Rodents and Restios:

Rodents and the fates of *Willdenowia incurvata* (Restionaceae) seeds
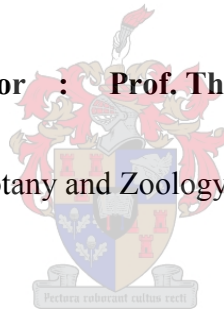
by
Benjamin Weighill

Thesis presented in fulfilment of the requirements for the degree Masters of Science in

Zoology at Stellenbosch University

Supervisor   :   Prof. Bruce Anderson

Co- supervisor   :   Prof. Theresa Wossler

Faculty of Science, Department of Botany and Zoology

Stellenbosch Univeristy

Private Bag X1

Matieland

7600

March 2017

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This study forms part of a larger study on rodent assisted seed dispersal in the fynbos which was initiated by Professor Bruce Anderson. The seed tagging method has been used previously by Bruce Anderson and his former student Ursina Rusch. The rest of the study was designed and executed by myself unless otherwise stated. The chapters in this thesis have been prepared for publication, therefore some duplication is unavoidable. Chapter 2: entitled "The nightshift: Seed dispersal and consumption differences by rodents before and after dark" has been submitted and accepted for publishing in the South African Journal of Botany and is included in this thesis with only minor edits.

Benjamin Weighill

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Abstract

The biodiversity hotspot of the fynbos offers a "natural laboratory" to study species diversification, particularly in flowering plants. Often it is the interactions with the surrounding biotic or abiotic environments that drive this diversification. Interactions between plants and seed dispersers are just one of these interactions.

In Chapter 1 of this thesis, recent studies pertaining to rodent assisted seed dispersal as well as other biotic seed dispersers in the fynbos were reviewed. This review emphasized the need for more studies of biotic seed dispersal in the fynbos despite the recent discovery of the first dung beetle assisted seed dispersal in the fynbos and the first investigations into seed dispersal by the Cape spiny mouse (*Acomys subspinosus*) and the hairy-footed gerbil (*Gerbilliscus paeba)*. In Chapter 2, the seed dispersing and consumption behaviour of the hairy-footed gerbil (*Gerbilliscus paeba*) and the four-striped mouse (*Rhabdomys pumilio*) was investigated. It was confirmed that *G. paeba* dispersed seeds of *Willdenowia incurvata* (Restionaceae) at night and only consumed a small percentage of seeds *in situ.* In contrast, *R. pumilio,* which was only active during the day, only consumed and never dispersed seeds.

In Chapter 3, the final data chapter, the effect of added elaiosomes from the closely related *Willdenowia glomerata* on rodents' reactions to *W. incurvata* seeds was investigated. It was found that the added elaiosomes deterred both *G. paeba* and *R. pumilio* from interacting with seeds of *W. incurvata*. Both rodent species preferred seeds without elaiosomes attached. *Gerbilliscus paeba* consumed and dispersed more seeds without elaiosomes attached than seeds that had elaiosomes attached when given a choice between seeds the two seed treatments. *Rhabdomys pumilio,* which does not disperse seeds, also consumed more seeds without elaiosomes attached than seeds with elaiosomes attached.

In the fynbos, one of the prevailing views regarding myrmecochory (seed dispersal by ants) is that it evolved as a response to heavy seed predation by rodents as seeds are rapidly removed from the soil surface by ants. However, the findings of this thesis suggest that not all rodents are purely seed predators and that some species may play an important role in seed dispersal. Consequently, it is more likely that myrmecochory evolved primarily to protect seeds from fire, through seed burial. Seed dispersal by rodents also involves burial and so it is likely that some rodents may perform a similar role to ants in protecting seeds from fire.

From a biodiversity perspective, the further study and identification of new interactions of this kind are key in understanding the processes that shaped the diversity seen in the fynbos today. In addition, such interactions are critical for maintaining ecosystem function. Protection of this biodiversity hotspot becomes extremely challenging when species interactions are unidentified or unstudied, emphasizing the importance of this study for conservation efforts. From an evolutionary ecology perspective, how scatter-hoarding in rodents evolved, as well as the evolution of plant strategies that enhance dispersal by rodents are highly contested. Evidence from fynbos studies could provide further insights for drawing conclusions regarding the evolution of this plant-animal interaction.

Opsomming

Die fynbos is 'n bioom ryk in biodiversiteit en bied 'n "natuurlike laboratorium" voor waarin die diversifikasie van spesies beter bestudeer kan word, veral in blomplante of angiosperme. Een van die drywers van hierdie diversifisering is die interaksie tussen plante en hulle omgewing asook ander organismes. Een van hierdie interaksies kom voor tussen plante en saad verspreiders en een interessante saad verspreider is knaagdiere.

In Hoofstuk 1 van hierdie tesis word onlangse studies van knaagdiere as saad verspreiders so wel as ander biotiese saad verspreiders in die fynbos opgesom. Alhoewel saad verpreiding deur miskruiers onlangs ontdek is en die eertse ondersokeing van saad versreiding deur twee knaagdiere, *Acomys subspinosus* ("Cape spiny mouse") en *Gerbilliscus paeba* ("hairy-footed gerbil"), gedoen is, bly daar 'n tekort aan studies van biotiese saad verspreiders in die fynbos. In Hoofstuk 2 word saad verspreiding deur *Gerbilliscus paeba* (Muridae) deuglik ondersoek. Daar word bevestig dat *G. Paeba,* wat slegs nagtelik aktief is, gereeld sade van *Willdenowia incurvata* (Restionaceae) versprei en vreet ook 'n paar daarvan. *Rhabdomys pumilio* ("four-striped mouse"), wat slegs in die dag aktief is, vreet slegs sade en het geen sade versprei nie.

In Hoofstuk 3, die finale data hoofstuk, word die effek van 'n bygevoegde lipiede-ryk aanhangsel of "elaiosome" (wat gewoonlik miere lok) aan knaagdiere se reaksie tot sade ondersoek.. Hier, is dit gevind dat *G. paeba* sade sonder elaiosomes verkies bo die wat elaiosomes bygevoeg het deur om meer gereeld sade sonder elaiosomes te versprei en te vreet. *Rhabdomys pumilio* verkies ook sade sonder elaiosomes en vreet meer van hierdie sade as die wat elaiosomes bygevoeg het.

Daar word gereken dat saad verspreiding deur miere as 'n reaksie tot saad predasie deur knaagdiere ontwikkel het. Sodoende word sade vinnig van die grond verwyder, weg van knaagdiere wat daaraan so vreet. Hierdie tesis wys egter dat nie alle knaagdiere saad vreters

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is nie en dat sommige knaagdiere ook saad verspreiders is. Saam hiermee word daar ook voorgestel dat die elaiosome self knaagdiere so ontmoedig om die sade te versprei of te vreet.

Van 'n biodiversiteit oogpunt, is die identifikasie en beskrywing van hierdie interaksies krities om die evolusie van die fynbos se diversiteit beter te kan verstaan. In byvoeging, is die funksionering van ekostelsels afhanklik van die interaksies binne die stelsel. Sonder om hierdie interaksies te identifiseer en te verstaan is die bewaring van die fynbos byna ontmoontlik. Laastens hoe die evolusie van knaagdiere se neiging om sade ondergronds te stoor as ook hoe plante hierdie diere se gedrag bemoedig (en sodoende saad verspreiding aan te moedig) begin het, is debatteerbaar. Studies soos hierdie uit die fynbos, kan moontlik bydrae om wêreldwye gevolgtrekkings te maak rakend die evolusie van hierdie plant-dier interaksie.

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Chapter 1: Introduction

Principles of seed dispersal

Plants are components of living, interactive ecosystems. The maintenance of interactions is important for the preservation of ecosystem function and community structures (Bond and Slingsby 1984, Holbrook and Schmitt 2004, Rudgers and Gardener 2004). One such interaction is that of a plant with its seed disperser. The interaction of a plant with its seed disperser determines where (and often if) the otherwise immobile seed will establish itself to form part of the population and plant community (Howe and Smallwood 1982, Forget 1992, Theimer 2001, Hirsch et al. 2012). One of the major threats to plant species persistence is thought to be the collapse of interactions between plants and their seed dispersers (Bond and Slingsby 1984, Bond 1994, Midgley and Illing 2009). From an evolutionary perspective, the loss of dispersers can alter selection on seed traits and these may consequently change after disperser loss (e.g. Galetti et al. 2013)**.** Disperser loss could also potentially lead to reduced gene flow (e.g. Carvalho et al. 2016) and perhaps even plant extinction (Bond 1994, Cordeiro and Howe 2003). Disperser-mediated extinction may be most likely for plant species with strong metapopulation dynamics where local extinctions are constantly tempered by dispersal and recolonization (Hobbs and Yates 2003, Verheyen et al. 2003, Standish et al. 2007). In addition, disperser-mediated extinction may also be likely in situations where dispersers move seeds to "safe sites." For example, in fire-prone habitats, the burial of seeds by dispersers helps plants to survive fires and the adult plants are usually killed leaving only the underground seeds to germinate (Moore and Vander Wall 2015). It is therefore important that dispersal vectors are identified and investigated to understand their roles played in species persistence, ecosystem function and community composition.

Even when seeds do not form obligatory relationships with their biotic dispersers (i.e. they do not need the seed disperser in order to germinate), seeds may still experience increased survival rates and higher fitness as a result of dispersal. Howe and Smallwood (1982) formalized the benefits of dispersal to plants in terms of two main hypotheses: (i) The "escape hypothesis" allows the seed to escape away from conspecific competition to a site of lower conspecific density and away from the parent plant and (ii) the "directed dispersal hypothesis" when dispersal moves seeds to sites which are more favourable for germination and survival than would be reached by random seed movement. Both of these hypotheses are also expected to reduce density and distance related mortality of seeds and seedlings and hypotheses are unlikely to be mutually exclusive.

Numerous studies have tested the escape and directed dispersal hypotheses and demonstrated that seeds benefit from being moved away from conspecifics (Wenny 2001, Hirsch et al. 2012) and to sites more suitable for germination (Hanzawa et al. 1988, Briggs et al. 1998). For instance the Central American agouti (*Dasyprocta punctate*) moves *Astrocaryum standleyanum* palm seeds to areas of lower conspecific density, supporting the escape hypothesis. In addition to lower conspecific density, these areas have an accompanying lower population of seed predators, resulting in increased germination and seedling establishment due to the directed dispersal of seeds toward these areas that seeds would not readily reach without their disperser (Hirsch et al. 2012). When the dispersal vector provides a fitness benefit to the plant or is a necessity for seed germination and survival, plants evolve traits that enhance or encourage dispersal. This ability of a dispersal vector to shape plant traits is well documented (Vander Wall 2010, Rusch et al. 2013a, Pesendorfer et al. 2016) and can cause unrelated plant species that share a similar dispersal vector to converge upon a similar sets of morphological traits, known as a seed dispersal syndrome (Forget and Milleron 1991).

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Seed dispersal vectors are generally classified as abiotic or biotic. Abiotic vectors are non-living for example wind or water. Biotic seed dispersal vectors are living and can be further classified as either active or passive. Active biotic vectors are animals that select seeds based on a set of traits while passive biotic vectors transport seeds accidently. This accidental transport occurs either externally on the body surface (ectozoochory) or internally when consumed with other foods (endozoochory) (Chambers and MacMahon 1994). Below, the literature on biotic seed dispersal in the fynbos is reviewed.

A review of biotic seed dispersal studies in the fynbos

Marloth (1894) provided the initial descriptions of the modes of seed dispersal in South Africa, but since then more means of seed dispersal have been discovered and studied. In the Western Cape of South Africa, lies the fynbos biome, with its mega-diverse angiosperm flora. This area acts as a "natural laboratory" for the study of angiosperm diversification, despite its relatively small area (Ellis et al. 2014). As one partial explanation for the mega-diversity of the fynbos, short dispersal distances may have facilitated adaptive divergence by limiting gene flow and population connectedness (Linder 1985). In addition, active biotic seed dispersal vectors may impose selection on seed traits (eg. Rusch 2013a), potentially shaping phenotypic divergence. However, the current paucity of seed dispersal studies in the Cape does not allow us to assess the validity of these hypotheses.

The primary abiotic seed dispersal syndrome in the fynbos is serotiny where wind disperses seeds released from cones usually after fire (e.g. Heelemann et al. 2008). Serotinous seeds are stored in cones that protect them from fire and often have wing appendages to aid in wind transport. Unrelated serotinous plant species share the common trait of wing appendages and cone storage. The primary biotic seed dispersal syndrome is ant-dispersal or myrmecochory. While myrmecochory still receives the majority of focus (e.g. Bond and Slingsby 1984,

Pierce and Cowling 2014, Slingsby and Bond 1985) recently rodent, dung beetle and bird mediated seed dispersal have received some much needed attention.

Myrmecochory

"The dispersal of seeds by ants (myrmecochory) appears to be the only biotic seed dispersal mechanism of any importance in the fynbos; plants with dispersal of seeds by birds and by attachments to animals are not found in significant numbers."

- *(Le Maitre and Midgley 1992)*

Myrmecochorous seeds have adapted to ant dispersal by evolving an elaiosome, a lipid-rich appendage which attracts ants (Beattie 1985, Bond and Breytenbach 1985, Handel and Beattie 1990, Bond et al. 1991). The ants take the seed into their nests, which may have a microhabitat favourable for germination (Beattie and Cluver 1983), remove the elaiosome to feed to their brood and leave the seed intact to germinate (Pfeiffer et al. 2010). These seeds are usually released annually rather than after fire and after being taken into the nest, they lay dormant underground and only germinate after fire. Over 11 000 plant species in 70 families worldwide have seeds that possess elaiosomes (Lengyel et al. 2010), illustrating the ability of this seed dispersal vector in shaping seed morphology. Unrelated myrmecochorous plant species share the common trait of an elaiosome which suggests convergence in the attractiveness of an elaiosome to different ant species (Hughes and Westoby 1992).

The fynbos differs from other parts of the world with this unusually high prevalence of myrmecochory which occurs in approximately 20 % of plants in the Cape Floristic Region relative to the 4.5 % global average (Bond et al. 1991, Lengyel et al. 2010). Ant dispersal is likely to translate into very short seed dispersal distances with an average of 0.97 m being recorded worldwide and 1.25 m in the southern hemisphere (Gomez and Espadaler 1998) which may have important consequences for speciation (Linder 1985, Ellis et al. 2014).

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Myrmecochory in the fynbos has allowed for the testing of the "directed dispersal" and "escape hypotheses". For example, ants disperse seeds of *Leucospermum conocarpodendron* by taking them into their nests at depths where they are safe from fire, a necessity in the fire-prone ecosystem of the fynbos (Bond and Slingsby 1983, Slingsby and Bond 1985). In addition, these seeds escape aggregations of conspecifics and reach sites more open than if seeds were passively dispersed. However, despite these benefits, Bond and Stock (1989) found that these sites were poorer in terms of nutrient content. Despite this and possibly as a result of lower competition, seeds that were ant dispersed into open areas were taller than those from passively dispersed seeds nearer the parent plant. While this study was interpreted as refuting the "directed dispersal hypothesis," it is doubtful that seeds would have even survived fires if they were not buried by ants. For myrmechochory in the fynbos, directed dispersal is unlikely to work through the process of seed movement to nutrient-rich sites and more likely to work through the process of movement to fire-safe sites.

Rodent assisted seed dispersal

The potential for rodent assisted seed dispersal was only relatively recently recognized in the fynbos (Midgley et al. 2002). This seed dispersal mechanism is well established in both temperate and tropical plant communities (e.g. Forget 1992 and 1993, Vander Wall 2001, Haugaasen 2010). In these communities, examples that support both the escape and direct dispersal hypothesis have been demonstrated (e.g. Hirsch et al. 2012).

Rodent assisted seed dispersal takes place via caching –storing of food items by animals in times of plenty to retrieve when conditions are less favourable (Vander Wall 1990, Rusch et al. 2014). Plants that make use of rodent hoarders as dispersers typically produce large, nut-like seeds to which rodents are attracted, rather than seeds with elaiosomes or wing appendages (Forget 1992, Brewer 2001, Vander Wall 2001, Gálvez et al. 2009). There are many such plant species that produce nuts without elaiosomes in the fynbos; approximately

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60 in the Proteaceae and Restionaceae families alone (Rusch et al. 2013a, Midgley et al. 2002). The thick hulls of these seeds make eating a seed *in situ* energetically inefficient for the rodent when there are large amounts of food available, while the large size represents a nutritional benefit that cannot be ignored (Vander Wall 1990, Brewer 2001). There are two seed caching strategies, namely, (i) larder-hoarding and (ii) scatter-hoarding. Larder-hoarders store the majority of their resources in one or two large caches which are regularly visited and actively defended against pilferers (e.g. Gerhardt 2005). These larder-hoarders are unlikely to effectively disperse seeds and benefit the plant, as the dense seed caches suffer from high competition and the majority of the seeds are predicted to be eaten (Vander Wall 1990).

Scatter-hoarders, in contrast, place seeds in numerous caches each containing a few seeds, which are not defended rigorously (e.g. Moore et al., 2007). The spreading out of resources sufficiently reduces the chance that the cache is discovered and raided by a pilferer (Vander Wall 1990, Jenkins and Peters 1992, Jenkins et al. 1995) and remains to be consumed when the hoarder next returns. As a result caches of higher value (i.e. more or larger seeds) are usually cached farther away from the source than lower value caches so that risks of pilferage are effectively reduced (Vander Wall 2003, Jansen et al. 2004, Xiao et al. 2005, Moore et al. 2007). However, these seeds cannot be dispersed indefinite distances, as there are energetic constraints associated with moving seeds and seeds should not be moved so far as to neutralize the benefit of consuming a seed (e.g. Muñoz and Bonal 2008).

In the fynbos, scatter-hoarding behaviour by rodents was initially suggested for the Cape spiny mouse (*Acomys subspinosus*) and the hairy-footed gerbil (*Gerbilliscus paeba*). These were the dominant rodent species found in association with *Leucadendron sessile* and *Willdenowia incurvata* seeds that had been buried (Midgley and Anderson 2004). Due to the nocturnal activity of these rodents, observing seed dispersal and confirming these suspected interactions proved challenging. Motion sensitive video footage has provided a solution for

this problem and *A. subspinosus* has since been shown to bury seeds of *L. loranthifolium* and *G. paeba* to bury seeds of *L. pubescens* and *L. sessile* (White et al. *in press*). In this study, both rodents typically buried seeds in caches containing only a single seed, suggesting this form of dispersal may assist seeds from escaping density related mortality. In addition *Willdenowia incurvata* (Restionaceae) has also been confirmed as being rodent dispersed by *G. paeba* using motion sensitive camera trapping and seed labelling (*see chapter 2:* Weighill et al. 2017). The only fynbos study that investigates the potential for rodent seed dispersers to select for seed traits was conducted by Rusch et al. (2013a). It was found that seeds of *L. sessile* with a medium thickness hull were buried more often than seeds with a thinner or thicker hull in a site where *A. subspinosus* was the dominant rodent species.

Since scatter-hoarding is potentially the only hoarding strategy that results in effective dispersal for plants, plants have evolved strategies and traits that encourage scatter-hoarding as well as caching of seeds rather than *in situ* consumption. These strategies were reviewed by Vander Wall (2010) and include plants imposing handling costs on the rodent, such as thick hulls that make *in situ* consumption inefficient and masting seed release. Masting seed release is the delay in seed release and retention of seeds over several seasons to release an extra-large seed crop in one season that encourages seed caching. Keeping in mind that the rodent cacher needs to recover its hidden food resource, plants may also produce seeds with weak olfactory cues. This reduces the likelihood of other seed predators from finding the caches and it may also lower the probability of seed retrieval, leaving more seeds to germinate (Hollander et al. 2012, Yi et al. 2016). Scatter-hoarders must also select an optimum number of seeds to cache: too many and the risk of pilferage becomes too high, while too few would render cache retrieval energetically inefficient (e.g. Geluso 2005). Reducing this risk of cache pilferage remains the most important factor influencing the

scatter-hoarder's likelihood of retrieving a stored cache rather than plant-driven factors such as seed size, quality and the timing of seed drop (Perea et al. 2015).

While most rodents are either larder-hoarders or scatter-hoarders, some rodents engaging in both strategies have been recorded elsewhere, but this has yet to be found in the fynbos. Deer mice (*Peromyscus maniculatus*), for instance, store Jeffrey pine seeds (*Pinus jeffrey*) in both their nests as larders as well as in shallow caches with one or two seeds in each (Vander Wall et al. 2001).

Numerous studies have observed rodent- seed interactions in the fynbos, yet historically (until 2002, see Midgley et al. 2002) rodents were treated purely as seed predators (e.g. Bond and Breytenbach 1985; Christian and Stanton 2004, Heithaus 2015). Consequently, myrmecochory was thought to have partially evolved as a response to intense rodent predation because ant dispersed seeds are rapidly removed from the soil surface and moved underground where they are protected from rodent seed predators. However this argument loses some credibility if some rodents act as seed dispersers. Furthermore, ants may consume some seeds (Pierce and Cowling 1991) just as rodents are likely to consume a large proportion over time suggesting both seed dispersal vectors result in some seed loss. The only common fynbos rodent that has consistently been shown to act purely as a seed predator thus far is *Rhabdomys pumilio* (White et al. *in press*, Weighill et al. 2017).

Plants that make use of rodents to disperse their seeds typically release seeds *en masse.* This strategy serves to satiate other seed predators and encourage the rodent seed dispersers to cache rather than consume seeds *in situ*. Since so many seeds are available and in *situ* consumption entails a handling cost, such as chewing through a thick seed hull, it becomes more efficient for the rodent to cache seeds for later consumption. When *en masse* seed release is not sufficient, caching by rodents is further encouraged by the intermittent seed

drop by plants known as masting, where seeds are stored for several years on a plant before being released simultaneously (Silvertown 1980, Kelly and Sork 2002, Vander Wall 2002 and 2010). Subsequently seeds are highly plentiful, but only available for an unpredictable and short period of time which encourages rodents to bury the seeds into caches (Forget 1993, Vander Wall 1990). Masting itself is also yet to be recorded in the fynbos for rodent dispersed plants.

Rodents and elaiosomes

Rodents use olfaction to locate seeds on and below the soil surface. It is thought that an elaiosome bearing seed produces a stronger olfactory cue than a seed that lacks an elaiosome. An elaiosome is always associated with myrmecochory (Berg 1975, van der Pijl 1982, Beattie and Hughes 2002, Edwards et al. 2006, Pfeiffer et al. 2010) and is yet to be found on seeds that are primarily rodent dispersed in the fynbos (Midgley et al. 2002, Rusch et al. 2013b). These stronger olfactory cues are implicated in the observed increased seed predation by rodents both on (Bond and Breytenbach 1985, Heithaus 2015) and below the soil surface (Christian and Stanton 2004) for seeds bearing elaiosomes in the fynbos. Elsewhere, the effect of adding elaiosome mimicking substances (Howard and Cole 1967, Howard et al. 1968, Jennings 1976) produced similar results as it too increased rodent seed predation. The reduction of rodent predation below the surface because of ants removing the elaiosome is thought to be one factor driving the evolution of myrmecochory in the fynbos (Beattie 1985). The seeds on the soil surface, however, are still at risk of rodent predation and rely on rapid ant response and removal to minimize rodent predation. Contrary to this, Hanzawa et al. (1985) found that removal of the elaiosome of *Corydalis aurea* (Fumariaceae) seeds, a North American resident, actually increased rodent predation and suggested the elaiosome may act as a rodent repellent. It was suggested that the elaiosome may be somewhat unpalatable or

that rodents associated the elaiosome cue with sparse seed aggregations due to rapid ant removal.

 Studies showing the benefits of elaiosomes are numerous; seeds are protected from predation (Bond and Breytenbach 1985, Christian and Stanton 2004, Heithaus 2015), placed in nutrient rich nest sites (Beattie and Culver 1983, Hanzawa et al. 1988) and escape conspecific competition (Handel 1978). However the benefits of lacking an elaiosome are poorly studied.

Dung beetle assisted seed dispersal

Active seed dispersal by beetles has only been confirmed in two instances worldwide, with other examples of beetle assisted dispersal typically involving seeds being mixed with dung and accidently moved by dung beetles passively (e.g. Estrada and Coates-Estrada 1986). One case is found in Spain where beetles ingest and disperse seeds of *Cytinus hypocistis* via endozoochory (De Vega et al. 2011). The second case presents a rare case of mimicry. Rather than ingesting seeds, dung beetles are fooled into dispersing seeds of *Ceratocaryum argenteum* (Restionaceae), a fynbos endemic, which has a strong scent, shape and texture which mimics ungulate dung (Midgley et al. 2015) (Fig. 1.1). This active seed dispersal, with the entire seed resembling dung with no reward to the dung beetle is unique. Two dung beetle species, *Epirinus flagellatus* as well as *Scarabaeus spretus* have both been observed being deceived by the seeds of *C. argenteum* (Town and Midgley 2016). So strong and accurate is this mimicry that dung beetles are attracted to the seeds within hours. The seeds are large, approximately 1cm in length, have no elaiosomes and have a hard seed coat, traits that would suggest rodent dispersal. However, unlike rodent dispersed seeds and indeed other members of the Restionaceae family, these seeds are strongly scented, similar to that of herbivore faeces. Typically strong scent is not associated with rodent dispersed seeds as this would increase the likelihood of relocation by the cacher (Hollander et al. 2012) and cache pilferers (Yi et al. 2016) reducing the possibility of germination. What makes this finding even more

interesting is that mimicry in seed dispersal has only been found in myrmecochory (Pfeiffer et al. 2010). These "dummy" seeds have oil-containing cells firmly attached to the epidermis or in the enlarged exocarp cells of the seed which still attract ants and trigger dispersal, however no elaiosome is present to be removed as a food reward for the ants. In contrast, floral mimicry is well documented worldwide and in the fynbos (Johnson 2000, Ellis and Johnson 2010, Johnson and Schiestl 2016).

Should the dung beetle return to the buried seed, to lay eggs in it or eat it (as it would with a dung ball), it would find both impossible with the hard coated seeds. The return of the dung beetle to the buried seed should therefore not influence the likelihood of germination. This is unique for a dispersal mechanism that involves the caching of seeds for later visitation. As a result, seeds may not have evolved traits that reduce the likelihood of seed discovery after dispersal such as those of rodent dispersed seeds that often evolve traits such as a weak olfactory cue that reduces post storage seed discovery to enhance the likelihood of germination (e.g. Howard et al. 1968, Hollander et al. 2012). The benefit of the germination site as well as success in seedling establishment following this mode of dispersal has yet to be investigated. In addition, the fynbos is typically lacking in large herbivores and very few species besides the eland (*Taurotragus oryx*) or bontebok (*Damaliscus pyragus*) roam De Hoop Nature Reserve and not in large numbers. The fact that these seeds have evolved such a mode of deception, suggests that these herbivores once roamed the fynbos more commonly.

Due to the inability of *C. argenteum* to resprout after fire the relationship it has with dung beetles is possibly obligatory as without burial, the seeds would perish in fire. There is even a third species of dung beetle, *Cirecellium bacchus* in the area, which elsewhere seeks out elephant dung, but as there are no elephants in the De Hoop National Park, it appears to seek eland dung as well and could potentially also be fooled. There are, however, no other seeds that are known to produce an herbivore faeces mimicking scent like *C. argenteum*, suggesting that this may be the only interaction of its kind.

Fig. 1. 1 Scale photographs of (a) a *Willdenowia incurvata* seed, potentially rodent dispersed, (b) a *Leucadendron sessile* seed, cached and dispersed by *Acomys subspinosus*, (c) a *Ceratocaryum argenteum* seed which is dispersed by dung beetles by mimicking (d) herbivore dung, in this photo, of a bontebok (Photographs by B. Anderson and Midgley et al., 2015).

Bird assisted seed dispersal

The importance of birds in the dispersal of seeds and their influence on subsequent dispersal patterns has been demonstrated elsewhere in the world (e.g. Howe and Primack 1975). However, the majority of these cases are for frugivorous bird species that ingest fruits as a form of endozoochory. Subsequently seeds are dispersed in the faeces, with the gut passage sometimes benefiting germination (e.g. Figueroa and Castro 2002), or by regurgitation of the intact seeds (e.g. Howe 1977). This ingestion may occur by accident in a mode of passive dispersal, although birds may actively select for specific seed traits such as a surrounding attractive fruiting body (Lord 2004). These fruiting bodies are somewhat of a rarity in the fynbos (Johnson 1992), but more common in the neighbouring thicket and forest biomes

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(Cowling et al. 1997) as well as coastal vegetation in the Cape (Glyphis et al. 1981) were fruit eating birds are also more common. It is possible that although birds are well known seed dispersers in other environments, they do not fulfil the role of burial for protection from fire required in inland fynbos. Evolving traits for both bird dispersal and subsequent burial is highly unlikely.

This has led to a lack of research into bird assisted seed dispersal in the fynbos and only two areas have been addressed: (i) the potential bird dispersal of invasive species (ii) and the potential of water-bird seed dispersal. The clumped seedling distribution of the highly invasive *Acacia cyclops,* around indigenous bushes and trees, can be attributed to birds, as they sit in the taller, mature indigenous trees and disperse seeds in the faeces while perched (Glyphis et al. 1981). The seeds of *A. cyclops* are suspended for a lengthy period from a red funicle, a colour birds find attractive when combined with the black opening of the seed pod (Turcek 1963), which could enhance the consumption of the seeds and subsequent dispersal. In addition, gut passage through birds enhances germination of *A. cyclops* by removing the hard seed coat while still allowing seeds to survive fire (Glyphis et al. 1981). Enhanced germination post gut passage is a trait often associated with bird dispersed seeds (Traveset 1998). Middlemiss (1963) observed as many as 21 bird species feeding on *A. cyclops,* including the Cape turtle dove (*Streptopelia capicola*) and the Cape bulbul (*Pycnonotus capensis*). Another noteworthy species which feeds on *A. cyclops* is the invasive European starling (*Sturnus vulgaris*) which suggests one invasive species may be assisting in the spread of another. In addition, the primarily granivorous bully canary (*Serinus sulphuratus*) and Cape weaver (*Xanthophilus capensis*) were also observed foraging on *A. cyclops.* Biological control agents which reduce the dense aggregations of *A. cyclops,* leaving fewer perch sites and seeds available, have the potential to diminish bird dispersal of *A. cyclops* seeds (Mokotjomela et al. 2015). The ability of birds to assist in the invasion of the fynbos by the

neighbouring thicket has also been investigated. Cowling et al. (1997) found that bird assisted dispersal of fleshy fruiting thicket species could result in the replacement of fynbos with thicket or forest in the absence of fire, as seeds of thicket and forest species perish in fire unlike those of *A. cyclops.*

Water-bird assisted seed dispersal may take place by both endozoochory (seeds are ingested and dispersed in the faeces or regurgitated) and ectozoochrory (seeds are attached externally to the birds' body or feet). Although fynbos studies of this dispersal mode are few, thus far dispersal has been shown to be highly species specific, depending heavily on the bird species as well as plant species and its associated seed traits (Reynolds and Cumming 2016b). Amongst 48 plant taxa identified by their propagules found in the faecal analysis or brushings of the birds, it is noteworthy that only two species have fleshy fruits, while five ectozoochorous species have barbed propagules for attachment to the birds body (Reynolds and Cumming 2016a). This would suggest that the majority of the endozoochorous species seeds are ingested accidentally. Subsequently seeds may be regurgitated or defecated intact after which they may germinate. It is unclear whether there are any benefits from engaging in this form of dispersal and whether selection pressures are strong enough for seed traits to have evolved for this specific interaction. *Elegia capensis* (Restionaceae) is a noteworthy inclusion on the list of ingested propagules, as this is a semi-aquatic fynbos endemic which water-birds certainly do move via endozoochory. However, while survival through gut passage is high for these propagules, germination is low (Reynolds, C, *pers. comm.*). This highlights the necessity for future studies regarding the effective germination and establishment of seeds dispersed by water-birds.

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Study rationale

Rodent assisted seed dispersal is understudied in the fynbos. Previously, rodents in the fynbos were considered as seed predators only. As a result it is thought that myrmecochory (seed dispersal by ants) had evolved in the fynbos as a strategy for seeds to escape heavy rodent predation. However, this is questionable if some rodents also disperse seeds. In addition, the investigation of rodents as seed dispersal vectors may shed light on a potential driver of speciation in the mega-diverse angiosperms of the fynbos. This could increase our understanding for how and why both plant and animal traits have evolved into the morphology we see today. From a conservation perspective, understanding the roles rodents play in seed dispersal may support the efficient protection and maintenance of this highly biodiverse region. The lack of knowledge of rodent assisted seed dispersal interactions is troubling as we may unknowingly lose keystone interactions despite our conservation efforts.

Study aims

Chapter 1: Provide a review of recent findings pertaining to biotic seed dispersal in the fynbos

Chapter 2: Compare behaviour of two rodents, *G. paeba* and *R. pumilio* toward seeds of *W. incurvata.*

Chapter 3: Investigate the effect an added elaiosome has on the reactions of *G. paeba* and *R. pumilio* to seeds of *W. incurvata.*

Chapter 4: Provide areas of further study for this and other rodent assisted seed dispersal interactions in the fynbos

Study species

Willdenowia incurvata (Thunb) HP Linder (Family: Restionaceae)

Willdenowia incurvata (vernacular name: Sonkwasriet) is an endemic fynbos plant, widely distributed within the Western and Northern Cape of South Africa, and represents a typical element of the Strandveld. *Willdenowia incurvata* plants occupy well-drained soil or sandy plains without rockiness (Linder et al. 1998). The adult plant has a rush-like appearance and stands between 0.5-1.5 m tall with a basal diameter of 0.3-3 m. Rhizomes are present, but very short.
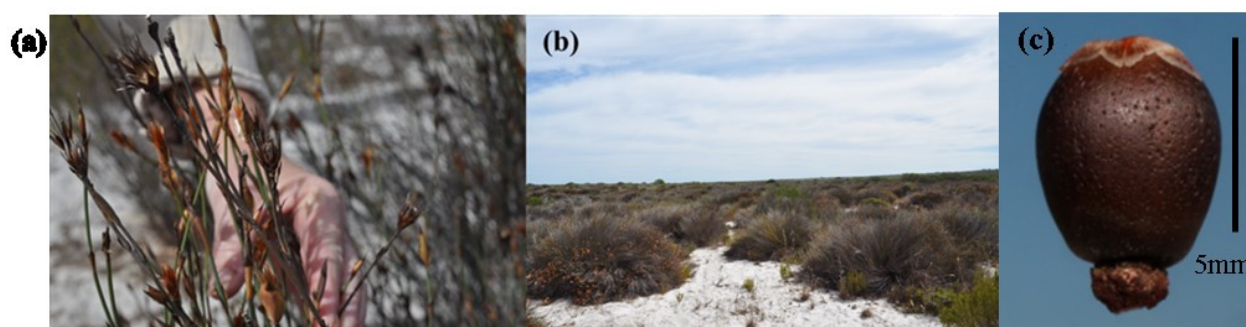
Fig. 1. 2 *Willdenowia incurvata* showing (a) reeds with distinct bracts, (b) adult plants in a sandy area with no rocks and (c) the nut-like seed with scale reference for seed size (Photographs by B Weighill and B. Anderson)

Plants are dioecious with male inflorescences forming an indefinite spikelet 20-35 mm long and 5-20 mm wide. Female inflorescences have only one, occasionally two, spikelets 10-35 mm long with a single flower and 5-10 sterile bracts. The flower is smaller than the nut making the nut surface (which is hard, woody and has a pitted wall) clearly visible. Nuts produced are 7-9 mm long, 5-6 mm in diameter, brown, elliptical in shape and possess neither an elaiosome or wing appendage (Fig. 1.2). Seeds are released *en masse* during the early summer months (October/ November).

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Large, nut-like seeds and a lack of traits adapted for another dispersal vector, suggest *W. incurvata* may be dispersed by rodents (Midgley and Anderson 2004). Post fire plants regenerate from these nut-like seeds or by coppicing from the rhizome or base. *Willdenowia incurvata* is commonly used for brooms and is heavily grazed by cattle which enjoy the female nuts and male inflorescences in particular (Linder 2013).

Willldenowia glomerata (Thunb) HP Linder (Family: Restionaceae)

Willldenowia glomerata is an endemic fynbos plant typically growing 0.2-1.5 m tall in a tufted or mat-like growth form. Seeds have pitted or smooth walls and possess elaiosomes 2-3 mm long (Fig. 1.3) (Linder et al., 1998).

Fig. 1. 3 *Willldenowia glomerata* showing (a) seeds nestled within distinct bracts, (b) adult plants in the foreground with mat-like growth form adjacent to *Willldenowia incurvata* in the background and (c) seed with distinct elaiosome with scale reference for seed size (Photographs by B. Weighill and B. Anderson)

Gerbilliscus paeba (Order: Rodentia, Family: Muridae)

Gerbilliscus paeba (Vernacular name: hairy-footed gerbil), formerly included in the genus *Gerbillurus* (see Knight et al. 2015, Monadjem et al. 2015), is a small, nocturnal rodent of the family Muridae occupying a range that includes the periphery of the southern Savannah, most of the South West Arid Zone and the South West Cape biotic zone (Davis 1974). Adults

typically weigh less than 30 g with no recorded sexual dimorphism apart from a behavioural dimorphism recorded by Stutterheim and Skinner (1973), who observed that laboratory held *G. paeba* females bury food stuffs in single larders, usually in the corner of enclosures; while males buried food stuffs in numerous places inside an enclosure.

Dorsal body colour is typically grey-brown (Fig. 1.4), but varies from reddish-orange to greyish-red between geographic regions with different colour substrates and within populations, while ventral body is white (Fig. 1.4) (Skinner and Smithers 1990).

Fig. 1. 4 *Gerbilliscus paeba* showing (a) grey brown dorsal body with an elongated head and (b) white ventral body with elongated tail (Photographs by B Weighill & A. Huysamer).

Typical *Gerbilliscus* features include an elongated head, tail and hind limbs. Tails, which are the same colour as the dorsal body parts, have a distinguishable slightly tufted tip. Hind feet are clawed and large, but diagnostically do not exceed 30 mm, while other gerbil species have hind feet exceeding 30 mm in length (Skinner and Smithers 1990). Diet has been recorded as seed dominant in the Kalahari and foliage dominant in the Karoo (Nel 1978, Kerley 1989) but overall diet also includes arthropods.

Rhabdomys pumilio (Order: Rodentia, Family: Muridae)

Rhabdomys pumilio (Vernacular name: striped mouse) is a small, diurnal murid rodent with a body mass of 40-80 g and a length of 20 cm including the tail although sizes vary with

locality (Schradin and Pillay 2003). *Rhabdomys pumilio* occupies a variety of habitats in southern Africa including grasslands, marsh, forests, semi deserts and deserts (Skinner and Smithers 1990). Body colour varies demographically from reddish brown to dark grey, however four distinct stripes from which the species derives its name, run down the back making identification easy (Skinner and Smithers 1990). Diet includes vegetation and arthropods; however seeds are the preferred diet when available. This species has been identified as an important pollinator of *Protea* species (Fleming and Nicolson 2002, Biccard and Midgley 2009).

Study site

Jakkalsfontein Private Nature Reserve is situated on the West Coast of southern Africa between the Dwars Rivers North and South, 60 km north west of Cape Town and 15 km south east of Yzerfontein. (33˚25'S 18˚15'E) and is an 1800 ha protected area previously used as coastal farmland until 2001. A diversity of vegetation types is found on the reserve including dune thicket, sandplain fynbos, Saldanha flats strandveld, Langebaan dunes strandveld, Hopefield strand fynbos, Atlantis sand fynbos, Cape seashore vegetation, Cape lowland freshwater wetlands and Cape inland salt pans (Low et al. 1998, Rebelo et al. 2006). Jakkalsfontein Private Nature Reserve represents an invaluable resource to ecologists and the public as an intact area of West Coast strandveld. The reserve experiences a typical Mediterranean climate, with rainfall (253 mm per annum) falling predominantly in winter with no snowfall or frost and the fynbos/thicket mosaic areas being fire prone, while the dune thicket area is less so.

The study was conducted on an 8 ha Atlantis and Hopefield sand fynbos section of the reserve, vegetation types classified as endangered and vulnerable respectively by Rebelo et al. (2006). This vegetation type is particularly susceptible to alien infestation by *Acacia*

saligna and *A. cyclops* while *W. incurvata,* the focal plant of this study, is one of the dominant native species of this vegetation type (Low 1990).

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Chapter 2 :The nightshift: Seed dispersal and consumption differences by rodents before and after dark

Abstract

Seed burial by dispersal vectors in fire-prone ecosystems is thought to increase seed survival rates by protecting them from fire and other seed consumers. In the fire-prone fynbos, seed burial is usually performed by ants. Historically, rodents in the fynbos were viewed purely as seed consumers; however, more recent evidence suggests that some species may also disperse seeds. This is done by scatter-hoarding the seeds in caches buried below the soil surface. Since seed dispersal and consumption by rodents usually takes place unobserved, it has been difficult to positively confirm which rodents disperse seeds and which ones only consume them. Here, the dispersal and consumptive behaviour of 2 rodents, *Rhabdomys pumilio* and *Gerbilliscus paeba*, were disentangled by using a combination of camera traps and the temporal compartmentalisation of seed fate patterns into day versus night in a depauperate, Cape strandveld rodent community. In this study, it was clearly demonstrated that the nocturnal hairy-footed gerbil (*G. paeba*) dispersed *Willdenowia incurvata* (Restionaceae) seeds. Although *G. paeba* also consumed seeds, they cached more seeds than they consumed during the period of observation. In contrast, the diurnal 4-striped mouse (*R. pumilio*) consumed but never cached seeds left out during the day. These results demonstrate that ecological roles of Cape rodents vary between species and that rodents in general cannot simply be regarded as seed consumers or dispersers as they have in the past.

Highlights

- *Gerbilliscus paeba* was only active at night and dispersed as well as consumed the seeds of *Willdenowia incurvata.*

- *Rhabdomys pumilio* was only active during the day and consumed, but did not disperse seeds.

- The combination of tagging seeds with fluorescent line and remote video evidence was shown to be an effective method of distinguishing between the relative roles of different rodent species.

Keywords: Cape strandveld; Gerbilliscus paeba; Scatter-hoarding; Seed banks; Seed caching; Rodent assisted seed dispersal; Seed predation

Introduction

Seed dispersers define the ecological context in which animal dispersed plants spend their lives (Howe and Smallwood 1982, Beattie and Culver 1983, Forget 1992, Theimer 2001, Hirsch et al. 2012b). This affects subsequent seed survival, seedling establishment, plant survival rates and consequently persistence and dynamics of populations (Vander Wall 1990, Theimer 2001, Rusch et al. 2013a). Furthermore, along with pollen transport, seed dispersal is the most important process promoting gene flow in plant populations, emphasizing its importance in shaping plant communities (van der Pijl 1982, Jordano et al. 2007, De Waal et al. 2014).

Seed dispersal vectors also have the ability to shape plant traits. The presence of elaiosomes or wings are two seed traits commonly found in plants of the Cape Floristic Region (CFR) (Le Maitre and Midgley 1992, Cowling et al. 1997). Several studies demonstrate that elaiosomes and wing appendages enhance dispersal by ants (Bond and Breytenbach 1985, Narbona et al. 2014) and wind (Matlack 1987, Cody and McC 1996, De Waal et al. 2014)

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respectively. This suggests that these dispersal vectors may have selected for the evolution of elaiosomes and wings. Seed traits of unrelated plants may converge because they share a similar dispersal vector. Such a suite of convergent traits are known as a seed dispersal syndrome (Forget et al. 1991). Elaiosomes are one of the most important traits associated with the ant dispersal syndrome, further suggesting that ants selected for the evolution of this trait (Pfeiffer et al. 2010). Similarly, many large seeded fynbos shrubs belong to the guild of serotinous plants which appear to be adapted to post-fire wind dispersal as their winged seeds are released into the wind from fire resistant cones only after fire has killed the parent plant.

In addition to ant and wind dispersal, rodent dispersal was recently recognized in the CFR (Midgley et al. 2002). While it appears as though relatively few plants in the CFR are rodent dispersed, it is important in temperate and tropical plant communities (Brewer 2001, Theimer 2001, Jansen et al. 2004a, Hirsch et al. 2012b). The rodent dispersal syndrome is generally associated with large nut-seeded plant species in the CFR which possess neither elaiosomes nor wing appendages (Midgley and Anderson 2004, Rusch 2013a). Rather these seeds possess a thick hull, are large in size and are released *en masse*. Rodents often store these seeds in times of plenty, enabling them to consume the seeds later when resources are less plentiful (Vander Wall 1990, Haugaasen et al. 2010, Rusch et al. 2014).

Several studies in the CFR suggest that rodents disperse seeds, although the evidence is all inferential and not direct (but see White et al. *in press*). Consequently, no rodent species have been unequivocally identified as dispersers and it is still unclear which rodents consume seeds without dispersing them. The best studied putative disperser in the fynbos is the Cape spiny mouse (*Acomys subspinosus*) (Muridae), which is thought to scatter-hoard *Leucadendron* (Proteaceae) seeds in very small caches, typically containing only a single seed (Midgley et al. 2002, Rusch et al. 2013a). *Acomys subspinosus* was implicated as a disperser, because it was one of the dominant rodents found in the community where the

tagged, nut-like seeds were being buried. They were also observed burying seeds in captivity (Vlok 1995, Midgley et al. 2002). Numerous nut-seeded plant species occur outside the habitats typically associated with *A. subspinosus*, and one study found that the hairy-footed gerbil (*G. paeba*) is a dominant rodent species found within the sandy habitats where the nut seeds of *Willdenowia incurvata* (Restionaceae) (*Thunb*) H.P. Linder were frequently buried (Midgley et al. 2004). The four-striped mouse (*Rhabdomys pumilio*) was recorded in both studies during the day, and the role that this rodent plays as a seed consumer or disperser is yet to be investigated. Seed dispersal by a diurnal rodent, such as *R. pumilio,* may be less likely because seed caching and retrieval during the day is probably more risky than during the night.

The primary aim of this study is to investigate the interaction between the rodent community of the strandveld and seeds of *W. incurvata* using remote camera footage and seed tracking. Preliminary, unpublished data suggested that *R. pumilio* and *G. paeba* are the dominant rodent species recorded during the day and night respectively and this study aims to test this observation. Considering the higher daytime predation risks, it is predicted that only *G. paeba* will be found adopting seed caching behaviour and consequently seeds will only be dispersed at night. In contrast it is predicted that *R. pumilio* will only consume seeds and that seeds left out during the day will not be buried. Secondarily this study aims to provide an ecological description of strandveld rodent behaviour including the burial depth of cached seeds, the distance seeds are moved and how many seeds are cached together. Furthermore, if seed dispersal occurs at night, it is hypothesized that *W. incurvata* may have evolved a nocturnal seed release strategy to minimize the exposure of seeds to diurnal seed predators.

In addition, scatter-hoarders have been found to cache higher value resources (i.e. more or larger seeds) further away from the food source so that risks of pilferage are reduced (Vander Wall 2003, Xiao et al. 2004, Moore et al. 2007). The costs of caching a seed should not

outweigh the nutritional benefit from the seed meaning seeds can also not be cached too far away from the source (e.g. Muñoz and Bonal, 2008). Should *G. paeba* indeed scatter-hoard seeds of *W. incurvata,* locating seed caches will answer whether *G. paeba* also adopts this cache defence strategy of caching higher value resources further away from the source.

Materials and methods

Study location and system

Jakkalsfontein Private Nature Reserve is situated on the West Coast of Southern Africa between the Dwars Rivers North and South, 60 km north west of Cape Town and 15 km south east of Yzerfontein (33°25'S 18°15'E). The reserve is an 1800 ha protected area previously used as coastal farmland until 2001. A diversity of vegetation types have been classified on the reserve and these include dune thicket and sandplain fynbos as well as Saldanha flats strandveld, Langebaan dunes strandveld, Hopefield strand fynbos, Atlantis sand fynbos, Cape seashore vegetation, Cape lowland freshwater wetlands and Cape inland salt pans (Low et al. 1998; Rebelo et al. 2006). The reserve experiences a typical Mediterranean climate, with rainfall (253 mm per annum) falling predominantly in winter.

The study was conducted on an Atlantis and Hopefield sand fynbos section of the reserve. These fire prone vegetation types are both classified as endangered and vulnerable respectively by Mucina and Rutherford (2006). *Willdenowia incurvata,* the focal plant of this study, is one of the dominant native species of these vegetation types (Low et al. 1998).

Study species

Willdenowia incurvata (Restionaceae) (vernacular name: Sonkwasriet) is an endemic CFR plant, widely distributed within the Western and Northern Cape of South Africa, and represents a typical element of the strandveld. Female plants produce a nut which has a hard surface and a pitted wall. These seeds are 7-9 mm long, 5-6 mm in diameter, and brown in colour, elliptical shape and possess neither an elaiosome nor wing appendage. The seeds also have a thick, hard hull and are produced in large quantities during the early summer months (October/ November) (Linder et al. 1998). These traits suggest *W. incurvata* may be dispersed by rodents (Midgley and Anderson 2004). *Gerbilliscus paeba* (Vernacular name: hairy-footed gerbil) (Muridae) is a small, nocturnal rodent whose range includes the South West Cape biotic zone (Davis 1974). Stutterheim and Skinner (1973) observed that laboratory-held *G. paeba* females bury food stuffs into single larders, usually in the corners of enclosures, while males buried food stuffs in numerous places inside an enclosure. Diet has been recorded as seed dominant in the Kalahari and foliage dominant in the Karoo, but overall diet also includes arthropods (Nel 1978, Kerley 1989). *Rhabdomys pumilio* (Muridae) is a small, diurnal murid rodent (Schradin and Pillay 2004). *Rhabdomys pumilio* occupies a variety of habitats in southern Africa including grasslands, marsh, forests, semi deserts and deserts. Diet includes vegetation and arthropods, however seeds are the preferred diet when available (Smithers 1986).

Study site

A stand of *W. incurvata* inside Jakkalsfontein Private Nature Reserve was chosen as the study site. This area was approximately 8 ha and dominated by mature *W. incurvata* bushes with clear sandy areas between them. This site was selected as it was not invaded and *W. incurvata* was common, but not dense enough to make seed tracking difficult. Inside this site, four

transects were laid down in straight lines approximately 200 m in length and spaced approximately 100m apart.

Determining the rodent community

To determine whether different rodents were active during different times of the day, 34 Sherman traps were placed into the field along four different transects. Ten traps were placed along the first transect, due to its slightly longer length, while the other three transects each had eight traps. Traps were spaced 20m apart and placed under *W. incurvata* bushes where rodent tracks were evident. Trapping was conducted for two sessions of seven consecutive days and seven consecutive nights during April/ May 2015. Trapping was conducted under ethical approval by the Research and Ethics Committee (REC): Animal Care and Use of Stellenbosch University, protocol number SU-ACUD15-00021 and with permission by Cape Nature, permit number 0056-AAA008-00056. Nocturnal trapping commenced when traps were set at sunset and ended at sunrise when traps where checked for a final time. Diurnal trapping commenced at sunrise and ended at sunset when traps were checked for a final time. Traps were baited with peanut butter and a trail of oats leading into the traps which contained a slice of apple inside for moisture to hydrate trapped rodents. Traps were checked every 3-4 h and occupants of traps were recorded to species level along with time of capture. The individual was released immediately and the trap was left shut until the next session commenced. Captures may not be independent as the same rodent could have been captured more than once. The differences between nocturnal and diurnal captures were analysed using a Pearson's chi-squared test in SPSS statistics (version 23).

Nocturnal versus diurnal seed fates

To determine whether the fate of seeds differed between night and day, two depots of ten tagged *W. incurvata* seeds were laid out inside each of the four transects spaced 40m apart on the soil surface for seven consecutive days and nights in April/May 2015 following rodent

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trapping. To track seeds, fluorescent yellow fly-fishing backing line tags (10 cm long) were attached to a sanded down portion of the *W. incurvata* seed using non-toxic and odourless Pratley's Putty® (described in Rusch et al. 2013a). This allowed the seed to be tracked using a UV torch during the night. Tags were barcoded using a coding system so that transect, depot number time of field placement and distance moved could be recorded for the seeds found. The fates of all seeds were recorded as (i) Buried: where the tag was located above ground and the seed still attached below ground. (ii) Eaten: the tag was found attached to a broken husk. (iii) Lost: the seed was not relocated. (iv) Unmoved; seeds remained at the depot. (v) Eaten *in situ* where the seed was eaten in its entirety at the depot site. Five of the seed depots also had a motion sensitive camera trained on them (Ltl Acorn 6210MC) to identify the animal associated with the seed fate. Cameras were placed 1 m from the seed depot and set to record 60 s video. Camera evidence was analysed and rodents recorded to species and behaviour recorded. Differences in the fates of seeds left out during the day versus the night were analysed using a Pearson's chi-squared test in SPSS statistics (version 23).

Once located, cache distance from the seed source (depot) was measured as well as the number of seeds in the cache. These data were analysed using a simple linear regression in SPSS statistics (version 23) to determine whether caches with more seeds were buried further from the seed source.

Timing of seed release

To determine whether *W. incurvata* releases its seeds nocturnally rather than diurnally, 30 plastic buckets (height = 295 mm, diameter = 500 mm) were placed under mature seed-bearing *W. incurvata* bushes and examined before and after sunrise and sunset for seed fall. The number of seeds in each bucket was recorded for seven consecutive nights and days in

peak seed-rain season (November / December, 2015) and the differences between day and night seed rain analysed using a paired samples t-test in SPSS statistics (version23).

Results

The two dominant rodent species captured were *R. pumilio* and *G. paeba.* Significantly more *G. paeba* (n = 65) captures were recorded nocturnally than diurnally (χ^2 = 56.733; *df* = 1 p < 0.001) with all 65 captures being recorded during the night (Appendix 2). Significantly more *R. pumilio* captures (n = 165) were recorded diurnally than nocturnally (χ^2 = 77.203; *df* = 1; p < 0.001) with all 165 captures being recorded during the day (Appendix 2). A small number of *Otomys irroratus* (southern African vlei rat) were recorded during the day (n = 4) and the night (n = 6), however there was no nocturnal-diurnal difference in its capture rates (χ^2 = 0.275; *df* = 1; p = 0.916) (Appendix 2).
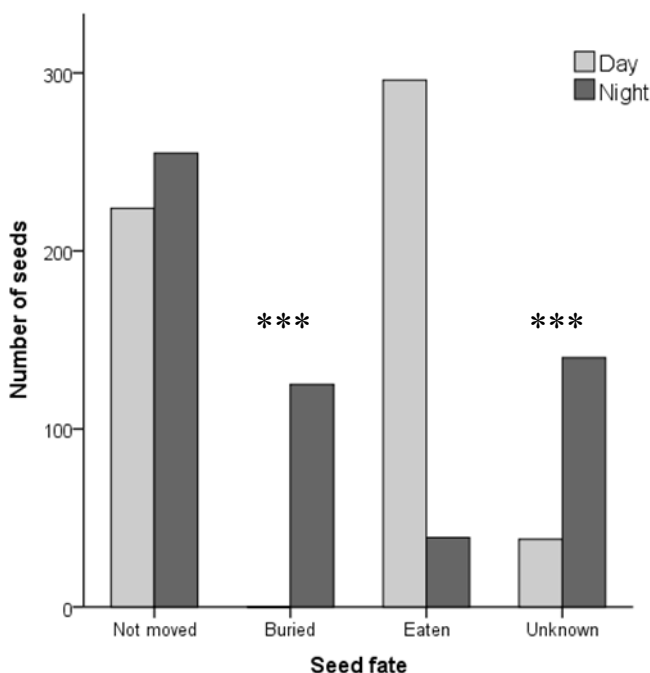

Fig. 2. 1 Different fates of seeds from depots placed on the soil surface during the day and night. Significant differences, based on χ^2 analysis, in the number of seeds succumbing to each fate during the day versus the night are indicated by *** (p < 0.001). Seeds were only buried during the night while the majority of seed consumption took place during the day.

Seed caching only occurred during the night, when *G. paeba* was active. Seed caches contained a mean ±SE of 5.4 ± 0.6 seeds with a maximum of 10 seeds cached together. Seeds were moved a mean distance ±SE of 4.7 m ± 0.3 m from their original depots (n = 118 seeds). Caching distances ranged from burial within the depots themselves to 11.5 m away. Seeds were buried a mean depth ±SE of 30 mm ±1 mm below the soil surface (n = 111 seeds) with a maximum depth of 70 mm. A total of 560 seeds were placed in seed depots for both the diurnal and nocturnal week of sampling, allowing for a direct comparison of seed fates. Significantly more seeds were buried nocturnally than diurnally (χ^2 = 46.086; *df* = 1; p < 0.001) (Fig. 2.1) with 22.3 % of seeds placed out at night being buried (n= 125), while none of the seeds placed out during the day were buried. Significantly more seeds were eaten diurnally than nocturnally (χ^2 = 1 24.086; *df* = 1; p < 0.001) (Fig. 2.1) with 52.9 % of the seeds placed out during the night being eaten (n = 296) and only 7.0 % of seeds placed out during the day being eaten (n = 39).

Video analysis confirmed seeds were removed and buried nocturnally by *G. paeba,* either by recording of seed removal and burial or recording of seed removal and tracking the seed using the barcode of the tag to infer how the seed was moved, while seeds were consumed diurnally by *R. pumilio.* Neither species appeared deterred by the presence of tags. Peak activity times of 21:00 and 04:00 were recorded for *G. paeba,* while a peak activity time of 11:00 was recorded for *R. pumilio* (Appendix 1). Video analysis also showed that significantly more seeds were buried nocturnally by *G. paeba* than diurnally by *R. pumilio* (χ^2 = 47.954; *df* = 1; p < 0.001) (Fig. 2.2). For seed consumption, significantly more seeds were eaten once taken away from the seed depot diurnally by *R. pumilio* than nocturnally by *G. paeba* (χ^2 = 114.879; *df* = 1; p < 0.001) (Fig. 2.2).
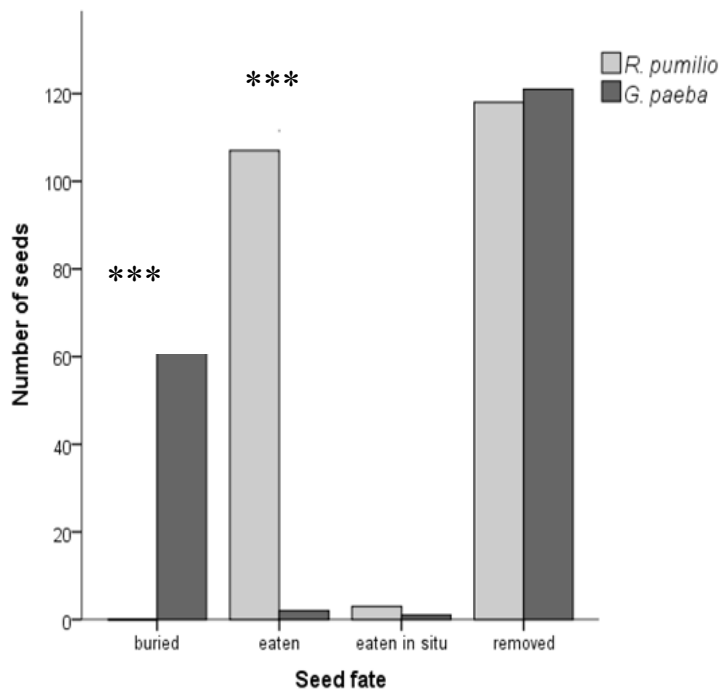
Fig. 2. 2 Video analysis of seed removal and subsequent seed fate from depots by *Gerbilliscus paeba* and *Rhabdomys pumilio*. Significant differences, based on χ^2 analysis, in the number of seeds succumbing to each fate indicated by *** (p < 0.001)
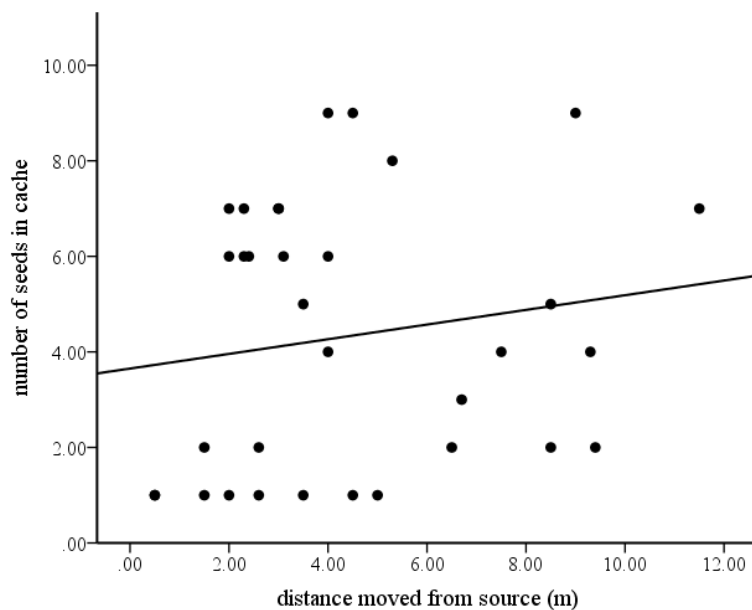
Fig. 2. 3 Linear regression showing a weak positive relationship between the number of seeds found in a cache and the distance moved from source by *Gerbilliscus paeba* (F(1,31) = 0.81, R^2 = 0.021, p = 0.44).

A weak, non-significant, positive relationship was found between the number of seeds cached together and the distance the seeds were moved from the seed source (F(1,31) = 0.81, R^2 = 0.021, p = 0.44 (Fig 2.3). Finally, a significant difference was found in the number of seeds

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that fell into the buckets underneath the *W. incurvata* bushes ($t_{(29)} = 3.298$, $p = 0.003$) with more seeds falling into the buckets during the day ($\bar{x} = 2.8 \pm 3.2$) than during the night ($\bar{x} = 0.8 \pm 0.9$).

Discussion

Using seed tracking and video footage, it was possible to confirm that the nocturnal *G. paeba* is a consumer and cacher of *W. incurvata* seeds. In contrast, the diurnal *R. pumilio* was confirmed as only a seed consumer. *Gerbilliscus paeba* can now conclusively be included as a disperser of *W. incurvata* seeds in the sandy areas of the CFR. It is further predicted that certain nut-seeded leucadendrons found on sandy flats (e.g. the rare *L. concavum*, known from only a single population, as well as *L. dubium*) are likely to be dispersed exclusively by *G. paeba*.

Following the predator satiation hypothesis (Howe and Smallwood 1982, Kelly and Sork 2002), the *en masse* seed release by *W. incurvata* suggests that seed predators may be satiated and predators are unable to consume all the seeds. This seed release strategy perhaps then serves a dual purpose: satiating the *R. pumilio* population while encouraging the seed dispersing *G. paeba* to cache seeds rather than consume them *in situ*. Most seed release by *W. incurvata* occurred during the day which showed that the time of seed release has clearly not evolved in response to the time of rodent disperser activity. Seeds are released in such vast quantities by *W. incurvata* that they may lay on the surface for several weeks (B. Anderson, *pers. obs*.). This suggests it may be irrelevant whether seeds are released during the day or night. Why only one of the rodent species (*G. paeba*) has evolved a scatter-hoarding strategy for *W. incurvata* seeds is unclear as both *R. pumilio* and *G. paeba* include the same seeds in their diet. One possible explanation is that scatter-hoarding by diurnal rodents such as *R. pumilio* may be selected against because of higher predation risks associated with caching

and retrieving seeds during the day. Another possible explanation is that rodents from more arid habitats, such as *G. paeba,* may be exposed to more variable food resources that fluctuate dramatically in availability, making scatter-hoarding more beneficial. Scatter-hoarding rodents are often associated with food resources that fluctuate with times of more plentiful resources encouraging food caching (Forget 1990, Haugaasen et al. 2010, Rusch et al. 2014).

Results from this study suggest that *G. paeba* does not bury higher value caches further from the seed source. This may be due to the possibility that cache value is determined rather by seed quality of individual seed mass as opposed to number of seeds. In addition, it may not be the distance from seed source that is important for reducing cache pilferage, but rather from the burrow of the individual. Instead of adopting behaviour to reduce pilferage, *G. paeba* may be a species that tolerates high cache pilferage by engaging in reciprocal pilferage of other individual's caches (see Vander Wall 2003). This would reduce the need for cache protection strategies such as spacing out of higher value caches.

This study allows for regional and worldwide comparisons to be made with other seed caching species. In this study, *G. paeba* usually opted to store seeds in caches of approximately 5 seeds. *G. paeba* was frequently recorded making caches of up to 10 seeds in this study. This may underestimate actual maximum cache sizes as depot sizes were limited to 10 seeds. The cache size recorded in this study is greater than those previously recorded by Midgley and Anderson (2004), who found caches of one or two *W. incurvata* seeds buried where *G. paeba* was a commonly trapped rodent. Cross-species comparisons within the CFR also suggest that the cache sizes of *G. paeba* are much larger than for caches associated with *A. subspinosus*. Where *A. subspinosus* was the associated caching rodent, caches were scatter-hoarded containing only single seeds (Rusch et al. 2013b), although these were the much larger seeds of *Leucadendron* (Proteaceae). Cache size choice by *A. subspinosus* is

thought to be a response to cache pilfering with smaller caches being used in places where the cases of pilfering are high and in wet soil where rodents are able to detect seeds more easily (Rusch et al. 2013b). In other parts of the world, cache size is thought to be a less important anti-pilferage strategy and increased cache spacing (Vander Wall 1990, Jenkins and Peters 1992, Jenkins et al. 1995) or even deceptive cache making strategies are deemed more effective (Steele et al. 2008, Perea et al. 2015).

In this study, numerous tags of seeds were found several days or even weeks later near the entrance of *G. paeba* holes suggesting that they may also store seeds in larders (as suggested by Stutterheim and Skinner (1973) for female gerbils). Alternatively, these seeds may be taken back to safety of the burrow for immediate consumption. Inspection of these tags showed all seeds had been consumed; however it is unclear whether they were consumed immediately or stored before consumption. The closely related Cape gerbil (*Gerbilliscus afra*) has also been recorded caching seeds in their burrows (Hes 1997) in what may be considered as a larder-hoarding strategy.

Comparing the seed dispersing behaviour of *G. paeba* with rodents from other continents shows some interesting similarities as well as differences. For instance the Kangaroo rat (*Dipodomys merriami*), a similar sized rodent from the United States, moves food items an average distance of 8m and as far as 49 m (Daly 1990), much farther than that of 4.7 m by *G. paeba* found in this study. *D. merriami,* like many other North American seed dispersing rodents has food pouches which enable them to move several seeds simultaneously. This could allow for more efficient dispersal energetics which could result in longer seed dispersal distances. *Gerbilliscus paeba* only moved seeds singly, suggesting either that the seeds are very large or that it has no pouches. This may have important consequences on energetic constraints regarding the movement of resources as well as memory capacity to return to the same site and relocating seeds. Comparisons can also be made across seed dispersal

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syndromes. The global average dispersal distance for myrmecochorous seeds is only 0.97 m and 1.25 m in the southern hemisphere (Gomez and Espadaler 1998) which is much shorter than the 4.7 m by *G. paeba* reported here. However, cases of long dispersal events for myrmecochorous seeds of up to 77 m have been reported in the southern hemisphere (Davidson and Morton 1981). When compared to fynbos myrmecochory such as the dispersal of *Leucospermum conocarpodendron* by *Anoplolepis steingroveri* and *Pheidole capensis, G. paeba* typically moved seeds twice as far (Slingsby and Bond 1985). It is important to note that comparisons between studies may be confounded by different seed tracking methods and missing seeds.

The seed tagging method used in this study was very successful as only 6.9 % of seeds were unaccounted for during the day and 25 % of seeds were unaccounted for during the night. This is equal or better than previous studies that make use of seed tagging. For example: Figures of only 37.4 % success in relocation of *Camellia oleifera* seeds tagged with 8 cm metal tags have been reported (Xiao et al. 2004). The only method of seed tracking that consistently yields more successful results in seed tracking is radioactive labelling that was first used by Vander Wall (1992) and radio telemetric thread tags (Hirsch et al. 2012a). It is not possible to speculate the precise fate of the unaccounted for seeds in our study, however the unaccounted for seeds are unlikely to change the main conclusion of this manuscript (i.e. seeds are only buried at night by *G. paeba*).

The circadian separation of *G. paeba* and *R. pumilio* activity times along with distinct differences in behaviour towards *W. incurvata* seeds presents a unique opportunity to further study the evolution of seed caching behaviour in the CFR. Only the presence of *O. irroratus* presented a grey area for rodent interactions with *W. incurvata* seeds, however this species was seldom captured in traps or on video and is known to consume vegetative matter as opposed to seeds (Curtis and Perrin 1979). Interestingly both *R. pumilio* and *G. paeba*

typically opt to remove seeds from depots first before consumption. This is perhaps a behavioural strategy to avoid competition for a located food source or escape predators around food sources.

Acknowledgements
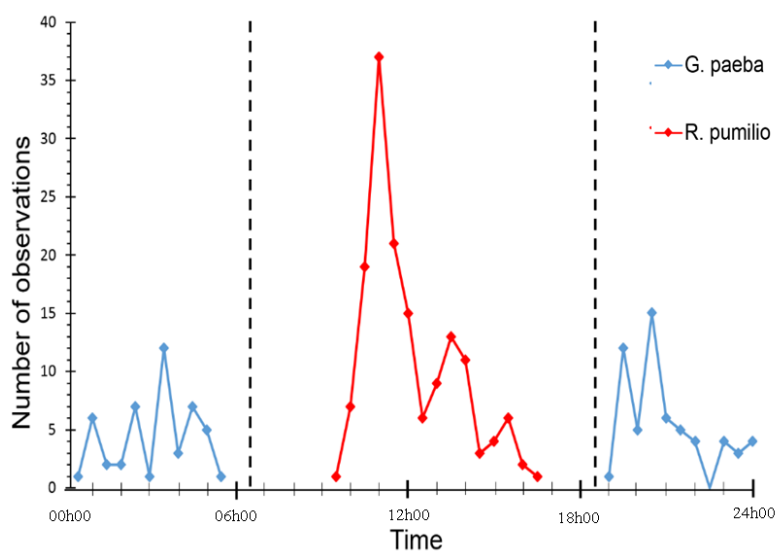
Appendix

Appendix 1 Video footage time stamps showing when *Rhabdomys pumilio* and *Gerbilliscus paeba* were most often recorded at seed depots. Dashed lines represent average sunrise and sunset times.
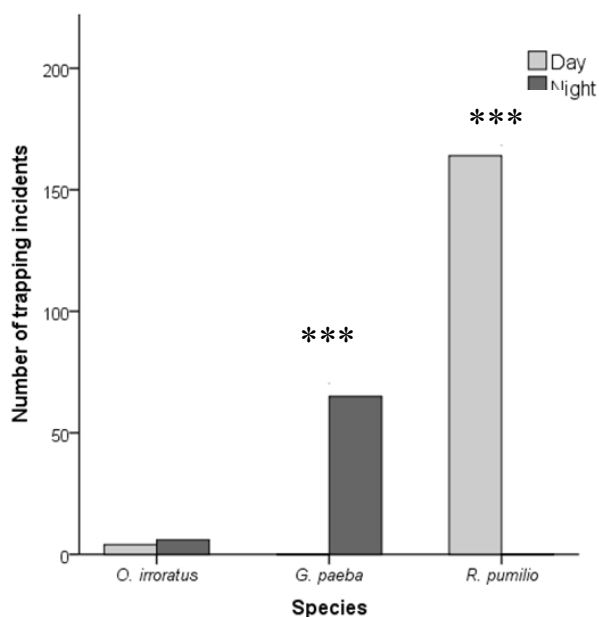
Appendix 2 Number of trapping incidents for each rodent species. Significant differences, based on χ^2 analysis, in the number of rodents caught during the day versus the night are indicated by *** ($p < 0.001$).

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Chapter 3: How do Cape rodents react to the addition of elaiosomes to *Willdenowia incurvata* (Restionaceae) seeds?

B. Weighill*, A. Huysamer and B Anderson. In prep: African Zoology

*Lead author collected and analysed the data and wrote the draft manuscript.

Abstract

The hairy-footed gerbil, *Gerbilliscus paeba* (Muridae) was recently recorded scatter-hoarding seeds of *Willdenowia incurvata* (Restionaceae) in the fynbos. Rodents are a poorly studied biotic seed dispersal vector in the fynbos, while ants (which disperse seeds which have elaiosomes) are well studied. While six species of the *Willdenowia* genus have elaiosomes and rely on ants to bury them, six do not, one of which is *W. incurvata*. This study found that when an elaiosome from a closely related myrmecochorous Restionaceae species, *W. glomerata*, was added to the seeds of *W. incurvata* it was less likely to be buried or consumed by *G. paeba* or consumed by the purely seed consuming *Rhabdomys pumilio*. This difference was attributed to elaiosomes being distasteful or to an association between elaiosomes and low seed reward quality. This finding may explain why some species of *Willdenowia* have elaiosomes while other closely related species within the same genus do not. This suggests that in some environments, rodent dispersal may be more preferential to ant dispersal.

Highlights

- Elaiosome addition deterred rodents from interacting with seeds of *Willdenowia incurvata.*

- *Gerbilliscus paeba* cached more seeds without elaiosomes than seeds with elaiosomes.

- *Rhabdomys pumilio,* which does not cache seeds, ate more seeds without elaiosomes than seeds with elaiosomes.

Keywords: Elaiosomes, Scatter-hoarding, Seed caching, Rodent assisted seed dispersal, Fynbos.

Introduction

Elaiosomes are lipid-rich seed appendages which ants are attracted to. Ants take elaiosome bearing seeds down into the nest, remove the elaiosome (which is typically fed to the brood) and discard the seed, intact, to germinate in the ant nest (Handel and Beattie 1990). Ant seed dispersal or myrmecochory, is widespread in over 3000 plant species in over 70 families worldwide; all of which possess elaiosomes (Hughes and Westoby 1992a, Beattie and Hughes 2002). Myrmecochory is also the dominant form of biotic seed dispersal in the highly biodiverse fynbos of the Western Cape, South Africa (Le Maitre and Midgley 1992). The elaiosome often acts as a food reward to the ants (Edwards et al. 2006) while it is specifically suggested that the oleic acid content of the elaiosome is responsible for the actual attraction and induction of carrying behaviour by ants (Westoby and Hughes 2009, Brew et al. 2010). Plants use this reward to manipulate ants into burying and dispersing their seeds. Dispersal benefits plants in many ways (reviewed by Howe and Smallwood 1982) and may include reduced distance and density related mortality. More specifically seed burial by ants may reduce rodent predation by rapidly moving seeds from the soil surface (Beattie 1985) and it can also protect seeds from fire (Moore and Vander Wall 2015).

Offering a reward to a disperser often comes at a cost. For example, the addition of an attractive appendage like an elaiosome, may also increase the risk of seed predators finding the seeds (Janzen 1969, Hughes and Westoby 1992b). In support of this, the removal of elaiosomes has been shown to reduce rodent seed predation both on (Heithaus 1981, Bond

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and Breytenbach 1985) and below the soil surface (Christian and Stanton 2004). In addition, adding fatty-acid material that mimics an elaiosome to seeds has been shown to increase seed predation by rodents (Howard and Cole 1967, Howard et al. 1968, Jennings 1976). A further cost of bearing an elaiosome is that the resources used to form it could be used elsewhere.

Despite the physiological cost of bearing an elaiosome and the possible increased risk of rodent predation, myrmecochory is clearly an effective seed dispersal strategy and beneficial for subsequent seedling establishment. This is due to the interaction it induces with ants and the fact that ants typically remove the elaiosome below the soil surface once inside the nest. By taking the seed down into the nest and removing the elaiosome, ant dispersal reduces competition for germination microsites (Handel 1978), prevents rodent predation (Christian and Stanton 2004) and facilitates germination in the nutrient rich nests (Hanzawa et al. 1988, but see Bond and Stock 1989). It is therefore interesting that several closely related species do not possess elaiosomes. Due to a lack of monophyly and phylogenetic resolution within the *Willdenowia* genus (Eldenäs and Linder 2000) it is difficult to make inferences regarding the evolution of elaiosomes.

In addition to ant attraction, the primary function of an elaiosome according to Hanzawa et al. (1985) is potentially to discourage rodents from consuming seeds . In their study rodents preferred *Corydalis aurea* (Fumariaceae) seed after their elaiosomes had been removed. This could occur if elaiosomes are distasteful or if seeds with elaiosomes are associated with low reward quality, abundance or long handling times. Hanzawa et al. (1985) found that elaiosomes were associated with low seed abundance because they were so rapidly buried by ants. In stark contrast to the findings of Hanzawa et al (1985), most other studies have found that the removal of elaiosomes reduced rodent seed predation, or that the addition of elaiosome mimicking substances attracted seed predators (Bond and Breytenbach 1985, Heithaus 1981, Howard et al. 1968, Howard and Cole 1967, and Jennings 1976). Elaiosome

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removal from seeds of *Leucospermum truncatulum* also reduces interactions between buried seeds and rodents in the fynbos (Christian and Stanton 2004) suggesting rodents are attracted to elaiosomes.

In all of these studies mentioned above, rodents were treated as purely seed predators and all seeds removed by rodents were considered consumed. However, some fynbos rodents behave as seed dispersers (Midgley et al. 2002, White et al. *in press, see Chapter 2*: Weighill et al. 2017). These rodents may in fact play an important role in seed dispersal in the fynbos as it may be expected that they disperse seeds slightly further than ants would have done. Both the predation and the dispersal of seeds by rodents are likely to affect the evolution of seed/plant phenotype. For instance, selection by rodents on seed hull thickness and seed size (Rusch et al. 2013) as well timing of seed release on a circadian scale (*see Chapter 2:* Weighill et al. 2017) has been investigated in the fynbos. However, how rodent consumers and dispersers may affect elaiosome presence or absence is yet to be tested.

This study aims to investigate the effect elaiosome addition to *Willdenowia incurvata* (Restionaceae) seeds has on the behaviour of both a seed caching and a seed consuming rodent. Elaiosomes were added to seeds of *W. incurvata* from the closely related and co-occuring *W. glomerata*. It is predicted that the seed consuming rodents will detect seeds with elaiosomes more easily and therefore consume more of them. In addition, it is predicted that seed caching rodents will avoid caching seeds with elaiosomes as these seeds are more easily detected underground and at a higher risk of being pilfered.

Study location and system

This study was conducted in Jakkalsfontein Private Nature Reserve on the West Coast of South Africa. The vegetation is typical of fynbos strandveld (Rebelo et al. 2006). The study was conducted on an Atlantis and Hopefield sand fynbos section of the reserve in an 8 ha

stand of strandveld which consisted primarily of adult *W. incurvata* bushes with several *W. glomerata* bushes in the area. The rodent community of this area is dominated by two species, the nocturnal *G. paeba* and the diurnal *R. pumilio*, which were trapped in Sherman traps in the area. Video analysis combined with seed tagging shows that *G. paeba* both consumes and caches *W. incurvata* seeds while *R. pumilio* only consumes them and displays no caching behaviour (*see chapter 2:* Weighill et al. 2017).

Study species

Plant species

Willdenowia incurvata (Thunb) HP Linder (Restionaceae) is endemic to but widely distributed within the fynbos of the Western and Northern Cape of South Africa, where it represents a typical element of the strandveld (Bergh et al. 2014). Female plants produce a large nut which has a thick, hard protective hull with a pitted surface. These seeds are 7-9 mm long, 5-6 mm in diameter, and brown in colour, elliptical in shape and possess neither an elaiosome nor a wing appendage (Fig 1.2). The seeds are released in large quantities during the early summer months (October/ November) (Linder et al. 1998). These seed traits and high nocturnal burial rates intimated that *W. incurvata* may be dispersed by *G. paeba*, the dominant nocturnal rodent in these sandy sites (Midgley and Anderson 2004). This was later confirmed using remote camera footage and seed tagging (*see chapter 2:* Weighill et al. 2017). The closely related *W. glomerata* (Thunb) HP Linder (Restionaceae) is also an endemic fynbos plant typically growing 0.2-1.5 m tall in a tufted or mat-like growth form. Seeds have pitted or smooth walls and possess elaiosomes 2-3 mm long (Fig 3.1) (Linder et al. 1998).

Rodent species

Gerbilliscus paeba (Vernacular name: hairy-footed gerbil) (Muridae) is a small, nocturnal rodent whose range includes the South West Cape biotic zone (Davis 1974). Stutterheim and Skinner (1973) observed that laboratory-held *G. paeba* females had a single larder, (usually in the corners of enclosures) where food was buried, while males buried food in numerous places inside an enclosure. Scatter-hoarding behaviour by *G. paeba* has subsequently been demonstrated in the fynbos for *W. incurvata* (*see chapter 2*: Weighill et al. 2017) and *L. pubescens* and *L. sessile* (White et al. *in press)*. Diet has been recorded as seed dominant in the Kalahari and foliage dominant in the Karoo, but overall diet also includes arthropods (Nel 1978, Kerley 1989). *Rhabdomys pumilio* (Muridae) is a small, diurnal murid rodent (Schradin and Pillay 2004). *Rhabdomys pumilio* occupies a variety of habitats in southern Africa including grasslands, marsh, forests, semi deserts and deserts. Diet includes vegetation and arthropods, however seeds are the preferred diet when available (Smithers 1986). This rodent consumes substantial numbers of seeds and has never been recorded burying seeds, despite two intensive studies that demonstrated dispersal by other rodent species (White et al. *in press*, *see Chapter 2:* Weighill et al. 2017).

Site choice

A stand of *W. incurvata* inside Jakkalsfontein Private Nature Reserve was chosen as the study site. This area was approximately 8 ha and dominated by mature *W. incurvata* bushes with clear sandy areas between them. This site was selected as it was not invaded and *W. incurvata* was common, but not dense enough to make seed tracking difficult. Inside this site, four transects were laid down in straight lines approximately 200 m in length and spaced approximately 100 m apart.

Materials and methods

Seed preparation

To track seeds, fluorescent yellow fly-fishing backing-line tags (10cm long) were attached to sanded down portions of the *W. incurvata* seeds used in the experiment. Pratley's Putty® was used for the attachment of the tags as it is non-toxic and odourless (see description in Rusch 2013). The seeds were recovered at night using a UV torch which made the tags glow in the dark. Once located, seeds were classified as either (i) Buried: where the tag was located protruding from the ground and the seed still attached below ground. (ii) Consumed: only the tag was found with seed hull still attached. (iii) Lost: the tag and seed were not relocated. (iv) Unmoved; seeds remained at the depot upon inspection. The dacron tags were barcoded using a coding system so that transect, depot and time of field placement could be associated with the located seed.

As *W. incurvata* does not possess an elaiosome, elaiosomes from another, co-occuring Restionaceae species, *W. glomerata*, were cut off with a scalpel and reattached to the *W. incurvata* seeds using a drop of superglue (Fig. 3.1). A small drop of superglue was also applied to *W. incurvata* seeds that did not have elaiosomes attached as a control. Prior to elaiosome removal, *W. glomerata* seeds were harvested by hand and stored whole, with bracts intact at -18 ˚C to prevent elaiosomes drying out. The elaiosomes were removed and attached to *W. incurvata* seeds just before they were used in the experiment.

Experimental design

Along each of the four transects, four depot sites were chosen under mature *W. incurvata* bushes where natural seed rain would occur. These depots were approximately 50 m apart. At these depots ten tagged seeds of *W. incurvata* were placed on the soil surface. Seeds were either placed in depots nocturnally when the seed caching *G. paeba* was active, or seeds were

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placed in depots diurnally when the purely seed consuming *R. pumilio* was active. With the circadian separation of the seed-caching *G. paeba* from the seed consuming *R. pumilio* (*see Chapter 2:* Weighill et al. 2017) it was possible to demonstrate the differences in these two rodents' reactions to *W. incurvata* seeds with and without elaiosomes

Single treatment experiments

During April/May 2015 two depots along each transect received ten *W. incurvata* seeds without elaiosomes added while the other two depots received ten *W. incurvata* seeds with elaiosomes added. For example, along a transect, depot one and three received 10 *W. incurvata* seeds without elaiosomes added while depot two and four received ten *W. incurvata* seeds with elaiosomes added. This alternated every 24 h for six consecutive days and nights so that no depot received the same type of seed treatment (i.e. no elaiosomes versus elaiosome added) for two days or nights in a row. Since each depot received equal numbers of seeds with and without elaiosomes, we were able to determine whether their fates differed in response to either *R. pumilio* or *G. paeba*. This experiment was unable to distinguish between the effects of elaiosomes on seed preference versus the ease of discovery by rodents, as the results potentially contain elements of both processes.

Mixed treatment experiments

During April/May 2016, ten *W. incurvata* seeds with elaiosomes and ten seeds without elaiosomes were placed at depots. The 20 seeds were placed randomly at specific transect depots so that rodents were able to actively choose between seeds with and without elaiosomes. Along a transect, only two of the four depot sites were used at a time. The same mixture of seeds was placed in the alternative depots on the following evening. This continued for six consecutive nights. For this experiment, seeds were only placed nocturnally because in 2016, minimal *R. pumilio* activity was recorded and almost no seeds were removed or consumed during the day.

Upon inspection, remaining seeds were collected and removed from the field. Ants were never observed removing experimental seeds either because they were uncommon or the seeds were too large to be removed. Since seeds with and without elaiosomes lie alongside one another in this experiment, differences in seed fate are unlikely to be the result of discovery differences but rather the result of preference differences.

A Wilcoxon Signed-Rank test in SPSS (version 23) was used to analyse the differences in the fates of seeds with and without elaiosomes. If a depot was not visited for two consecutive days or nights in 2015 it was discarded from statistical analysis. Unvisited mixed seed depots were also discarded from statistical analysis as it is impossible to determine whether a visiting rodent made any decision based on the seeds it encountered. In total, 2880 seeds were placed in depots during the study period.

Fig. 3. 1 (From left to right) A *Willdenowia glomerata* seed with a distinct elaiosome, a *W. incurvata* seed with no elaiosome added and a *Willdenowia incurvata* seed with the elaiosome from *Willdenowia glomerata* added. Images are to scale with a 5 mm reference bar. (Photographs by B. Anderson)

Results

In all of the comparisons, seeds without elaiosomes were buried more frequently by *G. paeba* and consumed more frequently by *R. pumilio* than seeds with elaiosomes. In diurnal seed trials, significantly more seeds without elaiosomes were eaten by *R. pumilio* than those with elaiosomes added (Fig. 3.2) (Z = -3.46, p = 0.001). No seeds were buried during the day.

During nocturnal seed trials using separate depots of seeds without elaiosomes and seeds with elaiosomes added, *G. paeba* buried significantly more seeds without elaiosomes than seeds with elaiosomes added (Fig. 3.3) (Z = -2.38, p = 0.017). However, there was no significant difference in the number of seeds eaten by *G. paeba* (Fig. 3.3) (Z = -0.18, p = 0.85).
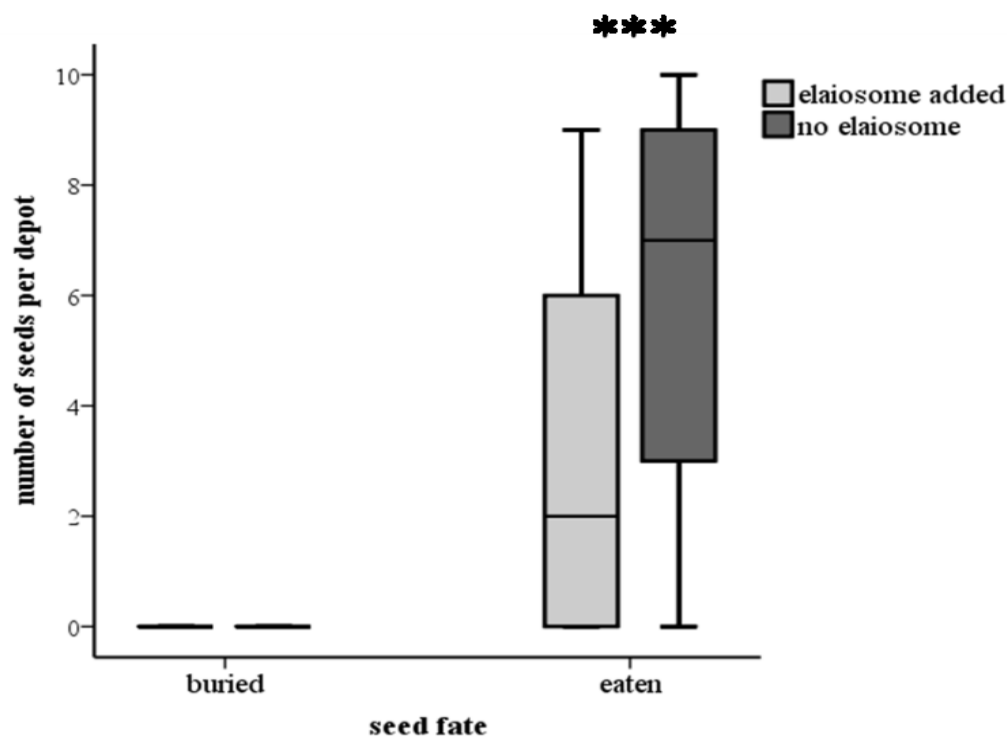

Fig. 3. 2 Diurnal seed consumption showed that *Rhabdomys pumilio* does not bury seeds and that it consumed more seeds from each depot without elaiosomes than seeds with elaiosomes (significance marked with a *** at p < 0.001).
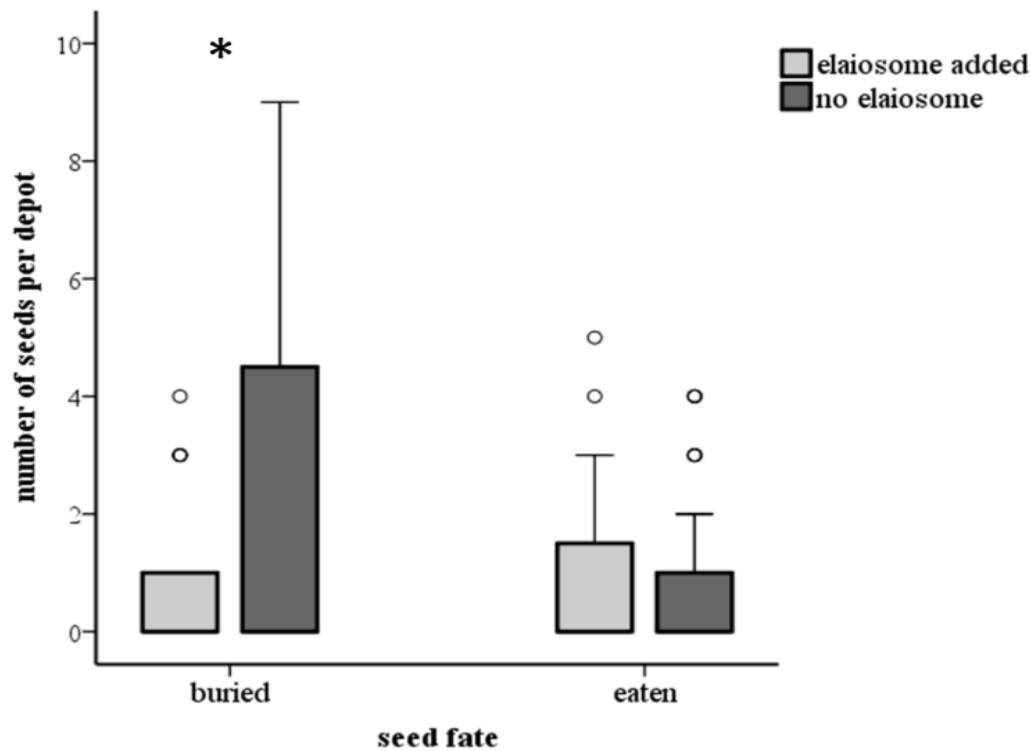
Fig. 3. 3 Nocturnal seed burial showed significantly more seeds were buried without elaiosomes than seeds with elaiosomes added by *Gerbilliscus paeba* (circles indicate outliers, significance marked with a * at $p < 0.05$).

During mixed seed trials, where rodents were able to make choices between seeds with and without elaiosomes, *G. paeba* again buried significantly more seeds without elaiosomes than those with elaiosomes added (Fig. 3.4) ($Z = -3.18$, $p = 0.001$). Significantly more seeds without elaiosomes were also consumed by *G. paeba* (Fig. 3.4) ($Z = -2.808$, $p = 0.005$). Following mixed seed trials, 43 caches were relocated of which 18 had no seeds with elaiosomes in them. In these caches, the mean number of seeds ±1SE found in the caches was 3.3 (± 0.41) seeds of which a mean of 2.3 (± 0.37) seeds had no elaiosome added and 1.0 (± 0.19) seed had an elaiosome still attached. Elaiosomes were still attached to the buried seeds that were found (*pers. obs.*).
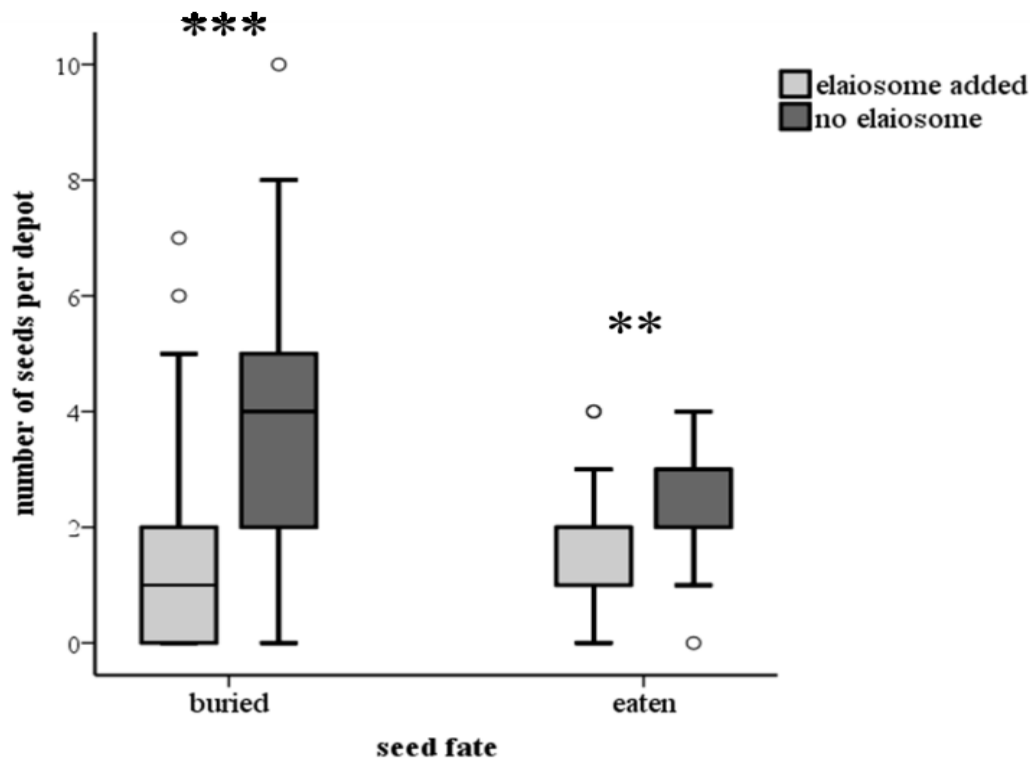
Fig. 3. 4 Nocturnal seed burial showed that *Gerbillscus paeba* buried and consumed more seeds without elaiosomes than seeds with elaiosomes when offered a choice of both seed types (circles indicate the outliers, significance marked with a *** at p < 0.001 and ** at p < 0.01).

Discussion

This study showed that both rodent species interacted more frequently with *W. incurvata* seeds that had no elaiosomes versus seeds with elaiosomes added. During the day, *R. pumilio* consumed more seeds lacking elaiosomes while during the night *G. paeba* buried, but also consumed more seeds without elaiosomes when given a choice in a mixed seed depot. There are five possible explanations for the greater burial of seeds by without elaiosomes by *G. paeba*: (i) rodents may prefer to bury seeds without elaiosomes if they are detected less easily by other rodents, (ii) elaiosomes may have a negative effect on seed longevity below the surface, (iii) rodents may associate elaiosomes with poor reward quality, (iv) rodents may find elaiosomes distasteful and (v) elaiosomes are associated with more sparse seed aggregations. Below, the merits of each of these possibilities are discussed.

i)   Elaiosome detectability

Elaiosome bearing seeds have been shown to produce stronger olfactory cues than seeds with elaiosomes removed (Heithaus 1981, Bond and Breytenbach 1985, Van Blerk 2016) . These elaiosome bearing seeds may consequently be more easily located by rodents both below (Heithaus 1981) and on (Bond and Breytenbach 1985, Christian and Stanton 2004) the soil surface. With pilferage levels recorded varying from 2-30 % in other scatter-hoarders (Vander Wall 2003), scatter-hoarders like *G. paeba* may be less likely to bury seeds that have a higher chance of being located and consumed by other individuals. However, this putative risk of pilferage cannot explain why *R. pumilio* also showed preference for seeds without elaiosomes as *R. pumilio* only consumed seeds and buried none of them. Similarly, *G. paeba* also consumed more seeds without elaiosomes than seeds without elaiosomes in the choice experiment, suggesting that the differences in seed fate are not the result of differences in detectability by pilferers.

ii)   Buried seed longevity

Elaiosomes have fleshy tissue that can persist for over a year (Pfeiffer et al. 2010) and may facilitate attack by pathogens (Christian and Stanton 2004). In contrast the hard and thick seeds hulls, like those associated with rodent dispersal (Midgley et al. 2002, Vander Wall 2010) and of *W. incurvata* are thought to assist in resistance to such pathogens and microbial attack (Mohamed-Yasseen et al. 1994). It is unknown over what time period *G. paeba* stores the seeds of *W. incurvata*, however the associated risk of microflora on elaiosome bearing seeds may be sufficient in deterring *G. paeba* from burying these seeds. However, this reasoning also does not explain why *R. pumilio*, which never buried seeds preferred to consume seeds without elaiosomes, and why *G. paeba* also consumed more seeds without elaiosomes.

iii) Poor associated reward

The elaiosomes used in this study come from seeds that are smaller in size than those that have no elaiosomes (Fig 3.1). If rodents associate the presence or absence of elaiosomes with seed reward size/quality then this could explain why both the seed consuming *R. pumilio* and the scatterhoarding *G. paeba* have preferences for seeds without elaiosomes. Large seed size is a trait specifically associated with rodent dispersal and in the fynbos, large seed size and rodent dispersal may have evolved in concert. Elsewhere in the world, rodents disperse large seeds further than small ones (Xiao et al. 2005, but see Brewer 2001), and they are removed and cached more readily (Jansen et al. 2004). This may be especially beneficial in environments where there is competition for germination sites and where fast germination is necessary.

iv) Distasteful elaiosomes

Hanzawa et al. (1985) is the only study thus far that suggests elaiosomes may deter rodents while attracting ants. It is possible that elaiosomes are distasteful to rodents, thus explaining the preference by both *R. pumilio* and *G. paeba* for elaiosome lacking seeds, although some seeds with elaiosomes attached were still eaten by both rodent species (Fig 3.2). Indeed, distastefulness could also explain why both seed consumers and dispersers interacted more frequently with seeds that did not have elaiosomes. Furthermore, distastefulness can also account for seed fate differences in both the choice experiment and in the experiments which did not give mice a choice between two kinds of seed.

v) Associated sparse seed aggregations

Lastly, the decreased attraction of rodents to elaiosome bearing seeds could be due to rodents avoiding areas with less dense seed aggregations. Hanzawa et al. (1985) suggested that since myrmecochory results in rapid seed removal from the soil surface, rodents may associate the elaiosome olfactory cue with a less dense aggregation of seeds. However, this is unlikely to explain the results of this study as seed fates differed in the choice experiments, suggesting

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that rodents were making active choices between different kinds of seeds and not simply avoiding certain areas associated with low seed abundance

The Cape strandveld, of which *W. incurvata* is a key component, is often subject to invasion by various *Acacia* species (Low et al. 1998). The maintenance of intact areas of indigenous vegetation is known to resist invasion by woody species such as *A. cyclops* and *A. saligna* (Morris et al. 2015). Many of these *Acacia* species produce elaiosomes and are myrmecochorous. This study shows that although these elaiosome bearing seeds are less likely to be cached and subsequently dispersed by rodents should elaiosome lacking species, such as *W. incurvata*, be present, rodents will not altogether ignore seeds that have elaiosomes attached.

In the past, it was thought that only ants provided burial protection against fire for seeds in the fynbos. It is now clear that rodents can also fulfil this role and hence have the potential to shape plant seed traits. This study presents the first evidence suggesting that a lack of an elaiosome may enhance dispersal by rodents in the fynbos. For this to occur, it is necessary that the benefits of rodent dispersal by *G. paeba* outweigh the costs of increased rodent predation by *R. pumilio* and *G. paeba* for seeds without elaiosomes. It is likely that the costs and benefits of having an elaiosome may vary with environmental context (e.g. abundance of seed dispersing and consuming rodents, abundance of seed dispersing ants, and also the advantages of large seed size associated with fast germination). This geographic mosaic of environmental conditions may explain why both the absence and presence of elaiosomes appears within the *Willdenowia* genus.

Acknowledgements

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Chapter 4: General conclusions and suggestions for future study

Summary of findings

While interactions such as pollination and myrmecochory are relatively well studied in the fynbos, research into rodent assisted seed dispersal is not, and has only recently begun to receive some much needed attention (e.g. Midgley and Anderson 2004, Rusch et al. 2013, Weighill et al. 2017, White et al. *in press*) This is encouraging as previously unidentified interactions are being identified and studied, which could play an important role in maintaining the ecosystem as well as provide opportunities to study the drivers of species diversification from the natural laboratory that is the fynbos.

In Chapter 2, a second case of rodent assisted seed dispersal was confirmed and then investigated. It was confirmed that *Gerbilliscus paeba*, which is strictly nocturnal, scatter-hoards the seeds of *Willdenowia incurvata* and consumes a few *in situ.* In contrast the co-occurring *Rhabdomys pumilio* is only active during the day and only consumes seeds without caching any. In this chapter we show that tagging seeds with fluorescent line attached with Pratley's putty is an effective method to follow seed fate as very few seeds were lost. It was also found that despite seed dispersal taking place exclusively at night and most of the seed consumption taking place during the day, *W. incurvata* does not release the majority of its seeds at night. Finally, contrary to predictions and findings by Xiao et al. (2005), Moore et al. (2007), Vander Wall (2003) and Jansen et al. (2004), it was found that there was not a significant relationship between the number of seeds found in a cache of *G. paeba* and the distance of the cache from the seed source.

In Chapter 3, elaiosomes from a co-occuring, closely related species, *W. glomerata* were added to the seeds of *W. incurvata* that do not possess elaiosomes. It was found that seed dispersing and consuming rodents tended to interact less frequently with seeds that had

elaisomes than with seeds that did not have eliaosomes. This is primarily attributed to elaiosomes being distasteful to rodents as suggested by Hanzawa et al. (1985), or alternatively an association between elaiosomes and poor seed reward quality.

Suggestions for future study

It is highly likely that there are other cases of rodent assisted seed dispersal in the fynbos that have not yet been identified let alone studied. Future study should therefore focus on identification and confirmation of possible interactions to initiate basic ecological investigations. As suggested by Midgley and Anderson (2004) both the Restionaceae and Proteaceae families have numerous species with large, nut-like seeds that are ideal candidates for rodent assisted seed dispersal. The rare *Leucadendron concavum* and *L. dubium* present two such Proteaceae species.

The role played by dispersal vectors in shaping plant traits is well documented, however many of these traits present a trade-off in the presence of additional seed predators, as is the case in this study. Future studies should therefore investigate the relative roles of predators versus dispersers in shaping seed traits. However in the fire-prone fynbos, protection from fire via burial may be more important than predator pressures.

As a new case of scatter-hoarding has been confirmed in this study, a whole new suite of studies become possible regarding the evolution of this behaviour. This includes investigation into anti-cache pilferage strategies and decision making (i.e. whether to cache or consume seeds *in situ*) by the scatter-hoarder. For instance the energetic constraint of caching seeds versus the reward should be studied as well as how *G. paeba* reduces the pilferage of its caches.

Concluding remarks

It has been suggested that myrmecochory in the fynbos evolved as a response to heavy rodent seed predation. However, it is clear not all rodents are merely seed predators. The key similarity between these two very different modes of seed dispersal (rodent assisted and myrmecochory) is seed burial. This suggests that above all else, biotic seed dispersal in the fynbos evolved to protect seeds from fire, safely beneath the soil surface. This need for burial as a fire protection strategy for seeds has been demonstrated in the California Floristic Province which also experiences regular fires (Moore and Vander Wall 2015). Any mode of dispersal that results in seeds being buried could, as a result, be highly beneficial if not obligatory for seed survival and establishment in the fynbos. This suggests plants in the fynbos will engage in these biotic interactions despite the heavy costs they may entail.

The fynbos is most certainly a natural laboratory, and rodent assisted seed dispersal a less explored wing thereof. Rodent assisted seed dispersal has low potential for being long distanced. It is therefore important that further studies of this type of biotic seed dispersal be conducted as this could add to the hypothesis that short dispersal distances have played an important role in allopatric speciation in the fynbos by limiting gene flow. This could add to our explanations for the mega-diversity in angiosperms in the fynbos seen today.

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Appendix: Collage of photos from fieldwork conducted during this thesis

Image 1: (left) B. Weighill releases a hairy-footed gerbil from a Sherman trap and (right) tagging seeds.
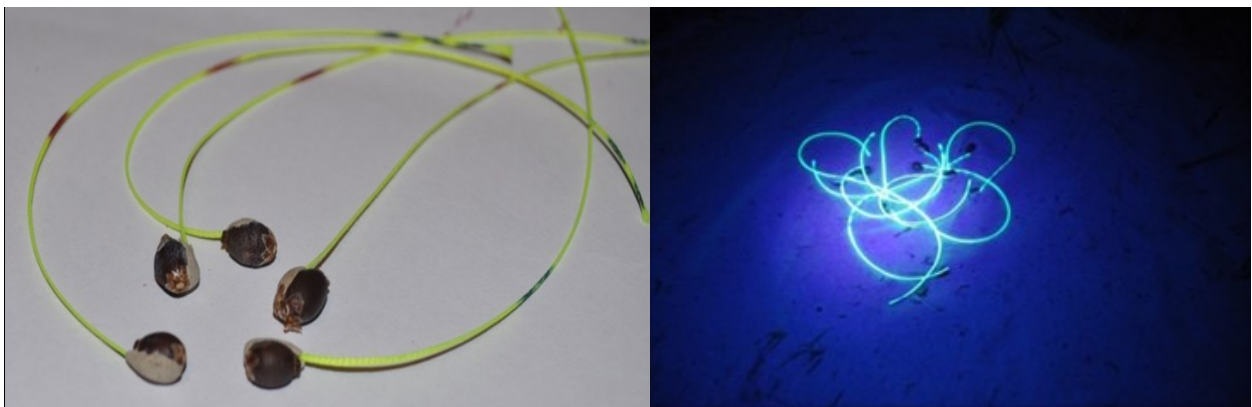

Image 2: (left) A close-up view of the tagged seeds and (right) fluorescent tags under UV illumination

Image 3: (left) Buried seeds with tags sticking out above the sand; (middle) tagging a found cache after burial by *Gerbilliscus paeba;* and (right) camera trap trained on a seed depot.