, *C. argenteum* was the only Fynbos nut to not experience any seed consumption (Table 2.2). It therefore appears that *C. argenteum* nuts are inedible to resident small mammals, despite being scatter-hoarded by *G. paeba* at Wolfdrif, although this is outside of the native range of *C. argenteum*. Recent studies observed dung beetles rolling and burying the nuts and suggest this is an under-studied dispersal mechanism (Midgley et al., 2015; Midgley & White, 2016).

Scatter-hoarding in the Fynbos Biome

Acomys subspinosus buried nuts of *L. sessile* irrespective of season at Sir Lowry's Pass (Rusch, Midgley & Anderson, 2014). At Aurora Mountain, *A. subspinosus* buried *L. loranthifolium* nuts in January, approximately two months after natural seed fall during a two-week period in November. *Gerbilliscus paeba* buried a variety of nuts, including *C. argenteum*, *L. sessile* and sunflower seeds, none of which are native to the study sites where it occurs, across all seasons (Figure 2.3d). Rodents that interact with non-native nuts may show atypical behaviours due to the novelty of the interaction (Vander Wall, 1990; Forget, 1993). However, *G. paeba* showed similar magnitudes of discovery, removal and burial of native (*L. pubescens* and *L. loranthifolium*) and non-native (*L. sessile*) nuts (Table 2.2). *Gerbilliscus paeba* individuals would not have previously encountered the non-native nut species, yet still scatter-hoarded. *Acomys subspinosus* and *G. paeba* both scatter-hoarded nuts regardless of season or plant species, suggesting that scatter-hoarding behaviour in these species is innate and not a learned behaviour based on experience. This suggests individuals will respond innately and opportunistically when finding energy-rich seeds in arid, resource-limited environments.

A potential bias in our data stems from presenting rodents with seeds having high energetic rewards and low processing costs (such as sunflowers), which may alter seed caching choices influenced by satiation levels (Rusch, Midgley & Anderson, 2013a). This potential bias was clearly manifest in our data as consumption of sunflower seeds by both *G. paeba* and *R. pumilio* was markedly higher, and burial typically lower, than any of the nut-fruited *Leucadendron* species. Results using sunflower seeds as a generic 'scatter-hoarding' resource, in place of native nuts that cannot be collected, must therefore be treated skeptically. Although perhaps suitable for screening field sites for the presence of rodent seed predators (including scatter-hoarders), this proxy method is prone to underestimate seed dispersal by rodents.

Gerbilliscus paeba occasionally removed nuts into their burrows (mean ± SE: 15.6 ± 1.9 cm, n = 5). This suggests larder-hoarding behaviour, but if so this was brief as only seed husks were found within burrows when they were excavated the day following seed removal. Such occurrences may instead have been the result of predator avoidance behaviour (i.e. consumption in a safe refuge). Given that the strings used for relocating nuts were only 10 cm long and burrows were typically much deeper than this length, it is possible that the proportion of nuts taken into burrows was under-represented in the data set.

Acomys subspinosus was uncommon at most sites and only interacted with nuts sporadically in rocky, fynbos habitats (e.g. no interaction with nuts at Drie Kuilen Nature Reserve, Helderberg Nature Reserve, Middelberg and Sir Lowry's Pass). At Sir Lowry's Pass, we found no evidence for *A. subspinosus* scatter-hoarding nuts of *L. sessile*, even though it has been trapped in the vicinity before and seed burial by this species has been reported (Midgley et al., 2002; Rusch, Midgley & Anderson, 2014). However, seed trials were only done in autumn (April) and over a short period of time. If seed stations were left *in situ* for longer periods, the likelihood of interactions would increase. Rusch et al. (2014) reported that variation in seed dispersal and predation behaviour by *A. subspinosus* is seasonal, with a peak in caching in summer/autumn during seed fall and predation peaks in winter/spring when cached seeds are depleted (Rusch, Midgley & Anderson, 2014). Our results suggest that scatter-hoarding by this species may be extremely spatially-localised at this site (Midgley & Anderson, 2005), and that local *A. subspinosus* populations may be depleted throughout its entire range. Our camera trap set-ups proved sensitive enough to detect insects moving into the field of vision (see Midgley et al. (2015)), so concern of under-estimation due to animal size (Tobler et al., 2008; Rowcliffe et al., 2011) is unlikely to be pronounced in our data.

A possible explanation for *A. subspinosus* being rare is that local rodent population abundances are thought to fluctuate spatially and temporally (Bond, 1984; David & Jarvis, 1985). Therefore, if rodents are only present in a localised plant population ephemerally, to what degree do nut-fruited plants rely on small mammal dispersers or how many seasons of effective seed dispersal are required during inter-fire periods for successful recruitment?

Primary dispersal distances (Figure 2.4) and burial depths recorded for *A. subspinosus* and *G. paeba* (Figure 2.5) were commensurate with those reported by other studies (Midgley et al., 2002; Midgley & Anderson, 2005). *Gerbilliscus paeba* moved nuts far greater distances when scatter-hoarding compared with consuming nuts. Spacing of nuts prevents naïve conspecifics from cache pilfering (Stapanian & Smith, 1978) and will vary depending on food abundance (Moore et al., 2007). These findings, however, are likely an underestimate of overall dispersal distances as they do not account for the potential of nuts being relocated to multiple other caches over time, thereby greatly increasing total dispersal distances from parents (Jansen et al., 2002).

Both scatter-hoarding species tended to remove nuts singly, and bury them mostly in single-seed caches (Figure 2.6a and b) (Midgley et al., 2002; Rusch, Midgley & Anderson, 2013b). Weighill et al. (2017) found *G. paeba* had a mean of five seeds per cache, showing there is considerable geographical variation in scatter-hoarding behaviours. Both species had more two-seeded caches than observations of two nuts removed per seed station visit, suggesting that approximately half of the two-seeded caches of *G. paeba* were established after repeated removals of one nut from the seed station(s). This would require *G. paeba* to remember the location of its caches in the short term (e.g. burying two nuts in one cache over two visits), as well as in the long term (e.g. cache retrieval), suggesting well-developed spatial memory acuity.

Scatter-hoarding provides nut-fruited plants in fire-prone ecosystems with a subterranean refuge from seed predators and fire (Moore & Vander Wall, 2015). Fire also stimulates germination directly and indirectly in many fynbos seeds (le Maitre & Midgley, 1992), especially nut-fruited *Leucadendron* species, which require heat shock to crack the hard seed husk and allow diffusion of oxygen to the embryo (Brown & van Staden, 1973). Midgley et al. (2002) found burial depths of *L. sessile* nuts at Sir Lowry's Pass (concurrent with cache depths found in this study) to coincide with the depths that seedlings emerged. This suggests that dispersal and burial of nuts by Cape scatter-hoarders in fynbos is crucial to seedling recruitment in nut-fruited plants, similar to other Mediterranean-like, fire-prone ecosystems (Moore & Vander Wall, 2015). Therefore, the absence of scatter-hoarding rodents at sites with rodent-dispersed plants remains an important aspect for future investigation.

Conclusions

The discovery of scatter-hoarding in the Cape (Midgley et al., 2002; Midgley & Anderson, 2005; Rusch, Midgley & Anderson, 2013b) indicates that serotinous and myrmecochorous seeds did not only evolve simply to avoid seed predation and fire (Bond, 1984; Bond & Breytenbach, 1985). However, it is apparent that small mammal granivores, as shown in previous studies and here, have a major effect on the seed reserves of several fynbos species, with some, such as *C. cuneata*, *C. phillipsii* and *W. cedarbergensis*, receiving no clear dispersal advantage. Conversely, the nut-fruited *L. loranthifolium*, *L. pubescens* and *L.*

sessile appear to have evolved large nuts for scatter-hoarding by resident small mammals to ensure successful recruitment in a fire-prone habitat.

2.6 Acknowledgements

Thank you to T Davis, J van Blerk, J Hansen, L du Toit, J Blount, A Nash, K Vanmali and G Molzen for assistance with data collection. We thank the National Research Foundation for funding and, lastly, we thank CapeNature for a permit to work with small mammals (0056-AAA041-00132).

2.7 Supplementary Material

Supplementary Video 2.1 can be viewed at the following link:
https://doi.org/10.6084/m9.figshare.4810798.v1

Supplementary Table 2.1 Fitted coefficients, the standard errors and *P*-values of the generalized linear mixed-model for probability of seed consumption (1) and seed burial (2) for (A) all small mammal species, for *Gerbilliscus paeba* across (B) seed type and (C) seasons.

	Fixed effects	Probability	S.E.	n	*t*	*P*-value
1) Seed consumption						
A) Small mammal species						
All	*Acomys subspinosus*	0.100	0.025	4	-2.084	0.040
	Gerbilliscus paeba	0.094	0.008	170	-0.620	0.536
	Micaelamys namaquensis	0.700	0.037	32	-0.040	0.969
	Rhabdomys pumilio	0.662	0.039	39	0.466	0.643
B) Seed type						
Gerbilliscus paeba	*Ceratocaryum argenteum*	0.000	0.000	36	0.000	>0.999
	Leucadendron loranthifolium	0.000	0.000	9	0.000	>0.999
	Leucadendron pubescens	0.000	0.000	9	0.000	>0.999
	Leucadendron sessile	0.037	0.013	80	-3.747	<0.001
	Sunflower	0.367	0.056	36	-1.773	0.079
C) Seasons						
Gerbilliscus paeba	Spring	0.000	0.000	12	0.000	>0.999
	Summer	0.000	0.000	17	0.000	>0.999
	Autumn	0.149	0.010	109	-8.126	0.078
	Winter	0.000	0.000	27	0.000	>0.999
2) Seed burial						
A) Small mammal species						
All	*Acomys subspinosus*	0.200	0.094	4	-1.839	0.069
	Gerbilliscus paeba	0.091	0.008	170	-7.739	<0.001
	Micaelamys namaquensis	0.000	0.000	32	0.000	>0.999
	Rhabdomys pumilio	0.000	0.000	39	0.000	>0.999
B) Seed type						
Gerbilliscus paeba	*Ceratocaryum argenteum*	0.033	0.005	36	-4.858	<0.001
	Leucadendron loranthifolium	0.211	0.029	9	-4.104	<0.001
	Leucadendron pubescens	0.178	0.026	9	-4.390	<0.001
	Leucadendron sessile	0.099	0.008	80	-6.962	<0.001
	Sunflower	0.083	0.010	36	-4.400	<0.001
C) Seasons						
Gerbilliscus paeba	Spring	0.233	0.000	12	-3.053	0.003
	Summer	0.194	0.000	17	-4.181	<0.001
	Autumn	0.053	0.000	109	-8.357	0.078
	Winter	0.100	0.000	27	-6.429	<0.001

Supplementary Table 2.2 Wald test of fixed effects of the generalized linear mixed-model for probability of seed consumption (1) and seed burial (2) for (A) all small mammal species, for Gerbilliscus paeba across (B) seed type and (C) seasons.

Fixed effects		X^2	Df	*P*-value
1) Seed consumption				
	A) Small mammal species	8.8	3	0.032
	B) Seed type	92.5	4	<0.000
	C) Seasons	21.2	3	<0.000
2) Seed burial				
	A) Small mammal species	12.9	3	0.004
	B) Seed type	9.9	4	0.041
	C) Seasons	13.2	3	0.004

Chapter 3

Dispersal of semi-fleshy fruits to rocky crevices by a rock-restricted rodent

---

3.1 Abstract

Seed dispersal allows successive generations of plants to be mobile in space and time. *Heeria argentea*'s unusual fruit and its ubiquity in extremely rocky habitats, suggests that this tree requires a specialist disperser. We therefore investigated the dispersal ecology of *H. argentea* and *Hartogiella schinoides*. We found *M. namaquensis* rapidly removed *H. argentea* and *H. schinoides* fruits, moving them short distances within and between rock outcrops, and consumed only the pericarps. Birds were observed consuming *H. schinoides,* but not *H. argentea* fruits, suggesting *M. namaquensis* is its sole, specialist disperser. Most *H. argentea* seeds (65%) with removed pericarps germinated successfully, while intact fruits did not. We show rock outcrops represent fire refugia, allowing *H. argentea* trees to grow to large sizes, with small stems and a co-occurring, wind-dispersed tree, *Widdringtonia nodiflora* found away from these sites. This rodent–tree mutualism is perhaps the clearest global example of directed dispersal and shows that these endemic trees are highly adapted for survival in the southwestern Cape habitat and are not tropical relicts.

Significance:

- The fruits of rock-restricted Cape trees are directly dispersed by rock rats to rock outcrops. This is the first description of rodent dispersal of fleshy fruits in South Africa.
- This species-specific interaction allows for rapid germination of seeds and protection from frequent fires for adults. This rodent–tree mutualism is perhaps the clearest global example of directed seed dispersal.

3.2 Introduction

Seed dispersal allows plants, via their seeds, to be mobile in space and time. The spatio-temporal locations at which seeds arrive have a major influence on the subsequent fitness of offspring, as location is a major determinant of seed and seedling survival. Many small patches of fire-avoiding forest exist in a wider sea of fire-prone shrublands and savanna across South Africa. In the southwestern Cape, these forests are restricted to growing in extremely rocky habitats, such as on cliffs and amongst rock outcrops and screes (Moll, McKenzie & McLachlan, 1980).

The dynamics of these forests are different to that of the adjacent shrublands (known as fynbos) where post-fire recruitment from long-lived seed banks of non-fleshy fruits dominates (le Maitre & Midgley, 1992). For example, many Proteaceae as well as *Widdringtonia nodiflora* (mountain cypress; Cupressaceae) are serotinous trees with dry, wind-dispersed seeds that grow in the open shrublands. In contrast, forest tree fruits are typically soft and non-dormant. *Heeria argentea* (rockwood) and *Hartogiella schinoides* (spoonwood) (sensu Islam et al. (2006)) are important constituents of these forests. *Heeria argentea* has pale, yellow-green, leathery-coated fruits up to 30 mm in diameter with a 3 mm thin, fleshy pericarp surrounding a single, soft, chlorophyllous seed (Figure 3.1a,b). Similarly, *H. schinoides* seeds are chlorophyllous but the fruit is smaller (diameter = 15 mm) and more visually distinct being a dark, red colour (Figure 3.1c,d).

Birds are unlikely dispersers of *H. argentea*, because the fruits are unattractive and unrewarding. Marloth (1925) suggested that *Procavia capensis* (rock hyrax) is the primary disperser of *H. argentea*. Presumably his main evidence was their overlapping distributions, both being rock-restricted, and the unusual fruit. Although there are no data, others have cited this hypothesis (von Teichman & van Wyk, 1996; van Wyk & van Wyk, 1997). We investigated the dispersal ecology of these fruiting species based on Marloth's hypothesis.

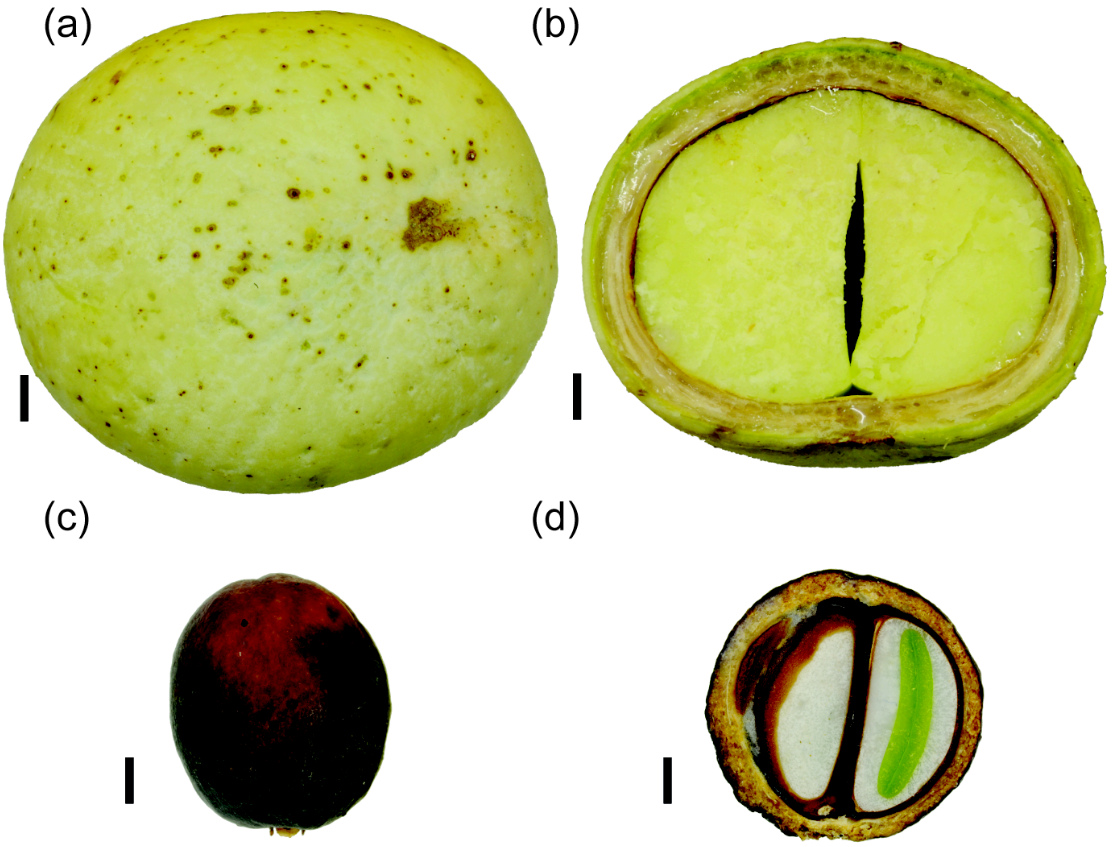


Figure 3.1 Intact and cross-sectioned fruits of (a, b) *Heeria argentea* and (c, d) *Hartogiella schinoides*, showing the thin, fleshy pericarps and chlorophyllous endosperm/embryo. Scale bar = 2 mm.

3.3 Methods

Data were collected in April and May 2016 at seven sites in the Limietberg Nature Reserve (33.620355°S, 19.106678°E) in the southwestern Cape, South Africa. *H. argentea* fruits were collected directly off trees in April 2016. The fruits ripen and fall to the ground over several months in the austral summer (personal observation). We placed 5–10 *H. argentea* fruits at depots for 3 days, with six depots per site (n_{total} = six sites; n_{total} = 330 fruits); four sites were in rock outcrops and two sites were in adjacent non-rocky proteoid shrublands. To locate moved fruits, each was attached to a reverse-wound bobbin with fast-setting non-toxic glue. At each depot (placed ± 50 m apart), we used a LTL Acorn 6210M remotely activated camera trap (Shenzen LTL Acorn Electronics Co., Shenzen, Guangdong, China) to monitor animal–fruit interactions. Cameras were set to record one photograph, immediately followed by a 30-s video. Each time an animal interacted with a novel fruit or entered/left the field of view it was considered a new event. For *H. schinoides,* we followed a similar, scaled-down experiment, placing out six depots at a single site with 10 fruits per depot. No camera traps were used in this experiment.

Germination trials were conducted on intact and handled *H. argentea* fruits (*n*=40 of each) over 6 weeks at the University of Cape Town's glasshouse. Fruits were placed in individual trays on the surface of a potting soil medium and watered once a week. We used a chi-squared test in the R programming language (R Core Team, 2018a) to compare overall germination of fruits.

We investigated fire impacts on *H. argentea* and *W. nodiflora* individuals 1 year post-fire. We measured the basal diameter (mm) of *H. argentea* and *W. nodiflora* adults in relation to distance within and from the outer edge of rock outcrops (defined here as a collection of rocks ≥2 m2). We estimated the fire damage by rating the proportion of the canopy dead in *H. argentea* and *W. nodiflora*. All individuals were surveyed within a 0.6-ha area that included both shrublands and rock outcrops. Beta regression (Cribari-Neto & Zeileis, 2010) in R was used to determine the relationships between tree size, distance to rock outcrops and fire damage. Reported values are mean ± standard deviation.

3.4 Results

Rock outcrops

Within only 3 nights, 6.63±3.85 fruits per depot were removed (total = 66%; *n*=159/240). Of these 159 fruits, 31% were located and collected, and 40% were not retrieved, but were tracked to a minimum distance until the thread either snapped or was dislodged. The remaining 29% were considered lost, likely as a result of thread failure.

Of the 159 moved fruits that left a thread trail, we determined that 90% of these had been moved at least 0.3 m into rock outcrops 3.26±3.77 m from the initial depot. Of these dispersed fruits, 92% had more than 75% of the pericarp removed (Figure 3.2a,b). None of the chlorophyllous seeds were visibly damaged. Seeds with pericarps did not germinate (*n*=0/40) whereas seeds with removed pericarps did (*n*=26/40, 65%; X2=31.5, d.f.=1, *p*<0.001; Figure 3.2c).

Figure 3.2 Cross-section of *Heeria argentea* fruits that are (a) intact and (b) with the pericarp removed showing the fleshy layer that Namaqua rock rats consume. (c) A germinated seed with the radicle breaking through a fruit without the leathery pericarp. Scale bar = 1 mm (a, b).

Despite observation of their droppings within 50 m of all sites, only one rock hyrax was recorded on camera at one depot and this individual did not interact with the fruits. Instead, *Micaelamys namaquensis* (Namaqua rock rat) was the most frequently viewed small mammal on camera trap observations (*n*=151) and was the only animal viewed removing fruits in the rock outcrop sites (*n*=100 events) (Supplementary Video 3.1). No bird visitation was documented. Fruits were typically moved out of the camera field of view but at one site we observed a Namaqua rock rat consuming the pericarp (Supplementary Video 3.2). We also viewed the following carnivores on the camera trap observations: *Genetta tigrina* (Cape genet) and *Galerella pulverulenta* (Cape grey mongoose), neither of which interacted with fruits.

Dispersal of *H. schinoides* appeared to follow the same pattern. Within 3 days, 80% of fruits (*n*total = 60) were removed and 58% of these were located (*n*=28), with 93% of those moved to rock outcrops and 68% had their full pericarp removed. We incidentally observed *Onychognathus morio* (red-wing starlings) feeding on *H. schinoides* fruits.

Shrublands

Fruits were rarely moved (2.25±2.31 per depot; 16% of *n*=90) at shrubland sites, with most located (*n*=11/14). Fruits occasionally showed the pericarp slightly consumed (*n*=11), with the pericarp never fully removed. All moved fruits were discarded in the open on the soil surface. Camera trap observations showed that the four small mammal species recorded usually ignored fruits (Supplementary Video 3.3). These species included *Rhabdomys pumilio* (four-striped grass mouse) (*n*=92), *Otomys irroratus* (vlei rat) (*n*=46) and *Acomys subspinosus* (Cape spiny mouse) (*n*=14), as well as occasionally *Hystrix africaeaustralis* (Cape porcupine), with only *R. pumilio* occasionally interested in *H. argentea* fruits (removing *n*=10 fruits).

Plant distribution and fire damage

Heeria argentea trees were distributed on the margin or within rock outcrops (64%), and 36% were found away from rock outcrops. Large adult *H. argentea* trees were typically found far within rock outcrops (-2.65±2.89 m) and lacked any fire damage. Small *H. argentea* trees were mostly found away from rock outcrops (7.83±8.94 m) and often received full top-kill from fire damage (53% of *n*=139 trees)(see Figure 3.3). *W. nodiflora* was never found within rocky refugia and always experienced full top-kill from fire damage. Both basal diameter (estimate ± s.d. = -0.04±0.01, z=-3.57, p<0.001) and distance relative to rock outcrops (0.06±0.01, z=4.58, p<0.001) were significant predictors of fire damage in *H. argentea* (pseudo R2 = 0.46).

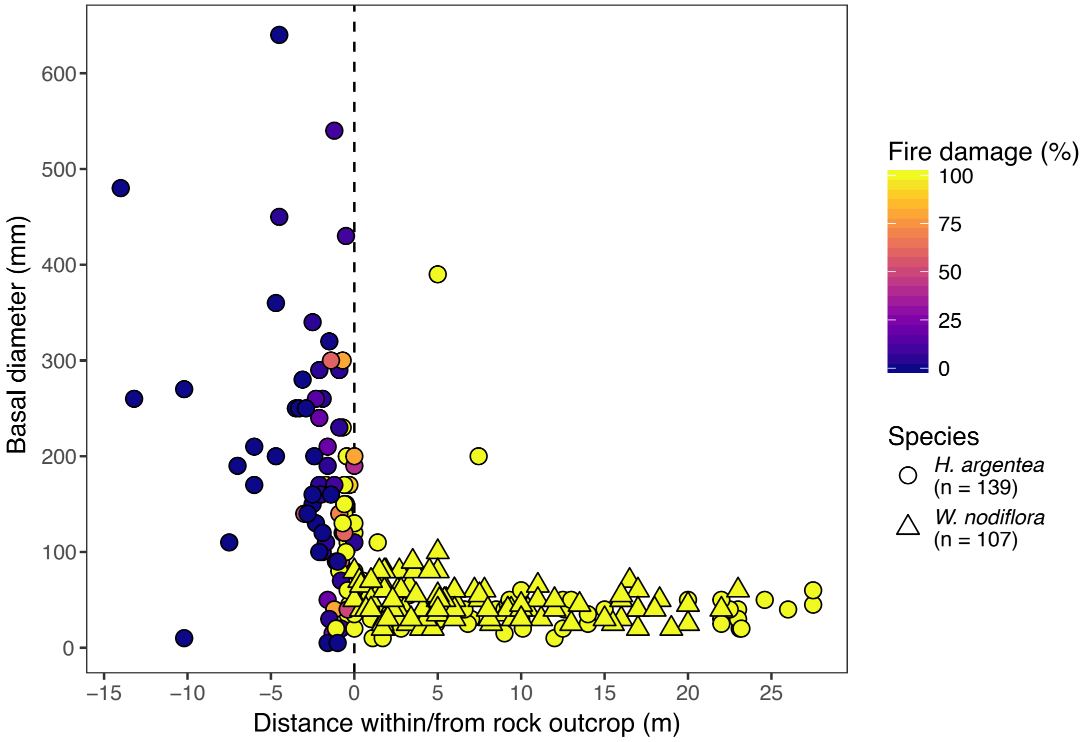


Figure 3.3 The proportion of canopy damage from a recent fire to *Heeria argentea* and *Widdringtonia nodiflora* trees of different sizes within (negative) and from (positive) rock outcrops.

3.5 Discussion

The rapid rate of fruit removal and pericarp consumption showed that the fruits of both *H. argentea* and *H. schinoides* are highly attractive and rewarding to Namaqua rock rats. Our findings thus lead to the rejection of Marloth's (1925) original hypothesis. As the seeds have no dormancy, germination is not stimulated by fire, or its products, unlike most seeds in fire-prone ecosystems. *H. argentea* is thus dependent on rock rats for dispersal and rapid initiation of germination. Avoiding predation by carnivores may explain why Namaqua rock rats take fruits to narrow and concealed crevices before consuming the pericarp layer.

The fruit dispersal patterns of *H. schinoides* also suggest Namaqua rock rat dispersal. Their fruits are smaller and dark red in colour – likely an adaptation for bird frugivory. We observed bird frugivory on *H. schinoides* fruits, but never observed birds feeding on *H. argentea*. The fruit of *Heeria* is likely too large for the gape of most local frugivorous birds and, as the seed is soft, pecking, biting or ingestion could destroy it. Further, birds would not likely disperse the seeds into crevices. As Namaqua rock rats also feed on *H. schinoides,* they are likely generalist frugivores.

The significance of dispersal to rock outcrops is not immediately apparent, as these may be poor sites for initial seedling growth. The large seed, and consequent large energy stores, of *H. argentea* may facilitate some growth in deep shade (Figure 3.4). Large *H. argentea* trees were mostly found within rocky refugia where they experienced almost no fire damage. In contrast, small stems were found away from fire refugia and generally experienced full canopy death. This difference suggests that individuals in less rock-protected sites are condemned to small size and likely reduced reproductive capacity as a result of frequent top-kill and consequent basal resprouting after repeated burning. Wind dispersal of *W. nodiflora* seeds appears to prevent arrival in rocky refugia because no individuals were observed within rock outcrops. Unlike *H. argentea* all *W. nodiflora* trees experienced full top-kill during the recent fire (Figure 3.3).

Figure 3.4 A *Heeria argentea* seedling emerges from a dark, rocky crevice at Limietberg Nature Reserve.

Aspects of the *H. argentea*–Namaqua rock rat interaction are unusual. Firstly, rodent dispersal of fleshy fruits is a novel dispersal mechanism in the Cape. Rodents may be dispersers of non-fleshy, dry seeds typically by scatter-hoarding (Vander Wall, 1990; White, Bronner & Midgley, 2017). Namaqua rock rat dispersal of *H. argentea* differs from most other small mammal dispersal. In other rodent frugivory cases, the fruits may be partially damaged (Steele et al., 1993; Mendoza & Dirzo, 2009; Shiels & Drake, 2011; Loayza et al., 2014). Globally, rodent frugivory, in which the seed is not ingested and left undamaged, has only been documented in a few studies (Feer et al., 2001; Shiels & Drake, 2011; Loayza et al., 2014). Exotic rodents have been documented to disperse seeds while only consuming the pericarp (Shiels & Drake, 2011). By consuming only the pericarp, Namaqua rock rats still achieve a reward while leaving the seed intact. Secondly, this is an example of directed dispersal in which fruits disproportionately arrive at non-random, fire-protected destinations that are highly favourable sites for survival (Wenny, 2001).

The three classic examples of directed dispersal are somewhat controversial. Mistletoe seedlings can only establish on thin, sun-exposed branches with only specific bird species, such as *Dicaeum hirundinaceum* (mistletoebirds), considered able to disperse seeds to these sites. That generalist birds, marsupials (Amico & Aizen, 2000), explosive seed release (deBruyn et al., 2015) and the wind (Tercero-Bucardo & Kitzberger, 2004) also effectively disperse mistletoe seeds weakens this argument. Myrmecochory (ant-dispersal) has been suggested as a mechanism for directed dispersal to ant nests (localised sites of high nutrients in nutrient-poor shrublands), but some evidence suggests otherwise (Rice & Westoby, 1986; Bond & Stock, 1989). Scatter-hoarding by rodents can be 'directed' to sites of low adult conspecifics (Hirsch et al., 2012). However, the benefit of these sites is escape from seed predators. *H. argentea* fruit dispersal is possibly the clearest global example of directed dispersal – too short-distance, site-specific and beneficial to established plants (rather than seedlings), to be explained by escape of seed or seedling predators or by colonisation of prime sites by seedlings (Wenny, 2001).

3.6 Acknowledgements

We thank Deon Rossouw of CapeNature for permission to work in Limietberg Nature Reserve. We also thank the South African National Research Foundation for funding. J.E. Quince provided assistance with data collection.

3.7 Supplementary Material

Supplementary Videos can be viewed at the following links:

- Supplementary Video 3.1: https://youtu.be/qOgylIJSKpI
- Supplementary Video 3.2: https://youtu.be/4jWIUrtzpUk
- Supplementary video 3.3: https://youtu.be/FtBmh-3oYi4

Chapter 4

The dispersal and germination of gifboom (*Hyaenanche globosa*: Picrodendraceae)

4.1 Abstract

Camera traps reveal that *Hyaenanche globosa* seeds were predominantly taken by *Acomys subspinosus*, a known scatter-hoarder. I found that some seeds are buried, presumably by *A. subspinosus*. In contrast, camera traps indicated that *Micaelamys namaquensis*, a known granivore, ignored seeds. *Hyaenanche globosa* is thought to be toxic, especially the pods and seeds. Therefore, directed toxicity may explain the latter observation but implies immunity by *A. subspinosus*. Cross-sections of seeds indicate a thin outer seed coat, then a pectin layer, a lipid layer and lastly a chlorophyllous embryo. Seeds germinate within five days after being soaked without need for cold treatment and more rapidly when the seed coat is experimentally ruptured. Germinating seeds produce copious mucilage, which is in part due to pectins. Pectin bodies of germinating seeds absorb water rapidly, increasing in volume by approximately 200-fold within five days. Water entry is mainly through the hilum. Seeds in which the hilum is blocked germinate more slowly.

4.2 Introduction

Hyaenanche globosa (Picrodendraceae) is a small tree up to 5 m tall and is endemic to a small mountainous area (the Gifberg, named after *H. globosa*) near Vanrhynsdorp in the Western Cape (Phillips, 1941). It is considered to be highly toxic; the common name gifboom is Afrikaans for poisonous tree and the generic name is derived from its supposed historical role in poisoning hyaenas.

There is some controversy over its toxic properties such as what the full range of active ingredients are and whether they are in pods or seeds (Momtaz et al., 2010). To date they are known to contain sesquiterpene lactones, hyaenanchin and tutin, the latter known to cause convulsions, delirium and coma in humans (Momtaz et al., 2010). There is some confusion in the literature in the terminology between pod, fruit and seed. Some studies have used entire *immature fruits* (Momtaz et al., 2010, Momtaz pers. comm.), while others have used *mature seeds* to isolate compounds (Henkel, 1858). Crushed seeds or seedpods were apparently added to carrion to kill predators by previous generations of livestock farmers (Watt & Breyer-Brandwijk, 1962). One of the earliest experiments on *H. globosa* showed the *immature fruits* are lethal when ingested by doves, rabbits and dogs (Henkel, 1858). Another common name used for the tree is wolveboontjie, meaning ′wolf seeds′ or more likely ′hyaena seeds′ (Marloth, 1925; Smith, 1955). This name suggests the *seeds* and not the fruits or pods were used to poison predators (Marloth, 1925).

Almost nothing is known about the ecology of the gifboom. For example, it has been reported that the seeds are black and shiny (see Phillips, 1941; Van Wyk et al., 2002). This is incorrect. The seeds resemble bean seeds and are a dull, red-brown colouration (Figure 4.1).

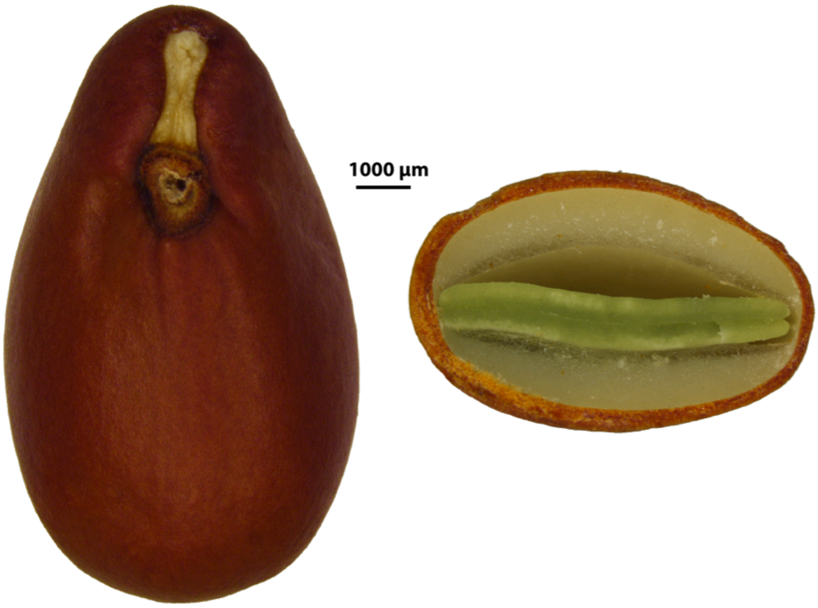


Figure 4.1 Cross-section of a *Hyaenanche globosa* seed showing the dull-red seed coat, hilum, epidermal layer, a fatty, pale lipid layer and the chlorophyllous endosperm.

Since the *H. globosa* fine-scale distribution is almost entirely restricted to rocks, as is *Heeria argentea* with which it co-occurs, my first question concerned whether this species is also dispersed to rocks by the Namaqua rock rat, *Micaelemys namaquensis* as was found by White and Midgley (2017). However, unlike *H. argentea* it has dry seeds, which are apparently toxic to mammals. The poisonous seeds could imply passive dispersal, as mammal granivores may be deterred. I also investigated the structure of the seed and its germination. The analysis of the seed indicates that, similar to *H. argentea*, it is chlorophyllous (Figure 4.1), which has not been reported before. Since the seeds are chlorophyllous they are likely to have minimal dormancy. This would be surprising for a species from such an arid area with a dry seed coat. Thus, my final question concerned germination of *H. globosa* seed. Therefore the hypotheses were 1) *H. globosa* seeds are dispersed to rocky sites by Namaqua rock rats; and 2) *H. globosa* seeds germinate rapidly without fire-related cues.

4.3 Methods

Data were collected in April 2017 on the Sewefontein farm on Gifberg (S31.737710°, E18.823065°) in the Western Cape, South Africa. *Hyaenanche globosa* is known from only one population, which occurs on the northern Bokkeveld Escarpment Mountain Plateau, in Bokkeveld Sandstone Fynbos (Mucina et al., 2014). The tree is found within a restricted elevation band, co-occurring with other rock-loving species, such as *H. argentea*, *Maytenus oleoides* and *Diospyros glabra*. The climate of the Gifberg is strongly Mediterranean, with predominantly winter rainfall and prolonged hot, dry summers. *Hyaenanche globosa* is multi-stemmed, presumably due to selective pressure from repeated disturbance, such as fire. It is dioecious and flowers in late spring (October to November), with the male flowers being conspicuously red despite being wind-pollinated. Large, 3-4 lobed fruits can be seen on plants year-round, as they take approximately 18 months to mature before seeds are dropped *en masse* in autumn (March).

To determine seed removal patterns and interactions with animals I used Ltl Acorn 6210M remotely activated trail cameras (Shenzen LTL Acorn Electronics Co., Shenzen, Guangdong, China) focused on seed piles. In case seeds were removed from the piles by small mammals, I attached seeds to reverse-wound cotton bobbins or 30cm long fluorescent UV threads (e.g. White et al., 2017). Fifteen sites were used; 13 had cameras with 5 seeds with bobbins and a further five with UV threads. Two additional sites had no cameras and 15 seeds each, with only UV threads.

Three cameras failed. They did not record animals interacting with seeds, despite seeds being removed from the sites and a further camera was knocked to the ground by an angulate tortoise, meaning interactions could not be recorded. For each camera, a new observation was defined as each time an animal interacted with a unique fruit or having entered/left the camera field of view. However, when two seeds were removed in one visit to a seed pile, this was considered one observation. Observations allowed me identify animals interacting with seeds, record the removal rates and the number of seeds removed per visit.

I measured distance to the nearest female for 50 *H. globosa* seedlings (defined here as under 50 cm in height), to record minimal seed dispersal distances. This may give insight into whether seeds are passively dropped and are mainly located below canopies (short distances, < 2 m) or actively dispersed away from canopies (longer distances, ≥ 2m). I searched for the seedlings and then measured distances to the nearest mature female plant. Mature female plants were identified by the presence of dried out pods below their canopies.

To determine whether the seed coat restricted germination I soaked 20 seeds with their seed-coat scarified (sand-papered away in a small area) in water at room temperature compared them to 40 untreated, soaked seeds. Germination was determined by the presence of a seedling radicle. To determine whether the main entry point of water is through the hilum I used a small spot of quick setting glue to close the hilum on 5 seeds. I compared germination over 5 days with 5 control seeds and 5 seeds with a sealed hilum.

During germination trials, it became apparent that vast amounts of mucilage area secreted. Typically, mucilage is produced by pectin, which can be detected by histochemical staining (Ruthenium red). To determine the water absorbing property of the pectin layer I weighed 5 seeds, soaked them continuously and then weighed them again after 5 days. Free water was removed from soaked seeds by dabbing them with filter paper several times before seeds were weighed. The size of hydrophilic pectin bodies was determined using scanning electron and compound microscopy. Using ImageJ (Schneider, Rasband & Eliceiri, 2012) I measured the area of 20 randomly selected pectin bodies before and after being soaked in water. As pectin bodies are almost spherical, I measured the shortest radius of each body to then estimate volume. To determine whether there could be a reward for a scatter-hoarder in the seeds I conducted the iodine test for starch and the sudan black for lipids. Where sample size allowed, swelling and germination differences were tested using Chi-squared tests and pectin body volume changes were tested using student t-tests. All statistical analysis and data presentation was done using the R programming language (R Core Team, 2018a).

4.3 Results

Seed removal and camera traps

Although most seeds were removed, determining the fate of these seeds (moved and buried or moved and eaten) was difficult because many seeds were moved significant distances into rock outcrops and threads attached to seeds snapped on rocks or plant material. Of the 160 seeds placed in seed piles, 125 seeds were removed (12 of 15 piles had all seeds removed) over 5 nights (Figure 4.2). Threads or strings were located for 84 of these seeds, with 81 of these being broken. Only 3 seed fates were determined, with two seeds being moved and consumed and only husks remained and one was moved and buried.

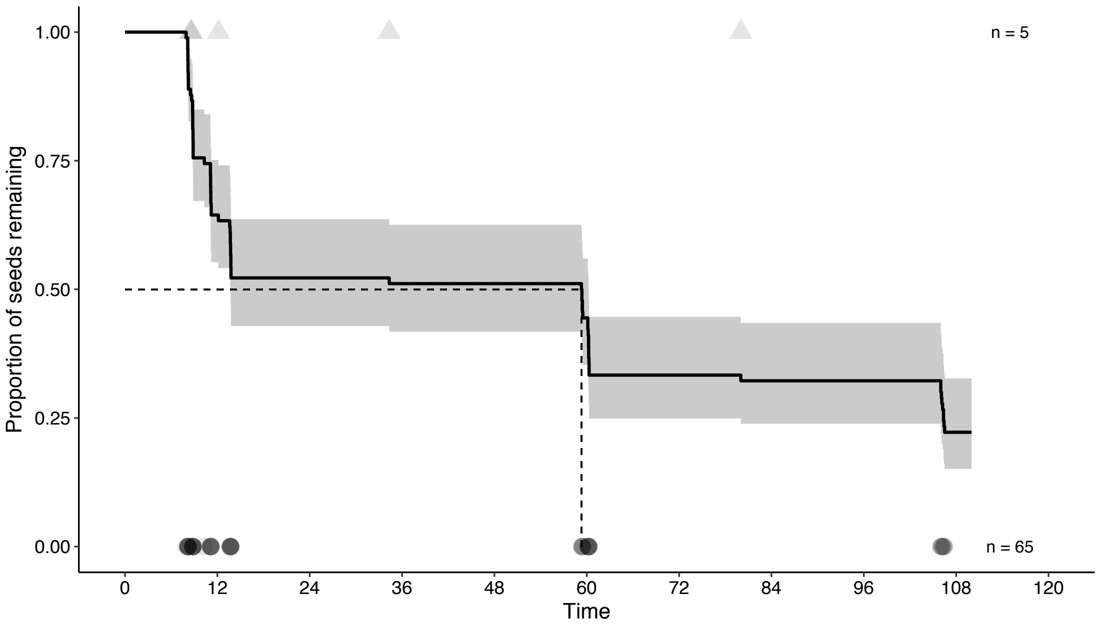


Figure 4.2 The proportion of *Hyaenanche globosa* seeds remaining at all seed piles and removal events by *Acomys subspinosus* (circles, n = 65) and *Micaelamys namaquensis* (triangles, n = 5) over the duration of the seed trial. Only data with seed removal at sites with functional cameras are shown here. Fifty % of seeds were removed after 59 hours.

Table 4.1 The frequency of different animal observations by camera traps and their respective behaviours to *Hyaenanche globosa* seeds.

	Animal species					
Behaviour	*Acomys subspinosus*	*Micaelamys namaquensis*	*Elephantulus edwardii*	Cape genet	Bird species	Reptile species
Ignored	0	13	1	1	8	7
Explored	0	5	0	0	0	0
Removed	65	5	0	0	0	0

By the end of the third night 50% of seeds were removed from seed stations, indicating rapid removal rates. Of the removal events noted as camera observations, 65 of these were by *Acomys subspinosus*, with *M. namaquensis* only observed in the remaining 5 removal events (Figure 4.2, Supplementary Video 4.1). *Micaelamys namaquensis* was frequently observed ignoring seeds altogether and occasionally exploring seeds (Table 4.1). When a seed pile was found by *A.*

subspinosus, it always removed all present seeds (n = 7 observations of full seed pile removed). *Acomys subspinosus* was observed on the cameras to remove a single seed per visit for 53% of removal events (n = 25), two seeds per visit 47% of removal events (n = 21) and rarely three seeds per visit (0.02%, n = 1).

Overall there were 105 independent animal observations on camera traps, dominated by *A. subspinosus* (62%) followed by *M. namaquensis* (22%) and then other animals (16%) (see Table 4.1), none of which interacted with the seeds.

Seedling distribution

The mean (± se) distance *H. globosa* seedlings were found away from nearest female adults was 4.66 (± 0.35) m. The maximum distance was 9.4 m (see Figure 4.3). These are minimum distances because the female parent and dispersal path is unknown. Most seedlings were found far away (≥ 2m) from adult females (92%, n = 45), with few found nearby (< 2m)(8%, n = 4).

Seed content

The seed is comprised of an outer epidermal layer, a pectin layer, a lipid layer and the chlorophyllous embryo (Figure 4.1). The presence of lipids was confirmed by the presence of a translucent patch when smeared on filter paper and that it stained black with sudan black. An iodine test was negative for starch.

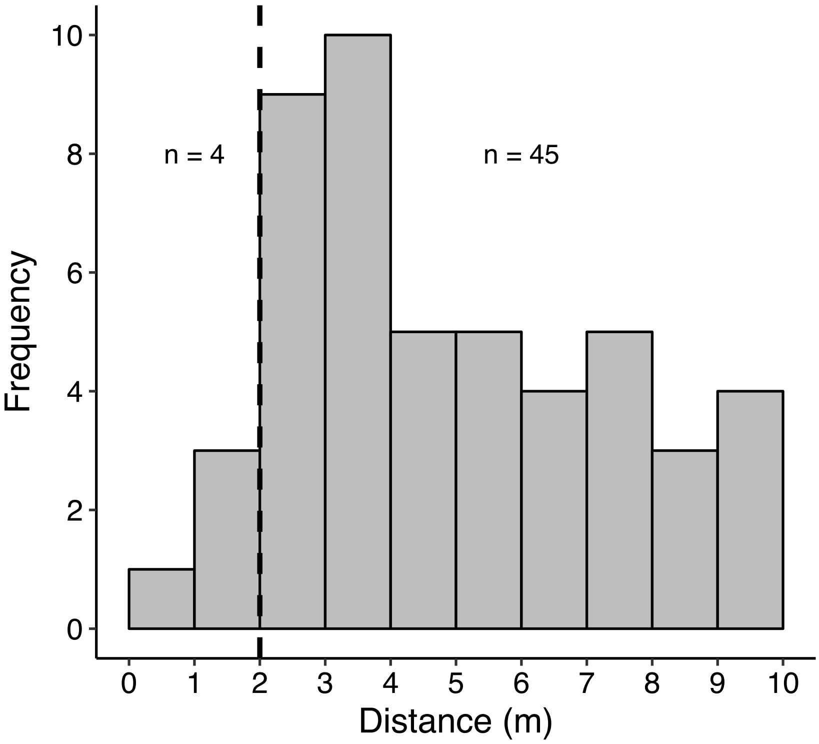


Figure 4.3 Distribution of the distances *Hyaenanche globosa* seedlings are found from nearest adult female trees. Vertical dotted line represents near (< 2m) vs. far away (≥ 2m) from adult females.

Germination

Scarified seeds swelled up more rapidly than control seeds; 50% (10/20) within 24 hours versus 15% (6/40) ($X^2 = 6.66$, df = 1, $p < 0.01$) and 80% (16/20) within 4 days versus 45% (18/40) ($X^2 = 5.30$, df = 2, $p < 0.05$). Germination is rapid; within three days 3 seeds had germinated (20% (1/20) sanded versus 5% (2/40) control seeds) and all germinants were from swollen seeds (19% (3/16) versus 0% (0/44)). Within four days, germination was 65% (13/20) for scarified seeds and 28% (11/40) for control seeds ($X^2 = 6.33$, df = 1, $p < 0.05$).

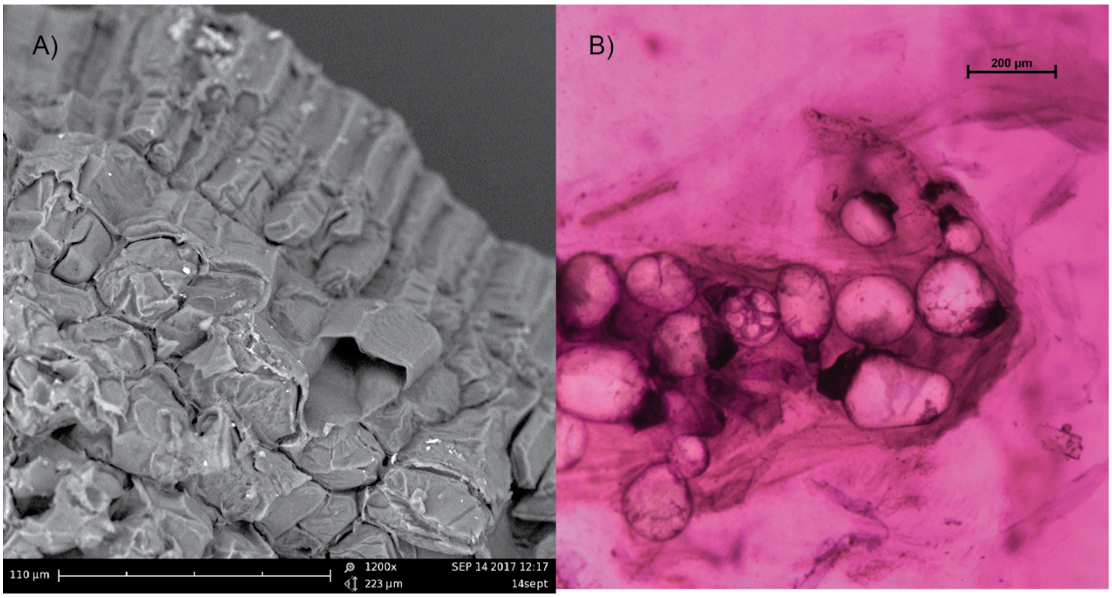


Figure 4.4 Pectin bodies in the inner layer of *Hyaenanche globosa* seed coat before (A) and after (B) being soaked in water. (B) shows the positive presence of pectins as the mucilage stains pink with ruthenium red.

Blocked hilum seeds did not germinate over 5 days (0/5) whereas all 5 control seeds germinated as did those with drop of super glue on the seed coat. Soaked seeds swell up and produce mucilage rapidly (Supplementary Video 4.2). The mucilage is not produced by the outer layer of the seed but from an inner layer. The mucilage stains pink with ruthenium red indicating the presence of pectins (Figure 4.4a and b, pectins pre- and post-soaking). Mean seed weight of unsoaked seeds was 0.22 g and this increased more than 4-fold to 0.93 g after soaking for 24 hours ($n = 5$). The mean volume for 20 randomly selected pre- and post-soaked pectin bodies was 8.03×10^{-6} mm^3 and 1.66×10^{-3} mm^3, respectively, amounting to a 207-fold increase in volume ($t = -10.75$, $df = 19$, $p < 0.01$).

4.4 Discussion

The lipid layer of the seed is likely the reward for the granivorous rodents. When discovered, seeds were rapidly removed from seed piles by *A. subspinosus* and rarely by *M. namaquensis*. Seedlings were found more than 2 m away from the closest adult female trees and typically found in rocky micro-habitats, which suggests animal dispersal of *H. globosa* seeds. Although removed seeds were rarely located, since substantial evidence shows that *A. subspinosus* scatter-hoards seeds (Midgley et al., 2002; Rusch, Midgley & Anderson, 2014; White, Bronner & Midgley, 2017) and it was captured on film removing seeds, it is the likely removal agent. The results of this study suggest that *A. subspinosus*, not *M. namaquensis*, is the sole disperser of *H. globosa* seeds.

Defending seeds from non-dispersing seed consumers is a common requirement for all plant species and defenses can be either physical or chemical (Vander Wall & Beck, 2012). However, this must be balanced between deterring antagonists and attracting mutualists. This is known as directed toxicity, where a secondary chemical may be a deterrent to antagonists, but remain non-toxic to mutualists (Cipollini, 2000). This has mostly been recorded in interactions with antagonists and mutualists with somewhat different metabolisms that the plant can exploit. For example, a cyanogenic glycoside found in *Prunus* and *Sambucus* species is toxic to invertebrates and mammals, while birds are not deterred (Struempf et al., 1999) and capsaicin in chillies is directed to mammalian seed predators and not bird dispersers (Tewksbury & Nabhan, 2001).

Figure 4.5 Two *Hyaenanche globosa* seedlings growing from the same micro-site in the soil. This species is not known to be polyembryonous.

Acomys subspinosus is a well-known scatter-hoarder (Midgley et al., 2002; White, Bronner & Midgley, 2017) and *M. namaquensis* is only known as a non-scatter-hoarding generalist granivore/frugivore (White & Midgley, 2017; White, Bronner & Midgley, 2017). That *M. namaquensis* did not remove seeds, while *A. subspinosus* did may indicate that there is a physiological limitation on seed predation with only the latter able to tolerate the seed coat poisons and that the toxins are directed at the non-scatter-hoarding generalist. However, unlike other cases of directed toxicity these animals likely have similar metabolisms as their diets are similar. Alternatively, *A. subspinosus* seed hoarding behaviour may allow it to control the toxicity of *H. globosa* seeds, by storing seeds and consuming them gradually, effectively metering out any toxic dosage. This is something the non-hoarding *M. namaquensis* is unable to do. Since the outer layer of the seed is considered to contain the poisonous layer, carrying two seeds in the mouth by *A. subspinosus* suggests some immunity to seed coat toxins as it does not have external cheek pouches.

Hyaenanche globosa seeds are released in autumn and because they germinate rapidly when soaked suggests that most buried seeds will germinate during winter rains. This suggests that the benefits of scatter-hoarding of seeds to *A. subspinosus* are of relatively short duration (a few months at maximum). I noted that *A. subspinosus* occasionally carried two seeds and this probably explains the production of twin seedlings at the same site (Figure 4.5). The occurrence of twin seedlings is possibly a diagnostic feature for potential scatter-hoarded plants in the Cape shrublands. For instance the carrying of more than one seed in the only other Cape scatter-hoarder *Gerbilliscus paeba* has been noted previously (White, Bronner & Midgley, 2017).

Once imbibed, seeds germinated quickly. Within a week, the seeds split open and produce copious amounts of mucilage (Supplementary Video 4.2). This mucilage is produced by the swelling of pectin bodies, which increase in volume by more than 200-fold. Mucilage is produced by seeds (myxospermy) from species from many families, especially in arid areas and various roles for mucilage have been suggested. For example, Yang et al. (2012) suggest mucilage helps to bury seeds beneath soil as well as to retain water. *H. globosa* is the first finding of mucilage production in the Picrodendraceae. Since I have shown this species is buried by scatter-hoarders, the main role of the mucilage may be to aid water retention. In the seasonally arid climate of the southwestern Cape, this trait would provide seedlings with retained moisture and a favourable micro-climate. Seeds are released from plants shortly before the winter rains and are buried in soil in rocky sites by *A. subspinosus*. Given they have low dormancy, they probably germinate in winter without need for fire-related cues. This is similar to the chlorophyllous seeds of *Heeria argentea* and *Hartogiella schinoides* that are moved to rocky micro-habitats by *M. namaquensis* (White & Midgley, 2017), although the latter species does not bury the seeds. Directed dispersal to rocky micro-habitats increased overall fitness of these trees by reducing exposure and damage to fire (White & Midgley, 2017).

Further research on *H. globosa* may investigate the role of directed toxicity in reducing seed predation, the benefit of rocky micro-habitats to tree survival, as well as determining the possible benefits of mucilage production to seedling survival.

4.5 Acknowledgements

I thank Bets and Dave Schlebusch for allowing us to work at Sewefontein and seed collection. Dr. Cornelia Klak assisted with German translation, Petra Muller assisted with microscopy and Megan Smith, Alex Connolly and Samantha McCarren assisted with fieldwork. Research was conducted under Cape Nature permit number 0028-AAA008-00270.

4.6 Supplementary Material

Supplementary Videos 4.1 and 4.2 can be viewed at the following link:

- https://www.dropbox.com/sh/odto40ynh12qls3/AABpWm2EWN8WBglRD3fV07eIa?dl=0

Chapter 5

Crypsis of serotinous Cape Proteaceae seeds on heterogenous post-fire soil environments

5.1 Abstract

Plant species with large seeds are susceptible to high levels of seed predation. The most common dispersal mechanisms in the Greater Cape Floristic Region (GCFR), serotiny, myrmecochory and scatter-hoarding, all play a role in reducing seed predation by keeping seeds away from non-dispersing seed predators. Post-dispersal, seeds of certain serotinous Proteaceae plants display what appears to be background matching, with polymorphic colours or ornamented surfaces making them difficult to detect on certain soil types.

Using field observations, seed removal trials and spectrophotometry data, I investigated whether seed crypsis (background matching) in Proteaceae seeds can reduce seed predation by visually cued avian seed predators.

I ran seed predation trials with *Leucadendron laureolum* winged seeds, with combinations of light and dark seeds and light and dark soils. In all cases, I found no difference in seed predation of winged seeds on different substrates by the only diurnal rodent granivore, *Rhabdomys pumilio*. I therefore ruled out rodent granivores as selective agents driving visual seed crypsis in Proteaceae.

Using seed spectral reflectance measurements, I then investigated whether birds could potentially discriminate between the polymorphic coats of serotinous *Leucadendron* winged seeds and the mosaic of post-fire substrates. I compared the reflectance of eight winged *Leucadendron* species, three species of *Protea* with hairy, parachute-like seeds and one hairy-seeded species for both *Aulax* and *Leucadendron*, with the reflectance spectra of their native substrates. I found that the lights seeds of most *Leucadendron* seeds showed clear background matching

with their native soils, with granivorous birds predicted to have difficulty distinguishing them apart. *Protea* seeds with parachutes generally do not background match and are likely not reducing their detectability from visually cued predators, with the exception of *Protea obtusifolia,* the limestone endemic, which matches its dark native soil well with dark-haired seeds.

This is the first description of background matching in the seeds of GCFR plants. I show that rodent granivores are not visually cued and that bird granivores are more likely to be the selective agent in driving visual seed crypsis of GCFR seeds. Seed colours are likely adaptations to substrate-specific seed predation biases by visually cued bird granivores. Empirically testing seed predation rates of polymorphic seeds on post-fire mosaic soils by bird granivores is a necessary next step.

5.2 Introduction

Large seeds that fall to the ground can suffer intense predation (Janzen, 1971) and so have evolved ways of minimizing it, such as being ant-dispersed (Bond & Breytenbach, 1985), scatter-hoarded (Gómez, Schupp & Jordano, 2018), having aposematic colouration (Endler & Greenwood, 1988), being retained in the canopy (le Maitre & Midgley, 1992), toxicity (Janzen, 1978) or by reducing their detectability (Lev-Yadun & Ne'eman, 2013). For example, background matching or disruptive colouration may affect detection by visually cued seed predators, reducing predation (Endler, 1978). Visually cued seed predators, such as birds, can have an important selection effect on seed appearance as less cryptic seeds that don't match their background will be more conspicuous and therefore more heavily predated on (Porter, 2013).

Animal colouration has long provided textbook examples of natural selection, with classics, such as peppered moth morph colour proportions fluctuating depending on their ability to match their backgrounds and avoid bird predation, which have changed due to environmental pollutants; (Tutt, 1891; Cook & Saccheri, 2013) or *Heliconius* butterflies, where a combination of aposematic colouration and mimicry occurs (Bates, 1862; Mallet & Singer, 1987). These examples generally show how traits in populations, such as colour, can respond to selective pressures, such as visually cued avian predators (Caro, 2005). Avian predation of small mammals offers a more specific comparison; *Peromyscus*, deer mice, pelage colouration varies by populations based on matching local substrate colours in order to reduce predation by birds (Dice, 1940). Mice suffer different rates of predations depending on their conspicuousness relative to their background, with populations tending towards stabilizing selection and pelage colour matching local substrate colour (Vignieri, Larson & Hoekstra, 2010).

There is a growing interest in plant defenses, such as camouflage, which was recently reviewed by Niu et al. (2018). An interesting case of plant defense using background matching is found in *Pinus* where seed colour polymorphisms offer differential fitness gains for seeds, as different colour seeds experience different rates of predation by birds on the mosaic of soil types in post-fire landscapes (Nystrand & Granström, 1997; Saracino et al., 1997; Saracino, D'Alessandro & Borghetti, 2004; Lev-Yadun & Ne'eman, 2013).

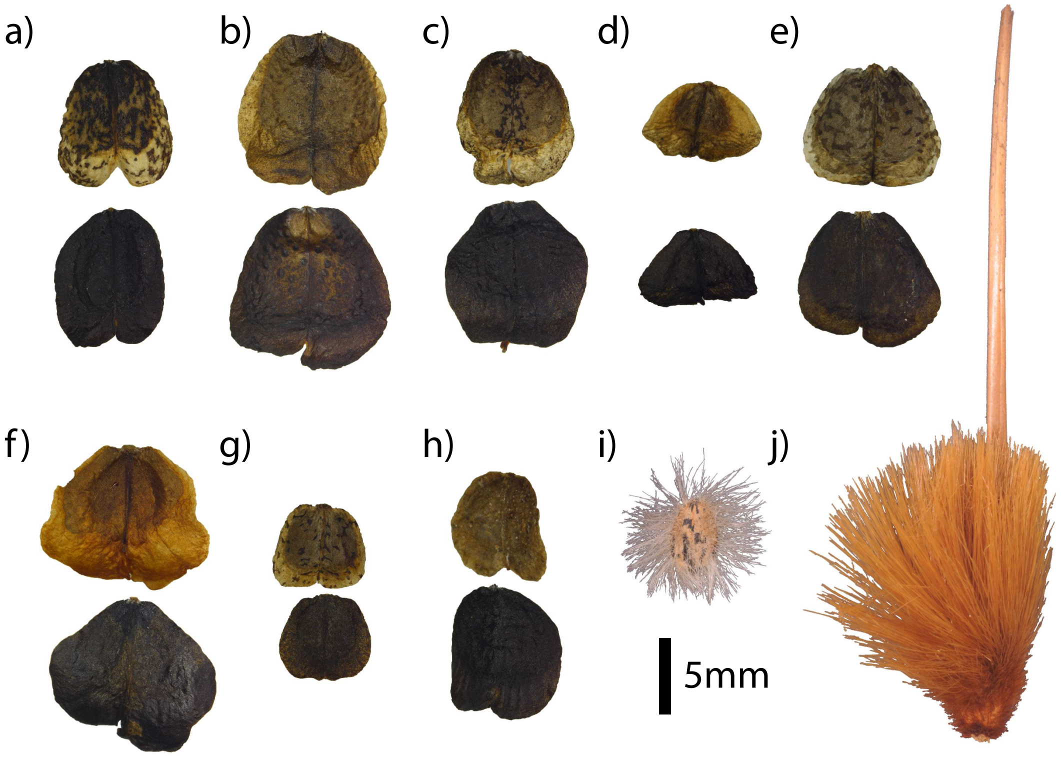


Figure 5.1 Photographs of the dark and light colour morph extremes of the flat-winged, serotinous seeds of a) *Leucadendron coniferum*, b) *L. laureolum*, c) *L. meridianum*, d) *L. modestum*, e) *L. muirii*, f) *L. salignum*, g) *L. teretifolium*, h) *L. xanthoconus*, and hairy seeds of i) *L. linifolium*, and j) *Protea repens*.

In the Fynbos Biome of the Greater Cape Floristic Region (GCFR), bird-dispersed plants and avian granivores are rare compared to adjacent vegetation types (Johnson, 1992). It has been suggested that this is due to the high nutrient cost of producing fruits on nutrient-poor soils (Fraser, 1990; Johnson, 1992; le Maitre & Midgley, 1992). However there are few reports that bird granivory may be intense on recently burnt Fynbos stands when serotinous seeds are released *en masse* (Chalmandrier et al., 2013). Most small mammal granivores in the GCFR are nocturnal with only one diurnal small mammal granivore *Rhabdomys pumilio*. However, there are at least ten GCFR granivorous bird species that could consume seeds of serotinous plants, though none are known to provide dispersal benefits to dry, serotinous seeds (Milewski, 1978; Fraser, 1989, 1990).

Seeds of a number of Cape Proteaceae species appear to display cryptic colouration for localized soil types, as seen in some *Pinus* seeds (Nystrand & Granström, 1997; Saracino et al., 1997). This is seen in seeds of serotinous flat-winged *Leucadendron* and hairy *Aulax, Leucadendron* and *Protea* species (Figure 5.1). These serotinous species rely on physical structures, such as plumes, parachutes or wings to slow primary dispersal fall rates and increase secondary wind dispersal (Schurr et al., 2005).

Leucadendron with flat-winged, seeds, such as *L. laureolum*, have seed colour polymorphisms ranging from mottled light gray/brown to charcoal black that are produced on different females within the same population (Figure 5.1). These seeds are released in post-fire landscapes generally composed of light, ash-soil mix patches and charcoal-litter mix patches, creating a mosaic of substrate patches immediately post-fire (Figure 5.2). Visually cued seed predators may detect and predate seeds at different rates if seeds are sufficiently cryptic by background matching, preferentially selecting more conspicuous seeds (Endler, 1978; Nystrand & Granström, 1997; Saracino, D'Alessandro & Borghetti, 2004).

Figure 5.2 Three weeks post-fire, fynbos substrates are generally composed of distinct light, ash-soil and charcoal-litter mixed patches, as seen here at Drie Kuilen Nature Reserve in March 2017, South Africa. Conspicuous clusters of orange-haired *Protea* seeds*, P. repens* in this case, tend to gather in depressions with charcoal-litter mixed patches.

I hypothesised that the seed colours of cryptic and polymorphic seeds are adaptations against visually cued seed predators on spatially heterogenous substrates. To determine whether small mammals can perceive differences in colour morphs across different substrates, I first ran seed removal experiments on *Leucadendron* wings of different seed colours on different substrates. Secondly, I used spectral reflectance measurements and visual models to test whether birds can perceive a chromatic difference between the seeds and their home substrates.

5.3 Methods

Collections

At Potberg, De Hoop Nature Reserve (-34.397638°, 20.548405°), *Leucadendron laureolum* seeds were collected and seed removal experiments conducted in February 2016; I additionally collected seeds of 14 Proteaceae species (see Figure 5.1) and approximately 100 ml samples of their native substrates in February 2018 for colour measurements. To assess colour patterns within and between individuals and populations, *Leucadendron laureolum* seeds were collected from three distinct populations: Viljoenspas (-34.087116°, 19.057083°), Jonaskop (-33.941279°, 19.524613°) and Potberg (300 seeds total). As seed coat and therefore colour is derived from the ovary, therefore being maternal tissue, seed colour is typically monomorphic for each female in *Leucadendron* (Midgley, 1987). Therefore a single cone was collected from each female/hermaphrodite within each subpopulation for dioecious (*Aulax* and *Leucadendron*) and hermaphroditic (*Protea*) genera. Cones were wrapped in paper to keep the seeds together and then dried for 48 hours at 60°C, releasing the seeds, and allowing me to determine ratios of dark:light seeds within species subpopulations. The vegetation types, which are intrinsically linked to their native substrates, included De Hoop Limestone Fynbos, Potberg Ferricrete Fynbos, and two sites in Albertinia Sand Fynbos. On limestone soils I collected three limestone soil endemic Proteaceae: *Leucadendron meridianum*, *L. muirii* and *Protea obtusifolia*. On ferricrete soils I collected *Leucadendron modestum* and *L. teretifolium*. On Albertinia sand soils I collected seed of *Leucadendron coniferum*, *L. laureolum*, *L. salignum*, *L. xanthoconus*, *Aulax umbellata*, *Protea neriifolia*, *P. repens*, *P. susannae*. Adjacent to a river channel, the Albertinia sand soil has a mix of small quartz stones, significantly changing the colour, (hereafter called riversand) where I collected *Leucadendron linifolium*.

Seed removal experiments

I placed five *L. laureolum* seeds of two extremes of the different colour morphs, lightest vs. darkest seeds, on two different substrates – their native soil (light) and charcoal (dark) in shallow petri dishes (i.e. two treatments with seeds that match and two treatments with seeds that *do not* match their substrates), making four treatments with five seeds each at each station. In total I placed out 15 stations, each ± 30 m apart, totaling 75 seeds for each treatment for three days. At each station I placed a single Ltl Acorn 6210M remotely activated trail camera (Shenzen LTL Acorn Electronics Co., Shenzen, Guangdong, China), set to capture three photographs when triggered. Removal rates were analysed using binomial regression with proportion of seeds removed used as the response variable and seed and substrate colour as independent variables.

Colour distances and visual models

Reflectance spectra of seeds and their native substrates were measured using a spectrometer (Jaz Spectrometer, Ocean Optics, USA) with fiber optic light source. I measured reflectance in 1 nm intervals from 300 to 700 nm. Measurements were made with the light and probe at 45° relative to the sample. The spectrometer was calibrated using light (WS-1, Ocean Optics, USA) and dark (STAN-SSL, Ocean Optics, USA) reflectance standards before and during measurements. All seeds were measured against a matt black background, while soils were measured in petri dishes. Three measurements were taken on separate sites on each sample and then a mean spectrum calculated for each sample.

For all visual models the blue tit *Cyanistes caeruleus* was used as a model species for cone sensitivity. *Cyanistes caeruleus* has comparatively similar cone photoreceptor abundances to most other Passerida (Hart, Partridge & Cuthill, 1998), which includes Cape granivorous birds. Following Maia et al. (2013) methods (in the R package 'pavo') to explore colour distances and visual perception, I calculated the quantum catches (amount of light capture) for each photoreceptor by using each sample's reflectance spectra and the modeled Blue tit cone sensitivities. Colour distances measured in Just Noticeable Differences (JNDs) can then be obtained by weighting the Euclidean distance of the quantum catches by the Weber fraction (0.1) of the Blue tit cones (Vorobyev & Osorio, 1998). JNDs represent a discrimination threshold between a chosen set of samples. Below one JND, two samples are considered indiscernible from one another for the receiver. Below four JNDs, receivers may have difficulty in distinguishing apart two samples, while four or more JNDs between two samples were considered discernable from one another. Mean JNDs and bootstrapped 95% confidence intervals were calculated for each pairwise comparison between seeds and their native substrates. Values for JNDs are shown as mean (lower estimate, upper estimate). All statistical analyses were conducted in R programming language (R Core Team, 2018b) using the pavo 2.0 package (Maia et al., 2018).

I started by 1) investigating patterns of colour variation in *Leucadendron* seeds that were considered light or dark by the human-eye, by measuring reflectance spectra of the abaxial and adaxial sides of five light and five dark winged *L. laureolum* seeds, with each seed from a unique female, at three distinct populations: Viljoenspas, Jonaskop and Potberg (30 seeds total). This allowed me to establish that the colour extremes of winged *Leucadendron* seeds are typically found on their adaxial surfaces (Figure 5.5). Thereafter I only focused on the adaxial surface of each seed measurement.

I then 2) investigated whether the colour of dark morph *Leucadendron* seeds matched dark, charcoal-litter mix patches. Using the adaxial surface of all *L. laureolum* seeds measured from the three distinct populations and compared this with dark soil patches collected three months post-fire (February 2017) at Redhill (34.194779°, 18.413208°).

Lastly, 3) to determine whether background matching was widespread for large seeded plants, I measured the spectra of seeds and their native substrates for 14 Proteaceae species at De Hoop Nature Reserve. At each site a single seed was collected from ten individuals (female or hermaphrodite) and ten 100 ml substrate and litter samples collected at the base of each individual. I randomly collected a loose stone in each of four sites in ferricrete (*L. teretifolium* and *L. modestum*) and limestone (*L. meridianum* and *L. muirii*) fynbos, where rockiness was above 25% within 1m^2 plots adjacent to each individual, and measured 10 samples for these species. Additionally, I measured total percentage rockiness for each 1m^2 plot.

5.4 Results

Collections

Flat-winged *Leucadendron* seeds showed a great amount of variation in the frequency of dark:light colour morphs across subpopulations of species (Figure 5.3). *Leucadendron teretifolium*, found on ferricrete soils with a high percentage of rocky ground cover, had the highest proportion of light to dark seeds sampled, while *Leucadendron salicifolium* and *L. procerum* had no sampled light seed colour morphs. Sixty-two percent of *Leucadendron* species investigated had at least 20% of each colour morph in the sampled population.

Seed Removal

Interactions with seed predators were recorded at thirteen (of 15 total) camera stations. Seven of these stations had recordings with positive identifications. All of these recorded only *Rhabdomys pumilio* interacting with and consuming seeds (Figure 5.4). Overall, for the matched treatments (dark seeds on charcoal soils and light seeds on native soils) 60 of 75 seeds were removed in total. While for the mixed treatments (dark seeds on native soils and light seeds on charcoal soils) 62 of 75 seeds were removed in total. Only two sites had no seed removal at all during the three day period; one site had no removal for matched treatments and two seeds removed for each of the mixed treatments; all other sites had all seeds of all treatments removed. No difference was found in removal between either treatment of seed or substrate colour ($z = 0.419$, $p = 0.675$), with a mean (± standard error) removal of 4.00 ± 0.53 and 4.13 ± 0.48 seeds at each station of matched and mixed treatments, respectively (Figure 5.5).

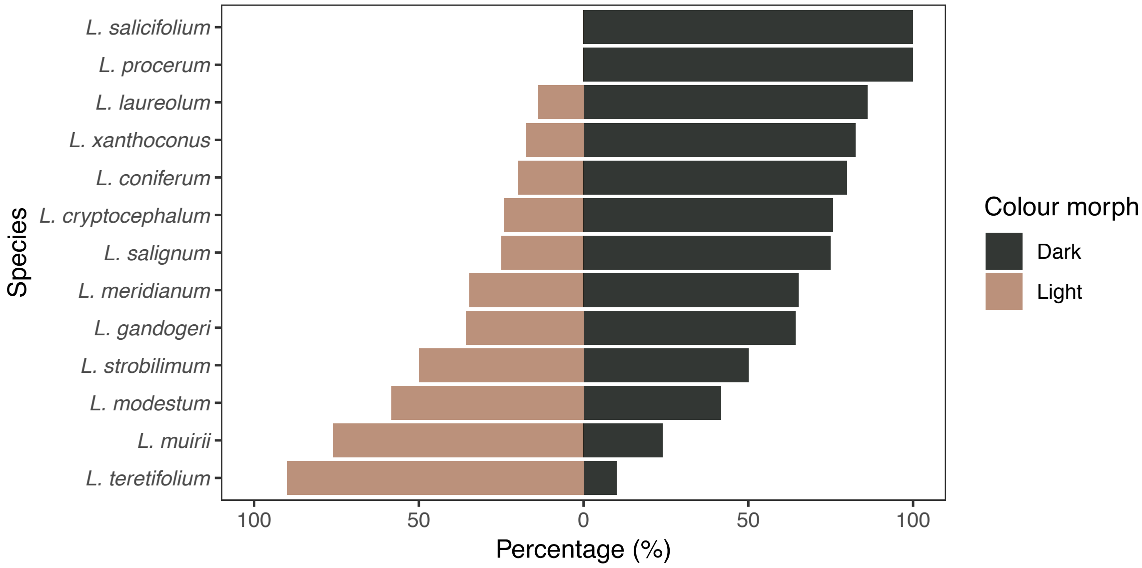


Figure 5.3 The percentage of each seed colour morph (categorised by eye) in subpopulations of 13 flat-winged *Leucadendron* species. The number of seeds collected from unique individuals for each species: *L. salicifolium* (n = 35), *L. procerum* (n = 30), *L. laureolum* (n = 36), *L. xanthoconus* (n = 40), *L. coniferum* (n = 40), *L. cryptocephalum* (n = 33), *L. salignum* (n = 40), *L. meridianum* (n = 49), *L. gandogeri* (n = 32), *L. strobilimum* (n = 16), *L. modestum* (n = 36), *L. muirii* (n = 25) and *L. teretifolium* (n = 30).

Figure 5.4 *Rhabdomys pumilio* investigating shallow petri dishes with *Leucadendron laureolum* seeds of different colour morphs placed on different substrate colours (charcoal and native soil) at De Hoop Nature Reserve, 5 February 2016.

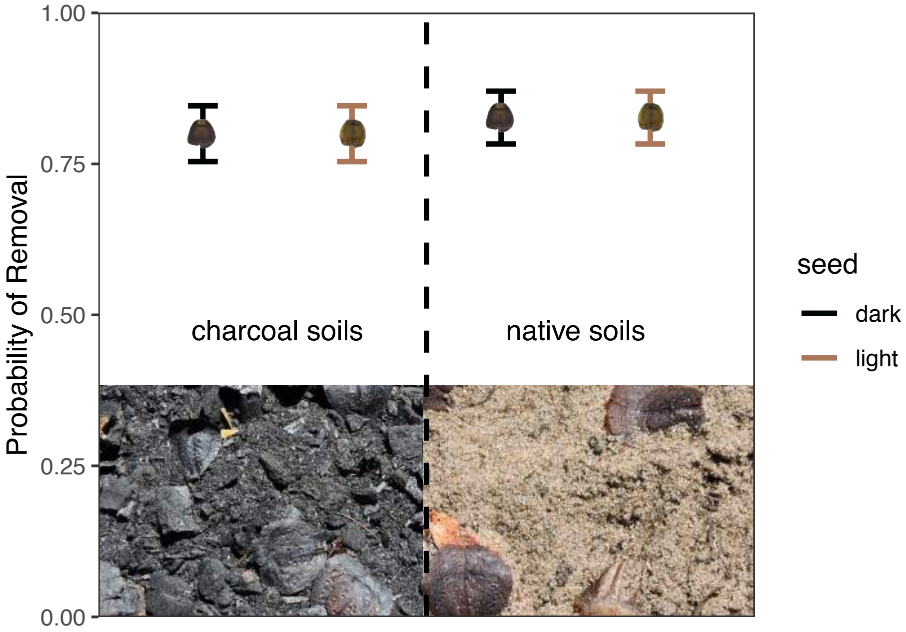


Figure 5.5 The predicted mean (±se) probability of seed removal of dark and light colour morphs of *Leucadendron laureolum* seeds on charcoal and native soils. No difference was found in removal of seeds between either combined treatment of seed or substrate (z = 0.419, p = 0.675).

Colour distances and visual models

Both light and dark seed colour morphs had their most reflective values on the adaxial side of the seed. Though the pre-determined colour morphs were generally well reflected by the colour spectra, it was clear that *Leucadendron* seed colour was not a dimorphic trait and seeds display a continuum of colour from dark to light (Figure 5.6).

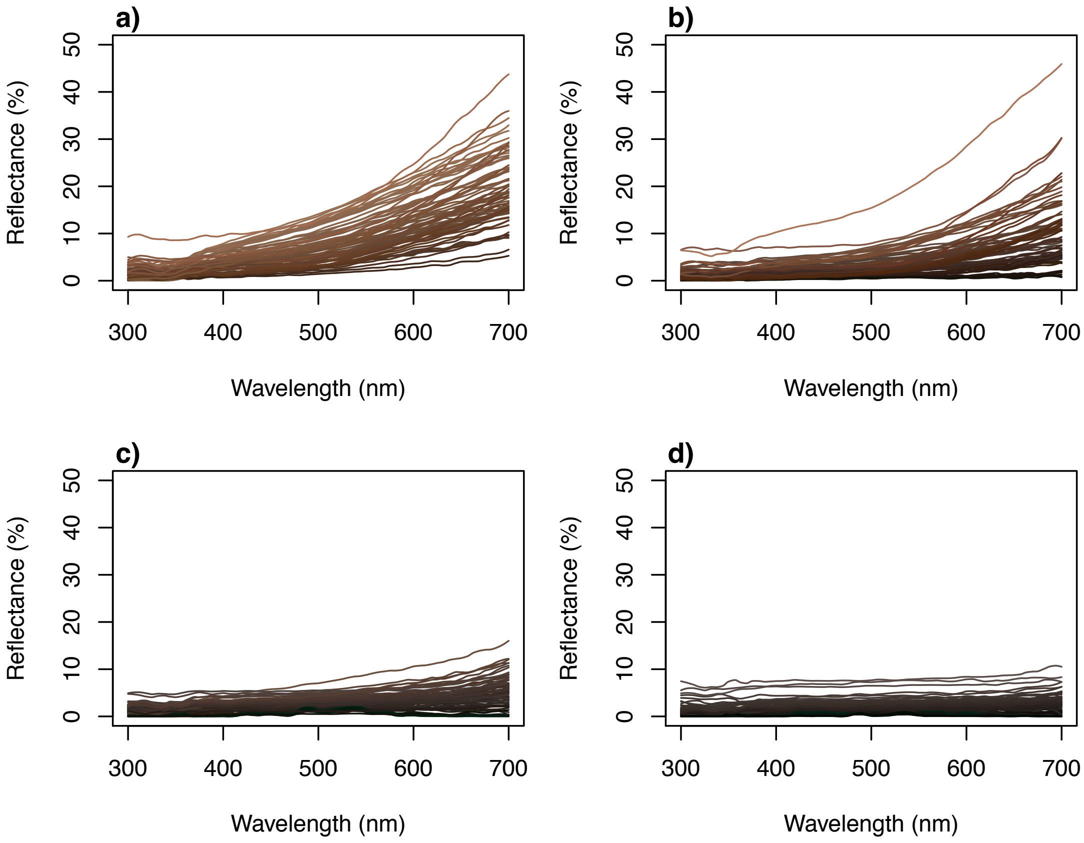


Figure 5.6 All seed colour measurements for the light morph a) adaxial and b) abaxial sides, and dark morph c) adaxial and d) abaxial sides. Light and dark morphs were categorised by eye in *Leucadendron laureolum* from three distinct sites.

Comparing the reflectance spectra of the adaxial surface of the dark seed colour morph with charcoal soils showed that both spectra have generally low reflectances peaking around 700 nm (Figure 5.7a). Visual models, assessing whether granivorous birds can distinguish between two samples under different conditions, suggest that under daylight conditions some dark seeds may be completely indistinguishable from charcoal soils (<1 JND) (Figure 5.7b). Bird granivores would likely find it difficult to distinguish between the dark seed and charcoal soils under most conditions (between 1-4 JNDs).

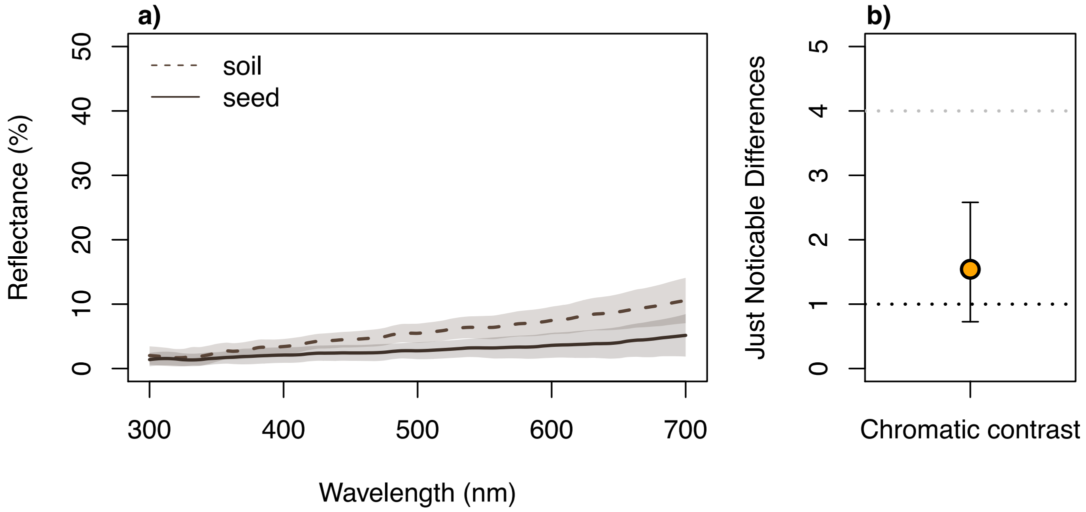


Figure 5.7 Comparison between: a) dark seed and charcoal soil reflectance spectra and b) their chromatic contrasts in JNDs relative to a Blue tit visual system.

Within soil types there was little variation in the reflectance of the native soil samples, for example the soil colour in Albertinia Sand Fynbos was consistent whether measured at *Leucadendron coniferum* or *Protea susannae* sites. This was evident for all soil types, including sand, limestone or ferricrete. Munsell colours for sand soils were brown to yellowish-brown; limestone soils were black to very dark greyish-brown; ferricrete soils were dark reddish-brown to dark brown; and lastly, riversand soils were light gray to pinkish-gray to white (Munsell Color, 2009). I found large variation in the reflectance spectra of rocks at the limestone and shale soils (Figure 5.8i,j,l,m), as well as variation in the overall rockiness of each site. For the limestone species, *Leucadendron meridianum* and *L. muirii* had mean ± se of 49.0 ± 25.6% and 93.2 ± 6.4% rockiness respectively, while the shale species, *L. modestum* and *L. teretifolium* had 60.0 ± 19.4% and 36.0 ± 19.3% rockiness, respectively.

All four flat-winged *Leucadendron* species found in Albertinia sand substrates showed light seed morphs with consistent reflectance spectra in shape and intensity (Figure 5.8a,b) that generally matched their native soils (Figure 5.9). *Leucadendron salignum* was the only Albertinia sand species to have a portion of values below 1 JND (mean (lower estimate, upper estimate)) (1.76 (0.54, 2.98)) while *L. xanthoconus* (3.18, 2.29, 4.61), *L. laureolum* (4.19, 3.06, 5.54) and *L. coniferum* (4.57, 3.19, 6.29) all had JND values between 2 and 6 JNDs.

Aulax umbellata (Figure 5.1i), with seeds covered in white hairs, had reflectance spectra that were a different shape to their native soils, but had a similar peak reflectance (Figure 5.8e) and had JND of 1.38 (1.07, 1.71) between their seed and their native soils. The red-brown, hairy seeds of *Protea repens* (Figure 5.1j) (9.74 (6.59, 13.06) JNDs)*,* *Protea susannae* (5.98 (3.94, 8.21) JNDs) and *Protea neriifolia* (13.65 (5.02, 25.90) JNDs) in sand soils showed reflectance spectra that did not match their native soils in shape of curve or intensity (Figure 5.8f,g,h). All species had JNDs above 4 between their seeds and native soils (Figure 5.9).

The small, flat-winged seeds of *Leucadendron meridianum* and *Leucadendron muirii* (Figure 5.1c,e) had very mottled seeds with pronounced dark and light patches. *Leucadendron muirii* seeds' reflectance spectra had a wide range of colours and generally matched the shape and intensity of the dark native soils (1.21 (0.83, 1.64) JNDs) and mosaic of rocks (1.19 (0.59, 1.86) JNDs) (Figure 5.8i, Figure 5.9). *Leucadendron meridianum*, though generally similar in appearance to *L. muirii*, did not match well with either the native soils (3.75 (2.97, 4.61) JNDs) or the rocks (4.45 (3.68, 5.39) JNDs) (Figure 5.8j, Figure 5.9). *Protea obtusifolia*, with dark hairs covering the seed, matched the native soils well in both the reflectance spectra (Figure 5.8k) and chromatic contrast between the seeds and the dark soils (2.97 (1.75, 4.47)) (Figure 5.9).

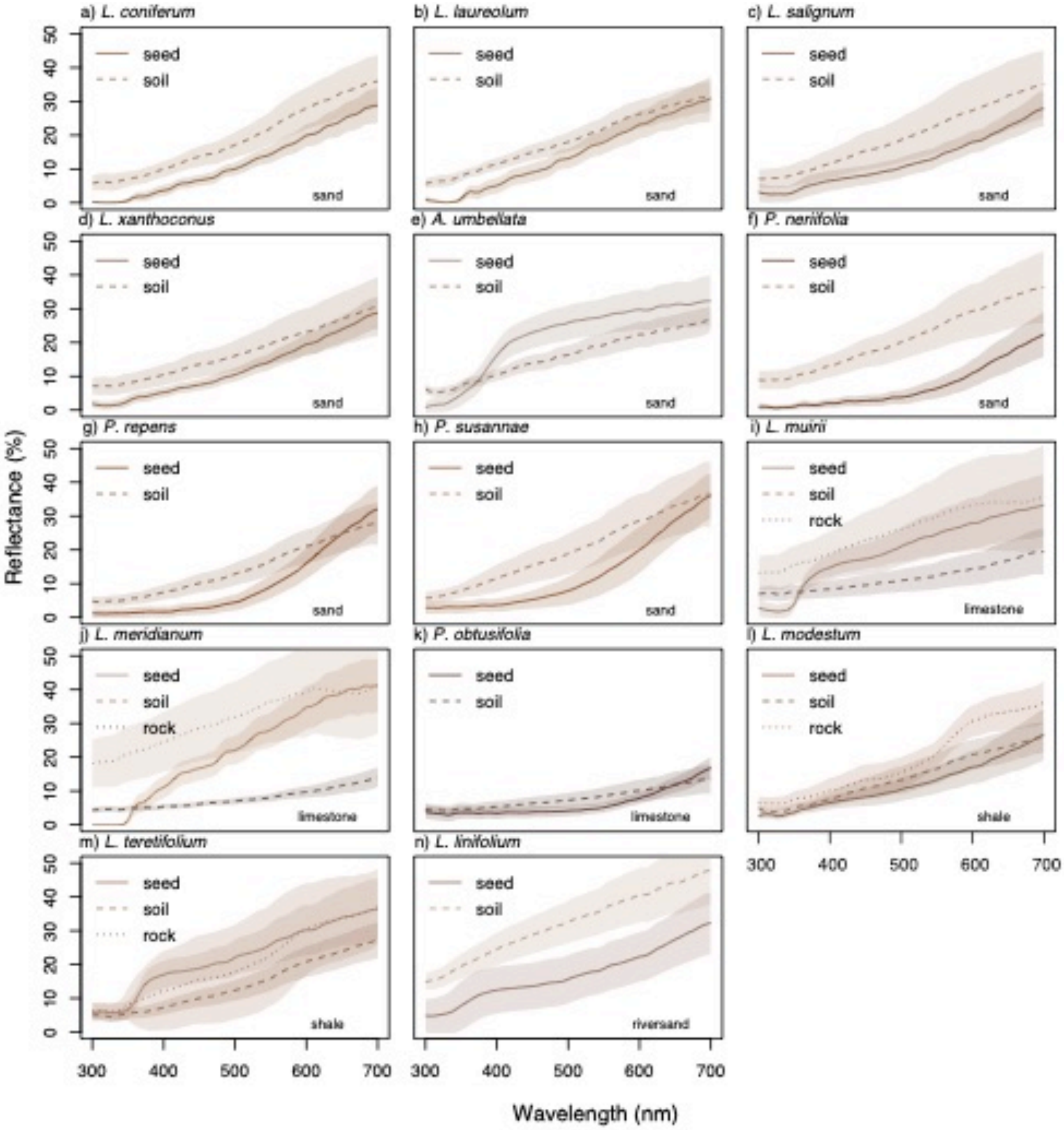


Figure 5.8 The reflectance spectra of 14 Proteaceae species seeds compared with their native soils and, in a few cases, native rocks. Measurements reflect the adaxial surface of light seed colour morphs for nine flat-winged *Leucadendron* species and a measurement from the center of the seed for the hairy *Aulax* and *Protea* seeds. Rock reflectance was only measured where overall rockiness >25%. The Proteaceae species include a) *Leucadendron coniferum*, b) *L. laureolum*, c) *L. salignum*, d) *L. xanthoconus*, e) *Aulax umbellata*, f) *Protea neriifolia*, g) *P. repens*, h) *P. susannae*, i) *L. muirii*, j) *L. meridianum*, k) *P. obtusifolia*, l) *L. modestum*, m) *L. teretifolium*, n) *L. linifolium*.

Leucadendron modestum, which grows on dark reddish-brown, ferricrete soils, was best matched chromatically to its native substrates, being almost always indistinguishable from both the soils (0.62 (0.21, 1.29)) and rocks (0.81 (0.17, 2.34)) for an avian granivore (Figure 5.8l, Figure 5.9). The mottled seeds of *Leucadendron teretifolium* were moderately well matched to its ferricrete substrates of soils (2.15 (1.68, 2.64)) and rocks (2.65 (1.20, 3.98)) (Figure 5.8m, Figure 5.9).

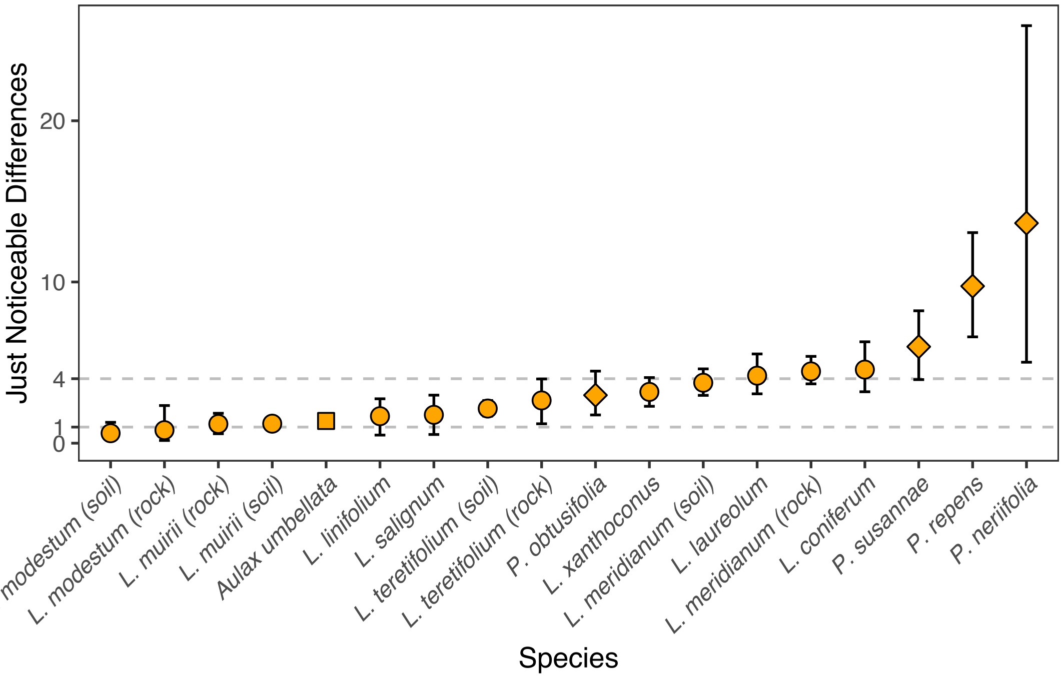


Figure 5.9 The mean and bootstrapped 95% confidence intervals of chromatic contrasts in Just Noticeable Differences (JNDs) between 14 Proteaceae species seeds (circles are *Leucadendron*, squares are *Aulax*, diamonds are *Protea*) and their native soils and rocks based on the visual model of bird granivores. Values below 1 JND suggest the objects are indistinguishable from one another for an organism with a similar visual system to the Blue tit. Values between 1 and 4 JND may occasionally be difficult to distinguish between samples for the bird under certain circumstances, while values above 4 JND would likely be easily distinguishable under all conditions from all distances.

Lastly, *Leucadendron linifolium*, which has white hairs covering the mottled brown seed (Figure 5.1i) and grows on light gray to white soils, had a reflectance spectra with generally lower intensity but similar curve shape (Figure 5.8n) and overall was well chromatically matched to their soils (1.68 (0.5, 2.75)) (Figure 5.9).

Chromatic values for each species' seeds were generally clustered for both native and surrounding soils. The first seven best native soil background matching seed species (Figure 5.9) (all species had < 3 JNDs between their seeds and native substrate), *Leucadendron modestum*, *L. muirii*, *Aulax umbellata*, *L. linifolium*, *L. salignum*, *L. teretifolium* and *Protea obtusifolia*, consistently had lower or within 1 JND compared to surrounding soils (Figure 5.10). *Leucadendron xanthoconus* and *L. meridianum* both had lower performing JNDs for surrounding soils compared with native soils, however, these values were ±2 JNDs between each other, yet all values were still within 4 JNDs overall (Figure 5.10). From *Leucadendron laureolum* on, all species seeds poorly matched their native soils and generally poorly matched surrounding soils too (with the exception of *P. susannae* matching surrounding soils better than its native soils).

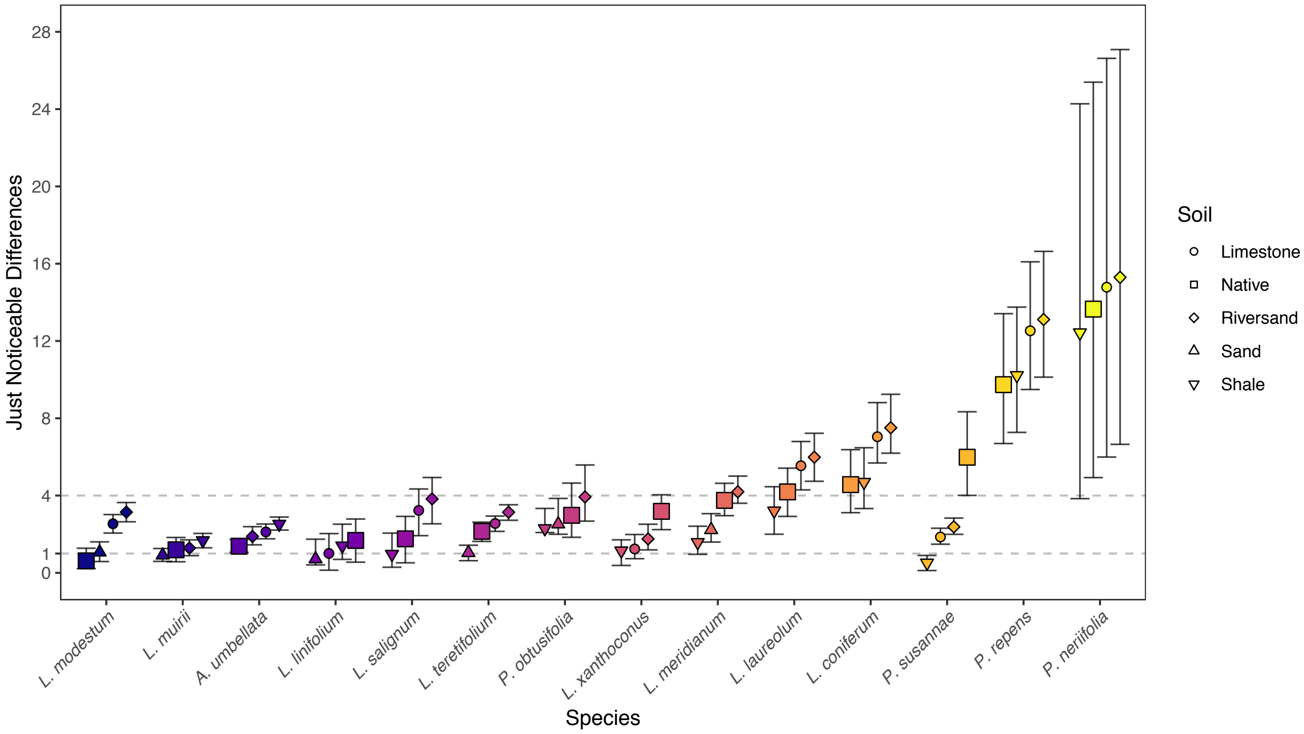


Figure 5.10 Mean chromatic contrast between light morph (*Leucadendron*) or hairy (*Aulax* and *Protea*) seed colour, their native soils (squares) and the surroundings soils (limestone = circle, riversand = diamond, sand = triangle, shale = inverted triangle). Native soils (squares) are enlarged for visual purposes only.

5.5 Discussion

All of the Proteaceae species investigated (except *L. salignum*) are obligate reseeders, so the time period between post-fire seed release and germination, generally induced by winter rains, is when seeds are most vulnerable to granivores (Bond, 1984; Lamont et al., 1991). Therefore, reducing seed predation by means of defensive traits, such as background matching or crypsis, would reduce the seeds' detectability and overall predation. This study represents the first systematic measurement and analysis of Proteaceae seed colours in an attempt to understand the potential impact of visually cued seed predation on defensive seed traits, such as seed crypsis.

Seed removal

Seed removal experiments showed that small mammal granivores do not discriminate between seeds based on seed colour or background matching (Figure 5.5), with no difference in removal rates between all combinations of dark and light seeds and soil. *Rhabdomys pumilio* is the only diurnal small mammal granivore in the GCFR (White, Bronner & Midgley, 2017) and was the only small mammal that visited seed stations (Figure 5.4). Nocturnal seed predators would not select for seed colour. Small mammals typically locate seeds by olfaction (Bond & Breytenbach, 1985; Vander Wall, 1993; Jorgensen, 2001) and would not primarily rely on visual cues to detect seeds. Additionally small mammal densities typically decline immediately post-fire (van Hensbergen et al., 1992), further limiting their role as selective agents of serotinous seed colour traits. This study could benefit from seed removal experiments immediately post-fire to quantify the relative removal rates of different seed colour morphs against heterogenous soil backgrounds and to quantify relative rates of bird seed predation.

Role of small mammals in seed predation

Small mammals play an important role in inter-fire seed predation, strongly influencing all large-seeded dispersal mechanisms (Bond, 1984; Christian, 2001; White, Bronner & Midgley, 2017). All large GCFR seeds are susceptible to high levels of seed predation by small mammal granivores. Myrmecochory and serotiny are both thought to in part be anti-rodent seed predation traits (Bond, 1984; le Maitre & Midgley, 1992): ants moving seeds into shallow burrows to consume the elaiosomes, before small mammals detect them on the surface (Bond & Slingsby, 1983); and woody cones storing seeds in protective cones for a few years, keeping seeds away from resident small mammal granivores, only releasing seeds post-fire to inundate any remaining or immigrant granivores with excess food (Lamont et al., 1991). Scatter-hoarding can similarly be viewed as an anti-predation mechanism: without scatter-hoarding rodents, which both consume and disperse seeds (White, Bronner & Midgley, 2017), small mammals that *only* consume seeds, such as *R. pumilio*, would decimate reseeding plant populations.

Despite their crucial role in the seed dispersal dynamics of large-seeded GCFR plants during inter-fire periods, their diminished presence post-fire and their dependence on olfactory cues effectively rule out small mammals as selective agents of the *Leucadendron* seed colour polymorphisms.

Avian seed predators

This points towards visually cued avian granivores, such as fynbos endemics *Crithagra leucopterus* (Protea seedeater) and *Crithagra totta* (Cape siskin), being important seed predators, especially in post-fire environments. There are reports of these species predating on *Protea*, *Leucadendron*, and *Widdringtonia* directly from the infructescences/cones both before and after fires (Milewski, 1978; Fraser, 1990; Chalmandrier et al., 2013). Chalmandrier et al. (2013) noted that granivorous birds were strongly associated with recently burnt stands of fynbos and suggested this was linked to the large amount of serotinous seeds released immediately post-fire.

Seed crypsis

As predicted by Lev-Yadun and Ne'eman (2013), the presence of multi-modal seed colouration in fire-dependent serotinous plants is widespread beyond *Pinus*, clearly displayed in the GCFR by flat-winged *Leucadendron* and hairy *Aulax umbellata* and *L. linifolium* seeds. I suggest that flat-winged, serotinous *Leucadendron* seed colour polymorphisms are a form of seed crypsis and are adaptive to the selective environment of visually cued bird seed predation on heterogonous post-fire soils, with dark seeds matching charcoal-litter mix patches and light seeds matching ash-native soil mix patches. White-haired seeds of *Leucadendron linifolium* and *Aulax umbellata* are well matched to their native backgrounds of light soil patches. In general, *Protea* seeds appear to not match their native backgrounds and likely rely on an alternative strategy to reduce seed predation. The exception is the limestone endemic, *Protea obtusifolia*, which closely matches the dark, gray limestone soils with a pronounced dark-black tinge to the typically reddish-brown *Protea* hairs.

Most flat-winged *Leucadendron* seeds better or closely (within 1 JND) matched their own substrate compared to surrounding soils. Values were often clustered, meaning highly cryptic seeds, like those of *L.* meridianum, matched their native soil and surrounding soils equally as well. Meanwhile, conspicuous species, such as *P. neriifolia*, were similarly poorly matched to their native soils and surrounding soils. This means that the seed crypsis is not substrate specific, with certain species more cryptic than others across all substrate types. This suggests that seed predation by avian granivores is unlikely to be a substrate-restricting mechanism, and possible speciation driver, for Proteaceae species.

The proportion of dark to light seeds changes significantly across species (Figure 5.3), with some flat-winged *Leucadendron* species only having dark seed colour morphs. Species on Albertinia sand soils (*L. laureolum*, *L. coniferum*, *L.*

xanthoconus, *L. salignum*) tended to have a lower proportion of light seeds, while the species on soils with a high percentage of rock cover (*L. modestum*, *L. teretifolium*, *L. meridianum*, *L. muirii*) showed a higher proportion of light seeds (>35%). Rocky habitats added an additional layer of visual complexity, with many lighter stones (Figure 5.8), likely promoting the persistence of light colour morphs in these populations.

The dark to light seed colour polymorphisms in flat-winged *Leucadendron* is likely maintained by the presence of heterogonous selective landscape of soil mosaics, which provide a significant challenge to bird granivores distinguishing seeds from their backgrounds, and allow different seed colours to differentially survive based on their seed colour matching their background. Avian granivores likely preferentially select for seeds that are mismatched from their background (e.g. dark seeds on light soils), which would further establish the polymorphisms.

A possible ecological explanation for the difference in degree of crypsis between hairy, conspicuous *Protea* seeds and winged, cryptic *Leucadendron* seeds may relate to seed set. *Protea* typically have very low seed set (1-30%) with many empty or inviable seeds, whereas *Leucadendron* (77%) and *Aulax* (94%) seed set is generally high (Collins & Rebelo, 1987). Though there are many factors that determine seed set, the high proportion of empty *Protea* seeds may increase foraging time for seed predators thus acting as a deterrent, while the highly fertile *Leucadendron* likely rely on seed crypsis as their primary deterrent against seed predators post-dispersal.

Conclusion

Cryptic colouration likely plays a crucial role in seed survival for both Proteaceae and other fynbos species in response to opportunistic post-fire seed predation by visually cued avian granivores, such as *C. leucopterus* or *C. totta*. This study on South African Proteaceae seeds adds to our growing understanding of the defensive colouration of seeds in response to visually cued, avian seed predators (Nystrand & Granström, 1997; Saracino et al., 1997; Jones, Krebs & Whittingham, 2006; Lev-Yadun & Ne'eman, 2013; Porter, 2013; Myczko et al., 2015). The results of this study suggest that the role bird granivores has been previously underestimated and they likely play an important role as selective agents of serotinous seed traits in the GCFR.

5.6 Acknowledgments

Thank you to everyone in BIO3015F who helped collect soil samples and to Anina Coetzee and Dawood Hattas for assistance with the spectrophotometer.

Chapter 6

Distributions of major seed dispersal modes in *Leucadendron* (Proteaceae) are strongly influenced by seasonal drought

6.1 Abstract

Understanding the drivers of species distributions is intergral to unraveling the ecology and evolution of important life-history traits, while also allowing us to make predictions on possible range changes under shifting climates and biotic interactions. Using the near-endemic Greater Cape Floristic Region genus *Leucadendron* I initially investigated the dispersal of a group of *Leucadendron* species with an unknown dispersal mode. I found that small, elaiosome-less and wing-less seeds ('nutlets') are not scatter-hoarded. I then investigated the role of spatiotemporally variable climatic and disturbance regimes in the distribution of rodent- and wind-dispersed species in *Leucadendron* using boosted regression tree models.

Serotinous species (wind-dispersed) were more frequently found in sites with less exposure to summer drought, while scatter-hoarded (rodent-dispersed) species are more often in sites with extreme summer drought. Similar results were found for responses to fire return interval, with scatter-hoarded species inhabiting sites with longer and more variable intervals and serotinous species prefering sites with shorter and less variable intervals. The distributions of mutualistic scatter-hoarding rodents did not significantly contribute to explaining either dispersal mode distributions.

Without persistent seed banks, serotinous plants are more vulnerable to local extinction from senescence due to long fire return intervals than scatter-hoarded plants. Further, serotinous seeds are small and generally require favourable conditions (reliable rainfall) for effective post-fire recruitment. Rodent-dispersed species may also be geographically restricted by seed predation

pressures, as increased summer and a general increase in soil moisture leads to increased release of seed volatiles and an inability to effectively hide seeds away from pilferers. In addition, rodent-dispersed species had significantly larger seed mass than all other dispersal modes (nutlets, ant- and wind-dispersed). The larger seed size is likely linked to increased seedling survival rates in drought prone areas and for facilitating scatter-hoarding by rodents.

These findings give context to the evolution of scatter-hoarding and serotiny in the GCFR. Serotiny is a plesiomorphic trait in *Leucadendron*, with rodent-dispersed species likely arising with the aridification and increased seasonality of the western part of the GCFR.

6.2 Introduction

Finding general rules underlying observed patterns in species distributions is a central tenet of biogeography and macroecology (Diaz, Cabido & Casanoves, 1998). This requires the study of distributions across space, time and taxa. Selective pressures of climate and edaphic conditions, disturbance regimes and biotic interactions determine plant distributions (Diaz, Cabido & Casanoves, 1998). Relating plant traits to these conditions can allow insights into the underlying mechanisms and processes of plant distributions (Heads, 2015). Using functional life-history traits rather than species allows a common currency to understand biogeography (Violle et al., 2014).

Plants have evolved myriad life-history traits to survive spatially and temporally variable selective pressures. For example, in fire-prone ecosystems, plants have evolved two different post-fire survival strategies – re-sprouting and re-seeding (le Maitre & Midgley, 1992). Within both strategies there are numerous seed dispersal mechanisms to ensure successful regeneration, each with a collection of mostly unique traits.

Seed dispersal is a mechanism that facilitates recruitment and survival of progeny as seedlings. Selective forces have led to the evolution of a great variety of different fruit or seed morphologies, which may take advantage of abiotic factors or biotic interactions for dispersal (Johnson, 1992). Plant taxa may share similar seed morphologies and dispersal syndrome due to either phylogenetic history or similar selective forces. Biotic dispersal mechanisms include antagonistic or mutualistic interactions with seed eating animals, while abiotic factors may be linked to interacting edaphic, disturbance and/or climatic factors (Howe & Smallwood, 1982).

There is significant interest in the genus *Leucadendron* as a clade to study the evolution of life-history traits, such as pollination or dispersal modes (Barker et al., 2004; Thuiller et al., 2004; Tonnabel et al., 2014, 2018). This is because *Leucadendron* is a moderately large, monophyletic genus (82 extant and 2 extinct species) and is almost entirely restricted to a single floristic region (the Greater Cape Floristic Region, GCFR), which helps to reduce issues associated with phylogenetic mismatching (Thuiller et al., 2004). Various life-history strategies are found in the genus, likely in response to the myriad environmental conditions found throughout the GCFR. Most species are killed by wildfires and thus rely on regeneration from seeds (le Maitre & Midgley, 1992). There are two dominant seed dispersal syndromes in this genus, wind- and rodent-dispersal (i.e. serotiny and scatter-hoarding), with four species being myrmecochorous or ant-dispersed (see Figure 6.1; Supplementary Table 6.1). All Cape serotinous species store seeds in protective structures and only release seeds post-disturbance where they are primarily and possibly secondarily dispersed by wind. Therefore serotinous *Leucadendron* species are referred to as wind-dispersed in this study, though this may not always be the case. The dispersal mode of a number of *Leucadendron* still remains unknown, particularly that of the small nuts or 'nutlets' (13, e.g. Figure 6.1c).

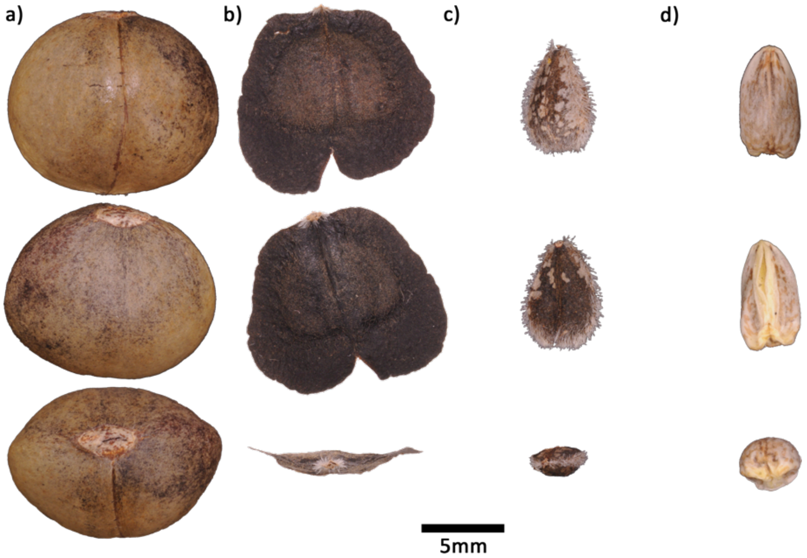


Figure 6.1 Vertical and side views of representative seeds of the four major dispersal modes in *Leucadendron* a) rodent-dispersed, *L. pubescens*, b) wind-dispersed, serotinous *L. laureolum*, c) nutlet, *L. laxum*, and d) ant-dispersed *L. sericeum*, covered in a white, fleshy elaiosome.

Understanding the environmental correlates of wind and rodent-dispersed plants can help answer questions related to drivers of distributions, evolution and future range shifts due to changing climates. By incorporating relevant biotic factors (e.g. mutualist species' distributions) and clarifying dispersal modes, this study aims to build on previous research on the biogeography of *Leucadendron* (Barker et al., 2004; Thuiller et al., 2004; Tonnabel et al., 2018) and add finer resolution to the understanding of the link between dispersal modes and species distributions. To clarify dispersal modes, I first investigated the dispersal mechanisms of *Leucadendron* species with nutlets. I hypothesized that nutlets are dispersed by rodents, as they are non-serotinous and do not have adaptations for wind-dispersal. Next, I investigated the link between seed mass and dispersal syndrome. I then used a machine learning approach to test the relative influence of climate, disturbance regimes and mutualists on the distributions of two major dispersal modes. As scatter-hoarded plants typically had larger seeds than serotinous plants, possibly buffering seedlings against a more extreme climate, I hypothesized that scatter-hoarded plant distributions are centered in seasonally arid regions, while serotinous plant distributions are typically found in more mesic habitats.

6.3 Methods

Dispersal of nutlets

To determine whether *Leucadendron* nutlets are rodent-dispersed, I investigated the dispersal of *L. laxum* and *L. elimense* seeds at the Elim Commonage (S 34.583269°, E 19.728444°). At seed depots, I placed out five, ten and twenty seeds of commercial sunflower, *L. laxum* and *L. elimense*, respectively. Commercial sunflowers were used to promote interactions, as they are highly attractive to small mammal granivores (White, Bronner & Midgley, 2017). I attached 15 cm long fluorescent UV threads (see Midgley et al., 2002) to seeds to track movement from seed depots. Two trials were run starting on 20 May and 27 June 2017, respectively. Ten seed depots were used during each trial and were left *in situ* for five days and nights, for a total of 100 camera trap nights over both trials. To record seed-animal interactions, each seed depot had a Ltl Acorn 6210M remotely activated trail camera (Shenzen LTL Acorn Electronics Co., Shenzen, Guangdong, China) focused on the seeds. Cameras were set to record a 30-s video when activated. Each time an animal interacted with a seed or entered/left the field of view it was considered a new observation.

Seed mass

I accessed seed mass data on *Leucadendron* from Royal Botanic Gardens Kew (2018). Hybrids were removed from the analysis, while I averaged together values for subspecies and varieties to give a single value per species. After this, values were available for 73 species. I measured seed mass of n = 20 ad-hoc collected *L. glaberrimum* with seed collected at Driehoek Farm (S 32.445225°, E 19.191380°) in December 2017. To account for heteroscedastic variances in seed mass, Kruskal Wallis tests and Spearman Rank Correlation analyses was used to investigate the relationship between seed mass and dispersal modes and other important predictor variables. Unless stated otherwise, all values are reported as mean ± standard deviation.

Spatial data

I obtained georeferenced presence/absence data for 81 *Leucadendron* species from the Protea Atlas Database, collected during a large citizen science mapping project for southern African Proteaceae (Rebelo, 2001). I merged subspecies and variety classifications to species level and two species awaiting description were merged to their closest allies (*L. crassulaefolium* to *L. arcuatum* and *L. touwsbergensis* to *L. tinctum*), as long as relevant life-history traits were consistent (e.g. serotiny). *Leucadendron immoderatum* was described after the Protea Atlas project ended, so spatial data was collected from iNaturalist (www.inaturalist.org) using the 'rinat' package (Barve & Hart, 2017) in R (R Core Team, 2018b). Overall data for 82 species were used in the analysis. To classify *Leucadendron* seed dispersal life history traits, I used Williams (1972) classifications of serotinous and myrmecochorous seeds, while classifying scatter-hoarded seeds using Midgley & Anderson (2005) criteria of > 5 mm length and 2 mm seed coat or those with previous research confirming interactions (Midgley et al 2002, Midgley & Anderson 2005, White et al. 2017), nutlets represented the remaining species with small seeds. In the analysis of seed dispersal syndrome distributions, only serotinous (n = 43) and scatter-hoarded (n = 22) syndromes were considered, with ant-dispersed (n = 4) and nutlet (n = 13) species excluded. The dispersal of nutlets was classified after *in situ* investigation into rodent interactions. Nutlets were not scatter-hoarded and were therefore excluded from the spatial analysis (n = 13, see Supplementary Table 6.1). Lastly, I only included species with a longitude of less than 27° to limit the analysis to species within the GCFR. This step effectively excluded all *L. pondoense* observations. After these exclusions, I used the spatial distributions of 81 species with 132729 observations.

During model development, I included a range of potential explanatory variables from different sources, including climate, edaphic, disturbance regimes, mutualist co-occurrence and vegetation types. I sourced climate variables from Fick and Hijmans (2017) and Abatzoglou et al. (2018), edaphic variables from Hengl et al. (2014) and fire return intervals from Merow et al. (2014).

I obtained bioclimatic variables from the WorldClim 2 dataset (Fick & Hijmans, 2017). The 19 variables represent the mean values over the available period 1970-2000 for annual trends, seasonality and extreme or limiting environmental factors for temperature and precipitation. The resolution is at approximately 1 km^2, effectively representing the mean climatic conditions over recent periods.

Edaphic properties were sourced from the ‘SoilGrids1km’ dataset (Hengl et al., 2014). This included soil organic carbon content (g kg^{-1}); soil pH (in water); sand, silt and clay fractions (% gravimetric), bulk density (kg m^{-3}), cation-exchange capacity (cmol kg^{-1}), coarse fragments (% gravimentric), soil organic carbon stock (t ha^{-1}), depth to bedrock estimate up to 2.4 m (cm) and bedrock probability of occurrence (0-100%). These data are at 1 km^2 resolution for six soil depths to 1.5 m deep and were averaged by depth-weighted averaging to make a two-dimensional layer.

Climatic water balance data were sourced from the ‘TerraClimate’ dataset (Abatzoglou et al., 2018). The TerraClimate dataset contains useful derived variables, including actual evapotranspiration (mm), potential evapotranspiration (mm), climate water deficit (mm), Palmer Drought Severity Index, soil moisture (mm), and vapour pressure deficit (kPa), with monthly values for each of these from 1958-2015. Spatial resolution is at ~4 km^2 and values were averaged over the available time period to represent the mean ‘terraclimatic’ conditions over recent times.

For estimates of fire return intervals, I used the Merow et al. (2014) dataset. Merow et al. (2014) used vegetation post-fire recovery time (Wilson et al., 2010; Wilson, Latimer & Silander, 2015) together with CapeNature and MODIS burned area polygons from 1980-2010 in a survival model framework to estimate fire return intervals at 500 m^2 resolution for most of the GCFR.

The annual average NDVI was calculated from the 10 days maximum-value composite NDVI images (250 m^2 resolution) obtained between 2001 and 2010. These were derived from eMODIS TERRA (US Geological Survey Earth Resources Observation and Science Center), which is corrected for atmospheric effects (Swets et al., 1999). The ASTER 30m resolution global digital elevation model (ASTER Global Digital Elevation, 2014; earthdata.nasa.gov) was used to quantify elevation and to calculate landscape slope and aspect. The 2012 vegetation map of South Africa, Lesotho and Swaziland (Mucina et al., 2014) was used to classify vegetation types and was accessed via the Biodiversity GIS website www.bgis.sanbi.org.

Species distribution models (SDM) for the known small mammal, scatter-hoarding mutualists, *Acomys subspinosus* and *Gerbilliscus paeba* (Midgley & Anderson, 2005; White, Bronner & Midgley, 2017), were produced using MaxEnt (as I had presence only data) (Phillips, Anderson & Schapire, 2006) using the 'dismo' package (Hijmans et al., 2017) in R (R Core Team, 2018b). Explanatory variables used were limited to climate, edaphic and vegetation types. Presence data for each species within the GCFR was collected from multiple sources including iNaturalist, Global Biodiversity Information Facility (GBIF.org, 2018) and thorough literature reviews for both species: *A. subspinosus* (Wiens & Rourke, 1978; Bond, Ferguson & Forsyth, 1980; Nel, Rautenbach & Breytenbach, 1980; Midgley & Clayton, 1990; van Hensbergen et al., 1992; Johnson, Pauw & Midgley, 2001; Fleming & Nicolson, 2002a,b; Midgley et al., 2002; Midgley & Anderson, 2005; Letten & Midgley, 2009; Turner, Midgley & Johnson, 2011; van den Heuvel & Midgley, 2014; Zoeller et al., 2016; White & Midgley, 2017; Kühn, Midgley & Steenhuisen, 2017; Lombardi et al., 2017; Wester, Karvang & Niedzwetzki, 2018) and *G. paeba* (Johnson, Pauw & Midgley, 2001; Midgley & Anderson, 2005; Kleizen, Midgley & Johnson, 2008; Johnson & Pauw, 2014; Weighill, Huysamer & Anderson, 2017; White, Bronner & Midgley, 2017). In total I collected 69 and 43 presence observations for *A. subspinosus* and *G. paeba*, respectively, from the various sources. To reduce spatial pseudoreplication, only one record of each species per site was included for modeling. I used MaxEnt's jackknife analysis to determine predictor variables relative influence on model robustness.

Before modeling, the resolution of all environmental variables was resampled to $\sim$1 km^2 using bilinear interpolation. FIRMS and TerraClim datasets were accessed and raster calculations performed using the Google Earth Engine (GEE) cloud-computing platform for satellite imagery and analysis of large datasets (Gorelick et al., 2017). Raster resampling and calculations for FIRMS and TerraClim and all other environmental variables were performed in R using the 'raster' package (Hijmans, 2017).

Boosted regression trees

To determine the relative influence of environmental variables on the spatial distribution of rodent and wind-dispersed *Leucadendron* dispersal syndromes I used the method of boosted regression tree (BRT) models, as outlined by Elith et al. (2008) using the 'dismo' (Hijmans et al., 2017) and 'gbm' (Ridgeway, 2017) packages in R. BRT models generate multiple relatively simple models, which are then combined for more robust predictions of the dependent variable (Elith, Leathwick & Hastie, 2008). These models allow for the inclusion of many types of predictor variables and can accommodate for missing values (De'Ath, 2007).

Before running the models, a convex hull buffer was drawn around all *Leucadendron* spatial points and the environmental variables were masked to this shape. To avoid non-independence of variables I evaluated them for collinearity using a threshold of $|r| > 0.7$ and selected one variable per highly correlated group (Dormann et al., 2013) (see Supplementary Figure 6.1).

To achieve minimum predictive error I ran a parameter optimization exercise and settled on a Bernoulli BRT models that ranked best for cross-validated deviance, cv AUC and AUC scores, leaving the model with tree complexity = 5, learning rate = 0.01, bagging fraction = 0.5, and cross-fold validation = 10 for both dispersal types. The total explained deviance was calculated as the difference between mean total and cross-validated deviance divided by the mean total deviance. The relative importance of variables is calculated as the number of times a variable is selected for recursive splitting during tree building, weighted by the squared improvement to the model after each split and averaged over all trees (Friedman & Meulman, 2003). BRT models were run on both rodent- and wind-dispersed *Leucadendron* modes.

6.4 Results

Dispersal of nutlets

After five nights, small mammal seed predators had encountered and removed nutlets at 12 of 20 depots. At these 12 depots, 45%, 53% and 46% of sunflower (2.25 ± 2.20 depot^{-1}), *L. laxum* (5.33 ± 4.71 depot^{-1}) and *L. elimense* (9.17 ± 9.07 depot^{-1}) seeds were removed overall, respectively. Of the 201 nutlets removed from depots, 149 were found (74%) with all found seeds consumed (based on discarded husks) and none buried. Seed removal distances ranged from 0.5 to 0.8 m, with 93% of seeds consumed *in situ* (<0.5m from depot).

Only three rodents were observed interacting with seeds: *Steatomys krebsii*, *Rhabdomys pumilio* and *Dendromus mesomelas*. *Otomys irroratus* (*n* = 9), a musk shrew (*Crocidura* spp.) (*n* = 1) and the birds *Mirafra fasciolata* (*n* = 2) and *Saxicola torquatus* (*n* = 1) were all observed at seed depots, but never interacted with the seeds. *Steatomys krebsii* accounted for 78.3% (*n* = 36 of 46) of all observed interactions, with *R. pumilio* and *D. mesomelas* making up 15.2% and 6.5%, respectively.

Seed mass

The mean mass of rodent dispersed seeds (mean ± sd, 136.66 ± 54.25 g/1000 seeds) was significantly larger than seeds of all other dispersal modes (ant: 26.61 ± 18.69 g/1000 seeds; nutlets: 19.58 ± 10.47 g/1000 seeds; wind: 25.11 ± 44.13 g/1000 seeds). Ant-dispersed and nutlet seeds were not significantly different in mean values from one another, while wind-dispersed seeds were the smallest (Supplementary Figure 6.2a). Nutlets were all smaller than 50 mg, while all scatter-hoarded nuts were greater than 50 mg. A significant, negative correlation was found between seed mass and precipitation in the driest month (R = -45, p < 0.01) (Supplementary Figure 6.2b).

MaxEnt predictions

Considering the small mammal distribution predictions, MaxEnt performed well according to the area under the receiver operation characteristic curve (AUC), threshold-based evaluation methods (Phillips, Anderson & Schapire, 2006) and had realistic outputs (Supplementary Figure 6.3). The *Gerbilliscus paeba* and *Acomys subspinosus* models had AUCs of 0.93 and 0.97, respectively. Jackknife analysis showed that soil moisture (22.24%) and precipitation seasonality (38.17%) were the variables with the most influence for *G. paeba* and *A. subspinosus*, respectively (see Supplementary Table 6.2). In general, the SDMs showed that *G. paeba* should be mostly found in the northwest and *A. subspinosus* should be in the high elevations of the GCFR.

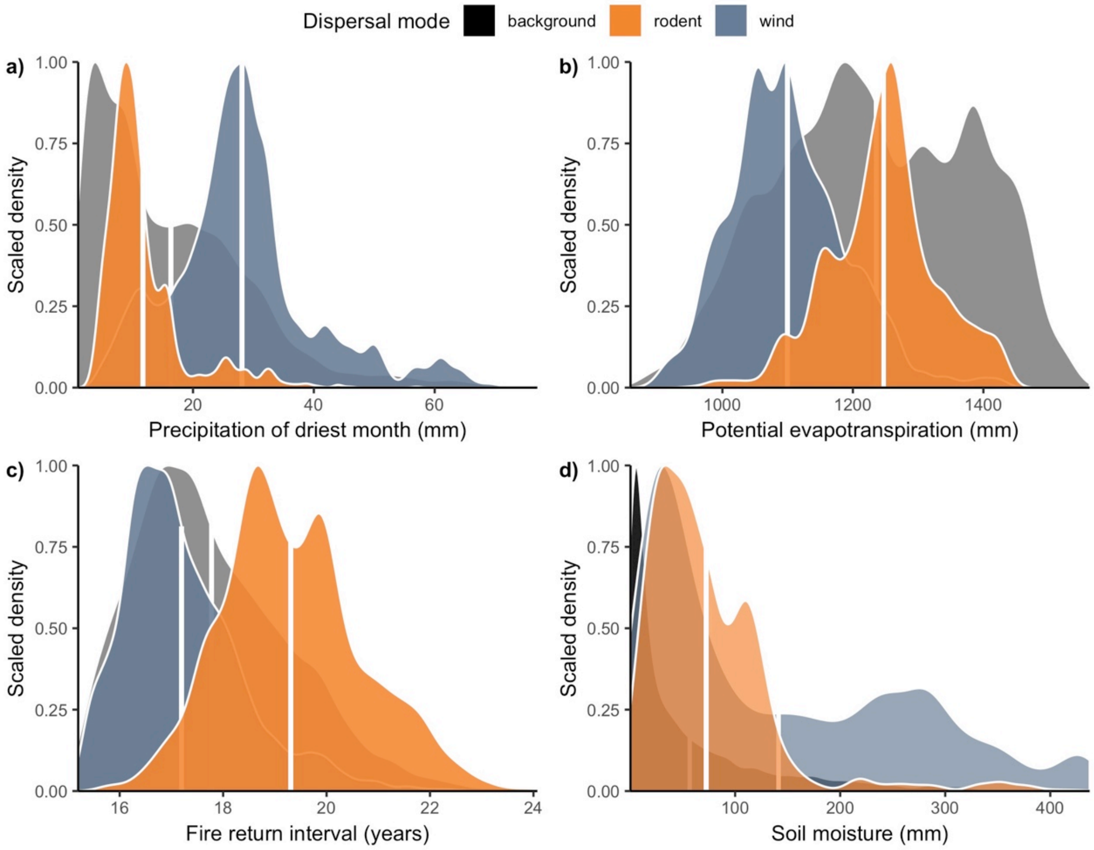


Figure 6.2 Scaled density distributions and mean values (white line) of top four most important variables in determining rodent- and wind-dispersed *Leucadendron* species' distributions, including a) precipitation in the driest month, b) potential evapotranspiration, c) fire return interval and d) soil moisture.

Boosted regression tree analysis

Considering the drivers of *Leucadendron* dispersal modes, boosted regression analysis produced a list of explanatory variables with precipitation in the driest month being the most important for both dispersal modes (Table 6.1). This finding was robust to the addition or removal of other variables (data not provided). The final simplified BRT model for rodent and wind dispersal modes included 2900 and 4100 trees; a training data correlation of 0.73 ± 0.1 and 0.76 ± 0.01 (mean ± se); and a cross-validation correlation of 0.48 ± 0.01 and 0.50 ± 0.01 (mean ± se), for each dispersal mode respectively.

The BRT models dispersal mode distributions explained 41 ± 0.01% and 49 ± 0.01% (mean ± se) of the deviance for rodent and wind dispersed *Leucadendron*, respectively (Table 6.1). Collectively, climatic variables had the largest relative influence accounting for 56.5% and 66.9%, with edaphic variables only accounting for 6.5% and 9.3% for rodent and wind dispersal, respectively (Table 6.1). Disturbance contributed a meaningful role, with fire return intervals accounting for 13.9% and 5.4%, respectively. Biological and topographic variables had little influence on the distribution of dispersal syndromes accounting for less than 5% for each variable in both cases (Table 6.1).

Table 6.1 Relative influence of variables and summary statistics from BRT models for both rodent and wind-dispersed *Leucadendron*.

	Relative influence (%)	
	Rodent	Wind
P_driest_month	29.38	41.77
Fire_return_interval	13.91	5.44
Soil_moisture	10.24	5.84
Potential_evapotranspiration	10.21	11.70
Mean_T_wettest_quarter	4.86	3.87
Acomys_subspinosus	4.18	2.51
Gerbilliscus_paeba	3.05	2.34
NDVI	2.51	3.36
Slope	2.21	2.24
PDSI	1.86	2.47
pH_index	1.85	2.41
Coarse_fragments_volume	1.71	1.23
Aspect	1.64	1.88
Silt_particles	1.62	1.62
Cation_exchange_capacity	1.32	1.41
Mean_T_driest_quarter	NA	1.19
Soil_organic_carbon_concentration	NA	2.66
Correlation mean (±se)	0.73 (0.01)	0.77 (0.01)
Deviance mean (±se)	0.41 (0.01)	0.49 (0.01)
Pseudo R2	0.53	0.55

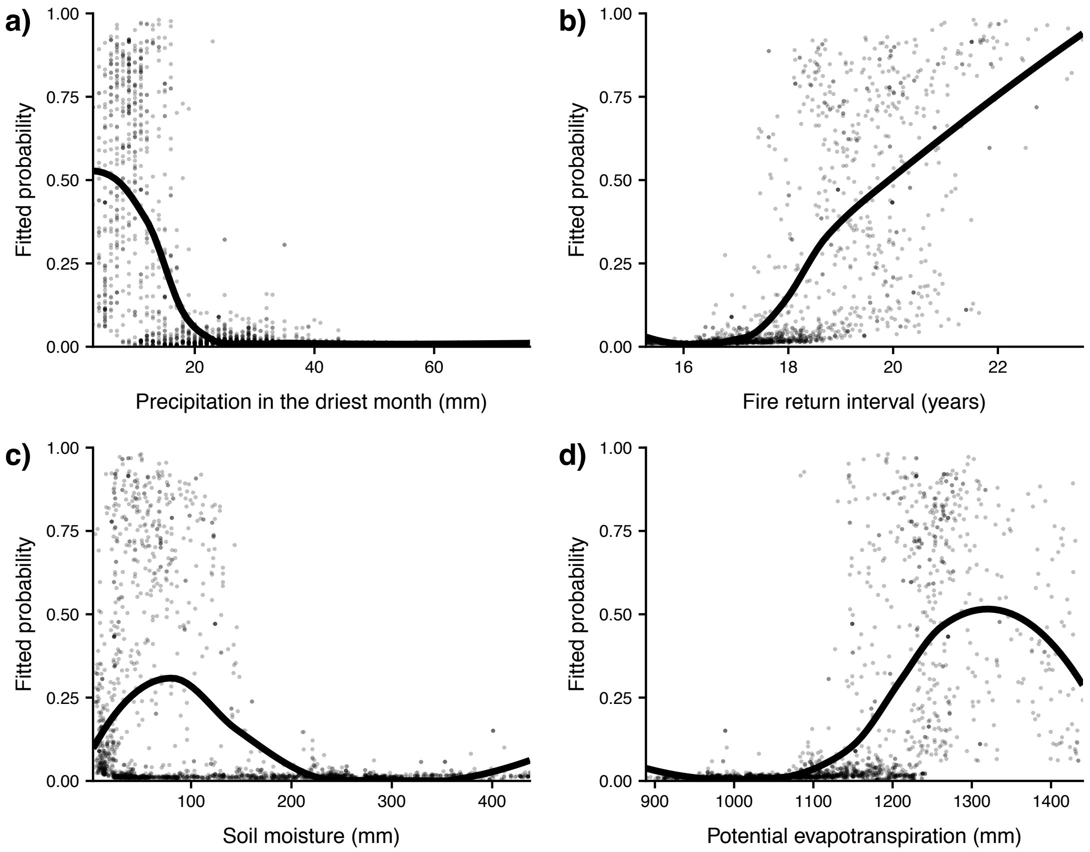


Figure 6.3 Fitted probabilities of the top four most important variables in determining rodent-dispersed *Leucadendron* species' distributions, including a) precipitation in the driest month, b) fire return interval, c) soil moisture and d) potential evapotranspiration.

Models for both dispersal modes (rodent and wind) yielded the same four environmental variables with the greatest relative influence: precipitation of the driest month (29.4% and 41.8%), potential evapotranspiration (10.2% and 11.7%), soil moisture (10.2% and 5.8%) and fire return intervals (13.9% and 5.4%) (Table 6.1). Raw observed data (Figure 6.2) and fitted probability plots (Figures 6.3) show that the probability of occurrence for rodent dispersed *Leucadendron* was greatest at low precipitation in the driest month (mean ± sd = 12.05 ± 6.65 mm), long fire return intervals (19.23 ± 1.32 years), low soil moisture (79.10 ± 62.33 mm), and high PET (1240.45 ± 80.86 mm). Conversely, wind dispersed *Leucadendron* were most likely to occur in habitats with greater precipitation in the driest month (28.11 ± 11.54 mm), shorter fire return intervals (17.19 ± 1.12 years), high soil moisture (146.13 ± 123.59 mm) and low PET (1098 ± 86.67 mm) (Figure 6.2, Figure 6.4). Spatial prediction showed rodent-dispersed *Leucadendron* have a geographical centre in the northwest of the genera's range (Figure 6.5a), while wind-dispersed species are best suited to the southern half of the GCFR (Figure 6.5b).

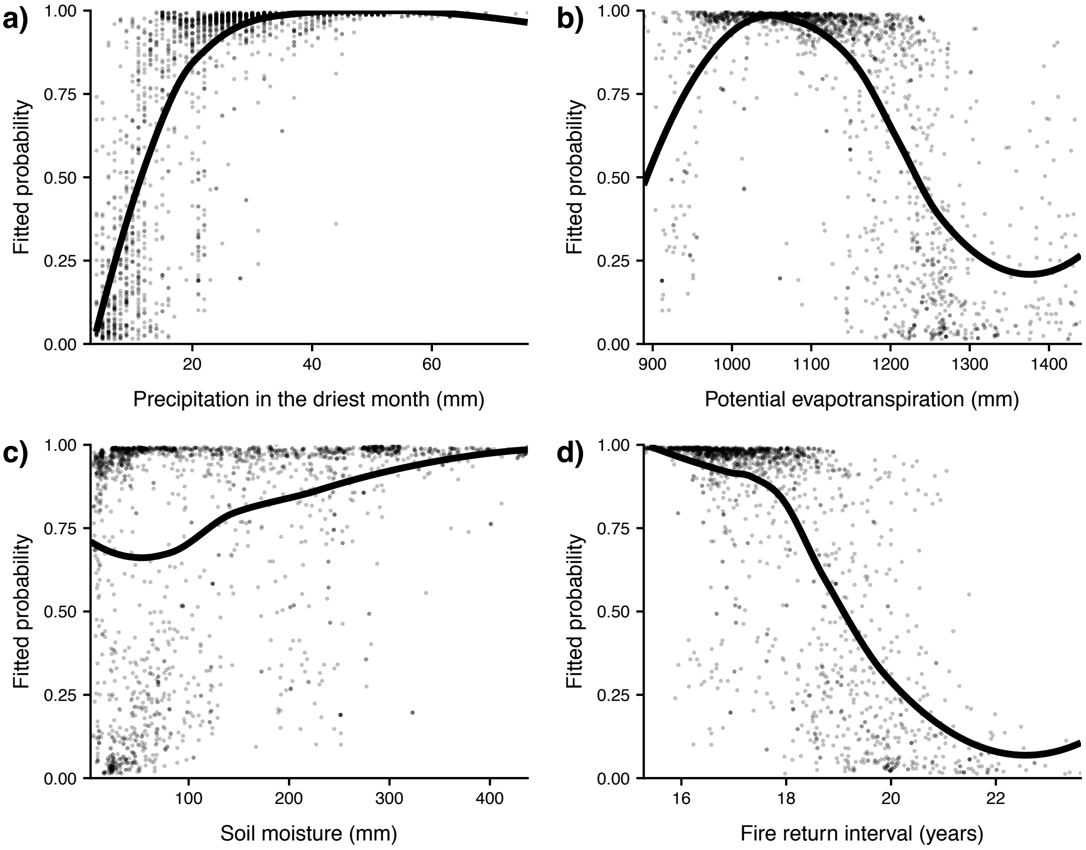


Figure 6.4 Fitted probabilities of the top four most important variables in determining wind-dispersed *Leucadendron* species' distributions, including a) precipitation in the driest month, b) potential evapotranspiration, c) soil moisture and d) fire return interval.

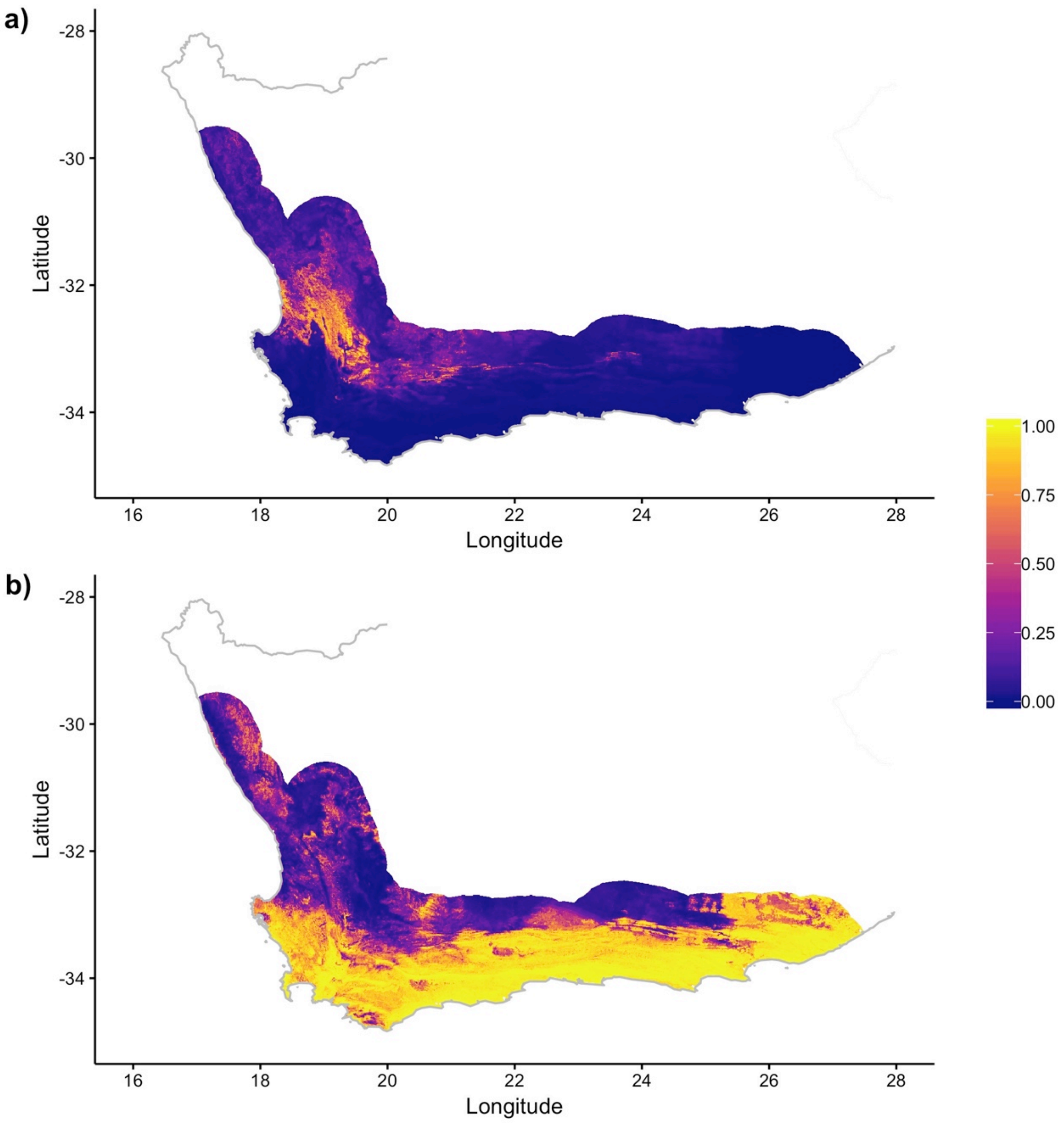


Figure 6.5 Predicted distribution likelihoods for a) rodent- and b) wind-dispersed *Leucadendron* based on BRT models across the range of *Leucadendron* in the Greater Cape Floristic Region, South Africa.

6.5 Discussion

Ecology of Nutlets

Natural history studies on the dispersal of nutlets, such as *L. elimense* and *L. laxum,* suggested these species do not rely on rodents for seed dispersal. The small mammals that interacted with *L. elimense* and *L. laxum* seeds are only known to consume seeds and have no recorded scatter-hoarding behaviours. However, the possibility that *Acomys subspinosus,* the only known scatter-hoarder in the southern Cape, does not cache nutlets cannot be discounted as the diet of this species shifts seasonally, from seeds in the summer/autumn to mainly insects, when trials in this study were done, with typically less scatter-hoarding in winter months (Rusch, Midgley & Anderson, 2014).

Nutlets, wind- and ant-dispersed species seeds are all relatively small (<50 mg) compared to rodent-dispersed seeds, with the major difference being the presence or absence of seed appendages. This suggests that small seeds with no dispersal appendage (e.g. elaiosome or plume) rely on an alternative dispersal strategy to rodent, ant or wind dispersed plants. With or without seed appendages, the dispersal distance of these plants will be predictably low, much like the vast majority of GCFR plants (Manning & Goldblatt, 2012). Most GCFR plants produce small seeds with no dispersal appendage (Manning & Goldblatt, 2012). This is the case for most species in the four largest plant families of the region, Asteraceae, Fabaceae, Iridaceae, and Ericaceae, suggesting poor seed dispersal capabilities are commonplace. Low seed dispersal distances are characteristic of the GCFR, with ant dispersal disproportionately represented with over 1000 species producing seeds with elaiosomes (Bond & Slingsby, 1983). These short dispersal distances could reduce gene flow between populations facilitating speciation and increasing risk of localized extinctions (Manning & Goldblatt, 2012).

An important adaptation to fire-prone environments is the burial of seeds below ground. Ants and rodents provide this service, moving seeds to safe sites protected from fire and seed predation in the soil bank (Bond & Breytenbach, 1985; Moore & Vander Wall, 2015). A gap in our understanding of GCFR seed dispersal is how seeds with no clear dispersal adaptation enter the soil bank and cope with fire and predation pressures. The dispersal, ecology and evolution of this group of seeds are not understood and require research attention.

Seed mass

Seed mass is important to overall plant fitness as it is linked to a trade-off between two key life history components (Coomes & Grubb, 2003). These are 1) seed mass is negatively correlated with the number of seeds a plant can produce; and 2) seed mass is positively correlated with seedling survival (Leishman et al., 2000). The implications are that smaller-seeded species may be better colonisers as they can arrive at more sites (due to producing more seeds), while larger-seeded species produce larger seedlings, which can better withstand a lack of available resources or disturbance (e.g. drought) (Coomes & Grubb, 2003). Within *Leucadendron* dispersal modes generally fall into broad seed mass classes. *Leucadendron* with rodent-dispersed seeds are significantly larger than wind-dispersed seeds and larger seeds are assumed to be more competitive at sites with severe drought (Supplementary Figure 6.2).

Drivers of dispersal modes

The best predictor of dispersal mode was seasonal drought. Seasonally dry habitats (less precipitation in the driest month, greater PET and lower soil moisture) with longer fire return intervals, concentrated in the northwest of the GCFR, have the greatest probability of occurrence for rodent dispersed *Leucadendron*. Wind dispersed *Leucadendron* were more likely to occur in wetter habitats (more precipitation in the driest month, lower PET and greater soil moisture) with shorter fire return intervals, as recruitment of serotinous seeds in the GCFR is maximized if predictable rains follow the austral summer/autumn fire season (le Maitre & Midgley, 1992). In areas where precipitation is less predictable, such as the arid northwest of the GCFR, more frequent droughts could lead to recruitment failure. These findings support the hypothesis that seasonal drought exerts a strong selective pressure for larger, rodent-dispersed seeds, which can better buffer against mortality in the variable environmental conditions during the seedling phase.

At a local scale, fire return intervals are variable, requiring plants to buffer against this variation with robust seed-storing mechanisms. Once sexually mature most fynbos species produce seed annually, with the most recent years seed crop contributing most to regeneration (Bond, 1985; van Wilgen & Viviers, 1985; Midgley, 1989). Underground seed banks and serotiny are two alternative strategies for regeneration under fire disturbance (le Maitre & Midgley, 1992).

For the obligate reseeders, both rodent- and wind-dispersed species are susceptible to local extinction events if fire intervals are shorter than time to first seed crop (Kruger, 1979; Keeley & Fotheringham, 2000). However, if fire intervals are too long, serotinous wind-dispersed plants may senesce before seed release, again leading to local extinction; whereas persistent seed banks may allow for more tolerance of long fire intervals.

My findings support those of Tonnabel et al. (2018) with wind-dispersed, serotinous species found in habitats with shorter, less variable fire return intervals. Tonnabel et al. (2018) similarly hypothesized that intensity of summer drought should drive fire-related traits in *Leucadendron*, however, they did not find a signal for this within their process-based niche evolution modeling framework. My results, as hypothesized, showed that the intensity of summer droughts is the most important factor in predicting the distribution of these fire-related dispersal traits and the likely evolution thereof. Thuiller et al. (2004) suggested that allometric scaling should predict *Leucadendron* with large leaves, cones and thus seeds in humid subtropical regions in the GCFR. My results contradict these findings, with large-seeded rodent-dispersed *Leucadendron* mostly absent from the lowlands of the southern Cape and instead centered in the northwestern Cape (Figure 6.5a).

The influence of mutualistic scatter-hoarding rodents was marginal in explaining the distribution of both dispersal modes, similar to findings in North America, where scatter-hoarder richness was mismatched from scatter-hoarded plant richness (Dittel, Moore & Vander Wall, 2019). This is more relevant for rodent dispersal, where one would expect rodent distribution to play an important role in rodent-dispersed plant distribution. Scatter-hoarding rodent distributions were effectively mismatched from rodent-dispersed *Leucadendron*. It is clear that both *Acomys subspinosus* and *Gerbilliscus paeba* occur outside of the known distribution of nut-fruited *Leucadendron*, suggesting they are not reliant on these species for persistence. However, nut-fruited *Leucadendron* are reliant on their mutualistic dispersers and are not found outside of areas occupied by these rodents. My findings therefore suggest abiotic factors are more important in determining the distribution of dispersal modes. A limitation of using presence only small mammal distribution maps is that they may not properly represent coevolutionary processes that are density dependent, such as seed dispersal.

Despite the BRT findings, rodents are likely to have played an important role in shaping large-seeded *Leucadendron* reproductive strategy. Seed mass is both heritable and sensitive to selection (Jansen et al., 2002). Via preferential seed removal, caching, re-caching and consumption, scatter-hoarders can act as selective filters on seed traits and, in turn, those traits influence how scatter-hoarders forage (Vander Wall et al., 2018). Many studies have found that rodents preferentially remove and bury larger seeds, while consuming smaller seeds, likely driving directional selection favouring large seeds (e.g. Vander Wall, 1995a, 2003; Xiao, Zhang & Wang, 2005; Wang & Chen, 2009; Steele et al., 2013). However, Rusch et al. (2013) found a stabilizing selection effect via seed predation by *Acomys subspinosus* on nut-like fruits, where small, thin-hulled seeds were preferentially consumed, medium-sized and –hulled seeds were scatter-hoarded and large, thick-hulled seed were ignored. This suggests there are both lower and upper limits on optimal seed size for scatter-hoarding rodents. If seeds are too small or too large, the energy spent foraging and handling seeds may exceed the energy gained from consuming the seed.

Nutlets, ant and wind-dispersed *Leucadendron* may be below the lower seed size required to induce scatter-hoarding in Cape small mammals, although they are still susceptible to predation. A plausible scenario in the evolution of nut-fruited *Leucadendron*, is that the drying of the winter-rainfall region of the GCFR led to increased drought-related environmental selective pressures on seedling survival. Consequently, this drove the evolution of large-seededness, which induced the interest and consequent selective pressures of seed-eating and -caching rodents.

There is a further restriction on the distribution of rodent-dispersed seeds. Scatter-hoarding will only occur where the hoarding animals have a net positive return on the energy spent acquiring new energy (Vander Wall & Jenkins, 2003; Brodin, 2010). Seeds are protected by scatter-hoarders hiding them in caches with low spatial densities, few seeds and deeper in the soil, yet are still susceptible to pilferage (Vander Wall, 1998; Vander Wall & Jenkins, 2003). Pilferers locate caches by olfaction and the primary environmental factor that influences seed detectability is moisture (Johnson & Jorgensen, 1981; Vander Wall, 1993, 1995b, 1998; Jorgensen, 2001; Rusch, Midgley & Anderson, 2013b; Paulsen et al., 2014). Seeds release volatile compounds and generally release more under wet conditions, making them more susceptible to predation (Vander Wall, 1998). I propose that a critical factor underlying the distribution of scatter-hoarded *Leucadendron*, and likely other nut-fruited plants in the GCFR, is seasonal dryness, which gives scatter-hoarders a competitive advantage over pilferers. Without this, cached seeds may be easily detected and scatter-hoarding would no longer provide a net return on energy spent. This effectively limits the distribution of scatter-hoarded plants to the regions that experience seasonal drought.

Evolution of dispersal modes

Looking at the evolution of serotiny globally, Lamont and Enright (2000) hypothesized that greater reliability of precipitation in Australian and South African Mediterranean-type ecosystems (MTE) would favour serotiny. In my findings, this prediction plays out at the regional scale for the GCFR, with wind-dispersed, serotinous *Leucadendron* found in milder climates with less severe summer droughts, aseasonal rainfall and shorter fire intervals. This means precipitation is reliable, ensuring effective post-fire recruitment as most serotinous seeds have no dormancy and need favourable conditions post-release (Lamont et al., 1991; Cowling et al., 2005). In addition, mild climates place less selective pressure on seed size, as water is available throughout the year allows smaller seeds to be competitive. Larger seeds have a competitive advantage where there is low rainfall in summer, as they produce larger, more resilient seedlings.

Serotiny is most prevalent where fire intervals are shorter, with the Australian kwongan and South African fynbos having the shortest intervals across all MTEs (Bradshaw et al., 2011). As shown in my and Tonnabel et al.'s (2018) results, longer fire intervals are predicted to select against serotiny. This is likely due to the risk associated with time to stand death exceeding the stand’s lifespan, leading to no recruitment. In this scenario, underground storage of seeds is favoured (Lamont et al., 1991).

Williams (1972) originally classified *Leucadendron* based on fruit characters, suggesting non-serotiny as the primitive state. Using molecular phylogenetics, both Barker et al. (2004) and Tonnabel et al. (2014) disagreed with Williams (1972), suggesting that serotiny is the likely primitive state of dispersal in *Leucadendron*. Similar results have been found in *Pinus* subgenus *Pinus*, with an inferred serotinous common ancestor ~89 Ma (He et al., 2012). Unfortunately, important nodes on the *Leucadendron* phylogeny remain poorly resolved reducing confidence in the frequency of trait transitions. Despite this, rodent-dispersal is inferred as a derived dispersal mode (Barker et al., 2004; Tonnabel et al., 2014).

A proposed scenario for the evolution of rodent-dispersed seeds in *Leucadendron* would be to link its origin with the shift to a more arid and seasonal climate in the northwest of the GCFR. The crown age of the Proteaceae subfamily Leucadendrinae (includes *Leucadendron* and nine other GCFR Proteaceae genera) is inferred to be between 22-39 Ma (Sauquet et al., 2009). This places the origin of *Leucadendron* long before the climatic changes that led to the characteristic Mediterranean climate of the GCFR (Verboom et al., 2014), but does not preclude the possibility of more recent radiation and extinction events that may be linked to these changes.

I propose two non-exclusive hypotheses for the association between scatter-hoarded *Leucadendron* and dry environments. Firstly, that large seeds, which have more resources can better survive drought and alternatively, that scatter-hoarding is more likely to occur in dry environments, because seeds are less susceptible to pilferage. Testing these hypotheses will help to establish their relative importance in driving the evolution and persistence of scatter-hoarding in the GCFR.

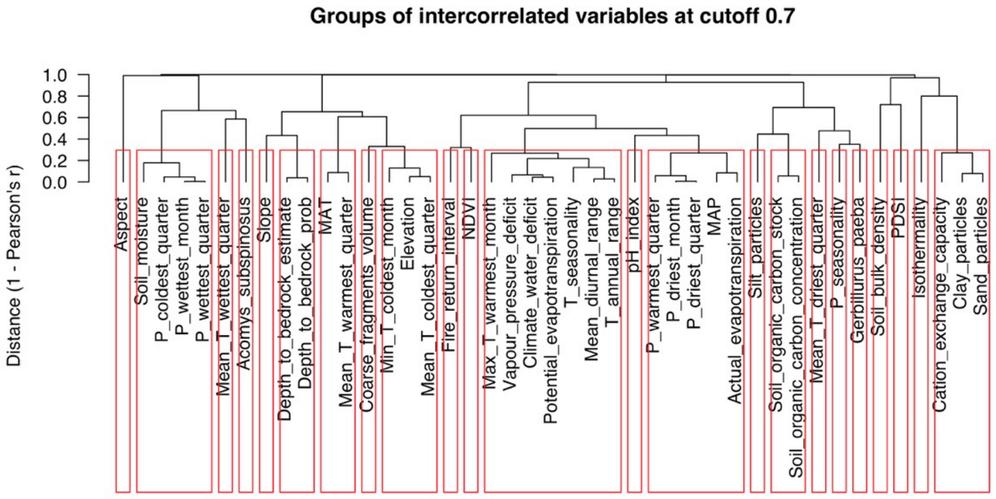
Conclusions

Overall, these results show that heterogenous climatic and disturbance ecotones can drive the persistence and evolution of dispersal syndromes and species distribution. With projections for a hotter and drier western GCFR; and hotter and stable or increased rainfall in the eastern GCFR (Altwegg et al., 2014), predictions can be made on possible range retractions or expansions based on dispersal syndrome. Other life history traits that may be similarly important in determining species distributions include resprouting ability and pollination syndromes (Tonnabel et al., 2014). The group of *Leucadendron* nutlets and other passively dispersal seeds require further attention in the GCFR to determine how these seeds effectively disperse and how this may influence their ecology.

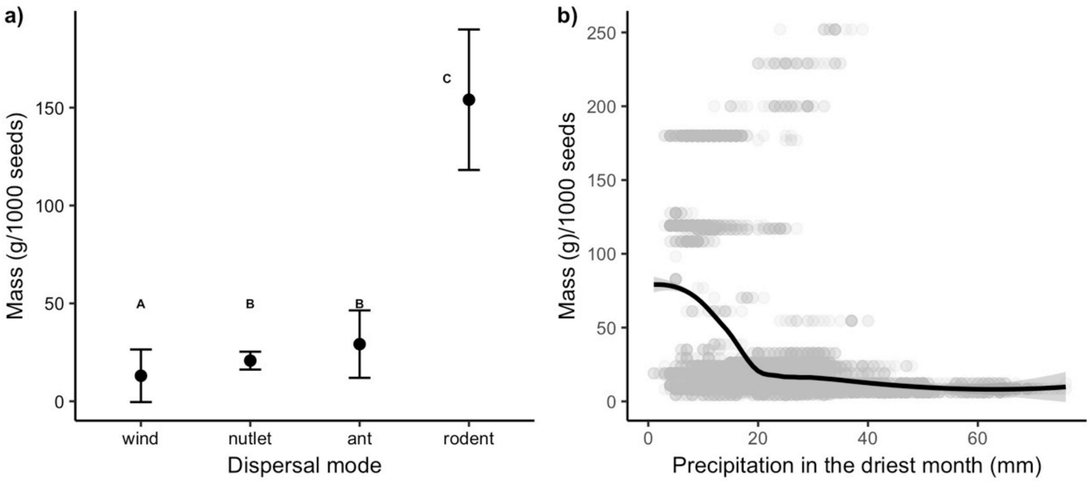
6.6 Acknowledgements

Thank you to Tony Rebelo for access and use of the Protea Atlas database. Thank you particularly to Michael Cramer for assistance with the boosted regression tree analysis and Jeremy Midgley and Gary Bronner for comments on the manuscript. Thank you to Chevonne Reynolds for assistance with bird identification.

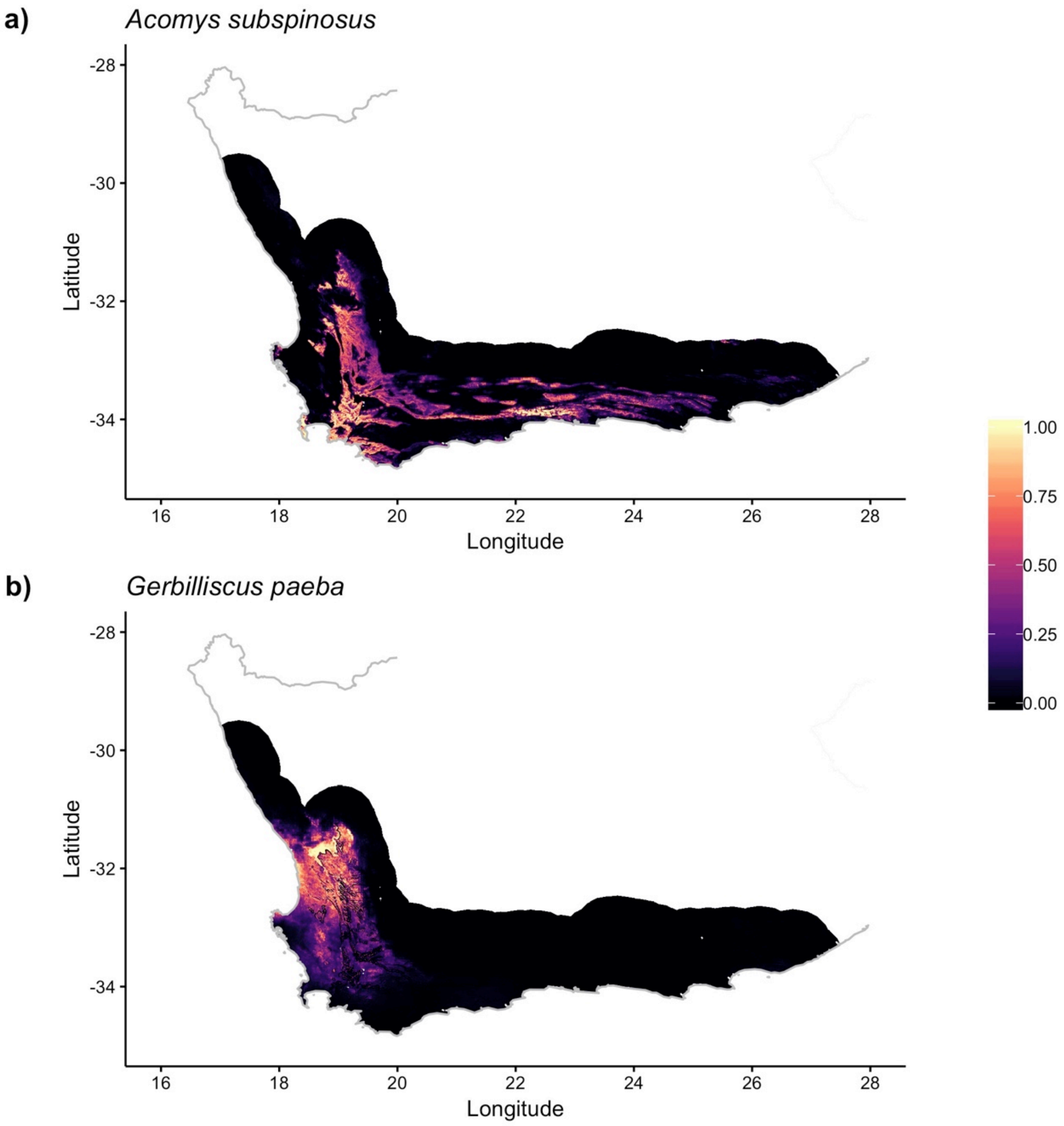
6.7 Supplementary Material



Supplementary Figure 6.1 Intercorrelated variables identified before used in distribution models. T = Temperature; P = Precipitation; MAT = Mean Annual Temperature; MAP = Mean Annual Precipitation.



Supplementary Figure 6.2 a) Seed mean mass (± sd) by dispersal mode. Wind-dispersed seeds were smallest, followed by nutlets and ants, while rodent-dispersed seeds were largest. b) The relationship between seed mass and the most important predictor of dispersal mode, precipitation in the driest month, represented with a smoothed loess curve.



Supplementary Figure 6.3 Predicted distribution likelihoods for a) *Acomys subspinosus* and b) *Gerbilliscus paeba* based on MaxEnt models of observations restricted to the range of *Leucadendron* in the Greater Cape Floristic Region, South Africa. The scale goes from 0, least likely, to 1, most likely. From the prediction, it is clear that *A. subspinosus* is almost entirely restricted to the mountainous habitats of the GCFR, and is completely absent from the lowlands. *G. paeba* is predicted to not occur in the southern Cape and is centered in the northwest in mostly sandy lowlands around Vanrhynsdorp and decreasing in likelihood further south to Atlantis.

Supplementary Table 6.1 All *Leucadendron* species used in this study with number of populations from the Protea Atlas (Rebelo, 2001), assigned dispersal modes, and descriptive statistics of important variables (mean ± sd).

Species	Number of populations	Dispersal mode	Seed mass (g/1000 seeds)	Precipitation of the driest month (mm)	Fire return interval (years)
L. album	668	serotiny	19.11	33.24 (5.23)	18.12 (1.02)
L. arcuatum	1249	rodent	116.52	11.72 (2.99)	19.66 (1.06)
L. argenteum	162	serotiny	252.01	33.04 (3.61)	16.57 (0.35)
L. barkerae	593	rodent	NA	19.12 (7.39)	18.9 (1.56)
L. bonum	13	rodent	NA	10.38 (1.26)	21.04 (0.23)
L. brunioides	5055	nutlets	19.34	7.38 (4.82)	19.79 (1.25)
L. burchellii	69	rodent	176.99	24.74 (1.69)	18.5 (0.45)
L. cadens	92	rodent	NA	15.22 (1.42)	20.39 (0.74)
L. chamelaea	189	nutlets	21.87	11.73 (2.32)	19.04 (0.8)
L. cinereum	231	serotiny	35.02	9.76 (2.68)	18.13 (0.47)
L. comosum	2712	serotiny	NA	27.77 (7.38)	17.67 (0.98)
L. concavum	57	rodent	76.83	9.07 (0.37)	19.74 (0.42)
L. conicum	473	serotiny	NA	48.73 (12.75)	15.81 (0.47)
L. coniferum	773	serotiny	10.47	21.25 (4.77)	16.67 (0.34)
L. cordatum	195	rodent	NA	24.68 (6.33)	19.23 (0.84)
L. coriaceum	35	nutlets	NA	29.8 (2.89)	16.88 (0.3)
L. corymbosum	374	nutlets	11.7	13.14 (2.4)	17.43 (0.79)
L. cryptocephalum	34	serotiny	NA	28.38 (3.28)	17.14 (0.33)
L. daphnoides	176	rodent	200	25.19 (2.47)	17.83 (0.36)
L. diemontianum	51	serotiny	7.42	10.33 (1.73)	19.35 (0.98)
L. discolor	110	serotiny	7.13	13.94 (3.01)	17.87 (0.26)
L. dregei	184	serotiny	16.5	34.92 (4.16)	18.71 (1.24)
L. dubium	203	rodent	61.06	11.31 (2.75)	20.22 (0.4)
L. elimense	2970	nutlets	17.72	21.88 (2.16)	16.71 (0.31)
L. ericifolium	222	myrm	54.58	32.26 (4.87)	16.74 (0.46)
L. eucalyptifolium	10138	serotiny	8.58	36.8 (14.28)	16.44 (0.75)
L. flexuosum	88	serotiny	9.51	12.66 (2.29)	18.2 (0.69)
L. floridum	58	nutlets	6.52	18.86 (3.28)	16.29 (0.03)
L. foedum	146	serotiny	9.28	6.83 (0.57)	17.99 (0.72)
L. galpinii	475	serotiny	18.5	28.16 (1.89)	16.68 (0.17)
L. gandogeri	1838	serotiny	23	27.5 (4.65)	16.87 (0.41)
L. glaberrimum	11961	rodent	180	10.07 (3.03)	19.75 (1.28)
L. globosum	39	nutlets	NA	27.18 (1.88)	17.03 (0.14)
L. gydoense	44	rodent	NA	11.84 (3.67)	19.33 (0.73)
L. immoderatum	3	serotiny	NA	30 (0)	18.3 (0)
L. lanigerum	4710	serotiny	25.15	15.81 (5.08)	17.37 (0.66)
L. laureolum	3445	serotiny	26.2	23.67 (5.5)	16.78 (0.71)

L. laxum	354	nutlets	11.43	20.86 (0.85)	16.77 (0.39)
L. levisanus	77	serotiny	70.2	17.78 (1.72)	17.53 (0.53)
L. linifolium	891	serotiny	12.8	21.86 (4)	16.53 (0.24)
L. loeriense	445	serotiny	5.69	32.5 (5.31)	17.36 (1.05)
L. loranthifolium	761	rodent	108.39	8 (1.9)	19.15 (0.65)
L. macowanii	35	serotiny	17.13	16.27 (0.63)	NA
L. meridianum	1424	serotiny	32.7	26.68 (4.3)	16.71 (0.27)
L. meyerianum	45	rodent	98.23	5 (0)	19.71 (0.19)
L. microcephalum	1038	serotiny	23.7	25.84 (3.8)	17.23 (0.61)
L. modestum	648	serotiny	12.61	20.9 (2.02)	16.71 (0.36)
L. muirii	601	serotiny	4.68	27.35 (3.47)	16.73 (0.29)
L. nervosum	66	serotiny	13.74	26.24 (1.86)	18.29 (0.54)
L. nitidum	417	myrm	17.71	10.3 (4.19)	20.94 (1.26)
L. nobile	374	serotiny	5.87	23.17 (7.68)	18.36 (0.92)
L. olens	57	myrm	15.57	37.25 (1.83)	16.34 (0.19)
L. orientale	35	rodent	NA	34.63 (2.33)	16.05 (0.44)
L. osbornei	141	serotiny	NA	18.67 (5.03)	18.24 (0.73)
L. platyspermum	257	serotiny	117.08	22.98 (2.08)	16.8 (0.35)
L. procerum	561	serotiny	10.9	5.63 (1.34)	19.3 (0.59)
L. pubescens	4065	rodent	119.16	7.71 (2.92)	19.65 (1.34)
L. pubibracteolatum	174	rodent	NA	37.72 (5.57)	17.34 (0.88)
L. radiatum	36	serotiny	NA	33 (3.12)	16.47 (0.28)
L. remotum	74	rodent	82.8	5.04 (0.31)	19.82 (0.25)
L. roodii	118	rodent	127.63	5.2 (0.87)	19.25 (0.6)
L. rourkei	105	serotiny	5.76	41.15 (2.95)	17.96 (0.96)
L. rubrum	4640	serotiny	23.94	20.9 (9.64)	18.39 (1.33)
L. salicifolium	1733	serotiny	19	23.18 (6.07)	17.58 (0.87)
L. salignum	24545	serotiny	8.3	24.84 (11.05)	17.43 (1.25)
L. sericeum	31	myrm	18.56	7.77 (0.43)	20.03 (0.28)
L. sessile	378	rodent	200	22.95 (6.44)	17.3 (0.8)
L. sheilae	90	rodent	NA	5.77 (0.62)	20.47 (0.98)
L. singulare	48	nutlets	NA	44.06 (1.9)	17.61 (0.28)
L. sorocephalodes	93	nutlets	NA	40.13 (4.7)	17.87 (0.8)
L. spissifolium	23115	serotiny	12.91	28.82 (9.47)	17.31 (0.97)
L. stellare	261	nutlets	29.38	10.23 (3.23)	17.41 (0.8)
L. stelligerum	123	serotiny	4.83	21.07 (0.62)	16.54 (0.16)
L. strobilinum	319	serotiny	11.3	32.14 (1.64)	16.26 (0.13)
L. teretifolium	1263	serotiny	4.29	16.35 (6.97)	18.86 (1.69)
L. thymifolium	78	nutlets	38.64	13.51 (1.63)	18.01 (0.73)
L. tinctum	668	rodent	229	24.19 (7.83)	17.77 (1.32)
L. tradouwense	34	rodent	NA	29.59 (0.66)	15.84 (0.18)
L. uliginosum	5400	serotiny	7.79	48.26 (8.81)	15.92 (0.42)
L. verticillatum	83	nutlets	NA	16.73 (0.5)	NA
L. xanthoconus	7193	serotiny	10.85	26.2 (5.4)	16.82 (0.47)

Supplementary Table 6.2 The relative influence of variables based on jackknife analyses from MaxEnt models for mutualistic small mammals, *Acomys subspinosus* and *Gerbilliscus paeba*.

	Acomys subspinosus	*Gerbilliscus paeba*
Soil_moisture	22.2409	29.1799
Slope	19.9347	5.4937
Soil_organic_carbon_concentration	16.1334	0.2698
Mean_T_wettest_quarter	15.1915	0.2406
Depth_to_bedrock_estimate	10.6999	0.0055
Coarse_fragments_volume	5.2561	1.6678
P_seasonality	4.2653	38.1653
pH_index	2.9121	0
MAT	0.8484	4.1351
Min_T_coldest_month	0.6015	0
NDVI	0.5204	0.1617
Isothermality	0.3912	1.5376
Potential_evapotranspiration	0.3362	12.268
P_driest_month	0.32	3.4205
Mean_T_driest_quarter	0.2207	2.5223
Silt_particles	0.0728	0
PDSI	0.0361	0.0497
Soil_bulk_density	0.0174	0.1779
Aspect	0.0014	0.1786
Cation_exchange_capacity	0	0.526

Chapter 7

Synthesis

7.1 Global hotspot of ecological interactions

The Cape flora is considered a global biodiversity hotspot (Myers et al., 2000; Mittermeier et al., 2011) and the most biodiverse Mediterranean-type ecosystem by area in the world (Cowling et al., 1996; Kreft & Jetz, 2007). As such, it follows that it is a hotspot for several types of ecological interactions, with many disproportionately represented in the Cape compared to other regions. This is particularly the case for a diversity of pollination modes, including pollination by sunbirds, long-proboscid flies, oil-collecting bees, monkey beetles and rodents (Steiner & Johnson, 2003; Anderson et al., 2014). Cape flora, and Southern African flora more generally, have an unrivalled specialization in their pollination interactions, with many plants relying on only a single or few animal species for effective pollination (Johnson & Steiner, 2000; Pauw & Stanway, 2015).

Much like the pollination interactions, seed dispersal shows a number of interesting ecological interactions in the region. Australian kwongan and the GCFR are the only regions where myrmecochory (1000+ species) are so well represented (le Maitre & Midgley, 1992). Scatter-hoarding is now well established as an important dispersal mechanism in Cape flora, with an estimate of up to 100 species with similar seed traits, such as large (>50 mg) and thick-walled, in the Proteaceae and Restionaceae alone (Midgley & Anderson, 2005). An interesting record of a small marsupial scatter-hoarding sandalwood seeds from Western Australia (Murphy, Garkaklis & Hardy, 2005), and the other similarities in environmental and climatic drivers between these floristic regions, warrants further exploration of scatter-hoarding, or the scarcity of it, in this region.

Although most myrmecochorous plants in the Cape have some dispersal redundancy, in that numerous ant species may disperse a single species, the same cannot be said for some of the pollinator (Johnson & Steiner, 2000) and scatter-hoarding systems (White, Bronner & Midgley, 2017), where species-specific reproductive mutualisms are more common and vulnerable to failure (Bond, 1994). Although the plants themselves may be resilient to any direct anthropogenic influence, indirect effects through mutualism disruptions can reduce ecological fitness or lead to co-extinctions (Aslan et al., 2013). This topic has been well explored for both pollination (Kearns, Inouye & Waser, 1998; Pauw, 2007; Potts et al., 2010) and seed dispersal (McConkey & Drake, 2006; Galetti & Dirzo, 2013; Valiente-Banuet et al., 2015) interactions.

7.2 Seed dispersal disruptions in the GCFR

Seed dispersal interactions are already known to be at risk in the GCFR. Bond and Slingsby (1984) and Christian (2001) have shown that invasion by Argentine ants, *Linepithema humile*, in the GCFR has led to displacement of native seed dispersing ants, such as *Anaplolepis* spp. and *Pheidole* spp., leading to less effective dispersal for ant-dispersed plants with important consequences for community dynamics. Maintaining the integrity of these interactions is crucial to conserving natural communities and populations.

Further research I have conducted on the critically endangered *Widdringtonia cedarbergensis* provides a relevant case study to mutualism disruptions in scatter-hoarded Cape plants. Seed trials at several sites throughout *W. cedarbergensis* range, only found seed predation by the non-scatter-hoarding *Micaelamys namaquensis* (White et al., 2017; JDM White, M Putz, JJ Midgley, unpublished data). Recent seed dispersal trials of *W. cedarbergensis* seeds at new sites found positive evidence for rodent dispersal, by the Cape spiny mouse, *Acomys subspinosus,* a known scatter-hoarder, as well as high levels of background seed predation by *A. subspinosus* but predominantly by the antagonist *M. namaquensis*. (JDM White, M Putz, JJ Midgley, unpublished data). Of particular interest is the link between extremely low recruitment rates (White et al., 2016) and the dependency on effective seed dispersal by *A. subspinosus,* with sites with scatter-hoarders present also having significantly more juveniles present (JDM White, M Putz, JJ Midgley, unpublished data). With only few recorded interactions or sightings of *A. subspinosus* and high levels of seed predation by the common *M. namaquensis*, there are indications of negative effects on *W. cedarbergensis* fitness due to seed dispersal disruption. As pressures on wild systems are already at ecological disaster levels, accurate identification of vulnerable pollination and seed dispersal interactions is a necessity for conservation planning, particularly in the Cape, where many vulnerable, species-specific ecological interactions still remain to be recorded.

7.3 Seed dispersal of large, dry Fynbos fruits

The main hypothesis of this thesis was that large, dry and semi-fleshy, fynbos fruits are scatter-hoarded by small mammals. To address this, I have investigated aspects of the natural history of several Fynbos species (6 plant families) with large fruits across the range of the biome. This collection of taxa and the chosen sites were never meant to be comprehensive, but represented a diverse collection of widespread, candidate plant species for scatter-hoarding.

This study had the primary advantage over previous seed dispersal research in the region in that remotely triggered camera traps were not readily available until recently. Camera traps have allowed me to non-invasively and efficiently document small, sometimes cryptic and often nocturnal, small mammal behaviours, providing a view into the lives of these animals never before possible. Using this valuable tool, this thesis documented several new ecological interactions with photographs or videos.

This included the first video observations in the wild and confirmation that *Acomys subspinosus* (this thesis) and *Gerbilliscus paeba* (see Weighill et al. (2017) for *G. paeba* video observations) do scatter-hoard seeds (Chapter 2, 4), playing the role of conditional mutualists, being both seed predators and dispersers (Theimer, 2005; Gómez, Schupp & Jordano, 2018). Additionally, a considerable number of observations showed that two of the most common fynbos small mammals, *Rhabdomys pumilio* and *Micaelamys namaquensis*, are antagonists to the many hard, dry nut-like fruits investigated, as they predated considerable proportions of seeds (Chapter 2, 5, 6). The dry, nut-like fruited fynbos plant species that are confirmed as scatter-hoarded include, *Leucadendron sessile*, *L. pubescens*, *L. loranthifolium* (Chapter 2) and *W. cedarbergensis* (JDM White, M Putz, B Coetzee, JJ Midgley, *in prep*).

In the case of semi-fleshy, recalcitrant fruits, such as *Heeria argentea* and *Hartogiella schinoides*, *M. namaquensis* has turned into the role of a mutualist frugivore, both dispersing and providing a germination service to the fruits (Chapter 3). It only consumed the thin, semi-fleshy pericarps while leaving the large, dormant intact embryos in food middens under rocks. I showed that these rocky sites provide protection from regular fires. This ecological interaction represents one of the clearest examples of directed dispersal in the natural world.

The dry, nut-like, yet recalcitrant fruits of *Hyaenanche globosa* were scatter-hoarded by *A. subspinosus* and provided an interesting new set of seed traits to consider for a possible Cape scatter-hoarding dispersal mode (Chapter 4). Additionally, this species provided clear evidence for seed predation defenses, with toxic seeds and evidence for directed deterrence/toxicity against the seed predator *M. namaquensis*. The usually voracious small mammal granivore never removed more than a single fruit per site, while the smaller, scatter-hoarding *A. subspinosus* readily removed all seeds they encountered, possibly managing the seed toxicity with behaviours not used by *M. namaquensis*, such as seed storage.

7.4 Seed predation and seed defenses

Small mammal seed predation is clearly an important ecological filter that fynbos plants with large seeds need to overcome. Both myrmecochory and serotiny are thought to be seed predation defenses to some extent (le Maitre & Midgley, 1992). This is mainly due to both dispersal modes providing the seeds with mechanisms to avoid rodent seed predation. Ants bury seeds with elaiosomes before small mammals can collect them (Bond & Slingsby, 1983), although they may still pilfer some of these seeds later due to their strong olfactory ability (Bond & Breytenbach, 1985). Serotinous plants with large seeds, predominantly in the Proteaceae (le Maitre & Midgley, 1992), retain their seeds in their canopy for several years avoid interaction with rodents, releasing them after fire or senescence when rodent populations crash (van Hensbergen et al., 1992; Kraaij & van Wilgen, 2014). Scatter-hoarding similarly provides a mechanism for predator avoidance. Although scatter-hoarding animals do predate many of the seeds they cache, a significant proportion of these seeds survive in the soil bank (White, Bronner & Midgley, 2017). The seeds that survived were likely buried in a position undetectable to either intra- or inter-specific small mammal granivore pilferers and the original scatter-hoarder must either have cached more seeds than it could feasibly consume, forgotten, been predated, or possibly was interrupted by a disturbance, such as fire. Thus, this interaction of small mammal granivores caching seeds in safe sites, undetectable to common, non-hoarding, seed predators and at a safe depth from fire heat damage, provides large fynbos fruits with a significant seed defense.

Although serotinous species provide their seeds with a safe storage space during inter-fire periods, kept away from small mammal seed predators, once they are released post-fire *en masse* into environments with no aerial cover, they are vulnerable to granivores. As rodent populations are thought to crash post-fire (van Hensbergen et al., 1992), the major seed predators are likely to be immigrating flocks of granivorous birds (Fraser, 1990). These visually cued seed predators are predicted to drive the evolution of seed colour polymorphisms that were observed in serotinous, flat-winged *Leucadendron.* The mosaic of substrate patches in post-fire environments, with pale, ash-sand and charcoal-litter mixed backgrounds, provides the evolution of seed colour with a selective landscape, upon which bird granivores are the agents of selection (Chapter 5). The large volume of seeds released ensures that many seeds will fall in cryptic sites where detection of the seeds against the heterogenous background by bird granivores is either time consuming, challenging or near impossible, ensuring effective dispersal for the seeds best matched to their background. Overall, by investigating the sensory ecology of bird granivore and seed interactions, I showed that background matching provides flat-winged *Leucadendron* and pale, hairy *Leucadendron* and *Aulax*, with an effective seed predation defense mechanism against visually cued predators (Chapter 5).

There are many Cape species that produce small, dry nut-like fruits (termed 'nutlets'), such as the *Cliffortia* and *Leucadendron* nutlets investigated in earlier chapters (Chapter 2, 6). These seeds are approximately 5 mm in length with considerably less mass than known scatter-hoarded seeds. Although their seed traits generally match those of scatter-hoarded seeds, being non-serotinous with no elaiosomes, and small mammals readily predate them, I found no evidence that they are scatter-hoarded. As these plants drop their seeds annually during inter-fire periods with dense vegetation, small mammal granivory is a larger risk than bird granivory. These nutlet species also tend to display a distinct seed fall phenology, compared to larger-seeded species. Scatter-hoarded and serotinous Cape plants drop their seeds *en masse* to satiate granivore populations (le Maitre & Midgley, 1992). In both *Leucadendron* (*L. elimense* and *L. laxum*) and *Cliffortia* (*C. cuneata* and *C. phillipsii*) nutlets, the seeds fall from the plants intermittently over a period of three to six months (JDM White, personal observation). This appears to be an alternative seed predation defense strategy to seed masting, where only a few seeds drop to the ground per week, in turn creating fewer aggregations of seeds and increasing foraging time for seed predators (Pyke, 1984). Ant-dispersed plants appear to employ a similar strategy, where seeds are dropped intermittently. The advantage for myrmecochorous plants is that ants are generally more ubiquitous than rodents and will likely detect a single seed before a small mammal can detect it first. Much like all seed dispersal modes, nutlets still suffer intense seed predation from ubiquitous small mammal granivores (Chapter 2, 6). How these seeds manage to find their way into the soil seed bank, where they will find protection from fires and predation, remains unknown. The determinants of effective dispersal of small-seeded Cape plants species remains almost entirely unknown and as the overwhelming majority of fynbos plants have small seeds, this should be noted as an important functional group that requires research.

7.5 Alternative dispersal strategies for large, dry nuts

In collaboration with my supervisors, Jeremy Midgley and Gary Bronner, and Steve Johnson, from the University of KwaZulu-Natal, we described a remarkable and unique, alternative strategy to scatter-hoarding for a large, dry nut-like fynbos fruit. The Cape restio, *Ceratocaryum argenteum*, produces large nuts with a strongly scented, outer tuberculate layer (Appendix 1, Figure 8.1). Due to its similar traits to many scatter-hoarded fynbos plants, including restios, such as *Willdenowia incurvata* (Midgley and Anderson 2005), we originally hypothesised that this species was scatter-hoarded by small mammals (Chapter 2), although this was later ruled out due the absence of scatter-hoarders in its range. After placement of seeds during seed removal trials, we frequently observed that two dung beetle species, *Epirinus flagellatus* (Appendix 1) and *Scarabeaus spretus* (Appendix 2) were attracted to *C. argenteum* seeds. In the case of the relatively small *E. flagellatus* (Appendix 1, Figure 8.1f), it rolled single seeds short distances before burying them ± 2 cm deep, providing an excellent dispersal service (Appendix 1, Supplementary Video 8.2), while the larger *S. spretus* tended to bury many seeds in a single hole at the site of first detection, which is high in quantity of seeds dispersed, though low in quality, due to the resulting intraspecific competition between related seedlings (Appendix 2, Supplementary Video 9.1).

Analysis of the scent of the seeds indicated the emission of numerous volatiles, many of which are also emitted by dung of local antelope (Appendix 1, Figure 8.2). Fresh seeds emit these dung-like volatiles at a greater rate than that of

Bontebok droppings and other restio seeds (Appendix 1, Supplementary Figure 8.1). This is likely aided by the tuberculated seed coat (Linder 2001), which greatly increases the surface area available for scent emission. Unlike *Leucadendron* seeds, which are more ovoid in shape and flattened, *C. argenteum* seeds are highly spherical and hard, which likely facilitates rolling by dung beetles, which cannot otherwise manipulate the seed into a ball-shape. The hard seed coat is not suitable for dung beetle consumption or ovipositing and therefore, this is a deceptive, non-rewarding interaction. *Ceratocaryum argenteum* seeds exploit the olfactory and possibly tactile sensory perception of dung beetles, through faecal mimicry, for effective dispersal of their seeds. See Supplementary Video 7.1 for additional video footage of the interaction between dung beetles and *C. argenteum* seeds.

Beyond the surprising nature of this dispersal interaction, we show that *C. argenteum* nuts, perhaps the largest nut-like fruit in the Fynbos Biome (Linder 2001), deter local small mammal granivores. Through multiple seed removal trials, small mammals never seriously damaged *C. argenteum* nuts (Appendix 1, White et al 2017). Under seed trial conditions, where multiple seed types were placed out together, small mammals would preferentially choose other seed species, before finally exploring and sometimes nibbling a *C. argenteum* seed coat, before ultimately ignoring them. When the hard, chemical-laden seed coat was manually removed, small mammals readily predated the large, chalky embryo (Appendix 1, Supplementary Video 8.1). This shows that the *C. argenteum* strongly scented, hard, tuberculate seed coat not only provides it with an excellent attractant for dung beetles dispersers, but also a physical and/or chemical deterrent to common small mammal granivores, a valuable seed trait in the Cape's seed predation selective landscapes.

7.6 The environmental drivers of major dispersal modes

To understand the possible environmental, disturbance or biological drivers of major Cape dispersal syndrome distributions, I used boosted regression tree modeling to identify the most important predictors of serotinous and scatter-hoarded *Leucadendron* occurrence. Precipitation in the driest month, soil moisture, potential evapotranspiration (all water related variables) and fire return interval were the top four most important predictors for both scatter-hoarded and serotinous *Leucadendron*. The model predicted that scatter-hoarded plants were in sites with less rainfall in the driest month, drier soils, greater potential evapotranspiration and longer fire return intervals while serotinous plants were in sites with the exact opposite climate and disturbance regime, with generally more moisture and shorter fire return intervals.

The predicted distribution map for scatter-hoarded *Leucadendron* (Chapter 6, Figure 6.5a) matches well to the sites where scatter-hoarding was observed throughout this thesis (Chapter 2, 4), commonly found in the arid, northwest of the region, while sites without scatter-hoarding (Chapter 2, 3, 5, 6), are in the south or southwest of the region, where conditions are more favourable to serotinous plants and scatter-hoarded plants are less common.

The predicted distributions of plants based on important life history traits can provide the basis for further research in either comparative- and/or conservation-based approaches. Plants' ability to respond to changing climates will, in many cases, largely depend on their physiological tolerances or plasticity, as well as on the integrity and flexibility of their interactions with important mutualists. Combining these in a modeling framework could help conservation planning at a regional and functional-type scale.

7.7 Conclusion

In this thesis, I have presented several seed predation and dispersal interactions new to science. The main hypothesis was shown to be generally applicable, as most large, dry nut-like fruits were scatter-hoarded by small mammals, with the important exceptions of small nutlets and the unique case of dung beetle dispersal. Overall, my data have contributed valuable insights into the natural history, sensory ecology and biogeography of large-seeded Cape plants. My findings show the importance of small mammals in fynbos community ecology, in maintaining and constraining populations of large-seeded species, through seed predation and dispersal. Natural history research has heuristic value and certainly widespread public appeal in popular science media and can provide valuable ecological data for the conservation of the plants, animals and communities studied in the Cape.

7.8 Supplementary Material

Supplementary Video 7.1 can be viewed at the following link:

- https://youtu.be/C_VFlAcsx10

Appendix 1

Faecal mimicry by seeds ensures dispersal by dung beetles

8.1 Abstract

The large brown, round, strongly scented seeds of *Ceratocaryum argenteum* (Restionaceae) emit many volatiles found to be present in herbivore dung. These seeds attract dung beetles that roll and bury them. As the seeds are hard and offer no reward to the dung beetles, this is a remarkable example of deception in plant seed dispersal.

8.2 Main text

Mimicry, deception and sensory exploitation of animals by plants are controversial, especially for seed dispersal (Schaefer & Ruxton, 2009). A few plant species produce colourful but hard seeds that are unrewarding to fruit-eating birds (Galetti, 2002; Schaefer & Ruxton, 2009). Evidence that this represents visual mimicry or visual sensory exploitation is weak: there is a general absence of co-occurring rewarding similarly coloured fleshy-fruited model plant species, seeds are often toxic and thus the colour is potentially aposematic (warning colouration) and birds largely ignore the seeds resulting in very poor seed dispersal (Galetti, 2002; Schaefer & Ruxton, 2009). Chemical deception in seed dispersal also appears to be equally rare, only being suspected in two myrmecochores (ant-dispersed plants) (Pfeiffer, Huttenlocher & Ayasse, 2010; Turner & Frederickson, 2013). Plants which produce seeds with low levels of the chemical attractant (oleic acid) do not effectively deceive ants; such seeds are as weakly dispersed as non-myrmecochorous plants without any oleic acid (Pfeiffer, Huttenlocher & Ayasse, 2010).

In the Potberg area of the De Hoop Nature Reserve (DHNR), in the southern Cape, we investigated the dispersal of *Ceratocaryum argenteum* Nees ex Kunth, an endemic Restionaceae plant species that occurs in local fire-prone shrublands on deep sands (Linder, 2001). Seeds of this species are unusual; its nuts are the largest in the family (1 cm in length), the nuts have no elaiosome for ant dispersal and instead of having a smooth, black seed coat typical of large nuts in the family, it has a rough, tuberculate and brown outer seed coat (Figure 8.1a–e). To the human nose the tuberculate layer has a pungent scent similar to herbivore faeces. Some Cape fynbos plant species with large nuts are buried by scatterhoarding small mammals (Midgley & Anderson, 2005). However this does not apply to *C. argenteum* as neither of the only two known Cape scatterhoarding small mammal species (*Acomys subspinosus* Waterhouse and *Gerbillurus paeba* A. Smith) occurs at this site. Here the dominant small mammal is *Rhabdomys pumilio* Sparrman (striped field mouse) (Skinner & Chimimba, 2005) (see Supplementary Table 8.1). It is a ubiquitous and widespread Cape omnivore that

consumes seeds (Radloff et al., 2010) but does not bury them (Midgley & Anderson, 2005). By using motion-activated cameras and fluorescent thread markers, we confirmed that despite being granivorous, *R. pumilio* never consumed or buried intact *C. argenteum* seeds. It frequently consumed dehusked *C. argenteum* seeds (Supplementary Video 8.1) and intact *Leucadendron sessile* seeds (Supplementary Table 8.1).

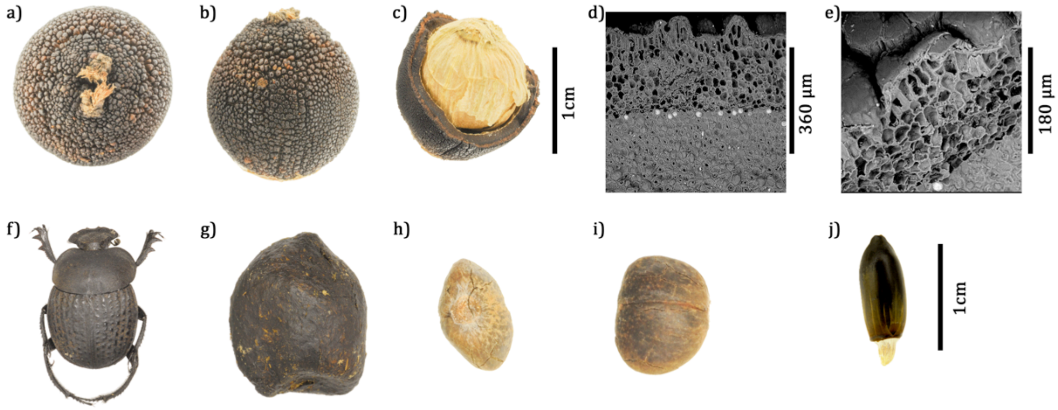


Figure 8.1. The similarities between *C. argenteum* seeds and a dung pellet and dissimilarities with other nut seeds. Vertical (a) and side (b) views of a *C. argenteum* seed as well as one that has been cracked open (c) showing the endosperm and thick woody inner seed-coat layer and the outer tuberculate layer which together form the husk. Scanning electron microscopy (SEM) of the (d, e) outer, tuberculate layer and inner seed-coat, with white silicon granules at the boundary between the two layers. (f) *E. flagellatus*. (g) Bontebok faeces. Vertical (h) and side (i) views of an *L. sessile* seed. (j) *Cannomois grandis* seed with white elaiosome.

A strong scent is unusual among nut seeds as it would facilitate the discovery, even of buried seeds, and thus predation by small mammals (Hollander, Vander Wall & Longland, 2012; Paulsen et al., 2013). It has been argued that hard-seededness (that is nuts) evolved to reduce scent emission and thus reduce consumption by small mammals (Paulsen et al., 2013). Therefore we hypothesized that the role of the strong scent of *C. argenteum* may be to chemically attract dung beetles and induce them to bury these seeds.

After the placement of experimental *C. argenteum* and *L. sessile* nuts in the field, we frequently (>20 occasions) observed that the dung beetle *Epirinus flagellatus* Fabricius (Scholtz & Howden, 1987) (Figure 8.1f) was attracted to the *C. argenteum* seeds. The beetles, seven individuals of which were collected for identification purposes, rolled these seeds in typical head-down dung-beetle fashion and then buried them by pulling them underground from below (Supplementary Video 8.2). Although these dung beetles are too small (1 cm in length) to activate motion-activated remote cameras, three dung beetles rolling seeds were incidentally filmed during three of the 214 video incidents that had been captured (Supplementary Video 8.3).

Dung beetles are typically more active during warm and moist periods in most South African ecosystems (Davis, 1996). To investigate the consequences of dispersal by the dung beetle *E. flagellatus*, we placed out five to ten *C. argenteum* seeds at 31 stations (195 seeds) after a rain event. Of these, 44% (n = 87) were removed from stations within 24 h. Using fluorescent threads as markers (Supplementary Video 8.3), we recovered 80% (n = 66) of the removed seeds, and of these 80% (n = 53) were found to be buried. This represents at least 27% of all experimental seeds being buried within 1 day, under these optimum moist conditions. *C. argenteum* seeds were typically moved 0.21 ± 0.08 m (mean ± s.e.m.) from experimental stations and buried 2.04 ± 0.21 cm (mean ± s.e.m.) deep. Seeds were mostly buried singly (n = 33), but occasionally also in larger caches (two seeds per cache n = 8; four seeds per cache n = 1). *C. argenteum* seeds are hard and thus provide no reward to small *E. flagellatus* dung beetles. We excavated all the located buried seeds within 24 h of burial and in no case did we capture any dung beetles or observe any beetle eggs on seeds or any damage to the seeds. This suggests that the deception is ‘discovered’ only when the dung beetle attempts to eat, or oviposit within, the hard seeds. As we were able to discount any seed burial due to small mammals, and frequently observed rolling and burial by dung beetles directly as well as capturing the behaviour on motion-activated videos, we are confident that this plant species is primarily dung beetle dispersed.

C. argenteum seeds look similar in size, shape and dark brownish colouration to the dung of local small antelope (Figure 8.1g). Seeds are remarkably circular (ratio of widest to narrowest seed dimension = 1.02; Figure 8.1a,b) whereas scatterhoarded nuts tend to be flattened (L. sessile ratio = 1.86; Figure 8.1h,i). This circularity would facilitate rolling. Seeds of most of the other *Ceratocaryum* and closely related *Cannomois* species are unscented, smaller, smooth, black and have elaiosomes for ant dispersal (Linder, 2001) (Figure 8.1j). We compared the volatile emissions of *C. argenteum* with those of seeds of several other Cape Restionaceae species and with the fresh dung of several African herbivores, using standard headspace sampling and gas chromatography mass spectrometry methods (see Supplementary Material). Currently at DHNR, the main small antelope is the bontebok, an obligate short-grass grazer (Radloff et al., 2010) and the most common large antelope is the eland, a mixed feeder (Radloff et al., 2010).

The total mass of the volatiles emitted by fresh *C. argenteum* seeds (<4 weeks after seed release) was almost 300-fold greater than that emitted by seeds of other Restionaceae species and even older *C. argenteum* seeds (>8 weeks after seed release) emitted a significantly greater amount of volatiles (Supplementary Figure 8.1). The total mass of volatiles from fresh bontebok faeces was intermediate between fresh and old *C. argenteum* seeds (Supplementary Figure 8.1). These findings were not altered even after emissions were corrected for mass or the surface area of droppings and seeds (Supplementary Figure 8.1).

The volatile composition of emissions from *C. argenteum* seeds is similar to that of the dung of large mammalian herbivores, particularly eland and bontebok (Figure 8.2). Compounds emitted from the seeds that are also emitted by eland and/or bontebok dung include various acids, the benzenoid compounds acetophenone, phenol, p-cresol and 4-ethyl-phenol, as well as the sulphur compound dimethyl sulphone (Supplementary Tables 8.2 and 8.3). Most of these compounds are well known as components of the scent of herbivore dung (Jürgens et al., 2013; Stavert, Drayton, et al., 2014) (see also Supplementary Table 8.3). Similarity in scent is not due to bontebok feeding on *C. argenteum* as this plant is unpalatable and bontebok feed instead on various grasses (Poaceae) (Radloff et al., 2010).

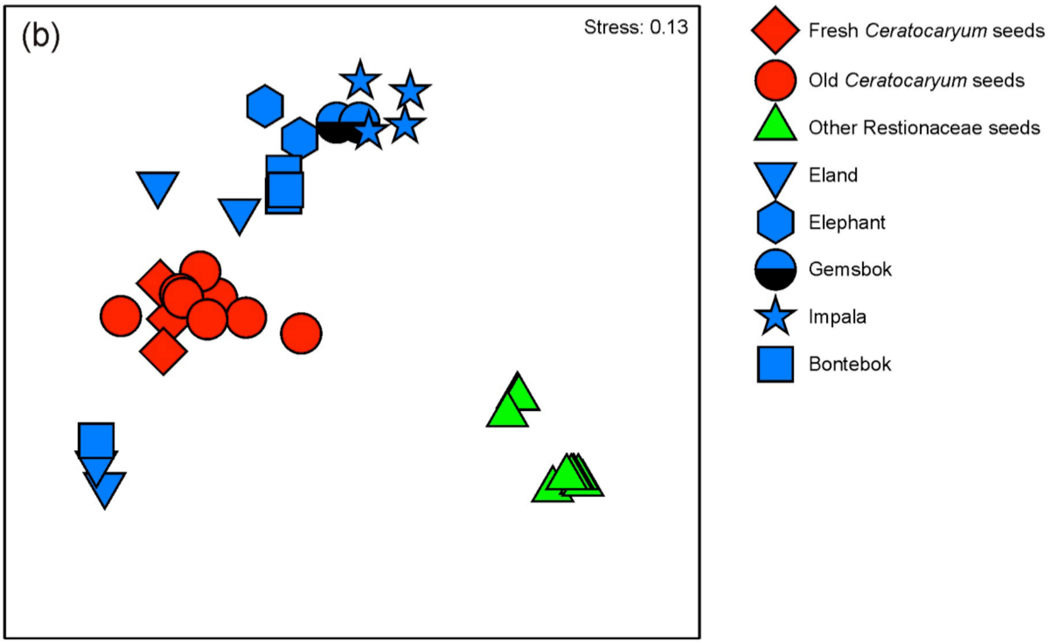


Figure 8.2 Comparisons of volatile emissions among seeds and large mammal droppings that indicate the similarity of *C. argenteum* to the dung of some herbivores. Similarity in the composition of volatile blends of seeds and animal droppings is based on non-metric multidimensional scaling. Symbols for other Restionaceae (Methods, Supplementary Table 8.3) that overlap are slightly offset for clarity. The composition of scent sampled from *Ceratocaryum* seeds is very similar (R = 0.75, P = 0.33) to that of dung of local herbivores (eland and bontebok), but differs markedly (R = 1.0, P = 0.028) from that of seeds of other Restionaceae (nested ANOSIM permutation test).

There are rare examples of dung beetle seed dispersal of dry and fleshy fruits and seeds. Acorns are rarely taken and buried by dung beetles (Pérez-Ramos et al., 2013), but such acorns are predominantly bird-dispersed or scatterhoarded by rodents. Also, dung beetles feed on these buried acorns (Stavert, Drayton, et al., 2014), so their positive demographic impact is very weak. Dung beetles roll the fleshy fruit-covered soft seeds of *Strychnos madagascariensis* and they are attracted to certain of its fruit scent molecules (Burger & Petersen, 1991).

However this is primarily a primate-dispersed plant species and this dung beetle is mostly associated with, and attracted to, herbivore dung (Burger & Petersen, 1991). Incidental seed dispersal by dung beetles of small hard seeds that are ingested by large animals and mixed within their dung also occurs (Vander Wall & Longland, 2004; Culot et al., 2011). For *C. argenteum*, however, the dispersal is not incidental via being mixed with dung. This plant species cannot resprout after fire and therefore depends on successful post-fire seedling establishment from an incineration-proof, buried seed-bank (Linder, 2001). In conclusion, *C. argenteum* exploits the visual and olfactory sensory perception of a dung beetle for dispersal and burial of its seeds. The interaction is deceptive with no reward offered for the dung beetles.

8.3 Methods

Data were collected in January and February 2014 in stands of *C. argenteum* in the Potberg section of DHNR, South Africa (−34.399907°, 20.554238°). *C. argenteum* nuts have a mean mass of 0.710 g (n = 20), comprising the tuberculate dry outer pericarp and thick seed-coat (combined mass 0.435 g), as well as a large (0.275 g) nutritious (57.8% protein) inner endosperm and embryo. *L. sessile* nuts were used as controls as these highly palatable seeds are known to be scatterhoarded (Midgley & Anderson, 2005). *L. sessile* nuts have mean seed mass 0.224 g (n = 20) and a mean endosperm mass 0.056 g that comprises 45% protein. Ltl Acorn 6210M remotely activated camera traps (n = 10) were used to observe the small mammal species interacting with the seeds. Videos were focused on seed depots from approximately 1 m away and were set at 1 min length videos, preceded by a photograph. We considered an event as an animal having an interaction (consuming, inspecting, removing or ignoring) with a seed, or a change in interaction or a seed, or an animal entering and leaving the field of view. Thus a single event may be spread over several 1 min video clips, for example as an animal chews the same seed. Also, several events may take place within a 1 min video clip, for example if an animal inspected and ignored several seeds. Using these definitions, a total of 214 events were recorded over 3 days, with 202 *R. pumilio*, nine *Otomys irroratus* Brants (a foliovore (Skinner & Chimimba, 2005) which ignored all seeds) and three *E. flagellatus* events. To determine whether removed seeds were consumed or buried or not we attached a 10 cm length of fluorescent thread to each seed using quick setting glue. Threads from buried seeds could be located with ultraviolet-emitting light-emitting diode torches. We placed five seeds of *C. argenteum* and *L. sessile* at ten stations, with each station being ±50 m apart. Video analysis and field observations showed that *C. argenteum* seeds mostly were ignored by *R. pumilio*, occasionally moved short distances but never consumed or buried (Supplementary Table 8.1). All field experiments involving small mammals were approved by the UCT Science Faculty Animal Ethics Committee (2013/V15/GB). To determine whether *C. argenteum* seeds are largely ignored because the endosperm is unpalatable, we compared the fate of five entire endosperms and five intact seeds at each of ten stations, at two separate sites, ±50 m apart, and again using video cameras. Video analysis indicated that dehusked *C. argenteum* seed removal and/or consumption was high (Supplementary Table 8.1), it was always due to *R. pumilio* and that it again largely ignored intact seeds (see Supplementary Video 8.1).

8.4 Acknowledgements

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8.5 Supplementary Material

Supplementary Videos can be viewed at the following links:

Supplementary Video 8.1 – *R. pumilio* consuming dehusked *C. argenteum* seeds and ignoring intact seeds, filmed in February 2014:

- https://media.nature.com/original/nature-assets/nplants/2015/nplants2015141/extref/nplants2015141-s2.mp4

Supplementary Video 8.2 – *E. flagellatus* (dung beetle) rolling and burying a *C. argenteum* seed at De Hoop Nature Reserve, filmed in February 2014:

- https://media.nature.com/original/nature-assets/nplants/2015/nplants2015141/extref/nplants2015141-s3.mp4

Supplementary Video 8.3 – Coincidental filming of *E. flagellatus* on remotely activated cameras triggered by *R. pumilio*, filmed in February 2014:

- https://media.nature.com/original/nature-assets/nplants/2015/nplants2015141/extref/nplants2015141-s4.mp4

Supplementary methods

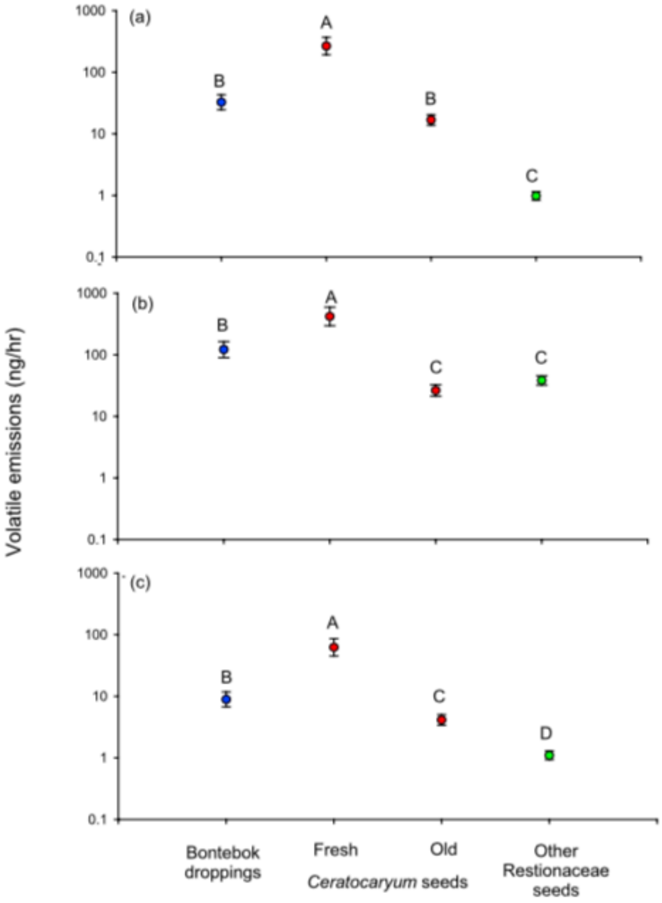
Volatile sampling

In addition to seeds of *C. argenteum,* we also sampled seeds of related Restionaceae species (*Cannomois parviflora*, *C. schlechteri*, *C. grandis*, *C. taylorii*, *Rhodocoma gigantea*, *Thamnochortus platypteris* and *Wildenowia incurvata*). Freshly collected dung of elephants (*Loxodonta africana* Blumenbach), gemsbok (*Oryx gazella* Linnaeus), impala (*Aepyceros melampus* Lichtenstein), bontebok (*Damaliscus pygargus ssp. pygargus* Pallas) and eland (*Taurotragus oryx* Pallas) was placed in sealable bags and frozen before sampling and freshly collected seeds were placed in paper bags before sampling. Seeds or de-frosted dung were then placed in a scentless Nalophan® oven bag (Kalle GmbH, Wiesbaden, Germany) and air in the bag was pumped for 60-120 minutes through a scent trap consisting of a glass micro vial with 1 mg of Carbotrap® mixed with 1 mg of Tenax® held in place with silanized glass wool. Air was pumped with a micro air sampler (Supelco PAS-500) at a realized flow rate of c. 150 ml/min. Controls in the same environment were taken from empty Nalophan® bag. The samples were thermally desorbed and analysed using a Varian CP-3800 GC (Varian, Palo Alto, CA, USA), with an Alltech EC-WAX column (30 m×0.25 mm internal diameter, film thickness 0.25 μm), coupled to a Varian 1200 quadrupole mass spectrometer. Thermal desorbtion was carried out using a Varian 1079 injector equipped with a Chromatoprobe device. The flow of helium carrier gas was 1 ml/min. The injector was held at 40 °C for 3 min with a 20:1 split and then increased to 200 °C at 200 °C/min in splitless mode. After a 3 min hold at 40 °C, the temperature of the GC oven was increased to 240 °C at 10 °C/min and held there for 12 min.

Volatile compounds were tentatively identified using NIST 11 mass spectral library (version 2.0), and were verified with retention times of authentic standards and published Kovats indices wherever possible. Compounds present at similar abundance in control samples were excluded from the analysis. To quantify scent emission rate per seed/dropping per hour, we injected a known amount of methyl benzoate (chosen because many of the key compounds were structurally similar benzenoids) into a scent trap which was then thermally desorbed under identical conditions to the samples. Rates of emission were compared among groups using nested generalized linear models with a gamma distribution and log link function, implemented in SPSS 22 (IBM Corp). Significance was tested using likelihood ratios. We used the sequential Sidak method for multiple comparisons among samples. To assess patterns of similarity in volatile composition among samples, we square-root transformed the percentages that each compound contributed to the overall scent for that sample and calculated a similarity matrix among samples using the Bray–Curtis method. This was then plotted in two-dimensions using non-metric multi-dimensional scaling (NMDS) implemented in Primer 6.1.6.

Supplementary Table 8.1. The number of seeds observed on remotely activated camera traps to be consumed *in situ* by *R. pumilio* in the two seed choice trials. (a) *C. argenteum* vs *L. sessile*. (b) Dehusked and intact *C. argenteum* seeds.

Seed Trials		No. of Seeds Consumed	Total no. of seeds removed	Total no. of seeds presented
a) Species	*C. argenteum*	0	7	50
	L. sessile	12	48	50
b) *C. argenteum*	Intact	0	17	100
Treatment	Dehusked	19	73	100



Supplementary Figure 8.1 Strong similarities in the volatile emissions of seeds and bontebok droppings. (a) Mean (± se) amount of volatile organic compounds emitted per hour. $X^2 = 136.2$, $P < 0.0001$. (b) Mean (± se) amount of volatile organic compounds emitted per gram per hour. $X^2 = 13.8$, $P = 0.001$. (c) Total amount of volatile organic compounds emitted per cm2 surface area per hour. $X^2 = 64.8$, $P < 0.0001$. Mean values that share the same letter do not differ significantly.

Supplementary Table 8.2 Volatiles emitted from eland and bontebok dung and from *Ceratocaryum argenteum* seeds. Values are proportions of total scent emission. See Supplementary Table 8.3 for full analysis.

Class	Compound	Eland	Bontebok	*Ceratocaryum* seeds	
				Fresh	Old
Aliphatic acids	Acetic acid	0.26	-	1.17	-
	Propanoic acid	0.23	-	32.08	5.75
	Isobutyric acid	0.34	-	0.26	-
	Butanoic acid	2.92	-	37.40	7.46
	Valeric acid	0.31	-	3.70	-
	Other acids	0.07	-	3.54	0.91
Aliphatic alcohols	Oct-1-en-3-ol		0.47	4.33	12.93
	Other alcohols	2.81	7.31	0.1.0	-
Aliphatic aldehydes		-	-	-	0.78
Aliphatic esters	Ethyl butyrate	3.45	-	2.71	-
	Other esters	-	-	1.44	0.782
Aliphatic ketones		-	2.90	-	0.76
Benzenoid compounds	Acetophenone	11.43	57.21	0.04	-
	1-phenylethanol	0.51	0.65	0.87	2.69
	Benzyl alcohol	0.05	0.61	0.14	2.59
	Phenol	0.15	1.48	0.03	1.44
	p-Cresol	-	0.20	0.42	0.17
	4-ethyl-phenol	-	0.07	10.13	35.51
	Other benzenoids	8.76	2.94	-	1.18

Carotenoid derivatives		-	1.06	-	2.29
Monoterpenes	(Z)-Ocimene	1.24	1.59	0.76	10.95
	Other monoterpenes	10.76	19.90	0.13	0.61
Monoterpene alcohols		0.03-	-	0.17	0.71
Sesquiterpenes		0.59	-	0.17	4.55
Nitrogen compounds		0.04	0.02	-	0.18
Sulfur compounds	Dimethyl sulfone	-	3.57	0.43	4.51
	Other sulphur compounds	-	-	-	0.29
	Dimethyl sulfone				
Unknowns		56.05	-	-	2.97

Supplementary Table 8.3 Volatiles emitted from dung of various mammalian herbivores and from the seeds of *Ceratocaryum argenteum* and other Restionaceae. Values are percentages of total emissions. Compounds are sorted by Kovats values within each compound class. The number of samples and replicates (in parentheses), the total number of seeds/droppings analysed is given below the name of each organism.

Compound name	Kovats	Identification¹	Eland	Elephant	Gemsbok	Impala	Bontebok	*Ceratocaryum fresh*	*Ceratocaryum* old	Other Restionaceae
			4 (8)	2 (2)	2 (52)	4 (70)	4 (114)	3 (130)	8 (240)	14 (268)
Aliphatic acids										
Acetic acid	1431	MK	0.26	-	-	-	-	1.17	-	0.71
Isopropylpyruvic acid	1458	M	-	-	-	0.52	-	-	-	-
Propanoic acid	1556	MK	0.23	-	-	-	-	32.08	5.75	-
Isobutyric acid	1571	M	0.34	-	-	-	-	0.26	-	-
Butanoic acid	1642	MKS	2.92	-	-	-	-	37.40	7.46	-
2-Methylbutanoic acid	1689	MK	-	-	-	-	-	2.40	-	-
Valeric acid	1728	MK	0.31	-	-	-	-	3.70	-	-
Isocaproic acid	1814	MK	0.07	-	-	-	-	-	-	-
isopropyl laurate	1863	MKS	-	-	-	-	-	1.14	0.76	-
Ethylcaproic acid	1955	MK	-	-	-	-	-	-	0.15	-
Aliphatic alcohols										
Isopentyl alcohol	1221	MK	2.46	-	25.70	17.26	4.56	-	-	-
n-Pentan-1-ol	1258	MK	-	-	-	-	1.80	-	-	-
Isohexanol	1327	MK	0.13	-	-	-	-	-	-	-
Hexan-1-ol	1363	MKS	-	0.15	4.45	-	-	-	-	-
2-Heptanol, 6-methyl-	1380	MK	0.11	-	-	-	-	-	-	-
(Z)-Hex-3-en-1-ol	1397	MK	-	0.10	-	-	-	-	-	-
Octan-3-ol	1404	MK	-	-	-	-	-	0.09	-	-
Oct-1-en-3-ol	1460	MKS	-	-	-	-	0.47	4.33	12.93	-
3,7-Octadien-2-ol, 2,6-dimethyl-	1471	M	0.11	-	-	-	0.95	-	-	-

(E)-Oct-2-en-1-ol	1632	MK	-	-	-	-	-	0.01	-	-
Aliphatic aldehydes										
(E)-Non-2-enal	1557	MK	-	-	-	-	-	-	0.78	-
Aliphatic esters										
Methyl isovalerate	1090	MK	-	-	-	12.28	-	-	-	-
Ethyl butyrate	1092	MKS	3.45	-	-	-	-	2.71	-	-
Propyl butyrate	1160	MK	-	-	-	-	-	1.44	-	-
Benzyl proponoate	1734	MK	-	-	-	-	-	-	0.02	-
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	1883	MK	-	0.31	-	-	-	-	0.54	-
1-(2-Hydroxy-1-methylethyl)-2,2-dimethylpropyl 2-methylpropanoate	1900	M	-	-	-	-	-	-	0.22	-
Aliphatic ketones										
Aliphatic ketone	1305	MK	-	-	-	-	2.90	-	-	-
Nonan-2-one	1408	MK	-	0.13	-	-	-	-	-	-
3,5-Octadien-2-one	1531	MK	-	-	-	-	-	-	0.76	-
Benzenoid compounds										
P-cymene	1291	MK	8.40	-	-	-	-	-	-	-
Ethyl cyclohexanoate	1435	MK	-	-	0.57	-	-	-	-	-
Anisole, m-methyl-	1453	MK	-	0.07	-	-	-	-	-	-
Benzaldehyde	1545	MKS	-	-	7.20	15.23	-	-	-	-
α,α-Dimethoxytoluene	1548	MK	-	7.48	5.04	32.88	1.26	-	-	-
Methylbenzoate	1650	MKS	-	-	0.38	-	-	-	0.11	-
Acetophenone	1676	MK	11.43	4.96	32.29	10.16	57.21	0.04	-	-
Estragole	1693	MK	-	-	-	-	0.31	-	-	-
1-Phenylethyl propionate	1766	M	-	-	-	-	-	-	0.56	-
1-phenylethanol	1818	MK	0.51	0.23	0.08	-	0.65	0.87	2.69	-
p-Cymen-8-ol	1863	MK	0.02	-	-	-	-	-	-	-
Guaiacol	1884	MK	-	-	-	-	1.33	-	-	-
Benzyl alcohol	1899	MKS	0.05	0.09	0.40	0.81	0.61	0.14	2.59	0.67
Phenylethyl alcohol	1938	MK	0.20	0.04	1.09	3.68	-	-	-	-
2-Methoxy-4-methyl-1-hydroxybenzene	1976	MK	-	-	-	-	0.04	-	-	-

Phenol	2030	MK	0.15	7.73	4.82	4.00	1.48	0.03	1.44	-
3-Phenylpropanol	2065	MK	0.02	-	-	-	-	-	-	-
(E)-Cinnamaldehyde	2066	MK	-	-	-	-	-	-	0.20	-
m-cresol	2089	MK	0.12	13.06	0.11	1.32	-	-	-	-
p-Cresol	2100	MKS	-	4.83	0.75	1.10	0.20	0.42	0.17	-
4-ethyl-phenol	2174	MK	-	0.02	-	-	0.07	10.13	35.51	-
Cinnamic alcohol	2300	MK	-	-	-	-	-	-	0.31	-
Carotenoid derivatives										
Dihydro-β-ionone	1842	MK	-	-	-	-	0.23	-	-	-
Irregular terpenes										
β-Cyclocitral	1647	MK	-	-	-	-	0.83	-	-	-
(Z)-Geranyl acetone	1853	MK	-	0.70	0.12	0.14	-	-	-	-
Dihydropseudoionone	1866	MK	-	-	-	-	-	-	2.29	-
Geranyl acetone	1881	MK	-	-	-	0.52	-	-	-	-
Monoterpenes										
α-Pinene	1092	MKS	-	24.92	-	-	-	-	-	-
Myrcene	1201	MK	10.23	-	-	-	19.90	-	-	-
Limonene	1228	MKS	0.53	18.47	16.54	-	-	-	-	-
(Z)-Ocimene	1257	MKS	1.24	-	-	-	1.59	0.76	10.95	-
(E)-Ocimene	1275	MK	-	0.24	-	-	-	0.13	0.61	-
Terpinolene	1306	MK	-	0.24	-	-	-	-	-	-
Monoterpene alcohols										
Linalool	1556	MKS	0.02	-	-	-	-	-	-	-
cis-Carveol	1840	MK	-	-	-	-	-	0.17	0.60	-
Nerol	1859	MK	0.01	-	-	-	-	-	-	-
(Z)-*p*-Mentha-6,8-dien-2-ol	1888	MK	-	-	-	-	-	-	0.11	-
Sesquiterpenes										
Caryophyllene	1622	MKS	0.46	9.72	-	-	-	0.17	4.55	-
Humulene	1697	MK	-	0.49	-	-	-	-	-	-
Tertiary terpene alcohol										
1,5,7-Octatrien-3-ol, 2,6-dimethyl-	1749	MK	0.02	-	-	-	-	-	-	-

Unknown sesquiterpenes			0.11	0.61	-	-	-	-	-	-
Nitrogen-containing compounds										
1,2-Benzisothiazole	1972	MK	-	-	-	-	-	-	0.18	-
Indole	2455	MKS	0.04	2.18	0.26	0.07	0.02	-	-	-
Skatole	2508	MKS	-	1.02	-	-	-	-	-	-
Sulfur-containing compounds										
Dimethyl sulfoxide	1596	MK	-	-	-	-	-	-	0.29	-
Methionol	1739	MK	-	-	0.19	0.02	-	-	-	-
Dimethyl sulfone	1916	MK	-	-	-	-	3.57	0.43	4.51	-
Unknowns	0	M	56.05	2.19	-	-	-	-	2.97	97.03

[1] M=mass spectrometer, K=Kovats, S=standard

Appendix 2

Two dung beetle species that disperse mimetic seeds both feed on eland dung

9.1 Abstract

Scarabaeus spretus zur Strassen was observed to roll and bury *Ceratocaryum argenteum* (Restionaceae) seeds in the sandplain fynbos of the Potberg area of the De Hoop Nature Reserve, South Africa. This species is the second dung beetle species found to be deceived by the faecal mimicry of *C. argenteum* seeds – the first species being *Epirinus flagellatus.* An isotopic analysis suggests that both these dung beetle species most likely feed on eland (*Taurotragus oryx*), not bontebok (*Damaliscus pygargus pygargus*), dung. Thus the model in this mimicry is eland dung; this interaction suggests large herbivores are an integral part of this fynbos.

9.2 Introduction

The dung beetle *Epirinus flagellatus* was observed to roll and bury seeds of the Cape plant *Ceratocaryum argenteum* (Restionaceae) at a site in the Potberg part of the De Hoop Nature Reserve in South Africa (Midgley et al., 2015). This primary dispersal of seeds involves chemical and visual mimicry because neither the dung beetle nor its larvae can eat these hard seeds. Chemically the seeds have characteristics of the dung of both of the most common large herbivores in the reserve: the eland (*Taurotragus oryx*) and the bontebok (*Damaliscus pygargus pygargus*) (Midgley et al., 2015). However, the seeds are more similar in shape and size to the smaller faeces of the bontebok, which is then the possible visual model that *C. argenteum* mimics. At the same Potberg site, during February 2016, we observed similar seed dispersal of *C. argenteum* seeds by another dung beetle, *Scarabaeus spretus* zur Strassen. The aim of this paper is to document this new burial behaviour and to investigate both dung beetle species to determine whether the faeces of the bontebok or the eland is the likely model of the mimic.

The bontebok is a short grass grazer whereas the eland is a mixed feeder (Skinner & Chimimba, 2005; Radloff, Mucina & Snyman, 2014). The two dominant grassland/ renosterveld grass species at Potberg are *Cymbopogon popschilli* (Andropogoneae) and *Cynodon dactylon* (Chloridoideae) (Radloff, Mucina & Snyman, 2014), to which can be added the relatively widespread *Themeda triandra* (Andropogoneae). All three species utilise the C4 photosynthetic pathway rather than the C3 pathway (Osborne et al., 2014). This pathway is common in tropical grasses whereas the C3 system is more common in woody plants and temperate grasses. The enzymes of these two different photosynthetic pathways produce different carbon δ13C signatures in their photosynthetic products. The relatively rare stable isotope of carbon 13C is slightly heavier than the more common 12C, which affects the ratios of these isotopes in different plants depending, for example, on enzyme preferences for the lighter isotopes. Fractionation is the process which reflects changes in relative proportions of isotopes, such as 13C:12C during C3 photosynthesis.

Fractionation can also occur in 15N because, as it is heavier than 14N, it may increase in tissues depending on factors such as levels of metabolism, catabolism and excretion. Thus animals are typically enriched by +3–5‰ in δ15N compared to their diet, although typically they are less than +1‰ enriched in δ13C (Peterson & Fry, 1987). During metamorphosis, larval tissue is broken down and then used to form new adult tissue and thus metamorphosis is also known to increase both δ13C and δ15N in the adult tissue in much the same way as would happen in adult tissue with an increase in trophic level (Tibbets, Wheeless & Del Rio, 2008). Thus the hypothesis that bontebok dung is the likely model *C. argenteum* mimics can be tested using the isotopic method for diagnosing animal diets, including those of dung beetles (Stavert, Gaskett, et al., 2014).

9.3 Methods

The study took place in the Potberg area of the De Hoop Nature Reserve (34.374420 S, 20.533060 E) in the sand plain vegetation type in which *C. argenteum* grows. During 3 days in early February 2016, we placed out 5 to 10 piles of *C. argenteum* seeds, with each pile comprising 10–20 seeds. Piles of seeds were 10 m apart on the edge of a 100-m stretch of a sand road through natural vegetation. We monitored the seed piles in the early morning for approximately 2 h (starting at about 08:00). This experiment took place after a 24-h rain event.

Dung samples were taken in various vegetation types in the Potberg reserve. These types were renosterveld (dominated by the shrub *Elytopappus rhinocerotis* (Asteraceae)), grassland (dominated by *Cynodon dactylon*), salt marshes (dominated by Chenopodiaceae), valley bottom fynbos (dominated by the Proteaceae shrubs *Leucadendron linifolium/ L. coniferum),* sand plain fynbos *(*dominated by *Leucadendron laureolum*, where *C. argenteum* occurs) and limestone fynbos (dominated by *Leucadendron meridianum*). Eland dung was found at all six sites whereas bontebok dung was found at all but the last two fynbos sites (sand plain fynbos and limestone fynbos). Previously, Radloff et al. (2014) noted that bontebok avoid fynbos whereas eland are found throughout fynbos, including limestone fynbos. To reduce chances of pseudoreplication, we sampled only a single pellet of bontebok or eland dung from a dung pile; only dung piles greater than 5 m apart were sampled and, as judged by colour, only relatively fresh samples were collected until a total of 10 pellets had been sampled from within each vegetation type. Dung pellets and dung beetle exoskeletons were dried and analysed for δ13C and δ15N, in ‰, using standard techniques at the Archaeometry Lab at the University of Cape Town. Dung beetle larvae have chewing teeth and are vigorous detritivores that depend on the plant remains that constitute the dung ball, rather than being dependent on microbiota associated with the ball (Byrne, Watkins & Bouwer, 2013). Although female beetles do select small fragments of plant remains from dung to constitute brood balls (Byrne, Watkins & Bouwer, 2013), this is not likely to significantly affect the isotopic signature of these balls nor the signature of the exoskeletons of adults that emerge from these balls.

Figure 9.1 (a) *Epirinus flagellatus* rolling a *Ceratocaryum argenteum* seed; (b) *Scarabaeus spretus* rolling a seed (the arrow indicates a sphaerocerid lesser dung fly); (c) the large hole made by *S. spretus* for burying several seeds (the arrow indicates the location of the dung beetle); and (d) a female sarcophagid fly on a seed.

9.4 Results

Dung beetles arrived at seed stations within a few minutes of placing seeds out; thus within 2 h each day, more than 10 beetles had arrived at seed piles along our short 100-m transect and had started burying seeds (Table 9.1 and Figure 9.1a–c). *E. flagellatus* crawled out of the vegetation towards seed piles, with only an occasional individual flying in, whereas all *S. spretus* individuals flew towards the seed piles. It was clear, based on the direct flight or crawling paths of both species to the seeds, that the attraction is strongly chemical. A *S. spretus* beetle even flew into a paper bag containing seeds. All cases of *S. spretus* burial involved limited movement of seeds (<0.25 m) from seed piles, whereas *E. flagellatus* moved seeds up to 2 m. *S. spretus* beetles were observed to frantically bury up to three seeds (*n*=2) and often five or more seeds (*n*=4) per excavated hole (see Supplementary Video 9.1). *E. flagellatus* was observed to only bury seeds individually, similarly to observations by Midgley et al. (2015). Flies of the Sarcophagidae were frequently observed to settle on *C. argenteum* seeds (Figure 9.1d), indicating that they too are deceived by the scent of the seeds. These 'flesh flies' are typically attracted to dung, carrion or rotting vegetation (Picker, Griffiths & Weaving, 2004). Lesser dung flies (Sphaeroceridae) were observed on *S. spretus* (Figure 9.1b).

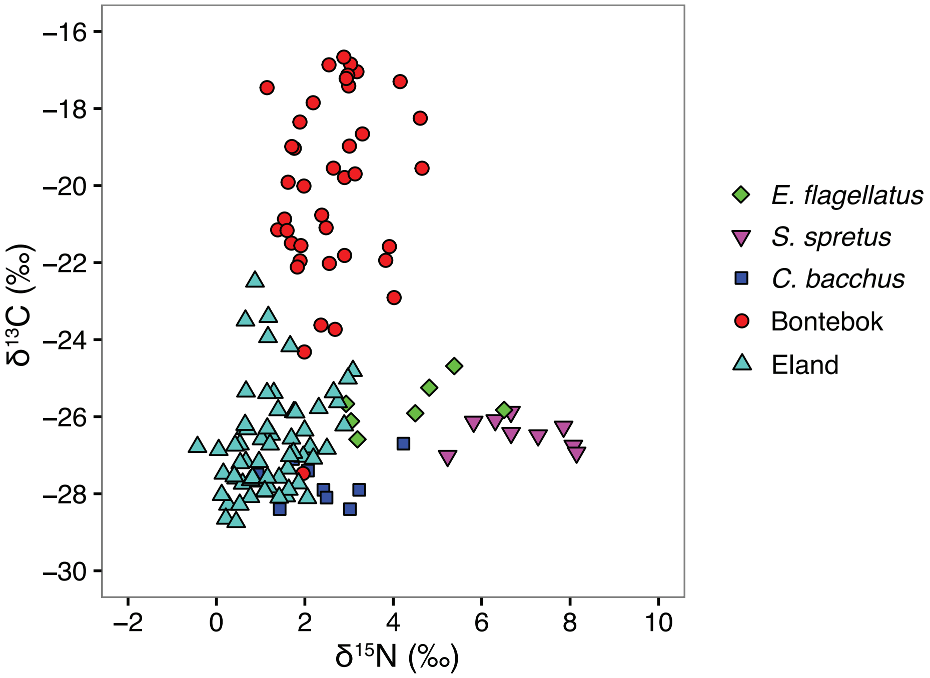


Figure 9.2 The distribution of isotopes (𝛿13C and 𝛿15N) of the dung of bontebok and eland as well as of three dung beetle species.

Samples of the grasses *Cynodon dactylon* and *Themeda triandra* from Potberg have a typical C4 isotopic signal (*n*=2 for each species, mean 𝛿13C of -13.61‰ and -14.29‰, respectively).

Table 9.1 Observations of dung beetles at *Ceratocaryum argenteum* seed stations at Potberg.

Date	**Duration (min)**	**Number of *Epirinus flagellatus* individuals**	**Number of *Scarabaeus spretus* individuals**
04 February 2016	120	10	3
05 February 2016	135	8	7
06 February 2016	90	7	3
Total	345	25	13

Dung of eland and bontebok are significantly different in both δ13C (U=52, $p<0.001$, Mann-Whitney test) and δ15N (U=309, $p<0.0001$, Mann-Whitney test) (Table 9.2). Bontebok graze a fairly equal mixture of C4 and C3 plants to create a mean δ13C value of -20.10‰, whereas eland are mostly eating C3 plants (Figure 9.2 and Table 9.2).

Table 9.2 Isotope analyses of dung beetles and dung from Potberg.

	n	**δ^{13}C**	**δ^{15}N**
Epirinus flagellatus	8	-25.76 (0.54)	4.48 (1.24)
Scarabaeus spretus	9	-26.45 (0.37)	6.92 (0.97)
Bontebok dung	40	-20.1 (2.44)	2.6 (0.89)
Eland dung	60	-26.71 (1.33)	1.23 (0.78)

9.5 Discussion

The increase in δ15N as a result of metamorphosis is in the range of +3‰ and +5‰ for a selection of insects ranging from Diptera to Coleoptera to Lepidoptera (Tibbets, Wheeless & Del Rio, 2008) and the increase in δ13C is about +1‰ (Peterson & Fry, 1987). Dung beetle adults whose larvae fed on bontebok dung should thus have values of slightly more than -20‰ δ13C but up to 7.6‰ δ15N. The mean δ13C values of both *E. flagellatus* and *S. spretus* clearly indicate a C3 dung diet and are thus much closer to that of the eland dung (Table 9.2 and Figure 9.2). The δ15N values are 3.25‰ and 5.7‰ above eland dung but only 1.9‰ and 4.32‰ above bontebok dung. The evidence from δ15N of the beetles is less equivocal about the larval food source because of the small 1‰ difference in dung between the two herbivore species. Overall the isotope results are compatible with eland being the main larval source of dung. Also bontebok dung is rare in the lowland fynbos habitat of *C. argenteum*. As these two dung beetle species are from different genera and are both attracted to *C. argenteum* seeds, these results indicate that the seed chemistry and deception by *C. argenteum* is not dung beetle species-specific. The deception appears most likely to be modelled on the chemistry of eland, rather than bontebok, dung.

Not much is known of the feeding biology of *Scarabaeus* or *Epirinus* beetles (Davis, Frolov & Scholtz, 2008). For both species, we observed diurnal activity and no pair formation at seed burial sites. *C. argenteum* seeds are about the same size as bontebok droppings and about half the size of eland droppings. That many seeds were buried per site for *S. spretus* suggests that several pellets of eland dung are typically used for feeding or egg laying, whereas *E. flagellatus* only buried a single seed per burial event. This distinctive burial behaviour of the two species likely results in differential recruitment patterns for *C. argenteum* seedlings. Single *E. flagellatus* burials would lead to lower intraspecific competition between seedlings compared with the multiple burials by *S. spretus*.

Many other dung beetles occur at Potberg, for example, the millipede-eating *Sceliages adamastor* (Davis, Frolov & Scholtz, 2008). The *δ*15N dung beetle values presented in Table 9.1 provide a framework to interpret those of *S. adamastor* to determine whether this beetle is an obligate insectivore. For example, *δ*15N values of an obligate millipede-eater should be a trophic level above herbivorous dung beetles such as *S. spretus* and *E. flagellatus* (i.e. they should have *δ*15N values greater than 7‰). The very large Addo flightless dung beetle (*Circellium bacchus*) also occurs at Potberg (Davis, Frolov & Scholtz, 2008); elsewhere it feeds on elephant dung (Davis, Frolov & Scholtz, 2008) but as there are no elephants at Potberg, its diet there is unknown and could too be clarified using the isotopic method. Being flightless, *C. bacchus* is often killed on roads and our analysis of nine roadkill individuals (mean *δ*13C of -27.70‰ and *δ*15N of 2.39‰) indicates eland dung is also its major larval food source.

The fact that *Ceratocaryum argenteum* is an element of deep sand fynbos (Linder, 2001), implies that sufficient quantities of large herbivore dung, such as that of eland, occurred in this vegetation. This would maintain the associated dung beetle species and the deceptive relationship between *C. argenteum* and these species. There is some debate as to whether large herbivores were once more common in fynbos and in this area of the Cape (Radloff, Mucina & Snyman, 2014). Our observation that *C. bacchus*, *E. flagellatus* and *S. spretus* utilise eland dung suggests that the eland is, and has been, a key species in this system and should be carefully managed as such. Finally, we suggest that there are now sufficient examples of seed dispersal by beetles for use of the term coleopterochory. This term would include primary dispersal such as that described above, as well as examples of beetle endozoochory (de Vega et al., 2011) in which small seeds are swallowed, as well as incidental or secondary dispersal in dung or with fruit (Midgley et al., 2015).

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9.7 Supplementary Material

Supplementary Video 9.1 can be viewed at the following link:

- https://youtu.be/C_VFlAcsx10

References

Abatzoglou, J.T., Dobrowski, S.Z., Parks, S.A. & Hegewisch, K.C. 2018. TerraClimate, a high-resolution global dataset of monthly climate and climatic water balance from 1958–2015. *Scientific Data*. 5:170191.

Altwegg, R., West, A.G., Gillson, L. & Midgley, G.F. 2014. Impacts of climate change in the Greater Cape Floristic Region. In *Fynbos: ecology, evolution and conservation of a megadiverse region.* N. Allsopp, J.F. Colville, & G.A. Verboom, Eds. Oxford, UK: Oxford University Press. 299–320.

Amico, G. & Aizen, M.A. 2000. Mistletoe seed dispersal by a marsupial. *Nature*. 408:929–930. DOI: https://doi.org/10.1038/35050170.

Anderson, B., Allsopp, N., Ellis, A.G., Johnson, S.D., Midgley, J.J., Pauw, A. & Rodger, J.G. 2014. Biotic interactions. In *Fynbos: ecology, evolution, and conservation of a megadiverse region*. N. Allsopp, J.F. Colville, & G.A. Verboom, Eds. Oxford, UK: Oxford University Press. 224–247.

Aslan, C.E., Zavaleta, E.S., Tershy, B. & Croll, D. 2013. Mutualism Disruption Threatens Global Plant Biodiversity: A Systematic Review. *PLOS ONE*. 8(6):e66993.

Barker, N.P., Vanderpoorten, A., Morton, C.M. & Rourke, J.P. 2004. Phylogeny, biogeography, and the evolution of life-history traits in Leucadendron (Proteaceae). *Molecular Phylogenetics and Evolution*. 33:845–860. DOI: 10.1016/j.ympev.2004.07.007.

Barve, V. & Hart, E. 2017. rinat: Access iNaturalist data through APIs. R package version 0.1.5. https://CRAN.R-project.org/package=rinat.

Bates, H.W. 1862. XXXII. Contributions to an Insect Fauna of the Amazon Valley. Lepidoptera: Heliconidæ. *Transactions of the Linnean Society of London*. 23(3):495–566.

van Blerk, J.J., West, A.G. & Midgley, J.J. 2017. Restio culm felling is a consequence of pre-dispersal seed predation by the rodent Rhabdomys pumilio in the Fynbos. *South African Journal of Botany*. 112:123–127. DOI: 10.1016/j.sajb.2017.05.017.

Bond, W.J. 1984. Fire survival of Cape Proteaceae: influence of fire season and seed predators. *Vegetatio*. 56(2):65–74.

Bond, W.J. 1985. Canopy-stored seed reserves (serotiny) Proteaceae. *South African*

Journal of Botany. 51:181–186.

Bond, W.J. 1988. Proteas as ‘tumbleseeds’: Wind dispersal through the air and over soil. *South African Journal of Botany*. 54(5):455–460.

Bond, W.J. 1994. Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical Transactions: Biological Sciences*. 344(1307):83–90.

Bond, W.J. & Breytenbach, G.J. 1985. Ants, rodents and seed predation in Proteaceae. *South African Journal of Zoology*. DOI: 10.1080/02541858.1985.11447928.

Bond, W.J. & Slingsby, P. 1983. Seed dispersal by ants in shrublands of the Cape Province and its evolutionary implications. *South African Journal of Science*. 79(6):231–233.

Bond, W.J. & Slingsby, P. 1984. Collapse of an Ant-Plant Mutualism: The Argentine Ant (Iridomyrmex humilis) and Myrmecochorous Proteaceae. *Ecology*. 65(4):1031–1037.

Bond, W.J. & Stock, W.D. 1989. The costs of leaving home: ants disperse myrmecochorous seeds to low nutrient sites. *Oecologia*. 81(3):412–417. DOI: https://doi.org/10.1007/BF00377092.

Bond, W.J., Ferguson, M. & Forsyth, G.G. 1980. Small mammals and habitat structure along altitudinal gradients in the southern Cape mountains. *South African Journal of Zoology*. 15(1):34–43.

Botha, S. 1989. Effect of sowing season and granivory on the establishment of the Clanwilliam cedar. *South African Journal of Wildlife Research*. 19(3):112–117.

Botha, P.W. & Pauw, A. 2017. Rodents and baboons reduce seed cone production of Protea neriifolia. *South African Journal of Botany*. 108:303–307. DOI: 10.1016/j.sajb.2016.07.020.

Botha, S. & le Maitre, D.C. 1992. Effects of seed and seedling predation by small mammals on seedling recruitment of Protea neriifolia in Swartboskloof, Cape Province. *South African Journal of Zoology*. 27(2):60–69. DOI: 10.1080/02541858.1992.11448263.

Bradshaw, S.D., Dixon, K.W., Hopper, S.D., Lambers, H. & Turner, S.R. 2011. Little evidence for fire-adapted plant traits in Mediterranean climate regions. *Trends in Plant Science*. 16(2):69–76.

Brits, G.J. 1987. Germination depth vs. temperature requirements in naturally dispersed seeds of Leucospermum cordifolium and L. cuneiforme (Proteaceae). *South African Journal of Botany*. 53(2):119–124. DOI: 10.1016/S0254-6299(16)31445-4.

Brodin, A. 2010. The history of scatter hoarding studies. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*. 365(1542):869–881.

Brown, N.A.C. & van Staden, J. 1973. The effect of scarification, leaching, light, stratification, oxygen and applied hormones on germination of Protea compacta R. Br. and Leucadendron daphnoides Meisn. *Journal of South African Botany*. 39:185–195.

Burger, B. V & Petersen, W.G.B. 1991. Semiochemicals of the Scarabaeinae, III: identification of the attractant for the dung beetle Pachylomerus femoralis in the fruit of the spineless monkey orange tree, Strychnos madagascariensis. *Zeitschrift für Naturforschung C*. 46(11–12):1073–1079.

Byrne, M.J., Watkins, B. & Bouwer, G. 2013. Do dung beetle larvae need microbial symbionts from their parents to feed on dung? *Ecological Entomology*. 38(3):250–257.

Carleton, M. & Happold, D. 2013. Order Rodentia. In *Mammals in Africa, vol. 3*. J. Kingdon, Ed. London: Bloomsbury Publishing. 27–38.

Caro, T. 2005. The adaptive significance of coloration in mammals. *BioScience*. 55(2):125.

Chalmandrier, L., Midgley, G.F., Barnard, P. & Sirami, C. 2013. Effects of time since fire on birds in a plant diversity hotspot. *Acta Oecologica*. 49:99–106. DOI: 10.1016/j.actao.2013.03.008.

Christian, C.E. 2001. Consequences of a biological invasion reveal the importance of mutualism for plant communities. *Nature*. 413(6856):635–639.

Cipollini, M.L. 2000. Secondary metabolites of vertebrate-dispersed fruits: evidence for adaptive functions. *Revista Chilena de Historia Natural*. 73(3):421–440. DOI: 10.4067/S0716-078X2000000300006.

Collins, B.G. & Rebelo, T. 1987. Pollination biology of the Proteaceae in Australia and southern Africa. *Australian Journal of Ecology*. 12(4):387–421.

Connell, J.H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In *Dynamics of populations*. P. Den Boer & G. Gradwell, Eds. Wageningen, The Netherlands: Centre for Agricultural

Publishing and Documentation. 298–312.

Cook, L.M. & Saccheri, I.J. 2013. The peppered moth and industrial melanism: Evolution of a natural selection case study. *Heredity*. 110(3):207–212. DOI: 10.1038/hdy.2012.92.

Coomes, D.A. & Grubb, P.J. 2003. Colonization, tolerance, competition and seed-size variation within functional groups. *Trends in Ecology & Evolution*. 18(6):283–291.

Cowling, R.M., Rundel, P.W., Lamont, B.B., Arroyo, M.K. & Arianoutsou, M. 1996. Plant diversity in Mediterranean-climate regions. *Trends in Ecology & Evolution*. 11(9):362–366.

Cowling, R.M., Ojeda, F., Lamont, B.B., Rundel, P.W. & Lechmere-Oertel, R. 2005. Rainfall reliability, a neglected factor in explaining convergence and divergence of plant traits in fire-prone mediterranean-climate ecosystems. *Global Ecology and Biogeography*. 14(6):509–519.

Cowling, R.M., Potts, A.J., Bradshaw, P.L., Colville, J., Arianoutsou, M., Ferrier, S., Forest, F., Fyllas, N.M., et al. 2015. Variation in plant diversity in mediterranean-climate ecosystems: The role of climatic and topographical stability. *Journal of Biogeography*. 42(3):552–564. DOI: 10.1111/jbi.12429.

Cribari-Neto, F. & Zeileis, A. 2010. Beta Regression in R. *Journal of Statistical Software*. 34:1–24. DOI: https://doi.org/10.18637/jss.v034.i02.

Culot, L., Mann, D.J., Muñoz Lazo, F.J.J., Huynen, M. & Heymann, E.W. 2011. Tamarins and dung beetles: an efficient diplochorous dispersal system in the Peruvian Amazonia. *Biotropica*. 43(1):84–92.

David, J.H.M. & Jarvis, J.U.M. 1985. Population fluctuations, reproduction and survival in the striped fieldmouse Rhabdomys pumilio on the Cape Flats, South Africa. *Journal of Zoology*. 207(2):251–276.

Davis, A.L. V. 1996. Seasonal dung beetle activity and dung dispersal in selected South African habitats: implications for pasture improvement in Australia. *Agriculture, Ecosystems & Environment*. 58(2–3):157–169.

Davis, A.L. V, Frolov, A. V & Scholtz, C.H. 2008. *The African dung beetle genera*. Protea Book House.

De'Ath, G. 2007. Boosted trees for ecological modeling and prediction. *Ecology*. 88(1):243–251.

deBruyn, R.A.J., Paetkau, M., Ross, K.A., Godfrey, D. V & Friedman, C.R. 2015. Thermogenesis-triggered seed dispersal in dwarf mistletoe. *Nature Communications*. 6(6262):1–5. DOI: https://doi.org/10.1038/ncomms7262.

Diaz, S., Cabido, M. & Casanoves, F. 1998. Plant functional traits and environmental filters at a regional scale. *Journal of Vegetation Science*. 9(1):113–122.

Dice, L.R. 1940. Speciation in Peromyscus. *The American Naturalist*. 74(753):289–298.

Dittel, J.W., Moore, C.M. & Vander Wall, S.B. 2019. The mismatch in distributions of vertebrates and the plants that they disperse. *Ecography*. 42(4):621–631. DOI: 10.1111/ecog.03876.

Dormann, C.F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J.R.G., Gruber, B., et al. 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography*. 36(1):27–46.

Elith, J., Leathwick, J.R. & Hastie, T. 2008. A working guide to boosted regression trees. *Journal of Animal Ecology*. 77(4):802–813. DOI: 10.1111/j.1365-2656.2008.01390.x.

Endler, J.A. 1978. A predator's view of animal color patterns. In *Evolutionary biology*. Springer. 319–364.

Endler, J.A. & Greenwood, J.J.D. 1988. Frequency-dependent predation, crypsis and aposematic coloration. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*. DOI: 10.1098/rstb.1988.0062.

Feer, F., Henry, O., Forget, P.-M. & Gayot, M. 2001. Frugivory and seed dispersal by terrestrial mammals. In *Nouragues. Dynamics and plant-animal interactions in a neotropical rainforest*. F. Bongers, P. Charles-Dominique, P. Forget, & M. Théry, Eds. Kleuwer Academic Publishers. 227–232. DOI: https://doi.org/10.1007/978-94-015-9821-7_21.

Ferrandis, P., Herranz, J́. M. & Martınez-Sánchez, J.J. 1999. Effect of fire on hard-coated Cistaceae seed banks and its influence on techniques for quantifying seed banks. *Plant Ecology*. 144:103–114.

Fick, S.E. & Hijmans, R.J. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*. 37(12):4302–4315.

Fleming, P.A. & Nicolson, S.W. 2002a. Opportunistic breeding in the Cape spiny mouse (Acomys subspinosus). *African Zoology*. 37(1):101–105. DOI:

10.1080/15627020.2002.11657160.

Fleming, P.A. & Nicolson, S.W. 2002b. How important is the relationship between Protea humiflora (Proteaceae) and its non-flying mammal pollinators? *Oecologia*. 132(3):361–368. DOI: 10.1007/s00442-002-0921-9.

Forget, P.-M. 1993. Post-dispersal predation and scatterhoarding of Dipteryx panamensis (Papilionaceae) seeds by rodents in Panama. *Oecologia*. 94(2):255–261. DOI: 10.1007/BF00341325.

Fraser, M.W. 1989. Short-term responses of birds to fire in old mountain fynbos. *Ostrich*. 60(4):172–182.

Fraser, M.W. 1990. Small mammals, birds and ants as seed predators in post-fire mountain fynbos. *South African Journal of Wildlife Research*. 20(2):52–56.

Friedman, J.H. & Meulman, J.J. 2003. Multiple additive regression trees with application in epidemiology. *Statistics in Medicine*. 22(9):1365–1381.

Galetti, M. 2002. Seed dispersal of mimetic fruits: parasitism, mutualism, aposematism or exaptation? In *Seed dispersal and frugivory: Ecology, evolution, and conservation*. D.J. Levey, W.R. Silva, & M. Galetti, Eds. Wallingford, UK: CAB International. 177–191.

Galetti, M. & Dirzo, R. 2013. Ecological and evolutionary consequences of living in a defaunated world. *Biological Conservation*. 163:1–6. DOI: https://doi.org/10.1016/j.biocon.2013.04.020.

Gómez, J.M., Schupp, E.W. & Jordano, P. 2018. Synzoochory: the ecological and evolutionary relevance of a dual interaction. *Biological Reviews*. DOI: 10.1111/brv.12481.

Gorelick, N., Hancher, M., Dixon, M., Ilyushchenko, S., Thau, D. & Moore, R. 2017. Google Earth Engine: Planetary-scale geospatial analysis for everyone. *Remote Sensing of Environment*. 202:18–27.

Hart, N., Partridge, J.C. & Cuthill, I.C. 1998. Visual pigments, oil droplets and cone photoreceptor distribution in the European starling (Sturnus vulgaris). *The Journal of Experimental Biology*. 201:1433–46. DOI: 10.1111/j.1752-0606.2009.00150.x.

He, T., Pausas, J.G., Belcher, C.M., Schwilk, D.W. & Lamont, B.B. 2012. Fire-adapted traits of Pinus arose in the fiery Cretaceous. *New Phytologist*. 194(3):751–759.

Heads, M. 2015. The relationship between biogeography and ecology: envelopes, models, predictions. *Biological journal of the Linnean Society*. 115(2):456–468.

Hengl, T., de Jesus, J.M., MacMillan, R.A., Batjes, N.H., Heuvelink, G.B.M., Ribeiro, E., Samuel-Rosa, A., Kempen, B., et al. 2014. SoilGrids1km—global soil information based on automated mapping. *PloS ONE*. 9(8):e105992.

Henkel, J.B. 1858. Beiträge zur Kenntniss der chemischen Bestandtheile der Früchte von Hyaenanche globosa Lamb., Euphorbiaceae. *Archiv der Pharmazie*. 144(1):16–37. DOI: 10.1002/ardp.18581440104.

van Hensbergen, H.J., Botha, S., Forsyth, G.G. & le Maitre, D.C. 1992. Do small mammals govern vegetation recovery after fire in fynbos? In *Fire in South African Mountain Fynbos*. B.W. van Wilgen, D.M. Richardson, F.J. Kruger, & H.J. van Hensbergen, Eds. Berlin, Heidelberg: Springer. 182–202.

van den Heuvel, I.M. & Midgley, J.J. 2014. Towards an Isotope Ecology of Cape Fynbos Small Mammals. *African Zoology*. 49(2):195–202. DOI: 10.1080/15627020.2014.11407635.

Hijmans, R.J. 2017. raster: Geographic Data Analysis and Modeling. R package version 2.6-7. https://CRAN.R-project.org/package=raster.

Hijmans, R.J., Phillips, S.J., Leathwick, J.R. & Elith, J. 2017. dismo: Species distribution modeling. R package version 1.1-4. https://CRAN.R-project.org/package=dismo.

Hirsch, B., Kays, R., Pereira, V.E. & Jansen, P.A. 2012. Directed seed dispersal towards areas with low conspecific tree density by a scatter-hoarding rodent. *Ecology Letters*. 15:1423–1429. DOI: https://doi.org/10.1111/ele.12000.

Hobbhahn, N. & Johnson, S.D. 2013. A new record of rodent pollination in the holoparasitic genus Cytinus. *South African Journal of Botany*. (86):168.

Hollander, J.L., Vander Wall, S.B. & Longland, W.S. 2012. Olfactory detection of caches containing wildland versus cultivated seeds by granivorous rodents. *Western North American Naturalist*. 72(3):339–347.

Holmes, P.M. 1990. Dispersal and predation in alien Acacia. *Oecologia*. 83:288–290. DOI: https://doi.org/10.1007/BF00317768.

Howe, H.F. & Smallwood, J. 1982. Ecology of seed dispersal. *Annual Review of Ecology and Systematics*. 13(1):201–228. DOI: https://doi.org/10.1146/annurev.es.13.110182.001221.

Islam, M.B., Simmons, M.P. & Archer, R.H. 2006. Phylogeny of the Elaeodendron group (Celastraceae) inferred from morphological characters and nuclear and plastid genes. *Systematic Botany*. 31(3):512–524. DOI: https://doi.org/10.1600/036364406778388610.

Jansen, P.A., Bartholomeus, M., Bongers, F., Elzinga, J.A., den Ouden, J. & van Wieren, S.E. 2002. The role of seed size in dispersal by a scatter-hoarding rodent. In *Seed Dispersal and Frugivory: Ecology, Evolution, and Conservation*. D.J. Levey, W.R. Silva, & M. Galetti, Eds. Wallingford, UK: CAB International. 209–225.

Jansen, P.A., Hirsch, B.T., Emsens, W.-J., Zamora-Gutierrez, V., Wikelski, M. & Kays, R. 2012. Thieving rodents as substitute dispersers of megafaunal seeds. *Proceedings of the National Academy of Sciences*. 109(31):12610–12615.

Janzen, D.H. 1970. Herbivores and the number of tree species in tropical forests. *The American Naturalist*. 104(940):501–528.

Janzen, D.H. 1971. Seed Predation by Animals. *Annual Review of Ecology and Systematics*. 2(1):465–492. DOI: 10.1146/annurev.es.02.110171.002341.

Janzen, D.H. 1978. The ecology and evolutionary biology of seed chemistry as relates to seed predation. In *Biochemical aspects of plant and animal coevolution*. J.B. Harborne, Ed. London, UK: Academic Press. 165–206.

Johnson, S.D. 1992. Plant-animal interactions. In *The ecology of fynbos: nutrients, fire and diversity*. R.M. Cowling, Ed. Cape Town: Oxford University Press. 175–205.

Johnson, C.M. & Pauw, A. 2014. Adaptation for rodent pollination in Leucospermum arenarium (Proteaceae) despite rapid pollen loss during grooming. *Annals of Botany*. 113(6):931–938.

Johnson, S.D. & Steiner, K.E. 2000. Generalization versus specialization in plant pollination systems. *Trends in Ecology and Evolution*. 15(4):140–143.

Johnson, T.K. & Jorgensen, C.D. 1981. Ability of desert rodents to find buried seeds. *Journal of Range Management*. 312–314.

Johnson, S.D., Pauw, A. & Midgley, J. 2001. Rodent pollination in the African lily Massonia depressa (Hyacinthaceae). *American Journal of Botany*. 88(1–12):1768–1773. DOI: 10.2307/3558351.

Jones, K.A., Krebs, J.R. & Whittingham, M.J. 2006. Interaction between seed crypsis and habitat structure influence patch choice in a granivorous bird, the chaffinch Fringilla coelebs. *Journal of Avian Biology*. 37(5):413–418. DOI: 10.1111/j.2006.0908-8857.03883.x.

Jorgensen, E.E. 2001. Emission of volatile compounds by seeds under different environmental conditions. *The American Midland Naturalist*. 145(2):419–422.

Jürgens, A., Wee, S., Shuttleworth, A. & Johnson, S.D. 2013. Chemical mimicry of insect oviposition sites: a global analysis of convergence in angiosperms. *Ecology Letters*. 16(9):1157–1167.

Kearns, C.A., Inouye, D.W. & Waser, N.M. 1998. Endangered Mutualisms: The Conservation of Plant-Pollinator Interactions. *Annual Review of Ecology and Systematics*. 29(1):83–112. DOI: 10.1146/annurev.ecolsys.29.1.83.

Keeley, J.E. & Fotheringham, C.J. 2000. Role of fire in regeneration from seed. In *Seeds: the ecology of regeneration in plant communities*. M. Fenner, Ed. Oxon, UK: CAB International. 311–330.

Kleizen, C., Midgley, J. & Johnson, S.D. 2008. Pollination systems of Colchicum (Colchicaceae) in Southern Africa: evidence for rodent pollination. *Annals of botany*. 102(5):747–755.

Knight, L.I., Ng, B.L., Cheng, W., Fu, B., Yang, F. & Rambau, R. V. 2013. Tracking chromosome evolution in southern African gerbils using flow-sorted chromosome paints. *Cytogenetic and Genome Research*. 139(4):267–275.

Kraaij, T. & van Wilgen, B.W. 2014. Drivers, ecology, and management of fire in fynbos. In *Fynbos: ecology, evolution, and conservation of a megadiverse region*. N. Allsopp & J.F. Colville, Eds. Oxford University Press, USA. 47–72. DOI: 10.1093/acprof:oso/9780199679584.003.0003.

Kreft, H. & Jetz, W. 2007. Global patterns and determinants of vascular plant diversity. *Proceedings of the National Academy of Sciences*. 104(14):5925–5930.

Kruger, F.J. 1979. South African heathlands. In *Heathlands and related shrublands of the world: descriptive studies. Amsterdam, Elsevier*. R.L. Specht, Ed. 19–80.

Kühn, N., Midgley, J. & Steenhuisen, S.L. 2017. Reproductive biology of three co-occurring, primarily small-mammal pollinated Protea species (Proteaceae). *South African Journal of Botany*. 113:337–345. DOI: 10.1016/j.sajb.2017.08.020.

Lamont, B.B. & Enright, N.J. 2000. Adaptive advantages of aerial seed banks. *Plant Species Biology*. 15(2):157–166.

Lamont, B.B., le Maitre, D.C., Cowling, R.M. & Enright, N.J. 1991. Canopy seed storage in woody plants. *The Botanical Review*. 57(4):277–317.

Leishman, M., R, Wright, I.J., Moles, A.T. & Westoby, M. 2000. The evolutionary ecology of seed size. In *Seeds: the ecology of regeneration in plant communities*. M. Fenner, Ed. Oxon, UK: CAB International. 31–57.

Letten, A.D. & Midgley, J.J. 2009. Rodent pollination in the Cape legume Liparia parva. *Austral Ecology*. 34(2):233–236. DOI: 10.1111/j.1442-9993.2008.01925.x.

Lev-Yadun, S. & Ne'eman, G. 2013. Bimodal colour pattern of individual Pinus halepensis Mill. seeds: a new type of crypsis. *Biological Journal of the Linnean Society*. 109(2):271–278. DOI: 10.1111/bij.12047.

Linder, H.P. 2001. Two new species of Ceratocaryum (Restionaceae). *Kew Bulletin*. 56(2):465–477.

Loayza, A.P., Carvajal, D.E., García-Guzmán, P., Gutierrez, J.R. & Squeo, F.A. 2014. Seed predation by rodents results in directed dispersal of viable seed fragments of an endangered desert shrub. *Ecosphere*. 5(4):1–9. DOI: https://doi.org/10.1890/ES13-00283.1.

Lombardi, G.C., Peter, C.I., Turner, R.C. & Midgley, J.J. 2017. The unusual, closed flowers of Erica lanuginosa (Ericaceae) are adapted for rodent-pollination and not cleistogamy. *South African Journal of Botany*. 111:189–193. DOI: 10.1016/j.sajb.2017.03.020.

Maia, R., Eliason, C.M., Bitton, P.P., Doucet, S.M. & Shawkey, M.D. 2013. pavo: An R package for the analysis, visualization and organization of spectral data. *Methods in Ecology and Evolution*. 4(10):906–913. DOI: 10.1111/2041-210X.12069.

Maia, R., Gruson, H., Endler, J.A. & White, T.E. 2018. pavo 2.0 : new tools for the spectral and spatial analysis of colour in R. *bioRxiv*. DOI: 10.1101/427658.

le Maitre, D.C. & Midgley, J.J. 1992. Plant reproductive ecology. In *The ecology of fynbos: nutrients, fire and diversity*. R. Cowling, Ed. Cape Town: Oxford University Press. 135–174.

Mallet, J. & Singer, M.C. 1987. Individual selection, kin selection, and the shifting balance in the evolution of warning colours: the evidence from butterflies. *Biological Journal of the Linnean Society*. 32(4):337–350. DOI: 10.1111/j.1095-8312.1987.tb00435.x.

Manders, P. 1986. Seed dispersal and seedling recruitment in Protea laurifolia. *South African Journal of Botany*. 52(5):421–424. DOI: 10.1016/S0254-6299(16)31506-X.

Manning, J. & Goldblatt, P. 2012. *Plants of the Greater Cape Floristic Region. 1: The Core Cape flora.* South African National Biodiversity Institute.

Marloth, R. 1925. *The flora of South Africa Vol. II, Section II.* Cape Town: Darter Bros. & Co.

McConkey, K.R. & Drake, D.R. 2006. Flying foxes cease to function as seed dispersers long before they become rare. *Ecology*. 87(2):271–276. DOI: 10.1890/05-0386.

Melidonis, C.A. & Peter, C.I. 2015. Diurnal pollination, primarily by a single species of rodent, documented in Protea foliosa using modified camera traps. *South African Journal of Botany*. 97:9–15.

Mendoza, E. & Dirzo, R. 2009. Seed tolerance to predation: evidence from the toxic seeds of the buckeye tree (Aesculus californica; Sapindaceae). *American Journal of Botany*. 96(7):1255–1261. DOI: https://doi.org/10.3732/ajb.0800297.

Merow, C., Latimer, A.M., Wilson, A.M., McMahon, S.M., Rebelo, A.G. & Silander, J.A. 2014. On using integral projection models to generate demographically driven predictions of species' distributions: development and validation using sparse data. *Ecography*. 37(12):1167–1183.

Midgley, J.J. 1987. The derivation, utility and implications of a divergence index for the fynbos genus Leucadendron (Proteaceae). *Botanical Journal of the Linnean Society*. 95(2):137–152. DOI: 10.1111/j.1095-8339.1987.tb01994.x.

Midgley, J.J. 1989. Season of burn of serotinous fynbos Proteaceae: a critical review and further data. *South African Journal of Botany*. 55(2):165–170.

Midgley, J.J. & Anderson, B. 2005. Scatterhoarding in Mediterraneaen shrublands of the SW Cape, South Africa. In *Seed fate: predation, dispersal and seedling establishment*. P.-M. Forget, J.E. Lambert, P.E. Hulme, & S.B. Vander Wall, Eds. CABI Publishing. 197–204.

Midgley, J.J. & Clayton, P. 1990. Short-term effects of an autumn fire on small mammal populations in southern cape coastal mountain fynbos. *South African Forestry Journal*. 153(1):27–30. DOI: 10.1080/00382167.1990.9629029.

Midgley, J.J. & White, J.D.M. 2016. Two dung beetle species that disperse mimetic seeds both feed on eland dung. *South African Journal of Science*. 112(7–8):7–9. DOI: 10.17159/sajs.2016/20160114.

Midgley, J.J., Anderson, B., Bok, A. & Fleming, T. 2002. Scatter-hoarding of Cape Proteaceae nuts by rodents. *Evolutionary Ecology Research*. 4(4):623–626.

Midgley, J.J., Gallaher, K. & Kruger, L.M. 2012. The role of the elephant (Loxodonta africana) and the tree squirrel (Paraxerus cepapi) in marula (Sclerocarya birrea) seed predation, dispersal and germination. *Journal of Tropical Ecology*. 28(2):227–231.

Midgley, J.J., White, J.D.M., Johnson, S.D. & Bronner, G.N. 2015. Faecal mimicry by seeds ensures dispersal by dung beetles. *Nature Plants*. 1:15141.

Milewski, A. V. 1978. Diet of serinus species in the southwestern cape, with special reference to the protea seedeater. *Ostrich*. 49(4):174–184. DOI: 10.1080/00306525.1978.9634096.

Mittermeier, R.A., Turner, W.R., Larsen, F.W., Brooks, T.M. & Gascon, C. 2011. Global Biodiversity Conservation: The Critical Role of Hotspots. In *Biodiversity Hotspots: Distribution and Protection of Conservation Priority Areas*. F.E. Zachos & J.C. Habel, Eds. Berlin, Heidelberg: Springer Berlin Heidelberg. 3–22. DOI: 10.1007/978-3-642-20992-5_1.

Moll, E.J., McKenzie, B. & McLachlan, D. 1980. A possible explanation for the lack of trees in the fynbos, Cape Province, South Africa. *Biological Conservation*. 17(3):221–228.

Momtaz, S., Lall, N., Hussein, A., Ostad, S.N. & Abdollahi, M. 2010. Investigation of the possible biological activities of a poisonous South African plant; Hyaenanche globosa (Euphorbiaceae). *Pharmacognosy Magazine*. 6(21):34–41. DOI: 10.4103/0973-1296.59964.

Monadjem, A., Taylor, P.J., Denys, C. & Cotterhill, F.P.D. 2015. *Rodents of sub-Saharan Africa. A biogeographic and taxonomic synthesis*. Berlin, Boston: De Gruyter.

Moore, C.M. & Vander Wall, S.B. 2015. Scatter-hoarding rodents disperse seeds to safe sites in a fire-prone ecosystem. *Plant Ecology*. 216(8):1137–1153. DOI: 10.1007/s11258-015-0497-1.

Moore, J.E., McEuen, A.B., Swihart, R.K., Contreras, T.A. & Steele, M.A. 2007. Determinants of seed removal distance by scatter-hoarding rodents in deciduous forests. *Ecology*. 88(10):2529–2540.

Mucina, L. & Rutherford, M.C. 2006. *The vegetation of South Africa, Lesotho and Swaziland.* South African National Biodiversity Institute.

Mucina, L., Rutherford, M.C., Powrie, L.W., van Niekerk, A. & van der Merwe, J.H. 2014. *Vegetation field atlas of continental South Africa, Lesotho and Swaziland*. South African National Biodiversity Institute Pretoria.

Munsell Color. 2009. *Munsell Rock Color Book*. Grand Rapids, MI.

Murphy, M.T., Garkaklis, M.J. & Hardy, G.E.S.J. 2005. Seed caching by woylies Bettongia penicillata can increase sandalwood Santalum spicatum regeneration in Western Australia. *Austral Ecology*. 30(7):747–755. DOI: 10.1111/j.1442-9993.2005.01515.x.

Myburg, A.C. & Rust, D.J. 1975. A survey of pests of the Proteaceae in the Western and Southern Cape Province. *Journal of the Entomological Society of Southern Africa*. 38:55–60.

Myczko, Ł., Skórka, P., Dylewski, Ł., Sparks, T.H. & Tryjanowski, P. 2015. Color mimicry of empty seeds influences the probability of predation by birds. *Ecosphere*. 6(10):1–7. DOI: 10.1890/ES15-00055.1.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A.B. & Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature*. 403(6772):853.

Nel, J.A.J., Rautenbach, I.L. & Breytenbach, G.J. 1980. Mammals of the Kammanassie Mountains, southern Cape Province. *South African Journal of Zoology*. 15(4):255.

Newton, R.J., Bond, W.J. & Farrant, J.M. 2006. Effects of seed storage and fire on germination in the nut-fruited Restionaceae species, Cannomois virgata. *South African Journal of Botany*. 72(1):177–180. DOI: 10.1016/j.sajb.2005.05.005.

Niu, Y., Sun, H. & Stevens, M. 2018. Plant Camouflage: Ecology, Evolution, and Implications. *Trends in Ecology and Evolution*. 33(8):608–618. DOI: 10.1016/j.tree.2018.05.010.

Nystrand, O. & Granström, A. 1997. Post-dispersal predation on Pinus sylvestris seeds by Fringilla spp: ground substrate affects selection for seed color. *Oecologia*. 110(3):353–359.

Osborne, C.P., Salomaa, A., Kluyver, T.A., Visser, V., Kellogg, E.A., Morrone, O., Vorontsova, M.S., Clayton, W.D., et al. 2014. A global database of C 4 photosynthesis in grasses. *New Phytologist*. 204(3):441–446.

Paulsen, T.R., Colville, L., Kranner, I., Daws, M.I., Högstedt, G., Vandvik, V. & Thompson, K. 2013. Physical dormancy in seeds: a game of hide and seek? *New Phytologist*. 198(2):496–503. DOI: 10.1111/nph.12191.

Paulsen, T.R., Högstedt, G., Thompson, K., Vandvik, V. & Eliassen, S. 2014. Conditions favouring hard seededness as a dispersal and predator escape strategy. *Journal of Ecology*. 102(6):1475–1484.

Pauw, A. 2007. Collapse of a pollination web in small conservation areas. *Ecology*.

88(7):1759–1769. DOI: 10.1890/06-1383.1.

Pauw, A. & Stanway, R. 2015. Unrivalled specialization in a pollination network from South Africa reveals that specialization increases with latitude only in the Southern Hemisphere. *Journal of Biogeography*. 42(4):652–661.

Pérez-Ramos, I.M., Verdú, J.R., Numa, C., Marañón, T. & Lobo, J.M. 2013. The comparative effectiveness of rodents and dung beetles as local seed dispersers in Mediterranean oak forests. *PLoS One*. 8(10):e77197.

Perrin, M. & Rathbun, G. 2013. Elephantulus edwardii. Cape sengi. In *Mammals in Africa, vol. 3*. J. Kingdon, Ed. London: Bloomsbury Publishing. 265–266.

Peterson, B.J. & Fry, B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*. 18(1):293–320.

Pfeiffer, M., Huttenlocher, H. & Ayasse, M. 2010. Myrmecochorous plants use chemical mimicry to cheat seed-dispersing ants. *Functional Ecology*. 24(3):545–555.

Phillips, E. 1941. *Euphorbiaceae. Flowering plants of South Africa. Vol XXI.* Van Schaik Ltd. Pretoria.

Phillips, S.J., Anderson, R.P. & Schapire, R.E. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling*. 190(3–4):231–259.

Picker, M., Griffiths, C. & Weaving, A. 2004. *A field guide to the insects of South Africa.* Cape Town: Struik.

Porter, S.S. 2013. Adaptive divergence in seed color camouflage in contrasting soil environments. *New Phytologist*. 197(4):1311–1320. DOI: 10.1111/nph.12110.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O. & Kunin, W.E. 2010. Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*. 25(6):345–353. DOI: https://doi.org/10.1016/j.tree.2010.01.007.

Pyke, G.H. 1984. Optimal foraging theory: a critical review. *Annual Review of Ecology and Systematics*. 15(1):523–575.

R Core Team. 2018a. R: A language and environment for statistical computing.

R Core Team. 2018b. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. https://www.R-project.org/.

Radloff, F.G.T., Mucina, L., Bond, W.J. & Le Roux, P.J. 2010. Strontium isotope analyses of large herbivore habitat use in the Cape Fynbos region of South Africa. *Oecologia*. 164(2):567–578.

Radloff, F.G.T., Mucina, L. & Snyman, D. 2014. The impact of native large herbivores and fire on the vegetation dynamics in the Cape renosterveld shrublands of South Africa: insights from a six-yr field experiment. *Applied Vegetation Science*. 17(3):456–469.

Rebelo, A.G. 2001. *A field guide to the proteas of southern Africa*. Fernwood, Vlaeberg, South Africa.

Rice, B. & Westoby, M. 1986. Evidence against the hypothesis that ant-dispersed seeds reach nutrient-enriched microsites. *Ecology*. 67(5):1270–1274. DOI: https://doi.org/10.2307/1938682.

Ridgeway, G. 2017. gbm: Generalized Boosted Regression Models. R package version 2.1.3. https://CRAN.R-project.org/package=gbm.

Roth, J.K. & Vander Wall, S.B. 2005. Primary and secondary seed dispersal of bush chinquapin (Fagaceae) by scatterhoarding rodents. *Ecology*. 86(9):2428–2439.

Rouget, M., Barnett, M., Cowling, R.M., Cumming, T., Daniels, F., Hoffman, M.T., Knight, A., Manuel, J., et al. 2014. Conserving the Cape Floristic Region. In *Fynbos: Ecology, Evolution, and Conservation of a Megadiverse Region*. N. Allsopp, J. Colville, & G. Verboom, Eds. Oxford University Press. 321–336. DOI: 10.1093/acprof.

Rovero, F., Zimmermann, F., Berzi, D. & Meek, P. 2013. "Which camera trap type and how many do I need?" A review of camera features and study designs for a range of wildlife research applications. *Hystrix*. 24(2).

Rowcliffe, J.M., Carbone, C., Jansen, P.A., Kays, R. & Kranstauber, B. 2011. Quantifying the sensitivity of camera traps: an adapted distance sampling approach. *Methods in Ecology and Evolution*. 2(5):464–476.

Royal Botanic Gardens Kew. 2018. *Seed Information Database (SID). Version 7.1. Available from http://data.kew.org/sid/*.

Rusch, U.D., Midgley, J.J. & Anderson, B. 2013a. Rodent consumption and caching behaviour selects for specific seed traits. *South African Journal of Botany*. 84:83–87. DOI: 10.1016/j.sajb.2012.09.007.

Rusch, U.D., Midgley, J.J. & Anderson, B. 2013b. Competing seed consumers drive the evolution of scatter-hoarding: Why rodents do not put all their seeds in one larder. *African Zoology*. 48(1):152–158. DOI: 10.3377/004.048.0115.

Rusch, U.D., Midgley, J.J. & Anderson, B. 2014. Seasonal fluctuations in rodent seed caching and consumption behaviour in fynbos shrublands: Implications for fire management. *South African Journal of Botany*. 93:217–221. DOI: 10.1016/j.sajb.2014.04.011.

SANBI. 2017. *Statistics: Red List of South African Plants version 2017.1*.

Saracino, A., Pacella, R., Leone, V. & Borghetti, M. 1997. Seed dispersal and changing seed characteristics in a Pinus halepensis mill. forest after fire. *Plant Ecology*. 130(1):13–19. DOI: 10.1023/A:1009765129920.

Saracino, A., D'Alessandro, C.M. & Borghetti, M. 2004. Seed colour and post-fire bird predation in a Mediterranean pine forest. *Acta Oecologica*. 26(3):191–196. DOI: 10.1016/j.actao.2004.05.002.

Sauquet, H., Weston, P.H., Barker, N.P., Anderson, C.L., Cantrill, D.J. & Savolainen, V. 2009. Using fossils and molecular data to reveal the origins of the Cape proteas (subfamily Proteoideae). *Molecular Phylogenetics and Evolution*. 51(1):31–43. DOI: 10.1016/j.ympev.2008.12.013.

Schaefer, H.M. & Ruxton, G.D. 2009. Deception in plants: mimicry or perceptual exploitation? *Trends in Ecology & Evolution*. 24(12):676–685.

Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*. 9(7):671–675.

Scholtz, C.H. & Howden, H.F. 1987. A revision of the southern African genus Epirinus Reiche (Coleoptera: Scarabaeidae: Scarabaeinae). *Journal of the Entomological Society of Southern Africa*. 50:121–154.

Schurr, F.M., Bond, W.J., Midgley, G.F. & Higgins, S.I. 2005. A mechanistic model for secondary seed dispersal by wind and its experimental validation. *Journal of Ecology*. 93(5):1017–1028. DOI: 10.1111/j.1365-2745.2005.01018.x.

Seufert, V., Linden, B. & Fischer, F. 2010. Revealing secondary seed removers: results from camera trapping. *African Journal of Ecology*. 48(4):914–922.

Shiels, A.B. & Drake, D.R. 2011. Are introduced rats (Rattus rattus) both seed predators and dispersers in Hawaii? *Biological Invasions*. 13:883–894. DOI: https://doi.org/10.1007/s10530-010-9868-7.

Skinner, J.D. & Chimimba, C.T. 2005. *The mammals of the southern African sub-region*. Cambridge University Press.

Smith, C.A. 1955. Early 19th century records of the Clanwilliam cedar (Widdringtonia juniperoides Endl.). *Journal of the South African Forestry Association*. 25(1):58–65.

Stapanian, M.A. & Smith, C.C. 1978. A model for seed scatterhoarding: coevolution of fox squirrels and black walnuts. *Ecology*. 59(5):884–896.

Stavert, J.R., Drayton, B.A., Beggs, J.R. & Gaskett, A.C. 2014. The volatile organic compounds of introduced and native dung and carrion and their role in dung beetle foraging behaviour. *Ecological entomology*. 39(5):556–565.

Stavert, J.R., Gaskett, A.C., Scott, D.J. & Beggs, J.R. 2014. Dung beetles in an avian-dominated island ecosystem: feeding and trophic ecology. *Oecologia*. 176(1):259–271.

Steele, M.A., Knowles, T., Bridle, K. & Simms, E.L. 1993. Tannins and partial consumption of acorns: implications for dispersal of oaks by seed predators. *The American Midland Naturalist*. 130(2):229–238. DOI: https://doi.org/10.2307/2426123.

Steele, M.A., Contreras, T.A., Hadj-Chikh, L.Z., Agosta, S.J., Smallwood, P.D. & Tomlinson, C.N. 2013. Do scatter hoarders trade off increased predation risks for lower rates of cache pilferage? *Behavioral Ecology*. 25(1):206–215. DOI: 10.1093/beheco/art107.

Steenhuisen, S. & Johnson, S.D. 2012. The influence of pollinators and seed predation on seed production in dwarf grassland Protea “sugarbushes” (Proteaceae). *South African Journal of Botany*. 79:77–83. DOI: 10.1016/j.sajb.2011.12.004.

Steenhuisen, S., Balmer, A., Zoeller, K.C., Kuhn, N., Midgley, J.J., Hansen, D. & Johnson, S.D. 2015. Carnivorous mammals feed on nectar of Protea species (Proteaceae) in South Africa and likely contribute to their pollination. *African Journal of Ecology*. 53(4):602–605.

Steiner, K.E. & Johnson, S.D. 2003. Specialized pollination systems in southern Africa. *South African Journal of Science*. 99(7):345–348.

Struempf, H.M., Schondube, J.E., Martinez, C. & Rio, D.E.L. 1999. The cyanogenic glycoside amygdalin does not deter consumption of ripe fruit by cedar waxwings. *The Auk*. 116(3):749–758.

Swets, D., Reed, B.C., Rowland, J. & Marko, S.E. 1999. A weighted least-squares approach to temporal NDVI smoothing. In *From image to information: 1999 ASPRS Annual Conference*.

Taylor, P.J. 2013. Otomys irroratus. Southern African vlei rat. In *Mammals in Africa, vol. 3*. J. Kingdon, Ed. London: Bloomsbury Publishing. 583–585.

von Teichman, I. & van Wyk, A.E. 1996. Taxonomic significance of pericarp and seed structure in Heeria argentea (Thunb.) Meisn. (Anacardiaceae), including reference to pachychalazy and recalcitrance. *Botanical Journal of the Linnean Society*. 122:335–352. DOI: https://doi.org/10.1006/bojl.1996.0066.

Tercero-Bucardo, N. & Kitzberger, T. 2004. Establishment and life history characteristics of the southern South American mistletoe Misodendrum punctulatum (Misodendraceae). *Revista Chilena de Historia Natural*. 77:509–521. DOI: https://doi.org/10.4067/S0716-078X2004000300010.

Tewksbury, J.J. & Nabhan, G.P. 2001. Directed deterrence by capsaicin in chillies. *Nature*. 412:403.

Theimer, T.C. 2005. Rodent scatterhoarders as conditional mutualists. In *Seed fate: predation, dispersal and seedling establishment*. P.-M. Forget, J.E. Lambert, P.E. Hulme, & S.B. Vander Wall, Eds. CABI Publishing. 283–295. DOI: 10.1079/9780851998060.0283.

Thuiller, W., Lavorel, S., Midgley, G.F., Lavergne, S. & Rebelo, A.G. 2004. Relating plant traits and species distributions along bioclimatic gradients for 88 Leucadendron taxa. *Ecology*. 85(6):1688–1699.

Tibbets, T.M., Wheeless, L.A. & Del Rio, C.M. 2008. Isotopic enrichment without change in diet: an ontogenetic shift in δ15N during insect metamorphosis. *Functional Ecology*. 22(1):109–113.

Tobler, M.W., Carrillo-Percastegui, S.E., Pitman, R.L., Mares, R. & Powell, G. 2008. An evaluation of camera traps for inventorying large-and medium-sized terrestrial rainforest mammals. *Animal Conservation*. 11(3):169–178.

Tonnabel, J., Mignot, A., Douzery, E.J.P., Rebelo, A.G., Schurr, F.M., Midgley, J.J., Illing, N., Justy, F., et al. 2014. Convergent and correlated evolution of major life-history traits in the Angiosperm genus Leucadendron (Proteaceae). *Evolution*. 68(10):2775–2792.

Tonnabel, J., Schurr, F.M., Boucher, F., Thuiller, W., Renaud, J., Douzery, E.J.P. & Ronce, O. 2018. Life-history traits evolved jointly with climatic niche and disturbance regime in the genus Leucadendron (Proteaceae). *The American Naturalist*. 191(2):220–234.

Turner, K.M. & Frederickson, M.E. 2013. Signals can trump rewards in attracting seed-dispersing ants. *PloS one*. 8(8):e71871.

Turner, R.C., Midgley, J.J. & Johnson, S.D. 2011. Evidence for rodent pollination in Erica hanekomii (Ericaceae). (1999):163–170.

Tutt, J.W. 1891. *Melanism and melanochroism in British Lepidoptera*. S. Sonnenschein and Company.

Valiente-Banuet, A., Aizen, M.A., Alcántara, J.M., Arroyo, J., Cocucci, A., Galetti, M., García, M.B., García, D., et al. 2015. Beyond species loss: the extinction of ecological interactions in a changing world. *Functional Ecology*. 29(3):299–307. DOI: 10.1111/1365-2435.12356.

de Vega, C., Arista, M., Ortiz, P.L., Herrera, C.M. & Talavera, S. 2011. Endozoochory by beetles: a novel seed dispersal mechanism. *Annals of Botany*. 107(4):629–637.

Verboom, G.A., Linder, H.P., Forest, F., Hoffmann, V., Bergh, N.G. & Cowling, R.M. 2014. Cenozoic assembly of the Greater Cape flora. In *Fynbos: ecology, evolution and conservation of a megadiverse region.* N. Allsopp, J.F. Colville, & G.A. Verboom, Eds. Oxford University Press, Oxford. 93–118.

Vignieri, S.N., Larson, J.G. & Hoekstra, H.E. 2010. The selective advantage of crypsis in mice. *Evolution*. 64(7):2153–2158. DOI: 10.1111/j.1558-5646.2010.00976.x.

Violle, C., Reich, P.B., Pacala, S.W., Enquist, B.J. & Kattge, J. 2014. The emergence and promise of functional biogeography. *Proceedings of the National Academy of Sciences*. 111(38):13690–13696.

Vorobyev, M. & Osorio, D. 1998. Receptor noise as a determinant of colour thresholds. *Proceedings of the Royal Society of London Series B*. 265(1394):351–358. DOI: 10.1098/rspb.1998.0302.

Vander Wall, S.B. 1990. *Food hoarding in animals*. Chicago: University of Chicago Press.

Vander Wall, S.B. 1993. Seed water content and the vulnerability of buried seeds to foraging rodents. *American Midland Naturalist*. 272–281.

Vander Wall, S.B. 1995a. The effects of seed value on the caching behavior of yellow pine chipmunks. *Oikos*. 533–537.

Vander Wall, S.B. 1995b. The influence of environmental conditions on cache recovery and cache pilferage by yellow pine chipmunks (Tamias amoenus) and deer mice (Peromyscus maniculatus). *Behavioral Ecology*. 11(5):544–549.

Vander Wall, S.B. 1998. Foraging success of granivorous rodents: effects of variation in seed and soil water on olfaction. *Ecology*. 79(1):233–241.

Vander Wall, S.B. 2003. Effects of seed size of wind-dispersed pines (Pinus) on secondary seed dispersal and the caching behavior of rodents. *Oikos*. 100(1):25–

34.

Vander Wall, S.B. & Beck, M.J. 2012. A comparisons of frugivory and scatter-hoarding seed-dispersal syndromes. *Botanical Review*. 78(1):10–31. DOI: 10.1007/s12229-011-9093-9.

Vander Wall, S.B. & Jenkins, S.H. 2003. Reciprocal pilferage and the evolution of food-hoarding behavior. *Behavioral Ecology*. 14(5):656–667. DOI: 10.1093/beheco/arg064.

Vander Wall, S.B. & Longland, W.S. 2004. Diplochory: are two seed dispersers better than one? *Trends in Ecology & Evolution*. 19(3):155–61. DOI: 10.1016/j.tree.2003.12.004.

Vander Wall, S.B., Dimitri, L.A., Longland, W.S. & White, J.D.M. 2018. Seed value influences cache pilfering rates by desert rodents. *Integrative Zoology*.

Wang, B. & Chen, J. 2009. Seed size, more than nutrient or tannin content, affects seed caching behavior of a common genus of Old World rodents. *Ecology*. 90(11):3023–3032.

Wang, B.C. & Smith, T.B. 2002. Closing the seed dispersal loop. *Trends in Ecology and Evolution*. 17(8):379–385.

Warzecha, B. & Parker, V.T. 2014. Differential post-dispersal seed predation drives chaparral seed bank dynamics. *Plant Ecology*. 215(11):1313–1322.

Watt, J.M. & Breyer-Brandwijk, M.. 1962. *The medicinal and poisonous plants of Southern and Eastern Africa*. 2nd ed. E. &S. Livingstone, Ltd, London.

Weighill, B., Huysamer, A. & Anderson, B. 2017. The nightshift: Seed dispersal and consumption differences by rodents before and after dark. *South African Journal of Botany*. 108:267–271. DOI: 10.1016/j.sajb.2016.11.005.

Wenny, D.G. 2001. Advantages of seed dispersal: A re-evaluation of directed dispersal. *Evolutionary Ecology Research*. 3:51–74.

Wester, P., Karvang, A.S.N. & Niedzwetzki, T. 2018. Loss of tail sheath (false autotomy) in South African Cape spiny mice (Acomys subspinosus). *African Journal of Ecology*. 56(3):656–658. DOI: 10.1111/aje.12521.

White, J.D.M. & Midgley, J.J. 2017. Dispersal of semi-fleshy fruits to rock crevices by a rock-restricted rodent. *South African Journal of Science*. 113(11):1–5.

White, J.D.M., Jack, S.L., Hoffman, M.T., Puttick, J., Bonora, D., Visser, V. & February, E.C. 2016. Collapse of an iconic conifer: Long-term changes in the demography of

Widdringtonia cedarbergensis using repeat photography. *BMC Ecology*. 16(1):1–11. DOI: 10.1186/s12898-016-0108-6.

White, J.D.M., Bronner, G.N. & Midgley, J.J. 2017. Camera-trapping and seed-labelling reveals widespread granivory and scatter-hoarding of nuts by rodents in the Fynbos Biome. *African Zoology*. 52(1):31–41. DOI: https://doi.org/10.1080/15627020.2017.1292861.

Wiens, D. & Rourke, J.P. 1978. Rodent pollination in southern African Protea spp. *Nature*. 276(2):71–73. DOI: 10.1038/276071a0.

van Wilgen, B.W. & Viviers, M. 1985. The effect of season of fire on serotinous Proteaceae in the Western Cape and the implications for fynbos management. *South African Forestry Journal*. 133(1):49–53.

Williams, I.J.M. 1972. *A revision of the genus Leucadendron (Proteaceae)*. Cape Town: Bolus Herbarium, University of Cape Town.

Wilson, A.M., Latimer, A.M., Silander, J.A., Gelfand, A.E. & De Klerk, H. 2010. A hierarchical Bayesian model of wildfire in a Mediterranean biodiversity hotspot: implications of weather variability and global circulation. *Ecological Modelling*. 221(1):106–112.

Wilson, A.M., Latimer, A.M. & Silander, J.A. 2015. Climatic controls on ecosystem resilience: Postfire regeneration in the Cape Floristic Region of South Africa. *Proceedings of the National Academy of Sciences*. 112(29):9058–9063.

van Wyk, B.-E. & van Wyk, P. 1997. *Field guide to trees of southern Africa*. Cape Town: C. Struik.

van Wyk, B.-E., van Heerden, F. & van Oudtshoorn, B. 2002. *Poisonous plants of South Africa*. Brizan Publications, Pretoria.

Xiao, Z., Zhang, Z. & Wang, Y. 2005. Effects of seed size on dispersal distance in five rodent-dispersed fagaceous species. *Acta Oecologica*. 28(3):221–229.

Yang, X., Baskin, C.C., Baskin, J.M., Liu, G. & Huang, Z. 2012. Seed mucilage improves seedling emergence of a sand desert shrub. *PLoS ONE*. 7(4):e34597. DOI: 10.1371/journal.pone.0034597.

Zoeller, K.C., Steenhuisen, S., Johnson, S.D. & Midgley, J.J. 2016. New evidence for mammal pollination of Protea species (Proteaceae) based on remote-camera analysis. *Australian Journal of Botany*. 64(1):1–7. DOI: 10.1071/BT15111.