# Seed dispersal by Black-backed Jackals (*Canis mesomelas*) and hairy-footed gerbils (*Gerbillurus* spp.*)* of !nara (*Acanthosicyos horridus*) in the central Namib Desert

by

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# Declaration

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# Dedication

I would like to dedicate this dissertation to Gobabeb - Namib Research Institute for the research experience acquired.

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# Abstract

This study investigated primary seed dispersal of !nara (*Acanthosicyos horridus*) by Black-backed Jackals (*Canis mesomelas*) and secondary seed dispersal by scatter-hoarding hairy-footed gerbils (*Gerbilliscus* (*Gerbillurus)* spp.*)* in the central Namib Desert. This was accomplished by examining visitation rates and fruit removal of !nara melons, primarily by jackals. In addition, I determined the viability and germination rate of !nara seeds collected from jackal scat. The results indicate that jackals were the dominant species to visit !nara (93.3%) and the only !nara frugivores recorded by camera traps over two !nara fruiting seasons. There was no difference in the viability of ingested seeds and control seeds, but germination rates of ingested !nara seeds were significantly higher (50.4%) than control !nara seeds (34%). This component of the study suggests that Black-backed Jackals are the main primary dispersers of !nara seeds in the central Namib Desert. I furthermore examined secondary seed dispersal by tracking !nara seeds to determine whether scatter-hoarding hairy-footed gerbils were caching or consuming seeds. I recorded the distance moved, depth of seed burial, recovery rate and the habitats in which seeds were buried in three habitat types. Hairy-footed gerbils removed 100% !nara seeds from experimental sites and cached 60.3 % of all the !nara seeds removed. The gerbils frequently retrieved the buried caches within two days (77% of the time) and re-cached them elsewhere. The majority of caches were in the open areas (83%) and only consisted of one (39%) or two seeds (45%). Only 1.7% of the cached seeds were not retrieved by the gerbils during the 30-day observation periods. !Nara seeds were moved an average distance of 29.1±1.6 m and buried at an average depth of 4±0.2 cm. Although there is high probability of cache retrieval, some of the cached seeds survived. As gerbil caches are at favourable locations for plant establishment, and as it is more likely that buried seeds will survive until suitable conditions for germination and seedling establishment, seed dispersal by hairy-footed gerbils is advantageous to !nara plants. Therefore, hairy-footed gerbil species in the central Namib Desert contributed to secondary seed dispersal of !nara. The combined interaction of endozoochory by Black-backed Jackals (*Canis mesomelas*) and synzoochory by hairy-footed gerbils (*Gerbillurus* spp.*)* in dispersing seeds of !nara plants (*Acanthosicyos horridus*) in the central Namib Desert suggest diplochory is highly likely.

**Key words:** primary seed dispersal, germination rate and viability, caching, secondary seed dispersal, scatter-hoarding

# Chapter 1: Introduction

## The study species: *Acanthosicyos horridus* (!nara)

!Nara (*Acanthosicyos horridus* Welw. ex Hook. f.), is a spiny, leafless, dioecious melon-bearing plant (Fig. 1) that belongs the family *Cucurbitaceae,* and is endemic to sandy areas of the Namib Desert (Meeuse, 1962; Maggs-Kölling *et al*., 2014). Its common name !nara is from the Khoekhoegowab language of the local ǂAoni Nama (also referred to as the Topnaar people) that have been harvesting its melons for centuries (Henschel *et al*., 2004; Gruntkoski & Henschel, 2004). It is a low-sprawling, perennial shrub that forms a hummock, which can cover large areas (1500 $m^2$; Maggs-Kölling *et al*., 2014) within the Aeolian dunes of the Namib Desert. It often grows at the base of the dunes and along perennial rivers where other vegetation may be sparse or absent, but is absent from the rocky plains of the desert.

**Figure 1.1**: A fruiting !nara (*A. horridus*) plant in the central Namib Desert. Photograph by Shikesho (2020).

The !nara is considered ecologically, economically and culturally valuable, providing food, shelter and water to both vertebrates and invertebrates (Henschel *et al*., 2004; Maggs-Kölling *et al*., 2014). It also provides the indigenous ǂAoni or Topnaar Nama community, who live along the Kuiseb River in the Namib Desert, with food, and traditional medicine. They also generate income by selling !nara products such as the seeds, fruit and oil (Henschel *et al*., 2004; Maggs-Kölling *et al*., 2014). The female plants (Fig. 1) usually produce only one crop of melons of 20–500 melons per plant between November and April every year (Berry, 1991). The melons are 10–20 cm in diameter and weigh 0.6–1 kg each (Klopateck & Stock, 1994; Berry, 2001). The !nara melon (Fig. 1.2) is round with a pale green, spiny, tough rind even when ripe, which encloses a highly nutritious, water rich pulp and hard-coated nutritious seeds (15–350 seeds per melon, pers. obs.).

**Figure 1.2**: !Nara melons. Photograph taken at Gobabeb in the central Namib by Shikesho (2019)

!Nara are likely to have evolved fleshy fruit to encourage seed consumption by vertebrates that will excrete or regurgitate seeds far away from the parent site (endozoochory), leading to seed dispersal. This might be particularly advantageous to the !nara because most cucurbits are known to produce germination suppressors (allelopathy) in the roots, poisoning the soil around the plant, or in the fruit pulp (Botha & Grobbelaar, 1981; Yu, 2001). Other plants, even other !nara, never co-inhabit the same area as individual !nara plants (pers. obs.). Thus, animals increase germination by removing the pulp around the seeds and moving the seeds in their gut away from the parent plant.

The distribution of !nara (Fig. 1.3) ranges from near Port Nolloth in South Africa to the Curoca River in Angola with extensive fields along the Kuiseb, Uniab and Curoca River deltas (Meeuse, 1962; Berry, 1991; Maggs-Kölling *et al*., 2014). !Nara is mostly known to be associated with ephemeral rivers and permanent groundwater that could indicate that groundwater is its main source of water (Klopatek & Stock, 1994). However, !nara is not always found in areas where the conditions seem suitable like along some of the ephemeral rivers in Namib Desert (Müller, 2002), for example the Tsondab River (pers. obs.).

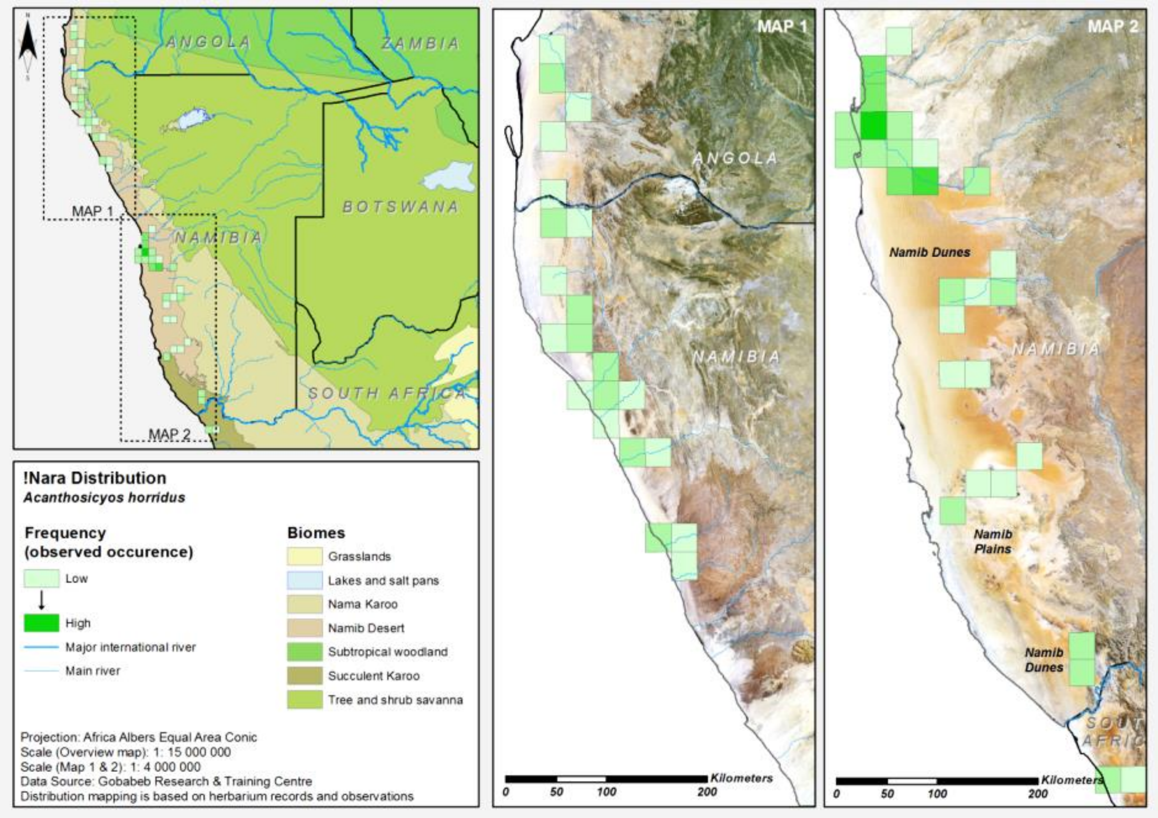

**Figure 1.3:** Distribution map of !nara within the Namib Desert (Thompson, 2018).

The !nara has a disjuct distribution with large gaps between the northern, southern and central Namib populations (Fig. 1.3). Each of these three major populations (Northern, Southern and central Namib) consists of fragmented populations which are quite far apart (pers. obs.). This distribution pattern and population dynamics of !nara may be a result of suitable habitats for !nara growth, but seed dispersal strategies used by the plant may be particularly important to explain its distribution. In the central Namib, Black-backed Jackals (*Canis mesomelas*) are considered the primary seed dispersers of !nara seeds (Müller, 2000; Chapter 2). Their movement and behaviour may help explain the disjunct and fragmented distribution of !nara, while the movement of seeds by scatter-hoarding gerbils (*Gerbilliscus* (*Gerbillurus)* spp.) as they cache and feed on seeds within the habitats may explain the distribution of plants within a population (Chapter 3).

## The importance of seed dispersal

Seed dispersal is one of the factors that influence plant fitness because it determines spatial and temporal movement of plant seeds into suitable habitats, usually with the aid of abiotic vectors such as wind and water and biotic vectors such as vertebrate animals (Wenny, 2001; Nathan *et al*., 2008). Seed dispersal is important to plants for many reasons, for example; (1) in periods of global change, it allows plant populations to track and shift together with suitable habitats (Cain *et al*., 2000), (2) it allows plant populations to migrate and colonise new suitable habitats where competition is lower and resources are abundant (Bilal, 2016), and (3) it provides escape from density and/or distance related mortality near conspecifics and prevents inbreeding within populations (Wenny, 2001). Within the current context of predicted climate changes, plant distributions are expected to shift into new favourable habitats (Vellend *et al*., 2006).

Seed dispersal is a multistep process, in which seeds are moved frequently and successively from the parent plant to the final areas where they germinate and recruit (Schupp *et al*., 2010). Seed dispersal can be broadly categorized into primary and secondary phases. In the primary phase, seeds will be moved directly from the parent plant to new areas, while the secondary phase is when previously dispersed seeds are moved to new areas (Schupp *et al*., 2010). Both primary and secondary seed dispersal phases provide benefits to the plant.

Animals, especially mammals and birds, are important dispersal vectors, particularly in long-distance dispersal because of their ability to travel across vast areas (Nathan *et al*., 2008). The availability, characteristics and behaviour of seed dispersers can be important for the persistence and distribution of plants. In some cases, plants develop a mutualistic relationship with a particular group of animals by providing food in exchange for seed dispersal (Ramos *et al*., 2006). In many cases, plants are dispersed in two or more phases with a different dispersal agent involved in each phase (diplochory; Van der Wall & Longland, 2004: Roth & Vander Wall, 2005). Thus, understanding seed dispersal is important for both the plants and animals involved.

## Previous !nara seed dispersal studies

!Nara is potentially dispersed in two phases. Phase one involves Black-backed Jackals (*C. mesomelas*) that feed on !nara melons and ingest !nara seeds (endozoochory) acting as a primary seed disperser. Phase two involves hairy-footed gerbils (*Gerbillurus* spp.) that collect previously dispersed seeds and bury them in caches elsewhere (synzoochory) as secondary dispersal agents. Müller (2000) did a preliminary study on !nara seed dispersal at Gobabeb in the central Namib Desert by collecting and examining animal faeces for intact !nara seeds. Of the faeces collected from six species, only those of the Black-backed Jackals had intact !nara seeds. Müller (2000) also examined secondary !nara seed dispersal by hairy-footed gerbils. The results concluded that hairy-footed gerbils cached !nara seeds, but all the cached were retrieved the following night (Müller, 2000).

For !nara, this suggests that jackals remove seeds, allowing them to escape density and/or distance dependent mortality (competition and predation) near parent plants, resulting in colonization of new areas. Gerbils may also harvest and move mature seeds away from the parent site, but they do not move seeds far enough to offer an escape from the parent community. In combination with other seed dispersers such as jackals, however, gerbils might move seeds to more favourable local microhabitats (directed dispersal), which may increases seed and seedling survival (Wenny, 2001).

Plants with fleshy fruit are most likely to be dispersed via endozoochory (Bilal, 2016). The fruit type diagnostic for the *Cucurbitaceae* family is a pepo, a large berry with a hard, leathery wall (Koekemoer *et al*., 2014). Although there is limited literature on their seed dispersal, the fleshy fruit have been reported to be dispersed through the digestive tracts of vertebrates (Meeuse, 1959; Steentoft, 1988; Kistler *et al.*, 2015). An interesting example is *Cucumis humifructus* (Aardvark cucumber), the seeds of which are dispersed by Aardvark (*Orycteropus afer*). This species is mostly found growing in old Aardvark holes and seeds have been recovered from Aardvark faeces (Meeuse, 1959; Hoffmann & Myburgh, 1995). Muskmelon (*Cucumis melo*) is believed to be eaten and dispersed by primates (Steentoft, 1988). Kistler *et al.* (2015) suggested that wild *Cucurbita* (squashes, pumpkins and gourds) were dispersed via endozoochory since their seeds were found in mastodon dung deposits and that their decline in the wild may be linked to the disappearance of mega herbivore mammals.

Seed-hoarding by rodents has been reported in *Marah macrocarpus*, when seeds are released and become available after fire events (Borchert, 2004). Chinese lard seed (*Hodgsonia heteroclita*) has been suggested as a target for scatter-hoarders (Schreiter *et al*., 2007), confirmed by local knowledge and known in the vernacular as “favourite squirrel food”. Many cucurbit taxa are of economic importance as a food source because of the large, fleshy fruit, thus human-mediated or anthropogenic seed disposal is probable.

## Study Site

This study took place in the central Namib, in the vicinity of Gobabeb - Namib Research Institute (Fig 1.4). The Namib is a long, narrow desert lying on the west coast of southern Africa. It is approximately 2 000 km long, running parallel to the coast of southern Angola to northern South Africa with its bulk in Namibia, and 140 km wide to the great western escarpment (Lancaster *et al*., 1983). The central Namib is one of the drier parts of the Namib. Average precipitation ranges between 5 mm/year in areas closer to the coast and 60 mm/year in areas further inland (Lancaster *et al*., 1983, Eckardt *et al*., 2013). Fog is another characteristic that defines the central Namib; it is more regular than rain and it reaches up to 100 km inland (Lancaster *et al*., 1983, Eckardt *et al*., 2013). The temperatures in the central Namib are extreme, ranging between -1.7 – 42.8°C (Lancaster *et al.*, 1983).

The Namib supports a variety of fauna and flora including a great diversity of endemic organisms. These organisms have behavioural and physiological adaptations to cope with extreme conditions of the Namib (Eckard *et al.*, 2013). The !nara, Black-backed Jackals, and hairy-footed gerbils are some of the organisms that are adapted to living in the harsh conditions of the Namib. Most animals get their water from the food they eat, for example, jackals get water from consuming a juicy !nara melon. Some plants have developed strategies to harvest fog water or depend on the underground aquifer (Klopatek & Stock, 1994).

**Figure 1.4**: Map of Namibia showing the Namib Desert running along the west coast (demarcated by red line) and Gobabeb-Namib Research Institute in the central Namib (09 September 2020, Google Earth).

## !Nara seed dispersers

The primary !nara seed disperser is considered the Black-backed jackal (*Canis mesomelas*, Fig. 1.5; Müller, 2000). It belongs to the family *Canidae*, and is endemic to east Africa and southern Africa (Kingdon, 2014). This species occurs in a wide variety of habitats including arid coastal deserts, savannah ecosystems, grasslands and farmlands (Kingdon, 2014). It is the only species of jackal inhabiting the Namib Desert. In the central Namib Desert at Cape Cross seal reserve, the jackals have a home range of 24.9 km$^2$ and they move about 7–12 km/day or more (Hiscocks & Perrin; 1998), which may vary with resource availability and landscape structure. These movement distances suggest that they may be excellent long-distance dispersers. They are unspecialised feeders, consuming vertebrates, invertebrates, and plant materials (Stuart, 1976; Hiscocks, 1987 Kingdon, 2014). Fruit have been recorded to be part of the jackal's diet in southern Africa (Stuart, 1976; Hiscocks, 1987; Kauda & Skinner, 2003; Goldenberg *et al.*, 2010; Humphries *et al*., 2015). The diet of jackals in the Namib Desert is reported to contain 50% plant materials, mainly made up of !nara seeds (*A. horridus)* and false ebony (*Euclea pseudebenus*) seeds during fruiting seasons (Stuart, 1976; Goldenberg *et al.,* 2010). The seeds left on the surface in jackal scat are likely to attract gerbils, which are considered secondary !nara seed dispersers as they collect and bury seeds in different microhabitats.

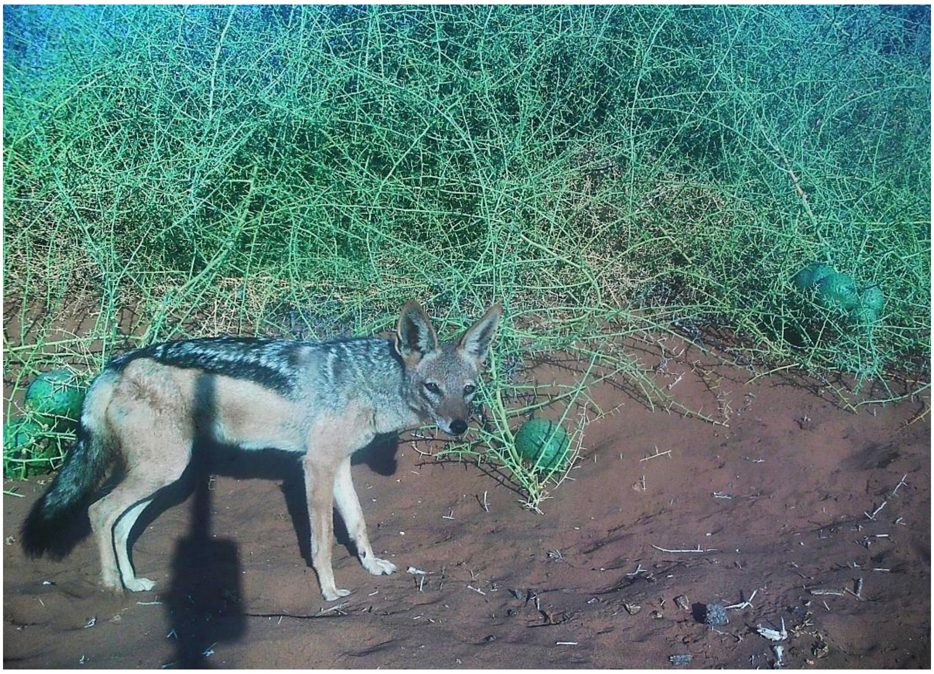

**Figure 2.5:** The Black-backed Jackal (*Canis mesomelas*). Photograph taken by a camera trap on a !nara plant at Gobabeb (2019).

Hairy-footed gerbils (*Gerbilliscus* (*Gerbillurus)* spp.) belong to the order Rodentia and family Muridae (Happlod, 2013). All species of this genus are endemic to Africa and occur in the Namib Desert. The most widespread species is *Gerbillurus paeba* that occurs from the southwestern arid biomes and Cape Floristic Kingdom into the savannas of southern Africa

(Perrin *et al*., 1999; Happlod, 2013). *Gerbillurus tytonis* and *Gerbillurus setzeri* are endemic to the Namib Desert. The distribution of *G. tytonis* and *G. paeba* (Fig. 1.6) overlaps with that of the !nara populations along the Kuiseb River in the central Namib Desert. Gerbils are omnivores and opportunistic feeders, consuming insects, foliage and seeds, and their diet will vary depending on the availability of food resources (Perrin *et al*., 1999; Happlod, 2013). All four species are believed to exhibit scatter-hoarding behaviour, caching seeds for later consumption. At Gobabeb, gerbils were recorded to predate on and scatter-hoard !nara seeds (Müller, 2000; pers. obs.).

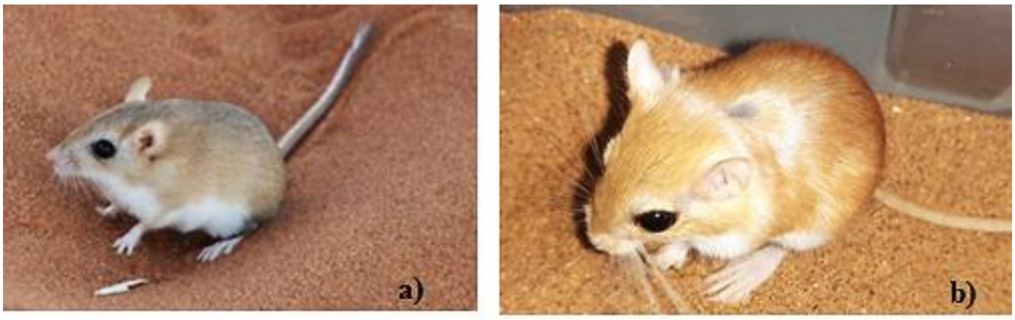

**Figure 1.6:** a) *G. paeba* and b) *G. tytonis* of the central Namib. Photographs by Handjaba (2020).

## Study objectives

Generally, the relative contribution of primary and secondary seed dispersers to plant distribution and plant population dynamics has received limited scientific attention (Ruiz *et al*., 2009). This is certainly the case for !nara. This study examined the respective roles of Black-backed Jackals and hairy-footed gerbils in !nara seed dispersal and whether they may be considered key dispersers of this valuable, endemic Namib plant species. Improved understanding of !nara seed dispersal and the behaviour of its seed dispersers may also contribute to understanding the disjunct and fragmented distribution of !nara in the Namib Desert by understanding factors such as distances that seeds were moved from parent sites, habitats in which seeds are deposited and seed viability.

Black-backed Jackals are well-known predators of !nara melons (Klopatek & Stock, 1994; Berry, 2001; Henschel *et al*., 2004), together with other animals such as donkeys, Oryx and hyenas (Marais, pers. comm., 2018). However, the degree to which they and other animals utilise ripe !nara has not yet been studied. I used camera traps to observe !nara frugivory by jackals. Undamaged seeds have been recovered from jackal scat (Muller, 2000; pers. obs.), but it is still unclear whether such seeds are indeed viable for germination. In addition, if endozoochory is a deliberate seed dispersal strategy for !nara, it can be expected that ingestion should also contribute to improved seed germination. These investigations, with details on the questions, methodology and results, are the focus of Chapter 2.

The second species of interest regarding !nara seed dispersal in the central Namib Desert are the hairy-footed gerbils (Müller, 2000). In particular, it is important to know to which degree they would detect and select !nara seeds; how far they would move the seeds and how deep they will bury the seeds; the recovery rate; and whether their scatter-hoarding behaviour would result in 'lost' seeds as is the case in other plant seeds dispersed by rodents (Weighill *et al.*, 2017; White *et al.*, 2017). The experimental trials and observations to examine the role of scatter-hoarding gerbils, including details on the methodologies and results, are discussed in Chapter 3.

# Chapter 2: !Nara seed dispersal by Black-backed Jackals

## Abstract

Black-backed Jackals (*Canis mesomelas*) may play a vital ecological role in the Namib Desert by feeding on !nara fruit and disseminating the ingested seeds to new sites. Such seed dispersal is important for maintaining population structures and dynamics, gene flow and reducing inbreeding within !nara populations. I examined the effectiveness of Black-backed Jackals as !nara seed dispersal agents in the central Namib. The data were collected from exclosures that only allow jackals and species of similar body size or smaller access to the !nara plant while excluding the large herbivores such as donkeys and Oryx. The effectiveness was determined firstly by calculating the visitation rate and fruit removal rate from camera trap monitoring data, and secondly by examining the viability of seeds removed from jackal scat by executing laboratory germination trials. Jackals were the dominant species to visit !nara with mature fruit (2.5 ±0.2 visit per day) and the only !nara frugivore (1.03±0.1 melons removed per day) recorded on the camera traps during two !nara fruiting seasons. Other behaviour such as urinating on melons and sniffing of melons was also noted. Scent samples from !nara melons were analysed to determine if ripe melons produce volatile compounds that may potentially influence the choice of melons by individual jackals. Results showed that 77.9% of the volatile compounds produced by ripe !nara melons was Pyrazole, while only minute quantities were detected in unripe melons. Other compounds produced by ripe melons, but not unripe melons, were Acetoine (11.2%), 2-Pyrrolidinone (2.1%), and Butan-1-ol (1.5%). The viability of !nara seeds extracted from jackal scat (72.8%) was not significantly different from that of control !nara seeds (71.4 %). The germination rate of !nara seeds from jackal scat was significantly higher (50.4%) than control !nara seeds (34%). Although viability did not change, ingestion of !nara seeds by jackals increased germination rate. This study provided evidence that Black-backed Jackals are key dispersers of !nara plants in the central Namib Desert.

**Keywords**: endozoochory, visitation rate, carnivore frugivory, germination rate, seed viability, Cucurbitaceae

## Introduction

The ecology and importance of seed dispersal by vertebrates such as primates, carnivores, birds and bats in many ecosystems have received extensive attention (Motta-Junior & Martins, 2002; Colon & Campos-Arceiz, 2013). Seeds from a large number of species, 67% gymnosperm and 27% angiosperm, (Traveset *et al*., 2007) are dispersed via the digestive tract of vertebrates, referred to as endozoochory or endozochorous seed dispersal (Traveset *et al*., 2007; Corlet, 2017; Spennemann, 2019). Animals feed on fresh fruit and defecate or regurgitate intact seeds away from the parent plant, often into suitable habitats for germination. Endozoochory has additional advantages for dispersal as seeds benefit from ingestion by seed coat scarification that may increase seed viability and germination rate (Travest *et al*., 2007; Colon & Campos-Arciez, 2013; Spennemann, 2019). Furthermore, endozoochory also contributes to increased dispersal distance, especially by animals with long gut retention time (Rost *et al*., 2012) and large home ranges. Once voided, seeds dispersed by endozoochory can be moved to micro-sites by rodents, ants and other seed harvesting animals, thus providing additional benefits such as increasing soil seed bank diversity, improved seed survival away from surface predators and higher chances of germination and seedling survival (Ruiz *et al*., 2010).

Dispersal by carnivorous mammals has attracted some attention by researchers (Herera, 1989; Motta-Junior &Martins, 2002; Rost *et al*., 2012). Canids also contribute to seed dispersal as they frequently consume fresh fruit and defecate undamaged seeds (Varela & Bucher, 2006), contributing to the distribution of plants. Endozoochory by canids has been examined mainly in foxes and wolves (Motta-Junior & Martins, 2002; Santos *et al*., 2003; Silver *et al*., 2005; Varela & Butcher, 2006) as well as in some small carnivores such as martens and badgers (Schaumann & Heinken, 2002; Otani, 2002; Zhou *et al*., 2008) in semi-arid, tropical and mediterranean regions. The role played by other canids such as jackals has received little attention, especially in desert and savanna ecosystems as in southern Africa.

Black-backed Jackals (*Canis mesomelas*), hereafter simply called jackals, belong to the family Canidae, genus *Canis* and are endemic to East Africa and southern Africa (Kingdon, 2014). This species occurs in a wide variety of habitats including arid coastal deserts (Kingdon, 2014), open savanna (Moenlman, 1983; Fuller *et al*., 1989), woodland savanna (Loveridge & Macdonald, 2002), grasslands and farmlands (Rowe-Rowe, 1982; Kingdon, 2014). In the Namib Desert, at the Cape Cross Seal Reserve, jackals have a home range of

24.9 km$^2$ and move about 7–12 km/day, or more (Hiscocks & Perrin; 1988). Home ranges and movement of jackals may vary depending on resource availability and habitat structure.

Jackals are unspecialised canids feeding on vertebrates, invertebrates, and plant materials. Fruit have been recorded to be part of its diet in southern Africa (Stuart, 1976; Hiscocks, 1987; Kauda & Skinner, 2003; Goldenberg *et al.*, 2010; Humphries *et al.*, 2015). Jackal diet in the Namib Desert is reported to contain 50% plant materials, mainly made up of !nara melons (*Acanthosicyos horridus)* and false ebony berries (*Euclea pseudebenus*) during fruiting seasons (Stuart, 1976; Goldenberg *et al.*, 2010). !Nara is a cucurbit plant that produces 20 – 500 melons per plant per season, with a mass of 0.6–1 kg (Berry, 2001), and contains 150–350 seeds per melon (pers. obs.). Many other animals are known to feed on the !nara melons i.e. Oryx, ostriches, donkeys, cattle, porcupines, elephants and other opportunistic fruit predators (Müller, 2000; ). The faeces of some of this species i.e. elephants has not been examined for !nara seeds, since they are not found in the central Namib where the study took place. For those that were examined (Oryx, ostriches, donkeys, jackals, cattle, and hyenas) undamaged seeds were only found in jackal scat, suggesting it is the only primary !nara seed disperser in the central Namib Desert (Müller, 2000). Other animal species where faeces contained pieces of !nara seeds were considered seed predators because they crushed seeds during feeding (Müller, 2000). Although the dietary composition of jackals has been examined (Stuart, 1976; Goldenberg *et al.*, 2010; Humphries *et al.*, 2016), little attention was paid to their potential role in plant seed dispersal. For instance, it is not known whether it is an opportunistic disperser or whether the !nara has evolved traits that specifically attract jackals.

Jackals may play a vital ecological role in the Namib Desert by feeding on !nara fruit and disseminating the ingested seeds to new sites. Such seed dispersal is important for plant communities by maintaining gene flow and reducing inbreeding within !nara populations (Rost *et al.*, 2012). Topnaar people residing along the Kuiseb River, in the Namib-Naukluft Park, Namib Desert, have reported that jackals harvest !nara melons and bury them away from the plant (Samuel Gowaseb, 2018; pers. comm.). Jackals are also known to cache surplus food for later use (Bothma, 1998; Kingdon, 2014). Caching of melons by jackals would also contribute to the dissemination of !nara seeds, especially if the cached melons were not retrieved.

The effectiveness of !nara seed dispersal by jackals will depend on feeding behaviour; fruit processing; the presence of viable seeds in scat; and seed germination rates, and seedling survival. Post-feeding movement of jackals (where faeces with seeds are deposited) may also affect the quality of this dispersal (Herera, 1989; Silver *et al*., 2005). While the qualitative component of likely jackal seed dispersal (presence of intact seeds in scat and germination ability of seeds) has been examined to some extent (Müller, 2000), there is little known about the quantitative component of this dispersal (visitation and fruit removal). Quantitative studies have been limited by methodology constraints (Herera, 1989; Prasad, *et al*., 2010; Campos *et al.*, 2018) but with modern advances in technology such as camera trapping it has become easier to observe animals without influencing their activity.

In this study, we quantify the role and effectiveness of Black-backed Jackals as !nara seed dispersers by using infrared-enabled cameras to monitor jackal visitation rate, !nara fruit removal, and other jackal behaviour associated with fruit selection and harvesting. Furthermore, !nara melon scent was analysed to determine if ripe melons produce volatile compounds that may contribute to melon selection by jackals. The fate of seeds dispersed by jackal via endozoochory was also examined through laboratory viability and germination trials as we expected higher viability and germination rates in seeds ingested by jackals compared to !nara seeds that were not ingested (control experiment).

## Materials and Methods

### Study site

The study was conducted between December 2018 and January 2020 in the central Namib Desert within the Namib-Naukluft Park along the ephemeral Kuiseb River in the vicinity of Gobabeb - Namib Research Institute (Fig. 2.1). The Namib Desert is situated along the eastern coast of the Atlantic Ocean and is characterised by low rainfall (5–60 mm/a, Lancaster *et al*., 1983; Eckardt *et al.*, 2013) and extreme temperatures (-1.7–42.8°C, Lancaster *et al*., 1983). There are three ecosystems close to Gobabeb: Gravel Plain; Aeolian Dunes Desert and  Kuiseb Ephemeral River linear oasis. !Nara populations occur along the outer banks of the Kuiseb River and at the base of the dunes.

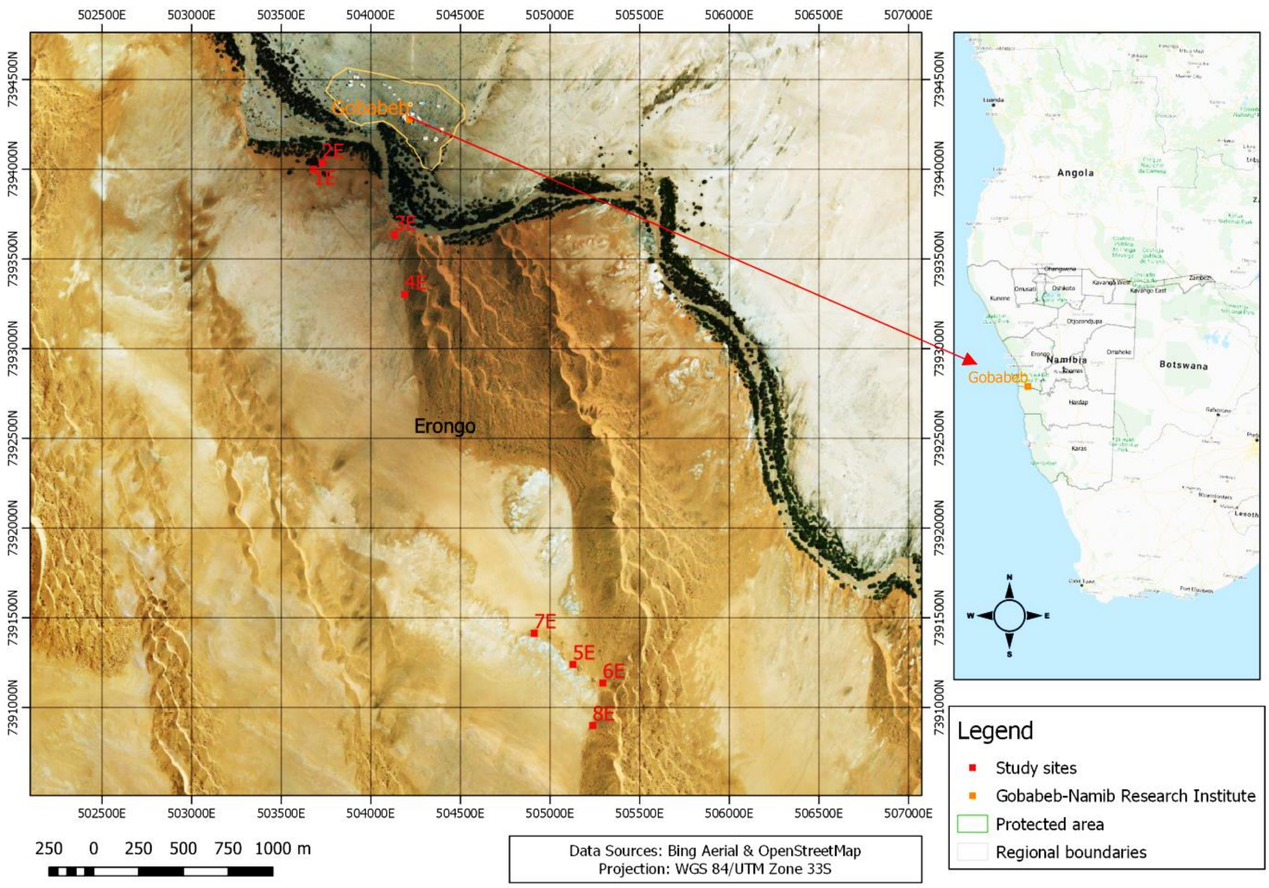

**Figure 2.1:** Satellite image showing the study site near Gobabeb. Eight plants that were monitored with camera traps are marked in red; some were close to the Kuiseb River, while others were at the base of dunes.

### Experimental procedures

The faeces of Oryx, donkeys, cattle and jackals were collected around the !nara plants and surrounding area and examined for intact seeds. In agreement with Müller (2000), we found that only jackal scat contained intact !nara seeds, allowing us to focus on jackals alone for this study. We therefore focussed on female plants in exclosures (Fig. 2.2) to exclude large herbivores such as donkeys, Oryx, cattle and springbok from accessing the plants and ripening melons, while still allowing access by jackals and other animals of similar or smaller body size.

**Figure 2.2:** A fenced-off !nara hummock. Large herbivores are excluded, while still allowing access by jackals and other animals of similar or smaller size (Fences sponsored by Dartmouth College, USA for a herbivory exclusion project).

### Visitations and Frugivory

To investigate !nara visitation, fruit removal and behaviour of jackals, Infrared (IR)-enabled CamPark motion sensor hunting trail camera traps (n = 14), model T45, were set up to monitor different fenced-off female !nara hummocks (n=8) around Gobabeb during two fruiting periods, December 2018 – March 2019 and again November 2019 – January 2020. The motion sensors of the cameras triggered infrared flashes at night that animals cannot detect, thus the nocturnal behaviour of jackals was not disrupted. The number of the cameras at each of the !nara hummocks was determined by the size of the hummock. These wide-angle trail cameras were secured to fence poles, or deployed on tripods at least 0.5 m above ground and 1 m away from the melons. The cameras were positioned to include as many melons as possible to maximise capture of visitations and fruit removal. The cameras were set as follows: three photos and a 60 seconds video per trigger; shot lag two minutes between triggers; middle sensitivity; 1280x720P video resolution; 14MP (4416x3312P) photo resolution; and 24 hours target capture time. The data recorded on the camera traps were downloaded every three weeks, at which time batteries were also checked to ensure adequate power.

A visit was defined as when an animal visited a plant, regardless of whether it removed melons or not. Visits may have occurred on multiple cameras at a hummock, thus records were considered as one visit if they were captured within two minutes of each other. The number of fruit that were removed was determined as the sum of melons removed during each visit to a plant. Other behaviour was noted such as sniffing of and urination on melons. Since each photo or video from the camera traps have a photo stamp of date and time, the daily activity patterns of jackals were also recorded (time at which the jackal visited the !nara) and analysed.

### Melon hoarding

To test whether jackals buried melons, two methods were used to track melons that were removed by jackals. Firstly, tracks around the !nara were followed in the morning to determine if jackals buried melons away from the plant for later use. Secondly, the ends of 200 m reverse-wound threads on bobbins were attached or glued to selected melons (n=23) while still on the plants. The bobbin allowed the thread to unravel without friction so that jackals could not feel that it is pulling on something when it leaves with a melon. The container with the bobbin was secured to the stem of the plant with a cable tie to make sure that the jackal did not drag the entire container with it. The thread trails were tracked on foot.

### Olfactory cues

We deduced from the camera trap data that jackals seem to use smell to select ripe melons (it is hard to visually differentiate between ripe and unripe melons as they have the same pale green outer colour. To determine scent differences between ripe and unripe fruit, four ripe melons and four unripe melons were harvested from two of the most frequently visited !nara plants for scent analysis. Melons were collected with the aid of an experienced local harvester (Jeffrey Khurisab, 2019). A Spectrex PAS-500 personal air sampler, scent traps and glass tubes were used for scent sampling. A glass tube with a scent trap was attached to the air sampler and placed in a plastic bag together with one of the !nara melons (Fig. 2.3). Both ends of the plastic bag were fastened with wool threads to prevent air from escaping or entering while sampling. The pump was left running for three hours as the melons seemed to have a weak scent. The scented traps were then sealed in a small glass vial and stored in a fridge. Each vial was labelled with the date, start time, end time, species name and whether the melon was ripe or unripe. Melons and scent traps were handled using latex gloves to prevent contamination of the samples. All the samples were then sent to the Johnson Pollination Research laboratory at the Kwa-Zulu Natal University in South Africa for analysis.

In addition, 16 ripe and 16 unripe !nara melons were harvested and buried, as a form of a “cafeteria” experiment, at 10 cm depth and distances of 100 m and 200 m away from the closest !nara plant to test whether jackals would locate the ripe melons using scent. Camera traps were used to monitor the melons that were buried (ripe versus unripe). Also whether the jackals first removed the ripe or the unripe melons and also whether they sniffed the melons first before picking one. Together these results may suggest the use of olfactory cue by jackals. This was done after we have observed on camera trap footage that jackals seem to sniff melons before picking one or moving away to another plant.WGS 84 and coordinates recorded with a handheld GARMIN etrex 10 version 3.40 GPS device. Melons were also handled using latex gloves.

**Figure 2.3:** A Spectrex PAS-500 personal air sampler being used to collect a scent sample from a !nara melon that was enclosed in a plastic bag to prevent any odour contamination.

### Seed viability and germination rate

Jackal scat containing intact !nara seeds were collected opportunistically between 2018 and 2020 to determine the viability and germination rate of ingested seeds. Additional seeds were manually extracted from 26 ripe melons by hand, using latex gloves, and then air-dried to be used as experimental controls. All seeds extracted from melons or scat were subjected to a floatation test to determine seed maturation. Only seeds that sank when submerged in water were used in experiments. All the extracted and tested seeds were air-dried and stored in paper bags at room temperature (±25°C) prior to further testing. The germination rate and viability of these seeds were assessed through laboratory experiments.

The seeds were divided into two sets for the germination trials: (a) control seeds (n=141; hand extracted from several melons as described above) and (b) ingested seeds extracted from jackal scat (n=141). All seeds were immersed in water for 24 h as a pre-germination treatment, after which sets of seeds were germinated according to the method described by Silva *et al.* (2005). Seeds were germinated in petri-dishes containing 1-Whatman filter paper saturated with 5 ml of deionized water. The temperature was kept constant at ±25°C, while natural cycles of 12 h light and 12 h dark were maintained. Each petri-dish contained eight seeds and was kept for 30 days in a sealed zip-lock bag to prevent drying out and to retain moisture. A seed was considered as having germinated if the radicle was observed within the trial period. The number of seeds that germinated was recorded for each set to calculate the percentage germination.

At the end of the trial period, seeds that failed to germinate within the 30 days were tested for viability (Harrington *et al.*, 2011; Varela & Butcher, 2006; Silva *et al.*, 2005) using 2,3,5-Triphenyltetrazoliumchloride (TCC). The seeds that went bad from both treatments during the germination trial were excluded from the viability test; therefore only 70 seeds were selected for each treatment. For this test, seed coats were removed and the embryos immersed in TTC and kept in the dark for 24 hours (Silva *et al.*, 2005). Seeds were considered viable if the embryo fully or partially turned pink or red and dead if the embryo remained white. The percentage viable seeds was calculated for each set of !nara seeds.

## Data analysis

All data were analysed using R (R Core Team, version 3.6.1, 2019). The daily jackal visitation rate was calculated as the total number of visitations divided by the total number of sampling days, while the fruit removal rate per visit was calculated as the total number of removed fruit divided by the total number of visitations. The average monthly visitation rate for each observed plant was also calculated as the total number of visitations divided by the number of sampling months. A generalized linear model (Poisson (log link)) under the package MASS (Venables & Ripley, 2002), was used to evaluate if the number of melons on the plant influenced the number of visitations. Visitation was the response variable and the number of melons per month was the predictor variable, while individual plants was used a random factor. A generalised linear model (binomial (logit link)) was used to test the proportion of seeds that germinated against those that did not for the two sets of !nara seeds (extracted from jackal scat vs control seeds). The response variable was proportion of seeds that germinated and the predictor variable was treatment (ingested seeds vs control seeds).

The data was visualised in the table using sjPlot (Lüdecke, 2020). The model that best fits the data was selected using Akaike information criterion (AIC, Appendix 2.4).

## Results

Eight fruiting !nara (*A. horridus*) plants in exclosures were monitored continuously with camera traps across two fruiting seasons for three months each, or a  total of 200 days (4800 hours), between 2018 to 2020 to observe visitation by medium body size species (i.e. jackals) and smaller body species (i.e. mongoose). The IR enabled motion sensor cameras recorded daily 24 hour activity of animals within 15 m of the camera at the !nara plants. We recorded all animals (examples in Fig. 2.4) that were captured on camera and classified them as (i) frugivores if they fed on !nara melons, visitors if they did not feed on !nara melons, or (iii) residents. Rodents were the most abundant animals observed around the !nara plants, but they were classified as residents, because they live within the !nara hummocks and did not interact with the melons. Black-backed Jackals (*C. mesomelas*) were the most frequent visitors (93.2%, n=508) and the only frugivore recorded on camera. Other visiting species that were not observed to consume any !nara melons included Cape Fox (*Vulpes chama*, 3 visits), Slender Mongoose (*Gelerella singunea*, 3 visits), Small Spotted Genet (*Genetta genetta*, 7 visits), hares (2 visits), African Polecat (*Ictonyx stiatus*, 1 visit), and various bird species.

**Figure 2.4:** IR-enabled camera photographs of (A) Black-backed Jackal (*C. mesomelas*) sniffing, and (B) carrying, a !nara melon . (C) Cape Fox (*V. chama*) and (D) Small spotted Genet (*G. genetta*) classified as visitors as they were not observed removing or consuming !nara melons.

### Visitation rate and frugivory

Only jackals were considered for frugivore analysis because they frequently visited the fruiting !nara plants and removed or consumed melons. On average, there were 2.5±0.2 visits per day for all eight plants (max=19 per day, n=200 days, mean of 0.32±0.2 per individual !nara plant) and a fruit removal rate of 1.03±0.1 per day (max= 6 melons per day, n=137 days) by jackals across all plants. We observed that jackals only harvest one melon per visit and carried it away from the plant for consumption. On rare occasions, they consumed the melon in close proximity to the plants that were being monitored.

### Visits and number of melons

The number of visitations per month seems to be negatively correlated to the number of melons on the plants (Fig. 2.5). However, this seems related to the outliers as the trend is appeared to be fairly random if outliers are removed. The generalised linear mixed effect model indicated the number of melons has a significant effect on the number of visitation ($p<0.001$, appendix 1.2).

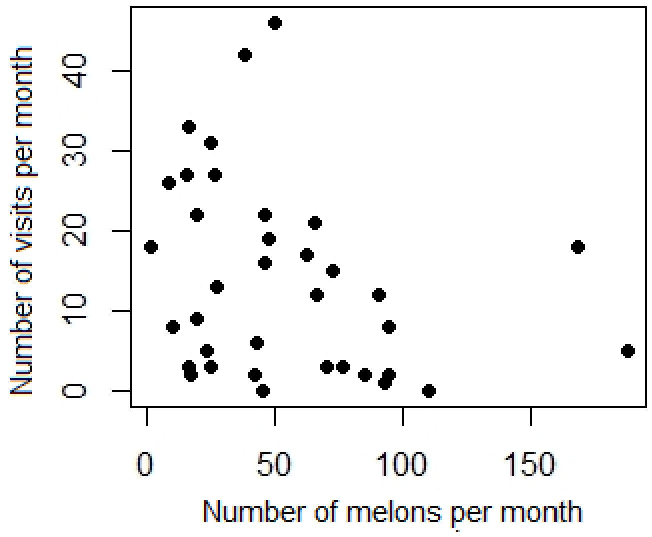

**Figure 2.5:** The relationship between the number of melons on each plant per month and the number of visits per month. Monthly visitation rate decreased with an increase in the number of melons.

### Activity and behaviour patterns

Jackals were mostly nocturnal with activity ranging between 16:00 and 09:h00, peaking from 19:00 to 22:00 (Fig. 2.6). No activity was recorded during the middle of the day (10:00 to 15:00), confirming the relevancy of camera traps for the study.

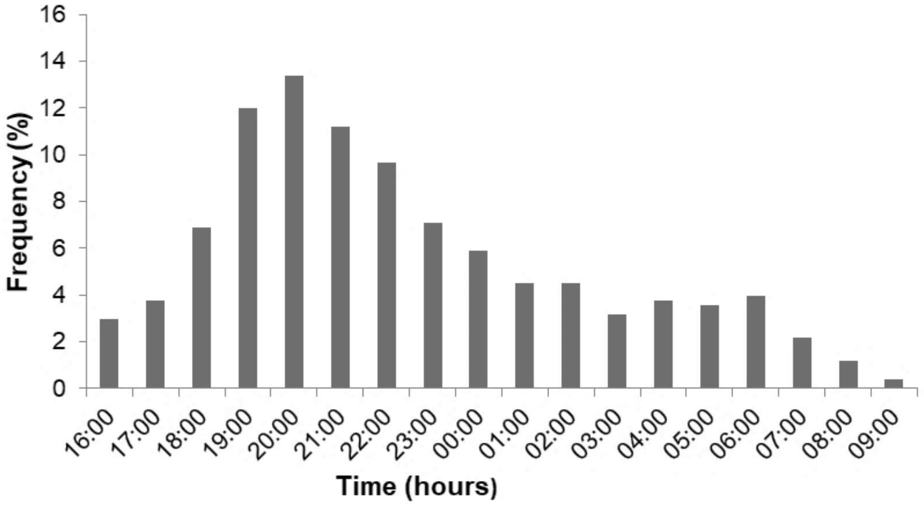

**Figure 2.6**: Temporal activity patterns of visits (n=508) by jackals to fruit-bearing !nara plants monitored by camera traps in the central Namib Desert.

Only a single jackal was observed at a time during visits - no pairs were recorded by the cameras. The most commonly observed behaviour was sniffing of melons (48.4%, n=508 visits) and the least observed behaviour was urinating on melons (1.4%, n=508 visit, Fig 2.7). We also observed that jackals dig out melons that were deep within a spiny !nara thicket.

**Figure 2.7:** Frequency of occurrence (%) of specific types of behaviour during visits by jackals (n=508) to fruit-bearing !nara bushes as observed by camera traps.

## Melon hoarding

No evidence was found that jackals cache !nara melons for later consumption from tracking footprint or threads from bobbins. The bobbin threads attached to melons indicated that most of those picked melons were consumed 50–100 m (n=17) away from the plant. No buried melons were found. Jackals sometimes carried the melons further than the bobbin thread length of 200 m (n=6), making it difficult to determine maximum dispersal distances of whole fruits. Given that jackals have large territories and that they may move long distances in a day, such whole fruit dispersal distances could be at the scale of kilometres.

## Olfactory cues

Jackals were observed to sniff melons, presumably to distinguish ripe melons from unripe melons. It is assumed they smell volatile compounds released by ripe melons. Camera trap footage showed that jackals were also able to locate buried melons (100%, n=32) and select ripe melons from unripe melons by sniffing. Jackals also urinated (6%) on some of the experimentally buried melons and then left the melon for a few days, before retrieving them. We were unable to identify whether the same individual that urinated on the melons was also the one to retrieve them.

**Table 2.1:** The volatile compounds produced by ripe (n=4) and unripe (n=4) !nara melons. It is assumed the unripe melons had a weaker scent as chemical analysis failed to isolate most of the compounds found in the scent of ripe melons. The dominant volatile compounds in ripe melons are highlighted in grey.

| **Compound class** | **Compound identification** | **Formal name for publications** | **Ripe (%)** | **Unripe (%)** |
|---|---|---|---|---|
| Aliphatic alcohol | 1-Butanol | Butan-1-ol | 1.5 | 0 |
| **Aliphatic ketone** | **2-Butanone, 3-hydroxy-** | **Acetoine** | **11.2** | **0** |
| Aliphatic acid | ACETIC ACID | Acetic acid | 3.3 | <0.001 |
| Sulphur compound | Dimethyl sulfoxide | | 0.0 | 0 |
| Aliphatic acid | Propanoic acid, 2-methyl- | Isobutyric acid | 1.4 | 0 |
| Aliphatic acid | Butanoic acid | Butanoic acid | 0.2 | 0 |
| lactone | Butyrolactone | | 0.5 | 0 |
| Aliphatic acid | Butanoic acid, 2-methyl- | 2-Methylbutanoic acid | 1.6 | 0 |
| **Nitrogen-containing compound** | **Pyrazole** | | **77.9** | **<0.001** |
| Nitrogen-containing compound | 2-Pyrrolidinone | | 2.1 | 0 |
| Nitrogen-containing compound | 3-Pyridinol?? | | 0.3 | 0 |

Analyses of volatile compounds producing the scent of ripe and unripe melons indicated that the most abundant volatile compound produced was Pyrazole (77%), followed by Acetoine (11.2%, Tab. 2.1, Steve Johnson, pers. comm., 2020**).** It is most likely that jackals use one or more of these compounds as olfactory cues for ripeness. Of course jackals may be using some other compound such as 2-Methylbutanoic acid, 2-Pyrrolidinone or other volatiles (Tab. 2.1) that were not detected in unripe melons as a cue, even if they only appear in small quantities. For human senses, unripe melons have low scent emission as was confirmed by the virtual absence of volatile compounds in scent traps.

### Germination rate and viability

We extracted 200 intact seeds from jackal scat (n=8) found during !nara fruiting seasons around Gobabeb. These scat samples were from the inter-dune and gravel plains adjacent to the !nara population. The furthest scat sample was 0.74 km away and the closest 0.07 km from the nearest !nara.

Laboratory germination trials indicated that more !nara seeds retrieved from jackal scat germinated (50.4%, n=141) within a 30 day period as compared to control seeds (34.0%, n=141). The generalised linear model (GLM) showed a statistically significant difference between the proportion of seeds that germinated from jackal scat than those from control seeds (p=0.0006, see Appendix 1.2).

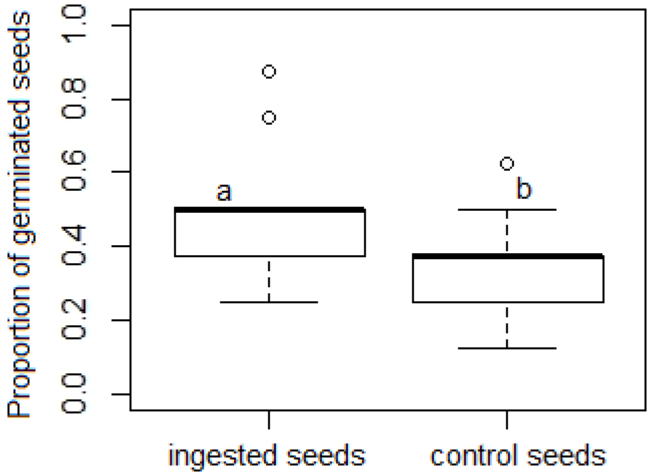

**Figure 2.8:** The proportion of seeds that germinated from seeds retrieved from jackals scat (ingested seeds, n=141) and seeds extracted by hand from ripe melons (control seeds, n=141). The letters represent significant differences at 95% confidence interval. The boxplots indicate the median, upper and lower quantile and 1.5 interquartile range.

The results from the viability test (2,3,5-Triphenyl Tetrazolium Chloride) after concluding the 30 day germination trial indicated that 72.8% (n=70) of the remaining seeds extracted from jackal scat were viable for germination, while 71.4% (n=70) of control seeds were viable for germination.

## Discussion

We recorded for the first time quantitative information on visitation rates, fruit consumption, daily activity period and behaviour of !nara frugivores by using camera traps in the central Namib Desert. Our results indicated that Black-backed Jackals, like other canids such as foxes and wolves (Silver *et al.*, 2007; Verela & Bucher, 2006), also consume considerable quantities (1.03±0.2 melons per day) of fruit when available, in this case !nara melons. !Nara melons may be an important source of food and water to the jackals in the central Namib, as 93.3% of all visits to fruiting exclosed !nara plants were by jackals . It was the only species recorded to feed on !nara melons for 200 days, over two seasons out of all observed animals which only included small and medium sized animal species (large herbivores where excluded from the experiment with exclusion fences). A logical conclusion is that jackals play an important dispersal role in consuming the pulp and ingesting this, with subsequent voiding of !nara seeds away from its parent plant.

Other species recorded at monitored !nara plants (Fig. 2.4) indicated that the plant also provides an important habitat for a variety of vertebrates in the study area. The dominance of jackals as !nara frugivores may be due to the tough, spiny rind of !nara melons, which may prevent small mammals and birds from opening the melons. Accessing !nara seeds require the removal of the pulp, which contain germination inhibitors (cucurbitacins) like most other cucurbits (Bothma & Grobbelaar, 1981). Although jackals were the only species recorded in this study as a !nara frugivore, large herbivores such as Oryx and domestic donkeys have been seen consuming !nara melons. However, they are not considered as seed dispersers because the seeds are crushed during feeding (Müller, 2000; pers. obs.) and were excluded from the fenced !nara plants that were monitored.

The average visitation rate per month does not increase with an increase in the number of melons (Fig. 2.5). This may be due to the number of ripe melons, more melons on a plant does not imply ripeness. Jackals may be attracted to !nara plants with ripe melons regardless of melon density. The jackal visitations occur mostly at night (19:00–06:00; Fig. 2.6), indicating that jackals are mainly nocturnal as in other studies (Nel *et al.*, 2012), and other ecosystems (Kauda, 2000; Ferguson *et al*., 1988), although they were also active in the late afternoons and early mornings. The camera traps record feeding behaviour at night, which provides a more accurate picture on the frugivorous dynamics of jackals on !nara melons, and an important insight on plant-animal interactions (Rivas-Romero & Soto-Shoender, 2015).

Jackals were observed to dig in order to gain access to some melons when the melon was deep in the plant thicket. !Nara plants have stems with copious sharp spines that are inter-twined to form a dense thicket. It can be painful and takes an effort to harvest melons that are deep in the thicket. Digging for !nara shows the extent to which  jackals will go to obtain !nara melons and thus the importance of !nara melons to jackals. Another interesting behaviour was that jackals urinated on melons (1.4%). Even if not common, urinating on melons could be an indication that jackals may mark particular melons in the same way that they mark territories and/or an attempt to hide the melon odour from other jackals that may visit.

Although no evidence was found that jackals hoard melons, they were able to locate experimentally buried melons, which indicate that they will be able to recover a melon using scent if jackals do indeed sometimes hoard melons. The absence of evidence might be a limitation in methodology as jackals may have buried !nara melons further than we could track them or carried them to their dens. In some cases (n=2) thread on the bobbins ended or snapped before the melon was consumed or hoarded. When the Topnaar people harvest unripe melons, they bury them for 2–3 days to allow them to ripen; they assert that this is an example they copied from jackals (Linda Bees, 2019, pers. comm.). Jackals have been previously reported to hoard other food items such as meat for later consumption (Bothma, 1998; Kingdon, 2014), thus hoarding of melons is a possibility.  If jackals do indeed hoard melons it may contribute even more to !nara seed dispersal if buried melons are not retrieved, or consumed in areas without existing !nara populations. Further studies or testing needs to be done using more refined methods to determine for certain if and when jackals would hoard !nara melons. Potentially using stronger and longer threads on bobbins or using tracking devices on melons, may allow tracking of melons carried far.

Jackals were very selective, seemingly using scent as a cue for selecting ripe melons as 48.4% of jackal visitations (n=508) included sniffing of melons (Fig. 2.7). The odour of ripe melons is clear and strong, even for humans. Chemical analysis of scent samples showed that the most abundant volatile compound found in ripe melons was Pyrazole (77.9%, Tab. 2.1), which is also found in watermelons (Tikuchi *et al.*, 2018). The other compound is Acetoine (11.2%), which has a pleasant, buttery odour and is produced by fermentative bacteria. Acetoine is used as flavoring in butter and found in apples, yoghurt, and wheat (Xiao & Xu, 2007; Xu *et al*., 2012 and Bao *et al*., 2015). Other minor compounds that were detected in ripe melons included 2-Pyrrolidinone, Acetic acid, 2-Methylbonanoic acid, and Butan-1-ol (Tab. 2.1). The rind of the melons does not change colour as an indication of ripeness, thus the volatile compounds produced by ripe !nara melons could be an indication of ripeness, since unripe melons had weak scent and low levels of volatile compounds. Other animals especially bats and primates have been reported to use scent as a ripeness signal (Luft *et al*., 2003; Nevo & Heymann, 2015; Nevo *et al*., 2016) and it has been suspected that olfaction in fruit may be an evolved signal rather than a cue (Nevo *et al*., 2016).

It is not clear if there is a specific compound that jackals may associate with ripeness or if they are just attracted to melons with a strong odour. Future studies should examine which volatile compounds jackals may associate with ripeness, if any. A cafeteria experiment using artificial compounds would show if jackals have a preference or not. In addition, more scent samples need to be analysed to determine if these compounds are only produced by ripe melons or if low concentrations of the same compounds in unripe melons just makes it hard to sample.

Intact seeds were extracted from jackal scat. Some 200 intact seeds were extracted from eight scat samples, which proved that many seeds are not damaged through mastication. The ability of !nara seeds to survive digestion can be attributed to the hardness of the seed coat and the lack of specialized digestive tracts or digestive enzymes in jackals for exploiting seeds (Varela & Butcher, 2006). Various authors (Stuart, 1976; Hiscocks, 1987; Goldenberg *et al*., 2010) reported that jackal scat contained more !nara seeds during the fruiting season, confirming that jackals feed extensively on !nara melons during this period. This may indicate a mutualistic relationship between !nara and jackals. !Nara provides water and nutrient rich melons to jackals, while the jackals disseminate !nara seeds away from the parent plants.

The presence and survival of large numbers of seeds, combined with the large respective home ranges (10–40 km$^2$) and the daily movements of 7–15.9 km per day (Bothma, 1998; Hiscocks & Perrin, 1998; Kauda, 2001), suggest that jackals might be effective primary as well as long distance !nara seed dispersers. Dispersal of seeds farther from the parent can benefit the plant by preventing distance dependent mortality, such as allelopathy and higher rates of seed predation near parent plants. Some cucurbit plant roots produces phytotoxins to prevent seeds from germinating too close to the parent plants (Yu *et al*., 2000; Yu, 2001; Hao *et al*., 2007).

Intact seeds in scat and the daily movement of jackals are important factors when assessing the role of jackals as seed dispersers, but so are viability and germination rate of ingested seeds. A laboratory trial found no significant difference between the viability of !nara seeds extracted from jackal scat and those manually extracted from ripe fruits, but there was a difference between the germination rate of the ingested seeds and control seeds (Fig. 2.9). Even though the viability was the same, germination rate increased when jackals ingested !nara seeds. This may be due to: (a) chemical scarification by digestive enzymes and physical scarification of the seeds by ingestion, (b) separation from chemical germination inhibitors that may be present in the pulp, and (c) reduction in the thickness of seed coat and endocarp, therefore increasing water and gaseous permeability (Motta-Junior & Martins, 2002; Silver *et al*., 2005; Varela & Butcher, 2006).

Dormancy breakage is another benefit associated with ingestion (Motta-Junior & Martins, 200; Varela & Butcher, 2006), in which seeds become ready for germination as soon as conditions are suitable. In arid areas like the Namib Desert where rain events are spatially and temporally unpredictable, that would mean seeds will germinate sooner when rain events do occur to exploit suitable conditions. But dormancy breakage may also have disadvantages – if rain does not occur following seed dispersal, the seed may desiccate from harsh conditions thereby reducing successful dispersal.

## Limitation and future studies

This study was carried out in only one population of !nara in the central Namib. Similar studies in other parts of the Namib such as the northern and southern Namib should confirm if jackals are the primary and long distance of !nara seeds in those parts of the Namib or other herbivores are involved. More camera traps to record visits and frugivory on more !nara plants within the central Namib and other populations simultaneously would show potential differences and similarities. In this study we were unable to identify if visits to particular plants were by one or more jackals. Populations close to farms or settlements should also be examined to determine if jackals feeding on !nara melons may attract jackals away from livestock to reduce human-wildlife conflicts during the !nara fruiting season. Jackals are considered problem animals by farmers, who have implemented preventive measures to keep jackals away from settlements. It should also be investigated as to how harvesting of melons by people may affect the dispersal process of !nara and possibly instigate more livestock predation by jackals.

## Conclusion

The behavioural repertoire of Black-backed Jackals in relation to the !nara suggests this may be a co-evolved mutualism. For example, jackals seem to carefully choose ripe melons, they are able to detect experimentally buried ripe fruits and they urinate on !nara melons as a possible marker. Jackals are highly attendant to !nara plants that start to bear ripe fruits and their scat contained intact !nara seeds that may have originated from far away. Critically, other meso carnivores and herbivores ignore the melons. From the plant's perspective, only the Black-backed Jackal is a disperser in the central Namib- other known !nara frugivores are more likely to destroy the seeds, but this may differ between the Northern and Southern Namib, where elephants and other animals may be involved. The !nara of the central Namib Desert therefore depends solely on Black-backed Jackals as the primary and long distance dispersal agent. Seed ingestion by jackals also seemed to increase the germination rate of !nara seeds. In combination, this suggests that the jackals are key disperser for !nara plant seeds and contributes to the persistence and population dynamics of !nara in the Namib Desert. Jackals are known as conflict animals to subsistence and commercial farmers (Humphries *et al*., 2015), including the Topnaar community along the lower Kuiseb River in the central Namib.  Since the Topnaar depend on both livestock and !nara melons, non-lethal livestock protection measures must be implemented to ensure the survival of both species.

# Chapter 3: !Nara seed dispersal by hairy-footed gerbils.

## Abstract

*Gerbilliscus* (*Gerbillurus*) spp. (hairy-footed gerbils) play a dual role by acting as both seed dispersers and seed predators, typical for all scatter-hoarders. Whether the seed will be predated or dispersed depends on food availability, seed traits and habitat. The role of gerbils in !nara (*Acanthosiycos horridus*) seed dispersal was examined in the central Namib Desert. This was accomplished by tracking !nara seeds to determine whether seeds were cached or consumed, as well as the distances over which seeds were moved, the depth of seed burials, and the habitats in which seeds were buried. In addition, we examined recovery rate of cached !nara seeds by hairy-footed gerbils. The results indicated that gerbils removed (100%, $p<0.001$) and cached (60.3 %, $p<0.001$) more !nara seeds than Camelthorn (9% removed; 40% cached; *Vachellia (Acacia) erioloba*) and Ana tree (35% removed; 53.9% cached, *Faidherbia albida*) seeds. !Nara seeds were moved an average distance of 29.1±1.6 m and buried at the average depth of 4±0.2 cm. The distance from the source increased with each re-caching event. Gerbil caches typically contained one to three seeds and 83% of the caches were buried in open areas away from vegetation cover. Furthermore, cached seeds offer short-term food benefit to the gerbils as 77% of the seeds were retrieved within 48 hours. Only 1.7% of the cached seeds were not retrieved over the 30-day observation period. This indicates that even though there is high probability of cache retrieval, some seeds survived predation. Gerbil species are therefore synzoochorous seed dispersers of !nara in the central Namib Desert.

**Key words**: synzoochory, melon dispersal, scatter-hoarding, !Nara, cache recovery rate, seed preference, seed fate, cache retrieval

## Introduction

Some rodent species store or hoard food for later consumption when resources are scarce, a behaviour that has been observed in many different ecosystems (Midgley *et al.*, 2002; Beck & Vander Wall, 2010; White *et al*., 2017; Gomez *et al*., 2019). Some of these rodent species bury seeds in spatially scattered caches containing one or two seeds; known as scatter-hoarding (Forget & Vander Wall, 2001; Giannoni *et al*., 2001; Cao *et al*., 2016; Wang & Corlett, 2017). Other species store seeds in one or two large caches, representing larder-hoarding (Wang *et al*., 2018). Scatter-hoarding behaviour is considered as a strategy to minimise cache theft by intraspecific and interspecific competitors in species that cannot actively protect and defend large caches (Geluso, 2005).

The fate of scatter-hoarded seeds can have two consequences: (a) cached seeds may be retrieved and consumed or (b) not retrieved and thus added to the soil seed bank (Geluso, 2005). Cached seeds that are not retrieved have the potential to germinate when conditions are suitable and contribute to plant recruitment. Burial of seeds by scatter-hoarding animal species is referred to as synzoochory (Gomez *et al.,* 2019). This form of seed dispersal, like other animal-meditated dispersal strategies, is considered to be the result of numerous adaptations in both plants and animals. On the plant's side, it endeavours to secure effective seed dispersal, while animals strive to utilise plant resources efficiently (Gomez *et al*., 2019). In synzoochory, the seeds themselves are the reward for the animal rather than secondary traits, thus plants invest in producing highly nutritious seeds to attract synzoochorous seed dispersers such as rodents.

Gomez *et al.* (2019) reported that synzoochory has been recorded in 1339 plant species, belonging to 641 genera and 157 families. Synzoochory is widely distributed across the world and carried out by over 30 animal families, which shows the importance of scatter-hoarding animals in plant distribution, recruitment and species survival (Gomez *et al.*, 2019). Scatter-hoarding rodents play a vital role in plant dynamics via seed predation and dispersal (Roth & Vander Wall, 2005). The scatter-hoarding behaviour of a particular species can play an important role in plant communities by moving seeds to more favourable microhabitats and increasing the distance away from parent sites where germination success may be lower (Razafindratzima, 2017; Wang & Corlett; 2017). In addition, scatter-hoarders reduce surface predation pressure on seeds, reduce competition for resources between seedlings, and increase the size of the soil seed bank (Wenny, 2001).

Many studies have looked at synzoochory seed dispersal by rodents such as gerbils (Midgley *et al*., 2002; Roth & Vander Wall, 2005; Weighill *et al*., 2017; White *et al*., 2017). *Gerbillurus paeba* is the only gerbil species from the genus *Gerbillurus* in southern Africa to have been recorded as a seed disperser via scatter-hoarding (Midgley & Anderson, 2004; Weighill *et al*., 2017; White *et al*., 2017).

Hairy-footed gerbils (*Gerbilliscus (Gerbillurus)* spp.), hereafter simply called gerbils, belong to the order Rodentia and family Muridae (Happold, 2013). All species of this genus are endemic to Africa. There are three *Gerbillurus* species occurring in the Namib Desert. The most widespread species is *G. paeba* that occurs from the southwestern arid biomes and Cape Floristic Kingdom into the savannas of southern Africa (Perrin *et al*., 1999). *Gerbillurus tytonis* and *Gerbillurus setzeri* are endemic to the Namib Desert. *Gerbillurus tytonis* is restricted to the sand dunes of the Namib Sand Sea, from Swakopmund to Lüderitz in Namibia, and *G. setziri* inhabits the gravel plains of the central Namib from the Kuiseb River northwards (Perrin *et al*., 1999; Happold, 2013). The habitats of these species overlap in some areas, such as at Gobabeb in the central Namib, and their distribution overlaps with !nara populations. Happold (2013) reported that these three gerbils are similar in colour and are differentiated mainly by the length of their tail and size of the hind-feet (Perrin *et al*., 1999; Happold, 2013) where they are sympatric.

Gerbils are omnivores and opportunistic feeders, consuming insects, foliage and seeds, and their diet will vary depending on the availability of food resources (Perrin *et al*., 1999; Happold, 2013). All three species are believed to exhibit scatter-hoarding behaviour, caching seeds for later consumption. At Gobabeb, gerbils were observed to predate on and scatter-hoard !nara seeds (Müller, 2000). !Nara ( *Acanthosicyos horridus*) produces large, hard-coated seeds that are enclosed in the pulp of the !nara melons. The seed contains an embryo and soft cotyledons (food storage), which is highly nutritious, containing 57% oil and 31% protein (Maggs-Kölling *et al*., 2014).

Other rodent species in the central Namib Desert such as the four-striped mouse (*Rhabdomys pumilio*) also feed on !nara seeds but have not been recorded to hoard seeds. They are therefore strictly seed predators (Weighill *et al*., 2017; White *et al*., 2017). Gerbils can be considered both primary and secondary seed dispersers referred to as diplosynzoochory - as they can collect seeds directly from the source as well as seeds that were dispersed first by other animals. In the case of !nara, Black-backed Jackal is considered a primary seed disperser in the central Namib desert, which may deposit seeds that may then collected and cached by gerbils (Chapter 2).

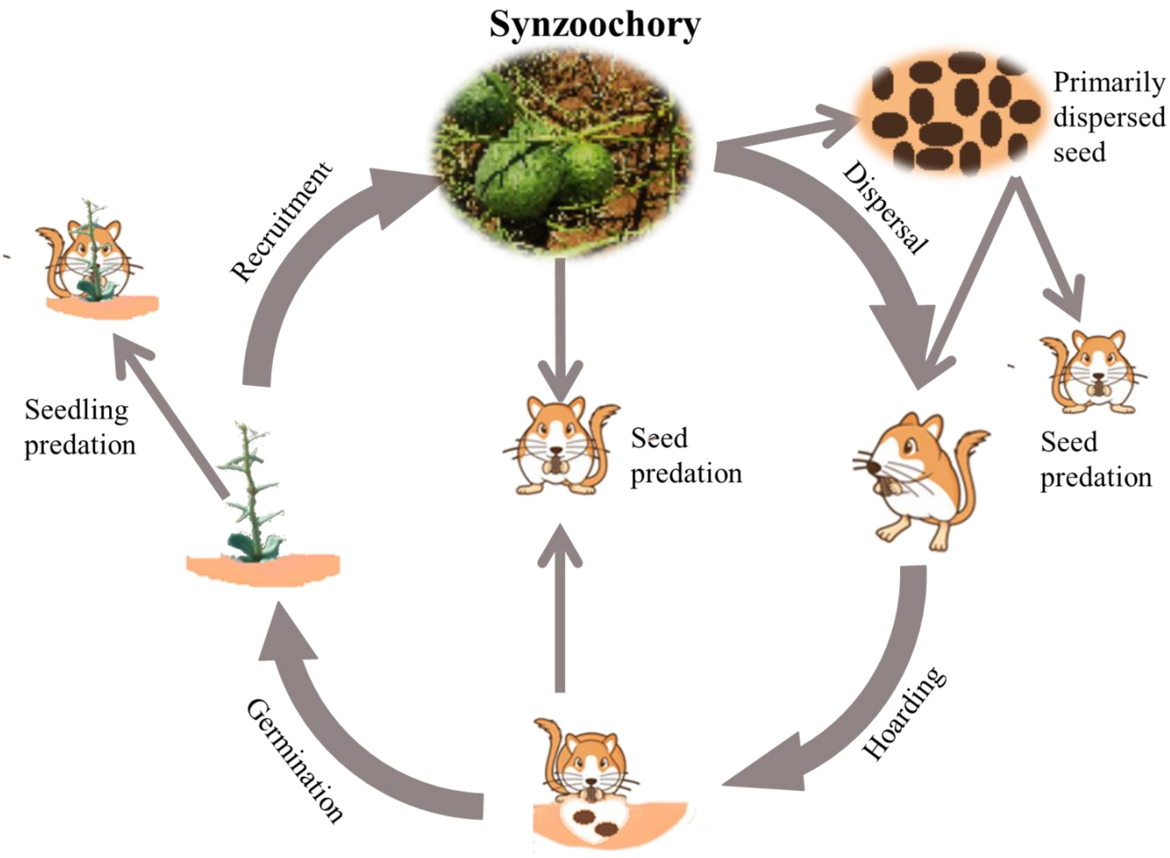

**Figure 3.1:** Seed fate in the context of diplosynzoochorous seed dispersal by seed caching hairy-footed gerbils.

Gerbils play a dual role by acting as both seed dispersers and seed predators, typical for all scatter-hoarders (Fig. 3.1). Whether the seed will be predated or dispersed depends on food availability, seed traits and habitat. In many ecosystems, scatter-hoarders play a vital role in seed dispersal (Beck & Vander Wall, 2010), but post-dispersal seed handling (predation versus caching) differs in different habitats (Razafindratzima, 2017). The habitat in which the seeds are scatter-hoarded and the recovery rate of seed caches play a major role in the quality and effectiveness of this dispersal mechanism (Wang & Smith, 2002; Ruiz *et al*., 2010).

The fate of seeds is often linked to seed recovery in scatter-hoarding species. Rapid recovery of caches means that seeds may not survive to germinate, while slow recovery rates allow some seeds to survive until conditions are suitable for germination. However, seeds recovered from caches may not be consumed immediately upon retrieval but rather re-cached at different sites. Re-caching of seeds may prolong the life-span of the seeds. Some studies indicated that both olfaction and memory are crucial for cache recovery; with some species relying heavily on memory alone for caches or seeds with a weak odour (Li *et al*., 2018; Yi *et al*., 2016). Food preferences and the nutritional value of the seeds may also influence the recovery rate and likely fate of recovered seeds, i.e. whether seeds are eaten or re-cached (Becker & Vander Wall, 2010; Wang & Smith, 2002).

The main aim of this study was to determine whether local *Gerbillurus spp* (hairy-footed gerbils) scatter-hoard seeds of !nara in the central Namib Desert. We hoped to advance the preliminary studies of Müller, (2000) by using marked seeds and infrared-enabled motion sensor cameras. Firstly, we investigated seed preference of gerbils for three common plant species occurring together: !nara (*Acanthosicyos horridus*), Camelthorn (*Vachellia (Acacia) erioloba* ), and Ana tree (*Faidherbia albida* ). Secondly, we investigated the fate of cached !nara seeds, the microhabitats for caches, the distances that seeds are being moved, and the depth at which seeds are buried. In addition, we also looked at the recovery rate and detection of cached !nara seeds.

We hypothesised that gerbils will move and cache a larger proportion of !nara seeds compared to the other two species as !nara seeds are larger in size, highly nutritious, and easily handled, since they have softer shells and cotyledons than the other two species. In addition, we expected that scatter-hoarding of !nara seeds would be for longer-term benefit with slow recovery rates as a food reserve when other food resources are scarce. Better information on !nara seed dispersal by secondary agents in the Namib Desert, where zoochorous seed dispersal of plant species is under-studied, is an important component to understand !nara distribution patterns and population recruitment as well as its importance to *Gerbillurus* spp.

## Materials and Methods

### Study site

The study was conducted in the central Namib Desert, within the Namib-Naukluft Park, in the vicinity of the Gobabeb-Namib Research Institute (Fig. 3.2) from June 2019 to February 2020. Each of the three local gerbil species is primarily associated with one of the three ecosystems at Gobabeb. *Gerbillurus paeba* is mainly associated with the ephemeral Kuiseb River, *G. setzeri* with the central Namib Gravel Plains and *G. tytonis* with the sand dunes of the Namib Sand Sea (Happold, 2013). The local distributions of these gerbil species are overlapping with !nara populations as well as with each other. The study was carried out in three distinct habitats on the river bank, the interdune plains and at the base of large dunes (Fig. 2.3). The gravel plain was excluded, and most likely *G. setzeri*, as !nara plants are not found on the gravel plains north of the Kuiseb. Five experimental sites were located in each habitat, each with seed stations, to determine the scatter-hoarding behaviour of the hairy-footed gerbils.

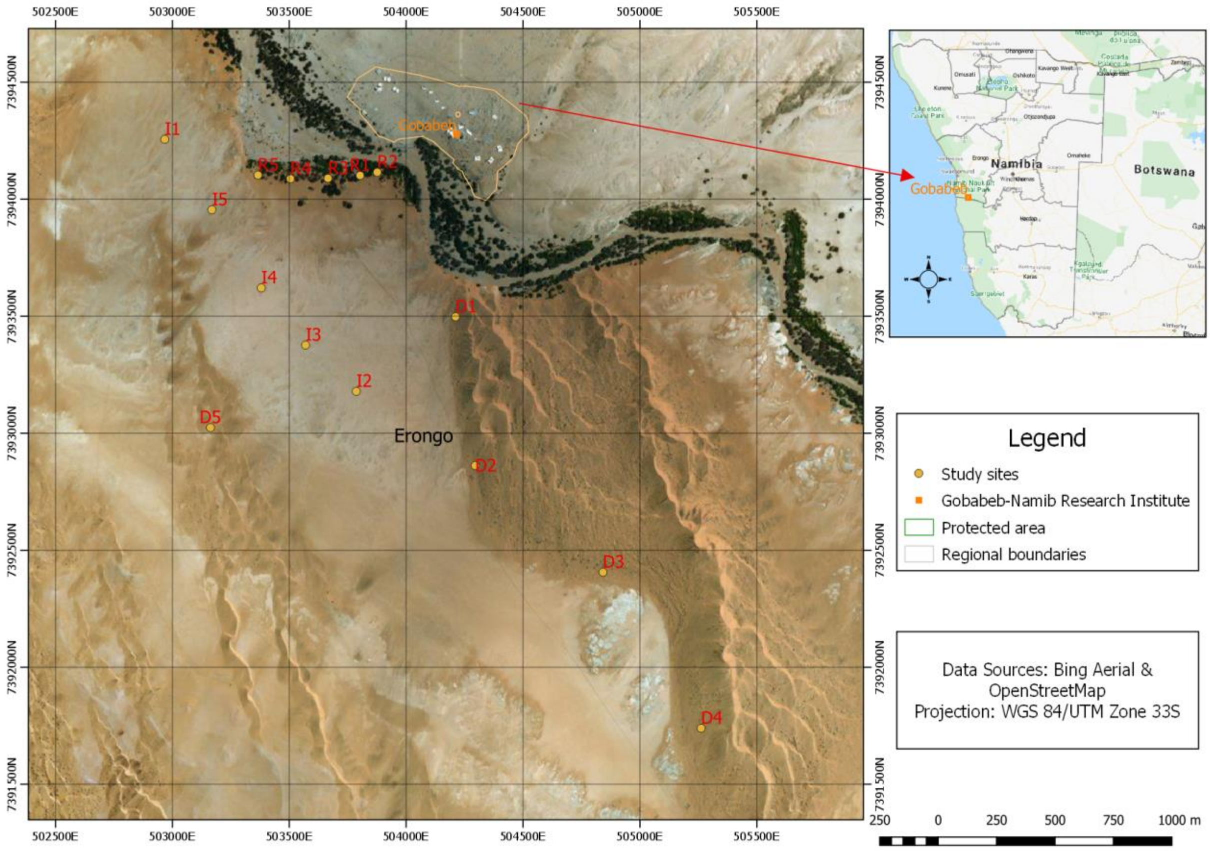

**Figure 3.2:** Study site near the Gobabeb - Namib Research Institute. Three habitats were selected: a riverbank habitat (R), the interdune habitat (I) and the sand dune habitat (D). The numbers in red represent different experimental seed sites for each habitat, where scatter-hoarding experiments were carried out.

## Experimental procedures

### Namib seeds

An experiment was designed to determine if hairy-footed gerbils are scatter-hoarders for all larger seeds of plant species present at Gobabeb. This included !nara seeds (*A. horridus*, average mass = 0.33 g, average length = 1.4 cm and average width = 0.93 cm), Camelthorn seeds (*V. erioloba*, average mass = 0.16 g, average length = 0.32 cm and average width =

0.12 cm) and Ana tree seeds (*F. albida*, average mass = 0.15 g, average length = 0.23 cm and average width = 0.098 cm). Seeds were extracted from fresh, mature melons and pods collected around Gobabeb, at which time physical attributes for a proportion of the collected seeds were also measured. We used a total of 390 !nara seeds, 225 Camelthorn seeds, and 225 Ana tree seeds for the scatter-hoarding experiments in the three habitats.

The seeds were handled with latex gloves to eliminate human scent as a factor that may influence the behaviour of the hairy-footed gerbils. In each habitat, five seeds of each plant species, thus 15 seeds, were provisioned at each the five seed stations on the ground surface (Table 2.1). This experimental procedure was replicated three times, this giving a total of 15 stations in each habitat. In addition, within each habitat, six stations containing 10 !nara seed only were provisioned to further examine whether caches were recovered by scent or memory and to further explore recovery rate. Seed stations were placed at least 50 m apart in each habitat. The coordinates (WGS84 datum) of each station were recorded using a handheld Garmin etrex 10 GPS device. The fate of the seeds was monitored daily over a minimum of three to seven days at each station.

**Table 3.1:** Summary of habitats and sample sizes used at each respective seed station. The data collection occurred during the !nara non-fruiting season (June and July) and during the !nara fruiting period (November and December).

| **Date** | **Habitat** | **Habitat type** | **Species** | **No. of stations** | **Seeds per station** | **Total seeds** |
|---|---|---|---|---|---|---|
| Jun-19 | River | Riverine | !nara | 21 | 5; 10 | 130 |
| Jul-19 | | | | | | |
| Nov-19 | | forest, | Camelthorn | 15 | 5 | 75 |
| Dec-19 | | Sandy | Ana tree | 15 | 5 | 75 |
| Jun-19 | Interdune | Rocky | !nara | 21 | 5;10 | 130 |
| Jul-19 | | | Camelthorn | 15 | 5 | 75 |
| Nov-19 | | | | | | |
| Dec-19 | | | Ana tree | 15 | 5 | 75 |
| Jun-19 | Dunes | Sandy | !nara | 21 | 5; 10 | 130 |
| Jul-19 | | | Camelthorn | 15 | 5 | 75 |
| Nov-19 | | | | | | |
| Dec-19 | | | Ana tree | 15 | 5 | 75 |

### The fate of seeds

Each seed was marked with a 15 cm ultraviolet (UV) fluorescent thread and an individually numbered tag that was glued to the seed with clear, non-toxic cyanoacrylate adhesive or super glue. This allowed us to track the fate of the seeds after being removed by gerbils (White *et al*., 2017). Each night we noted whether the seeds were ignored or removed (White *et al*., 2017), after which we tracked removed seeds by using an ultraviolet-emitting LED torch to locate the UV thread by searching around the seed station for up to a distance of 100 m. We recorded which seeds from each species were cached, consumed, or not found at all. The experimental procedures were replicated three times in each habitat between June and December 2019 (Table 3.1).

The percentage of seeds removed for each plant species of seeds discovered by gerbils from each habitat and station were calculated from the field data (Appendix 2.1). Out of the total number of seeds removed for each plant species, the percentage of seeds consumed, the percentage of seeds cached, and the percentage of seeds not recovered (uncertain fate as these could not be found) were also quantified from each habitats and station (White *et al*., 2017).

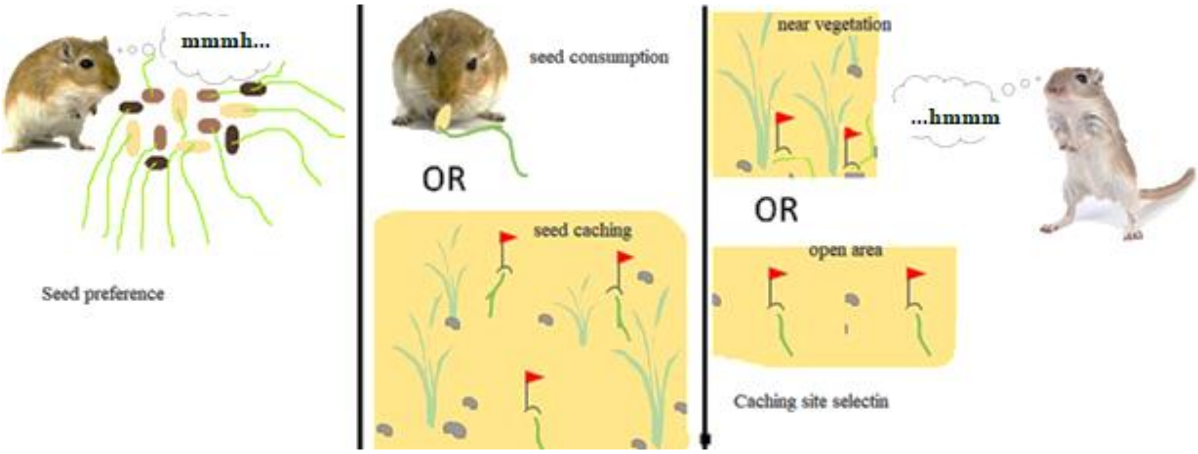

**Figure 3.3:** Graphic summary of the experimental trials on seed preference and seed fate decisions by scatter-hoarding gerbils. The experimental design allowed reconstruction of decisions made by the gerbils in each habitat as to which seeds to cache or to consume, as well as where to cache the seeds such as in the open or near vegetation.

### Camera traps

Infrared-enabled (IR) CamPark hunting trail cameras activated through motion sensors (n=6), model T45, were set up as camera traps at two seed stations in each habitat, for each replicate and some caches in each habitat, in order to confirm that seeds were indeed taken by hairy-footed gerbils. The cameras were secured on tripods and focused directly on the seeds on offer or a cache. The cameras were set to capture three photographs and a 30-second long video per trigger, with five second intervals between trigger events for 24 hours. All gerbils are strictly nocturnal, but other seed predators such as birds and other rodent species are diurnal or crepuscular. The cameras emit infrared light while photographing or recording videos at night that does not disrupt the behaviour of gerbils. The photographs and videos were examined on a notebook computer to determine visitors to the station. Night-time photographs and video under IR light are in grey scale, which made it difficult to distinguish between the different species of gerbils. *Gerbillurus* species are usually identified by measuring weight, the size of the hind feet and the tail features as well as subtle colour differences. We grouped all gerbils, which are distinct from other closely related genera, together as *Gerbillurus* spp. (gerbils) for the purpose of this study.

### Scatter-hoarding of !nara seeds

A marker peg with a conspicuous flag was used to mark each cache located by tracking the UV fluorescent threads at night and its coordinates recorded with a handheld Garmin etrex 10 GPS device. The next morning, when there was sufficient light to minimise disturbance, we recorded parameters such as the number of seeds in a cache, the distance from the seed station, the depth and species of buried seeds, and attributes of the cache microhabitat such as open area versus vegetated. The distances over which seeds were moved were measured in a straight line from the cache or re-caching locations to the seed stations where the seeds were taken from by using a 50 m measuring tape. The depth of seed burial was measured by excavating next to the cache and exposing the length of the thread without disturbing the seed. The taut length of thread from the soil surface to the seed was measured as depth with a 30 cm ruler. We used latex gloves when excavating caches, while seeds were left in their caches in the way we found them for further monitoring. The fate of cached !nara seeds, coded as re-cached, eaten or untouched and cache recovery rate, were monitored over a period of 30 days to determine if scatter-hoarding of seeds by gerbils is for short-term or long-term benefits. Several iterations of re-caching were monitored. Camera trap monitoring allowed us to confirm that cached seeds were retrieved by gerbils and not by other rodent species in the area. However, we were unable to determine whether caches were retrieved by the same individual that cached the seeds.

In order to determine whether gerbils recover their caches using memory and/or scent, we buried two additional !nara seeds 50 cm away from each of the 60 caches created by gerbils, thus 120 seeds, to determine if a gerbil will detect the new cache by smell alone as they would have no memory of it. Camera traps were used to monitor if gerbils return directly to the cache site or they return to the general area and then use scent to find the cache. If gerbils returned directly to the exact cache location without searching, then it was considered as using memory, while if they sniffed around the site before pinpointing the exact cache location, is considered as using both memory and scent to locate the caches. The percentage of additional buried seeds that were discovered by gerbils was calculated.

### Data analysis

The data were analysed using R (R Core Team, version 3.6.1, 2019)). The differences in the median proportion of seeds that were removed amongst the three species, across three habitats, were tested through a Kruskal-Wallis test under the package pgirmess (Patrick, 2018). The proportion of seeds that were removed was arcsine transformed. Generalised linear mixed effect models (GLMMs) by maximum likelihood (binomial (logit link) under the package lme4 (Pinheiro *et al*., 2019), were used to analyse the results for the following scatter-hoarding behaviour parameters: the differences in proportion of seeds cached or consumed amongst the three plant species. The proportion of seeds cached or consumed from each seed station was the response variable and the species, and habitats were regarded the predictors, while individual seed stations was considered to be a random effect. The distance over which !nara seeds were moved from seed stations and the burial depth of !nara seeds at the three habitats were compared using a linear mixed effect model by maximum likelihood (lme) model under the package nlme (Bates *et al*., 2015). Distance and depth were the response variables while habitats was the predictor variable. The number of !nara seeds per cache (cache size; response variable) from each habitats (habitats as a predictor) was analysed by using a generalised linear model (Poisson) under the package MASS (Venables & Ripley, 2002). The rate of cache recovery (response variable) in each habitat (habitat as a predictor variable) was analysed using the generalised linear model (Poisson). An anova test was run on each model to determine the factor significance and the Tukey HSD post hoc under the agricolae package using emmeans function (Mendiburu, 2020) was run on the models to determine the significant difference between categorical variables. The data was visualised in tables using sjPlot (Lüdecke, 2020). The best model was selected by using the Akaike Information Criterion (AIC, appendix 2.16).

## Results

Only hairy-footed gerbils were observed to be active at the seed stations. These are most likely to be either *G. paeba* or *G. tytonis,* the dominant and most common two gerbil species that inhabit the riverbank, interdune and sand dune habitats (Happold, 2013). It is unlikely that *G. setziri* was present at the seed stations, with the possible exception of the interdune habitat, as their preferred habitat is the open desert pediplains. Gerbils discovered 47 out of 65 seed stations, thus could theoretically have encountered 290 !nara seeds (out of 390), 165 Camelthorn seeds (out of 225) and 165 Ana seeds (out of 225).

### Seed removal

From all the seed stations, gerbils removed 100% (n=290) of available !nara seeds (*A. horridus*), 9% (n=165) of Camelthorn seeds (*V. erioloba*), and 35% (n=165) of Ana tree seeds (*F. albida*). We noted from camera trap monitoring that once gerbils discovered a seed station, they first removed all the !nara seeds before removing seeds from the other two species, if any at all. Gerbils clearly preferred !nara seeds across all habitats, followed by Ana tree seeds and lastly Camelthorn seeds (Fig. 3.4). We excluded seed stations that were not discovered by gerbils, in order to calculate how many of the discovered seeds were removed.

We carried out a Kruskal-Wallis test as a generalised linear model. It did not perform well for this analysis as it had large standard errors (appendix 2.2). The Kruskal-Wallis test indicated species has a significant effect on the proportion of removed seeds ($p<0.0001$). The post hoc test showed a difference between !nara and the other two species (Camelthorn and Ana), but not between Camelthorn and Ana tree (appendix 2.3). The proportion of removed !nara seeds was significantly higher than those of Camelthorn and Ana tree across all habitats. The species, thus, had an effect on the number of seeds removed.

There is no significant difference in the median proportion of seeds removed in each habitat ($p=0.83$). Thus, the habitat had no effect on the number of seeds removed.

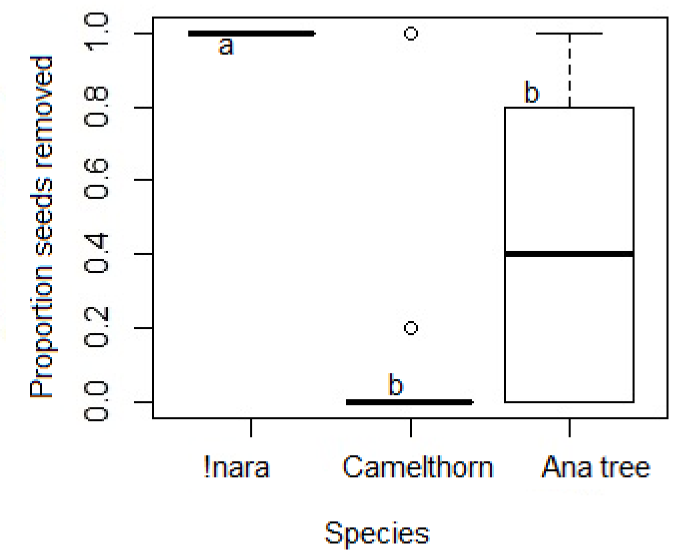

**Figure 3.4:** Proportion of removed seeds for each species (!nara, Camelthorn and Ana Tree) across all habitats (sand dune, river, interdune). The boxplots indicate median, lower and upper quantile and 1.5 interquartile range. The letters represent significant differences between species (Kruskal-Wallis, at 95% confidence interval).

### Fate of removed seeds

We found a higher proportion of !nara seeds that were cached (54.9%) in the sand dune habitat compared to caches in the riverbank (36.6%) and interdunes (8.6%) habitats (Fig. 3.5c). Gerbils only discovered seven of the 21 seed stations that were provided in the interdune habitat, so proportionally fewer seeds were removed in this habitat.

**Table 3.2:** A summary of the percentages of seeds that were cached, consumed or not found for each species out of all seeds that were removed (!nara, n=290; Camelthorn, n=20; and Ana, tree n=70) in all the habitats. The percentage of seeds that were removed, cached and consumed in each habitat for each species is provided in appendix 2.1.

| Species | Cached (%) | Consumed (%) | Not found (%) |
|---|---|---|---|
| !Nara | 60.3 | 8.0 | 31.7 |
| Camelthorn | 40.0 | 5.0 | 55.0 |
| Ana tree | 53.9 | 5.0 | 41.0 |

The generalised linear mixed effect model indicated that the probability of seeds being cached was significantly different among species and habitats (($p<0.0001$; appendix 2.5). Both species and habitats had an effect on the proportion of seeds cached. The proportion of cached !nara seeds was significantly higher than that of Camelthorn and Ana tree seeds in all the habitats (Fig. 3.5a).

The probability that seeds would be consumed was not significantly different among species (p=0.96), and significantly different habitats (p<0.06 appendix 2.7). The generalised linear model indicated that both species and habitat have no effect on the proportion of seeds consumed.

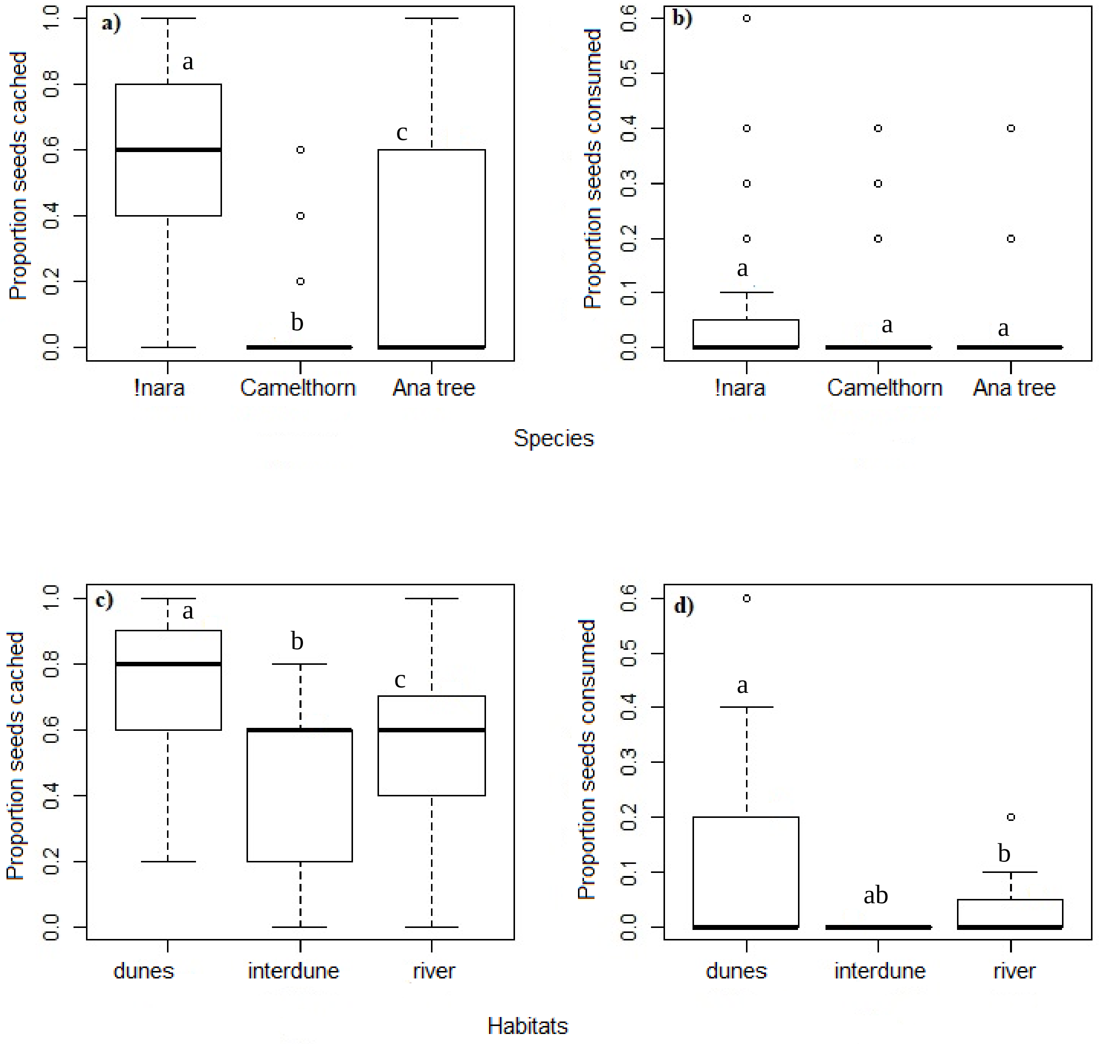

**Figure 3.5:** Proportions of seeds cached and consumed by hairy-footed gerbils for three plant species (a & b); and proportion of !nara seeds only cached and consumed in three habitats (c & d) . The boxplots indicate median, lower and upper quantile and 1.5 interquartile range. The letters indicate significant differences seeds cached and consumed for between species and habitat at the 95% confidence interval.

The primary behavioural response of gerbils was to cache !nara seeds, consuming a few, while Camelthorn and Ana tree seeds were largely ignored. Gerbils did not retrieve cached seeds of Camelthorn and Ana trees for more than four weeks, while cached !nara seeds were retrieved much sooner. The results suggests that gerbils value !nara seeds much more than the other two  commonly available seeds.

We therefore only considered !nara seeds for analysing results regarding removal distances, burial depth, number of seeds in a cache, the fate of cached seeds, microhabitats for caches and cache recovery rate. There was not enough data for Camelthorn and Ana tree seeds to deduce meaningful results due to the small number of seeds that were removed by gerbils.

### Distance moved and burial depth

Gerbils moved the !nara seeds over an average distance of 30.3±1.91 m (max=87.31 m) in the dunes, 29.8±2.02 m (max=68.8 m) on the riverbank, and 18.4±2.88 m (max=37.18 m) in the interdune. The linear mixed effect model showed that the removal distance is significantly different between the three habitats (p=0.003).The post hoc test indicated a significant difference between the sand dunes and interdune (p=0.002) and also between riverbank and interdune (p=0.006), but not between riverbank and sand dune habitats (p=0.88; appendix 2.9). The !nara seeds were moved further in the dunes and on the riverbank compared to the interdune habitat (Fig. 3.6a).

The !nara seeds were cached at a similar depth in all habitats, at an average depth of 4.0±0.21 cm (Fig. 3.6b). The linear mixed effect model indicated that depth was not significantly different between any of the three habitat (p=0.25; appendix 2.11).

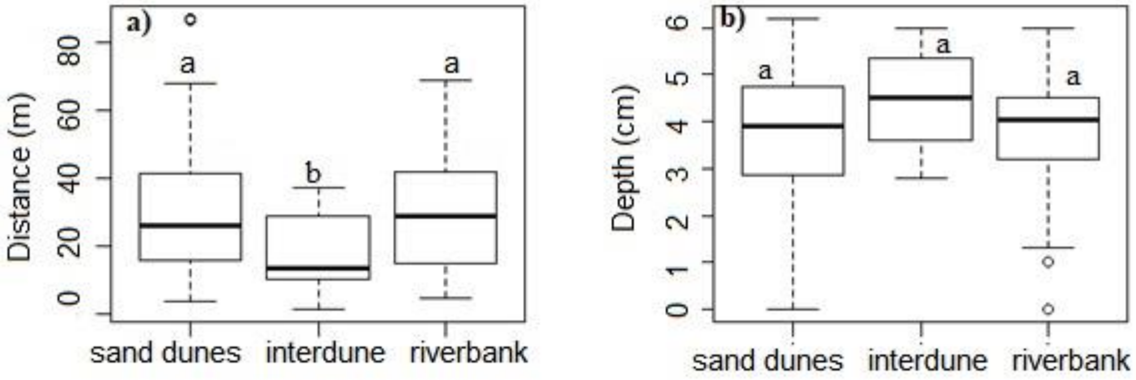

**Figure 3.6:** Boxplots indicating median, lower and upper quantile and interquartile range of (a) removal distance (m) and (b) burying depths (cm) of cached !nara seeds by hairy-footed gerbils in three habitats (dunes, n=96; interdune, n=15; and riverbank, n=64). Boxplots (a) shows the distance (m) over which seeds were moved from a seed station to a cache location, and boxplot (b) shows the depth (cm) at which cached seeds were buried. The letters indicate significant differences in removal

distance (appendix 2.9) and depth of burial between habitats (appendix 2.11) at the 95% confidence interval.

### Cache microhabitats

Most of the !nara seeds were buried in an open sandy area (83%; Fig. 3.7) away from any vegetation. Only a few seeds (17%) were buried next to or within 50 cm of vegetation in all the habitats, mainly *Stipagrostis sabulicola* (Namib Dune Bushman-grass) and *Salvadora persica* (Mustard Bush). We observed that in the dunes, seeds were buried along the base of the dunes with only a few seeds buried on the dune plinth, while on the riverbank seeds were buried away from the main channel and dense riparian vegetation towards the open interdune plains.

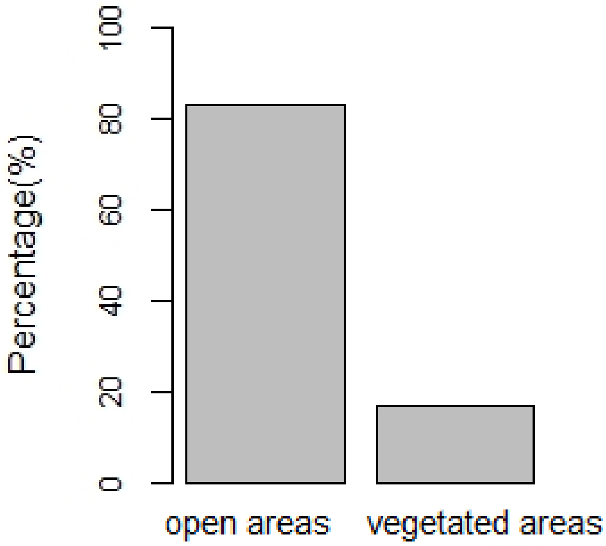

**Figure 3.7:** The percentage of !nara seeds buried in open areas and within 50 cm of vegetation (n=175).

### Scatter-hoarding of !nara seeds

We found most seeds were buried in pairs (45%) or singly (39%) in all of the habitats. Larger caches of three or more seeds were rare (16%), though more common in the riverbank habitat (Fig. 3.8). Cache sizes were not significantly different between the three habitat ((dune, interdune, river; p=0.18; appendix 2.13).

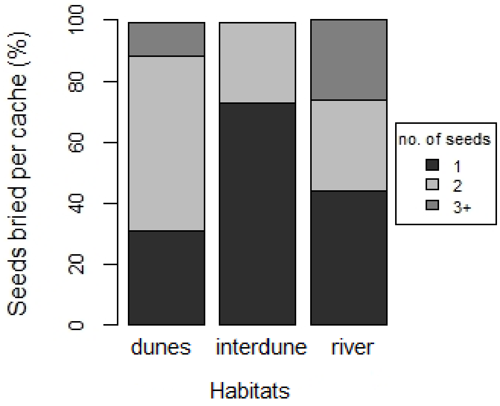

**Figure 3.8:** Percentage of !nara seeds per cache in the sand dune (n=96), interdune (n=15) and riverbank (n=64) habitats.

### Fate of cached seeds

Most !nara seed caches (84%) survived only for two days (48 hours) in all the habitats before being retrieved by gerbils. A few caches (2.9%) lasted for up to 14 days (Fig. 3.9) and only 1.7% of caches were not retrieved during the 30-day monitoring periods. Habitat type has a significant effect on the rate of recovery of !nara cached seeds (p<0.0001). The Tukey HSD post hoc test found the significant difference between the dunes and interdune (p<0.0001) and interdune and river (p<0.0001), but not between the dunes and the riverbank habitats ((p=0.25), appendix 2.15). No seeds remained undetected for more than five days in the interdune site.

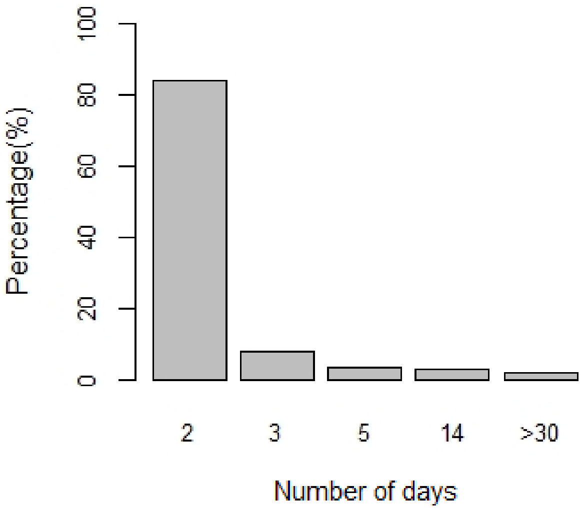

**Figure 3.9:** Percentage of cached seeds retrieved within a specific number of days by hairy-footed gerbils.

When the gerbils retrieved cached seeds, some of the seeds were re-cached (40%), a few were consumed (10%), but the fate of most seeds was unclear (50%). It is assumed that the gerbils may have taken the seeds into their burrows rather than beyond our search radius. In most cases, secondary caches were again recovered and some seeds re-cached. We located up to four caching events for some seeds, especially in the dune habitat (Fig. 3.10). We did not continue to search for re-cached seeds beyond a fourth cache as the number of seeds that were re-cached progressively declined to the stage where little additional information would have been obtained.

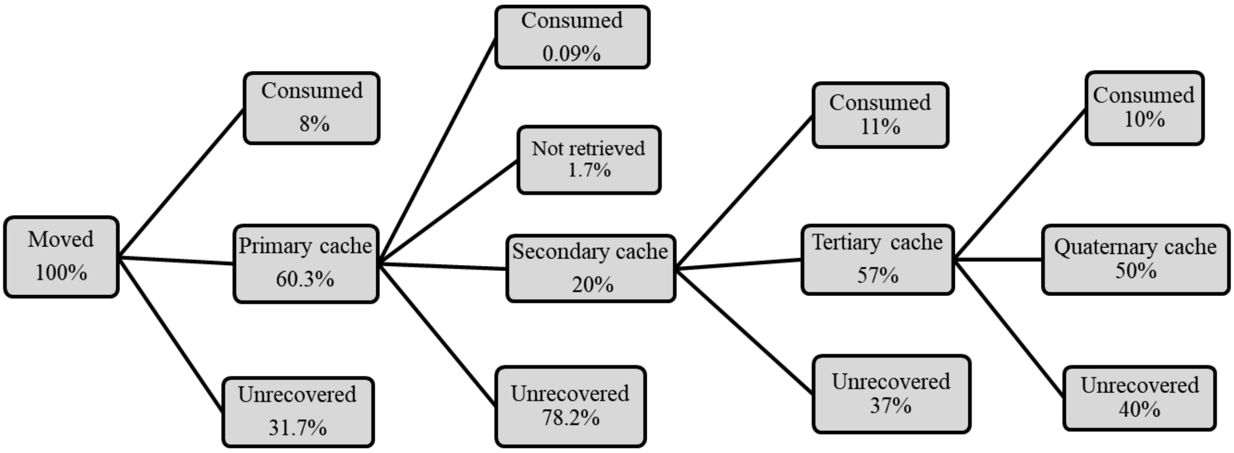

**Figure 3.10:** Fate of cached !nara seeds in all habitats. Seed caches and seed fate were tracked until the quaternary cache. The number of !nara seeds in caches declined after every retrieval as seeds were moved through primary caches (n=175), secondary (n=35), tertiary (n=20), and quaternary (n=10) caches.

The hairy-footed gerbils re-cached seeds progressively further away from the initial experimental seed station (Fig. 3.11). After the primary and secondary cache removals, which were directionally away from the seed stations, removal distances and directions to tertiary and quaternary caches became more random. This result was not anticipated, thus the number of seeds that remained to be tracked did not allow quantitative observations.

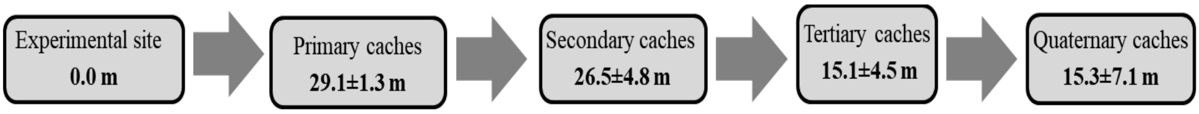

**Figure 3.11:** Consecutive removal distances between cache locations over the observation period. Re-caching of !nara seeds showed a progressive increase in the distance from the seed station whenever it occurred, in all the habitats.

### Memory versus Scent

Video observations from the camera traps showed that gerbils did not run straight to a cache. They entered the general area where they buried the seeds, which suggests partial memory, before searching and sniffing until they find the cache, which suggests that they use scent. As a control experiment, 91.8% of the !nara seeds (n=110) buried in experimental caches 50 cm of a gerbil cache were found and recovered by gerbils (Fig. 3.12). Only 8.2% of seeds (n=10) in experimental caches were not found by gerbils, which proves that gerbils can use scent to locate experimentally buried seeds.

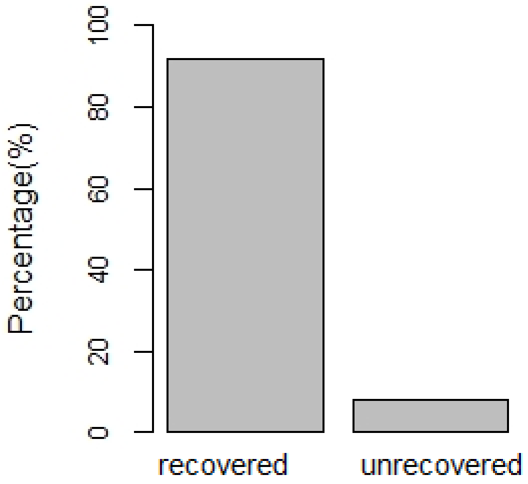

**Figure 3.12:** Percentage of !nara seeds that were found and recovered by gerbils, and those not found, after being buried in experimental caches 50 cm from a gerbil cache. We buried 120 !nara seeds at 60 experimental caches in the dune and riverbank habitats.

## Discussion

*Gerbillurus* spp. (hairy-footed gerbils) showed a clear preference for !nara seeds as 100% of the available !nara seed were removed at all the seed stations that were discovered by gerbils (Fig. 3.4) regardless of the habitat. This indicates that seeds from !nara melons (*A. horridus*) were valued more than seeds of the other two species, namely Camelthorn (*V. erioloba*) and Ana tree (*F. albida*), which are also supplying copious amounts of large seeds to habitats in the study area. Gerbils may choose seeds that provide greater nutritional value such as the highly nutritious !nara seeds (Maggs-Kölling *et al.*, 2014). Wang & Yang (2014) indicated that both seed size and energy content have an effect on rodents' foraging behaviour - larger seeds with high energy content being removed rapidly. Other traits such as outer coat hardness and tannin content may also play a role in seed selection by rodents (Wang & Chen, 2012; Cao *et al.*, 2016; Wang & Yang, 2020). As !nara seeds are larger than Camelthorn and Ana tree seeds and have a relatively softer coat and cotyledons, this may also play a role. Since all seeds were marked and treated in the same way by handling with latex gloves and gluing UV fluorescent thread to the seeds, these treatments should not have had a major effect on the results.

The fate of seeds collected by gerbils depends on their subsequent behaviour. Seeds are either consumed immediately or cached for later consumption. Müller (2000) proposed that gerbils scatter-hoard !nara seeds, while this study showed that gerbils cache most !nara seeds they encounter ( Fig. 3.5, Tab. 3.2) in small-sized caches (Fig. 3.8) and quite far from the seed source (Fig. 3.6). It also showed the caching sites preferred by gerbils (Fig. 3.7) and cache recovery rate (Fig. 3.9). This study also confirms that gerbils plays a dual role as both seed predators and seed dispersers of !nara (Fig. 3.5).

White *et al.* (2017) argued that seed caching behaviour in *G. paeba* is innate as they are most likely to cache preferred seeds regardless of season or plant species. Similar to seed caching studies by *G. paeba* in other ecosystems (Weighill *et al.*, 2017; White et al., 2017), *G.paeba* may also be an important seed disperser in the central Namib Desert where it occurs along ephemeral rivers (Happold, 2013). It also penetrates for short distances, up to 10 km, along well vegetated dune base corridors into the Namib Sand Sea. However, the Namib Hairy-footed Gerbil, *G. tytonis*, which is endemic to the Namib Sand Sea, is common along the plinth of the dunes and at large sand hummocks adjacent to the course of the Kuiseb River. Both *G. paeba* and *G. tytonis* were probably active in the riverbank habitat and may forage on the interdune habitat, but *G. tytonis* is the dominant species in the dune habitat. *G. paeba* and *G. tytonis* probably contributed equally to the results from this study, weighed towards *G. tytonis* in the dune habitat.

Some of the seeds removed from seed stations were not found (Tab. 3.2). These were most likely moved into impenetrable thickets on the riverbank or into burrows. It is more unlikely, though still possible, that seeds were moved further than our search radius (>100 m), or buried too deep. The fate of those seeds may have resulted in minor changes to the probability of seeds being cached or consumed.

The distances over which !nara seeds were moved to a caching site provide insight on seed dispersal distances - the threshold for long-distance dispersal is >100 m (Wang *et al.*, 2019), and whether gerbils move seeds into suitable micro-habitats for germination. The !nara seeds in the interdune habitat were moved over shorter distances (Fig. 3.6a), which could be a result of the landscape structure. The open plains without any cover may pose greater risks to gerbils having to travel further and a risk that seed caches may be discovered by competitors. There was no difference in the average depth of 4 cm at which !nara seeds were buried (Fig. 3.6b), even though the interdune habitat has a hard rocky surface typical for desert pediplains in comparison to the soft loose sand at the other two habitats. This may also be the optimal depth for gerbils to bury seeds in the central Namib Desert - deep enough that other seed predators cannot discover the caches but not too deep for the cache owner to be able relocate it.

The removal distance and burial depth may be related to food abundance, seed size, seed energy content and the energy cost of carrying food greater distances and burial at greater depths (Xiao *et al.*, 2005; Moore *et al.*, 2007). If this is the case, then removal distances and burial depth may change depending on conditions in the Namib, either spatially or in different years. In terms of dispersal benefits to plants, the depths at which seeds are buried can affect the amount of light and water that reach the seeds, therefore impacting on seed germination. Seed burial may also prevent seed desiccation by reducing water loss in the hot, dry desert by reducing exposure to direct sunlight on surface. Studies have determined the optimal burial depth for germination to be between 2 cm and 5 cm (Vander Wall, 1992; Chambers & McMahon, 1994; Murphy *et al.*, 2005). Gerbils in the central Namib Desert buried seeds within this range, which may be ideal for eventual germination and seedling survival of !nara.

Most !nara seeds were buried in the open microsites and away from vegetation (Fig. 3.7). No seeds were found buried in sand hummocks associated with any !nara plant even though a number of !nara plants occurred within the search radius of several seed stations along the riverbank and dune base habitats. It is likely that gerbils may avoid caching seeds where competitors may be foraging or sheltering, in order to minimise cache pilferage. Most gerbil burrows in the habitats where the experiments took place are found at the base or inside perennial vegetation where the soil is stable enough for starting burrows. Gerbils in those habitats also often forage around vegetation (pers. obs.). Gerbils may therefore avoid such areas when caching seeds.

It can be predicted that gerbils are likely to choose caching sites that maximise seed recovery by the owner, reduce energy expenditure during recovery, and minimise pilferage by intraspecific and interspecific competitors. In the dunes, seeds were buried along the base of the dune and on the open interdune surface. Along the riverbank, seeds were buried away from the dense vegetation towards the open interdune area. These locations would also be advantageous to the seeds as they are buried away from potential root competitors (other plants). !Nara plants are mainly found at the base of sand dunes and along the outer margin of riparian vegetation. From a !nara perspective, the seeds seems to be buried in the most conducive habitats for !nara establishment.

In the central Namib Desert, gerbils buried one to three seeds in spatially scattered caches (Fig. 3.8). This would minimise seed pilferage by intraspecific and interspecific competitors (Gu *et al*., 2017). It was suggested that scatter-hoarding behaviour is exhibited by animals that are unable to actively defend and protect large caches, so they scatter the caches in hope of reducing cache theft by others (Geluso, 2005). This description fits *G. paeba* and *G. tytonis* quite well as they are small and solitary. Scatter-hoarding may also benefit the plants if recovery rate of caches is slow or if the owners forget their cache sites. Our results are comparable to those of Müller (2000) and White *et al.* (2017), but the cache size in this study is smaller than the five to 10 seeds per cache of *G. paeba* in the study by Weighill *et al.* (2017). The cache size may be affected by the habitat type, the species, food availability, and intraspecific and interspecific competitors.

A few !nara seed caches survived for two weeks; though most were recovered by gerbils after only two days (Fig. 3.9). We cannot confirm if the caches were retrieved by owners or pilferers. The rapid cache recovery seems to imply that scatter-hoarding of !nara seeds by gerbils is for short-term benefits. It may also be due to limited food supply as 2019 was a very dry year in the Gobabeb area.

Following the predator satiation theory (Kelly & Sork, 2002), there may not be enough !nara seeds available to satiate the gerbils and encourage them to leave their caches intact for longer periods. The !nara plant does not release seeds directly into the environment, but bears large melons with a tough rind that require a large frugivore to open the fruit first before seed extraction can take place. Black-backed Jackals carry out that service in the central Namib Desert (see Chapter 1) and they defecate intact !nara seeds on the soil surface making them available for gerbils consumption.

Müller (2000) did not investigate whether gerbils in the Gobabeb area retrieve all their caches or re-cache seeds. This study provides conclusive evidence of frequent seed re-caching and that some caches were not retrieved (Fig.3.10). Even though seed recovery rate was high, the small percentage of seeds that were not retrieved after burial may survive until conditions are favourable for germination.

Gerbils re-cached some of the !nara seeds from those that were previously cached, reaching up to four successive dispersal movements (Fig. 3.10), each time at some distance from the previous cache (Fig. 3.11). Gerbils may relocate the seeds to minimise the probability of the caches being discovered by pilferers. Wang *et al.* (2018) concluded that re-caching and caching seeds in open areas are strategies for reducing pilferage. Furthermore, caches may be relocated more frequently to ensure that the cache owner does not forget the location of the caches over time. Seed re-caching benefits dispersal by increasing the dispersal distance (Fig. 3.11) and prolongs storage and ultimately benefits seed germination and seedling establishment (Perea *et al*., 2011; Wang *et al*., 2014; Wang *et al*., 2019). Although frequent cache retrieval and re-caching may be beneficial for seed dispersal, it also decreases the probability of survival for seeds as it may increase seed consumption (Perea *et al*., 2011; Wang *et al*., 2019). Results from this study confirmed many of these predictions: !nara seed caches were frequently retrieved and re-cached, with recurrent consumption of some seeds and progressively greater dispersal distances. Some caches were not recovered, which may ultimately benefit the !nara plants.

Camera trap recordings at cache sites indicate that gerbils relied on both memory and scent to relocate their buried !nara caches. Gerbils never went straight to the cache; they always went to the general area and then searched and sniffed in the vicinity until they located the cache. They also managed to discover most of the experimental caches of !nara seeds that we buried 50 cm away from existing gerbil caches (Fig. 3.12). This is a strong indication that gerbils also rely on scent. We are unable to confirm whether it was the owner of the cache that also recovered it, but the behaviour of the gerbils recorded on camera seems to suggest that it is. It would be expected that a gerbil unfamiliar with the general location of the cache would investigate a larger area, or exhibit general foraging behaviour, before homing in on the cache location. The ability of the gerbils to relocate caches by using both scent and memory would give them a competitive advantage over intraspecific and interspecific competitors.

Despite the low survival rate of !nara seeds in caches from this experiment, gerbils would still play a role in the !nara population structure and regeneration. The low percentage of !nara seeds that survive gerbil predation may have competitive advantages by being located in suitable microhabitats at some distance from conspecific competitors due to repetitive seed movement through re-caching. In order to fully understand the role of gerbils in !nara distribution patterns and population dynamics, monitoring natural or artificial gerbil caches during wet periods to determine germination and seedling survival probability will be a logical next step. !Nara seed germination depends on sufficient rain, and since rain is an unpredictable event in the Namib Desert, such an experiment may require careful planning. In addition, studying seed pilferage may add to the understanding of cache survival rate, this can be accomplished by burying seeds at random locations where gerbils may establish caches and determining the probability of the caches being discovered. Since scent does play a role in cache detection, further investigations as to how scent may be affected by environmental conditions would also be relevant.

## Limitations and Future studies

We only examined shared elements of scatter-hoarding by hairy-footed gerbil species in the central Namib Desert as we were unable to identify the species of gerbils from camera trap images. We therefore do not know if all hairy-footed gerbil species that occur in the central Namib were involved, or just some of the species. We also cannot identify species specific traits in scatter-hoarding behaviour, with particular reference to potential differences between *G. paeba* and *G. tytonis* that may explain some of the not significant variance in results from riverbank and dune habitats. Identification methods to identify gerbils to species level from camera trap images will be an advantage. Future studies should also look at scatter-hoarding of !nara seed in the southern and northern Namib and from areas further west (coastal Namib) and further east (inland Namib) where conditions are different. Rodent species that occur in those areas maybe different from the ones that occur in the central Namib and their behaviour may be influenced by environmental conditions at their geographic location, such as closer to the coast or further inland. Other factors such as seed size, seed traits and nutritional quality can be examined to determine their effect on scatter-hoarding by gerbils. Likewise, if burial depth affects seed recovery, setting up experimental buried seeds at different depths would indicate if gerbils are able to locate deep caches as may happen when sand accumulates during sandstorms. From a !nara seed dispersal perspective, the effect of seed burial on seed desiccation can be examined by testing seed viability and germination rate of buried seeds vs seeds left on the surface for a certain period of time. Testing whether open habitats reduce seed pilferage will be also be advantageous in explaining open habitats selection as a caching site by gerbils. We also did not succeed in collecting enough jackal scat (n=8, see Chapter 1) to test whether gerbils will extract !nara seed from fresh or degraded jackal scat to confirm diplochory (Vander Wall & Longland, 2004)).

## Conclusion

This study confirmed synzoochory by hairy-footed gerbils (*Gerbillurus* spp.) of !nara (*A. horridus*) in the central Namib Desert. Local gerbils collect !nara seeds and bury them in scattered caches in the ecosystem. Our limited study suggests that enough of these caches may be 'lost' because gerbils, for various reasons, cannot locate a particular cache again. By collecting and scatter-hoarding seeds, gerbil move and bury the seeds that protect them from surface seed predation and minimise desiccation. By moving seeds, gerbils also improve their chances of germination and seedling survival by reducing density-dependent effects such as conspecific competition and higher predation risk (Ruiz *et al.*, 2010). Most importantly, gerbils removed 100% of !nara seeds they encountered. This may demonstrate a mutualistic relationship between the !nara plant and the gerbils where either melons in general or !nara specifically have evolved large nutritious seeds to encourage synzoochory. Furthermore, the results suggested that directed seed dispersal (Wenny, 2001) may be very likely as gerbils caches are commonly in locations that are most suitable for !nara plants. That would mean that hairy-footed gerbils play an important role in the distribution patterns, population structure and dynamics of !nara plants in the central Namib Desert.

# Chapter 4: Synthesis

This study examined the respective roles of Black-backed Jackals and hairy-footed gerbils in !nara seed dispersal and whether they may be considered key dispersers of this valuable endemic Namib plant species. We quantified the role and effectiveness of Black-backed Jackals as !nara seed dispersers by using infrared enabled cameras to monitor jackal visitation rate, !nara fruit removal, and other jackal behaviour associated with fruit selection and harvesting. Furthermore, !nara melon scent was analysed to determine if ripe melons produce volatile compounds which may contribute to melon selection by jackals. The fate of seeds dispersed by jackal via endozoochory was also examined through laboratory viability and germination trials.

In brief, the results indicated the jackal was the most dominant species to visit the !nara (93.3%). Although other animals such as Cape Fox, Slender Mongoose and Small Spotted Genet were recorded by the camera traps, only jackals were recorded as a !nara frugivore over two !nara fruiting seasons. Jackals were observed to sniff melons, presumably to distinguish ripe melons from unripe melons. It is assumed they smell volatile compounds such as Pyrazole, Acetoine, 2-Methylbutanoic acid, and 2-Pyrrolidinone, which are released by ripe melons. They are likely to use one or more of those as a cue for ripeness. The selection of ripe melons by jackals implies that the seeds that are dispersed are mature enough for germination. There was no difference in the viability of ingested seeds and control seeds that were manually harvested from melons, but germination rates of ingested !nara seeds were significantly higher (50.4%) than control !nara seeds (34%). The increase in germination rate can be due to separation from chemical germination inhibitors that may be present in the pulp, seed scarification and dormancy breakage. This study suggests that Black-backed Jackals are the primary dispersers - and indeed a key disperser of !nara seeds in the central Namib Desert.

The second species of interest regarding !nara seed dispersal in the central Namib Desert are the hairy-footed gerbils. We determined whether local *Gerbillurus* spp (hairy-footed gerbils) scatter-hoard seeds of !nara plants in the central Namib Desert. Firstly, we investigated seed preference of gerbils for three common plant species occurring together - !nara (*Acanthosicyos horridus*), Camelthorn (*Faidherbia albida*), and Ana tree (*Vachellia erioloba*). Secondly, we investigated the fate of cached !nara seeds, the microhabitats for caches, the distances that seeds are being moved, the depth at which seeds are buried and cache sizes (number of seeds in a cache). In addition, we also looked at the recovery rate and detection of cached !nara seeds.

In summary, the results show that local hairy-footed gerbils removed all !nara seeds (100%) from seed stations compared to those of Camelthorn (9%) and Ana tree (35%). This indicates that hairy-footed gerbils clearly preferred !nara seeds. Out of all removed !nara seeds, 60.3% were cached. Only 1.7% of the cached !nara seeds were not retrieved by the gerbils during a 30-day observation period. However, gerbils recovered 77% of their caches within two days (48 hours), and most of the others within a week. The 1.7% of caches not retrieved may be considered 'lost' caches. The low percentage of !nara seeds that survive gerbil predation may have competitive advantages by being located in suitable microhabitats at some distance from conspecific competitors due to repetitive seed movement through re-caching. We were unable to track all seeds, some of these seeds may have been moved into burrows.

!Nara seeds were moved an average distance of 29.1±1.6 m. The distance moved also increased with each re-caching events. Movement and increased distance of !nara seeds by gerbils to microsites may help explain the distribution of individual plants in a population. Seed were buried at an average depth of 4±0.2 cm, which is within the range (2–5 cm) considered optimal for seed germination (Vander Wall, 1992; Chambers & McMahon, 1994; Murphy *et al*., 2005). Some 83% of cached seeds were buried in open microhabitats (no vegetation within 50 cm of the cache), which is considered a strategy for avoiding areas with high potential of seed pilferage, but may benefit germination due to low intraspecific and interspecific competition. Although there is high probability of cache retrieval, some seeds survived predation. Therefore, hairy-footed gerbil species in the central Namib Desert contributed to secondary seed dispersal of !nara. Since all seeds were marked and treated the same method by handling with latex gloves and gluing UV fluorescent thread to the seeds, those treatments should not have had a major effect on the results. This study confirmed synzoochory by hairy-footed gerbils (*Gerbillurus* spp.) of !nara (*A. horridus*) in the central Namib Desert.

## Limitation and future studies

In the jackal study, we were unable to identify if visits to particular plants was by one or more jackals. More camera traps to record visits and frugivory on more !nara plants within the central Namib and other populations simultaneously would show potential differences and similarities in behaviours. It was also not clear if there is a specific compound that jackals may associate with ripeness or if they are just attracted to melons with a strong odour. For futures studies, a cafeteria experiment using artificial compounds would show if jackals have a preference or not. In addition, more scent samples need to be analysed to determine if these compounds are only produced by ripe melons or if low concentrations of the same compounds in unripe melons just makes it hard to sample.

For Gerbils, we only examined shared elements of scatter-hoarding by hairy-footed gerbil species in the central Namib Desert as we were unable to identify the species of gerbils from camera trap images. We therefore do not know if all hairy-footed gerbil species that occur in the central Namib were involved, or just some of the species. We also cannot identify species specific traits in scatter-hoarding behaviour, with particular reference to potential differences between *G. paeba* and *G. tytonis* that may explain some of the not significant variance in results from riverbank and dune habitats. Identification methods to identify gerbils to species level from camera trap images will be an advantage.  We were unable to confirm if the caches were retrieved by owners or pilferers.

## Conclusions

Black-backed Jackals provide primary seed dispersal service to !nara plants by consuming !nara melons and excreting intact !nara seeds (Chapter 2). These seeds are left on the surface that makes them available to seed hoarders and predators. Local hairy-footed gerbils may collect these seeds and bury them in scattered caches in the ecosystem as a secondary seed disperser (Chapter 3). Dispersal by gerbils will likely change the initial dispersal patterns of seeds, primarily dispersed by jackals. This would constitute diplochory in !nara, whereby plants have two distinct modes of dispersal - endozoochory by Black-backed Jackals and synzoochory by hairy-footed gerbils (Fig. 4.1). The combined benefits of diplochory (distance, depth and dispersal site) are crucial for allelopathic plants and generally important for plants such as !nara that occur in hyper-arid desert environments (Vander Wall & Longland, 2004).

!Nara plants may have evolved large nutritious melons and large nutritious seeds as a strategy to aid in primary seed dispersal by jackals and secondary dispersal by gerbils by providing food rewards to both dispersers. Jackals increased !nara seed dispersal distance and germination rate, while gerbils move seeds into suitable microhabitats where burial protects seeds from surface seed predation and minimises desiccation. Both jackals and gerbils play a vital role in explaining and determining the distribution patterns, population structure and dynamics of !nara plants in the central Namib Desert.

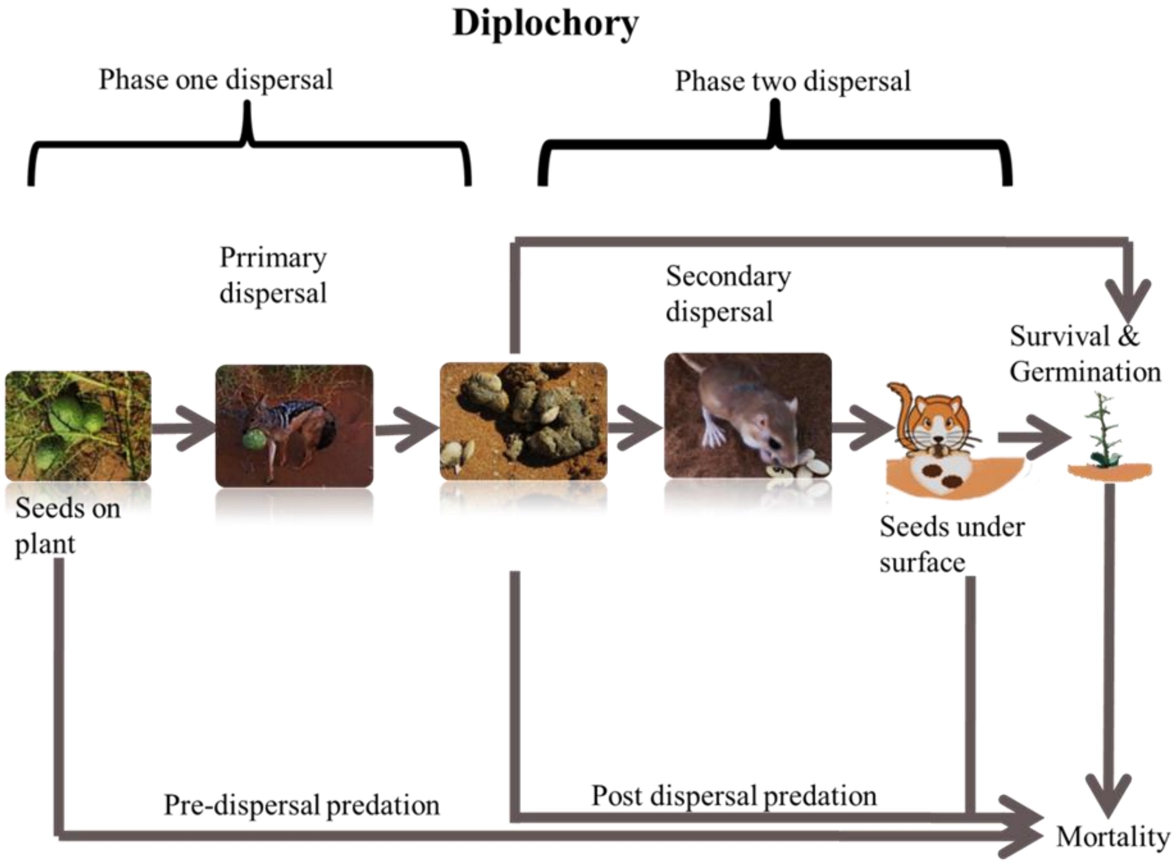

**Figure 4.1:** Proposed diplozoochory with phase one (primary) dispersal and phase two (secondary) dispersal and seed fate of !nara seeds. Black-backed Jackal consume !nara melons and defecate intact seeds (endozoochory), while scatter-hoarding hairy-footed gerbils harvest previously dispersed !nara seeds and bury them in caches (synzoochory).

The dispersal pattern that was observed in the central Namib may differ from the southern and northern Namib, where other species such as elephants and different rodent species may be involved. Anecdotal information indicates that !nara fruit are consumed, and seeds potentially dispersed by giraffe, elephant, porcupine, and domestic dogs in areas where these species occur. In the central Namib, jackals may explain the gaps between fragmented populations because they travel longer distances from populations and gerbils may explain the distribution of individual !nara plants within a population due to movement of seeds to different microhabitats within the area, but the effect of microhabitat on germination also needs to be examined. Future studies need to investigate !nara seed dispersal in the northern and southern ranges of !nara occurrence to fully understand the effect of dispersal on !nara distribution and population dynamics. Such studies may help to explain the large distances between populations in the northern, southern and central Namib Desert, which could have been the effect of seed dispersal by large frugivores such as elephants (Kistler et al., 2015).

Humans may also have played a role in !nara seed dispersal by harvesting !nara seeds for consumption and carrying them over long distances to other areas. !Nara is a cultural keystone species for the Topnaar people (≠Aonin), who harvest !nara melons (Henschel., 2014; Maggs-Kölling et al., 2014). They used to be nomadic pastoralists in the Namib Desert (Henschel et al., 2004), moving from one area to another. They may have carried !nara seeds with them, which may explain some of the unusual disjunction between populations. Conversely, human-mediated seed dispersal in the case of !nara may not be relevant, as most of the seeds together with the fruit pulp are cooked for consumption, which will affect the viability of the seeds. Fruit harvesting by people results in lower melons and seed crops that may influence mutualism between !nara, jackals and gerbils. It would be relevant to know how important the !nara is to these mammals and whether harvesting has any conservation impacts.

# Popular Science Summary

## It takes two to tango: The !nara relies on unusual partners for seed dispersal

Across the natural world, plants have evolved interesting and ingenious methods of seed dispersal. One of these is to bear delicious, fleshy fruit that attract animals, especially mammals. Hidden in these fruit are seeds that, once eaten and if they survive the digestive process, are dispersed via defecation, often a fair distance from the parent plant.

This is certainly the case with the Namib Desert specialist, !nara (*Acanthosicyos horridus*). This endemic plant occurs nowhere else in the world but in sandy areas at the base of dunes and along ephemeral rivers of the Namib. !Nara is a spiny, leafless, melon-bearing plant that annually produces 20–500 pale-green, grapefruit-sized, juicy melons, between the months of November and April. These nutritious and water-rich fruit attract an abundance of animals such as Oryx, domestic donkeys, hyenas, elephants, giraffes, domestic dogs and jackals, which are able to crack through the hard, spiny rind. Once opened, many smaller mammals, birds and insects will also partake in this feast. Many of these fruit-eaters damage the seeds through chewing. Only a few species, including elephants, dogs and jackals, defecate intact seeds, thus dispersing them to germinate and establish in new areas.

Gobabeb-Namib Research Institute has been studying and monitoring the nearby !nara population for years. The scattered and isolated occurrence of !nara across its entire distribution range from southern Angola to the northern Cape in South Africa, has perplexed scientists. I recently added to solving this mystery while conducting research on the seed dispersal of !nara by Black-backed Jackals and hairy-footed gerbils, two unusual accomplices, unwittingly working in tandem, to ensure the survival of this iconic Namib plant.

Using camera traps, I recorded jackals frequently visiting !nara plants - the only species I recorded picking, marking and eating melons during two fruiting seasons. Jackals only eat ripe melons and were observed sniffing the melons, presumably to detect ripeness. By consuming only ripe melons, it is guaranteed that the voided seeds are mature and able to easily germinate. Lab tests revealed that the germination rate of ingested !nara seeds, removed from jackal scat, were 20% higher than that of manually harvested seeds, while viability was the same in both groups. This may be due to softening and weakening of the seed coat through digestion, thus allowing better water absorption and gas exchange, or breathing, and potentially the removal of chemicals in the flesh of the fruit that prevent the seeds from germinating.

Jackals deposit their scat with !nara seeds are left on the soil surface. Seeds then become available to local hairy-footed gerbil species, which collect and cache seeds by burying them elsewhere for later use, almost like gardeners planting seeds. This behaviour was investigated by offering a choice of !nara, Camelthorn and Ana tree seeds in piles in different areas, and using camera traps to record what happens. Gerbils showed a clear preference for !nara seeds as they removed all of them compared to 35% of the Ana tree seeds and only 9% of the Camelthorn seeds. Gerbils cached most !nara seeds (60%) they harvested, moving the seeds about 30 m away and burying them at a 4 cm depth. Gerbils scatter their caches to minimise robbers from stealing their cache, since they cannot actively defend and protect one large cache. Gerbils recovered almost 80% of their caches. The few seeds that were forgotten may survive until a rainfall event and conditions are suitable for !nara germination.

The seed dispersal mechanisms by jackals and gerbils are equally important for !nara plants. Primary dispersal by jackals increases !nara seed dispersal distance and germination rate, and allows !nara to colonise new areas. On the other hand, secondary dispersal by gerbils move seeds into suitable sites and burial protects the seeds from predation and minimises seed desiccation and solar radiation damage. The combined seed dispersal actions of these animals may be key to explaining the distribution patterns within and between populations of !nara plants in the Namib Desert.

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# Appendix 1

**Appendix 1.1:** The number of visits and melons removed by visiting animals as observed on camera trap photographs (eight !nara bushes, 200 days).

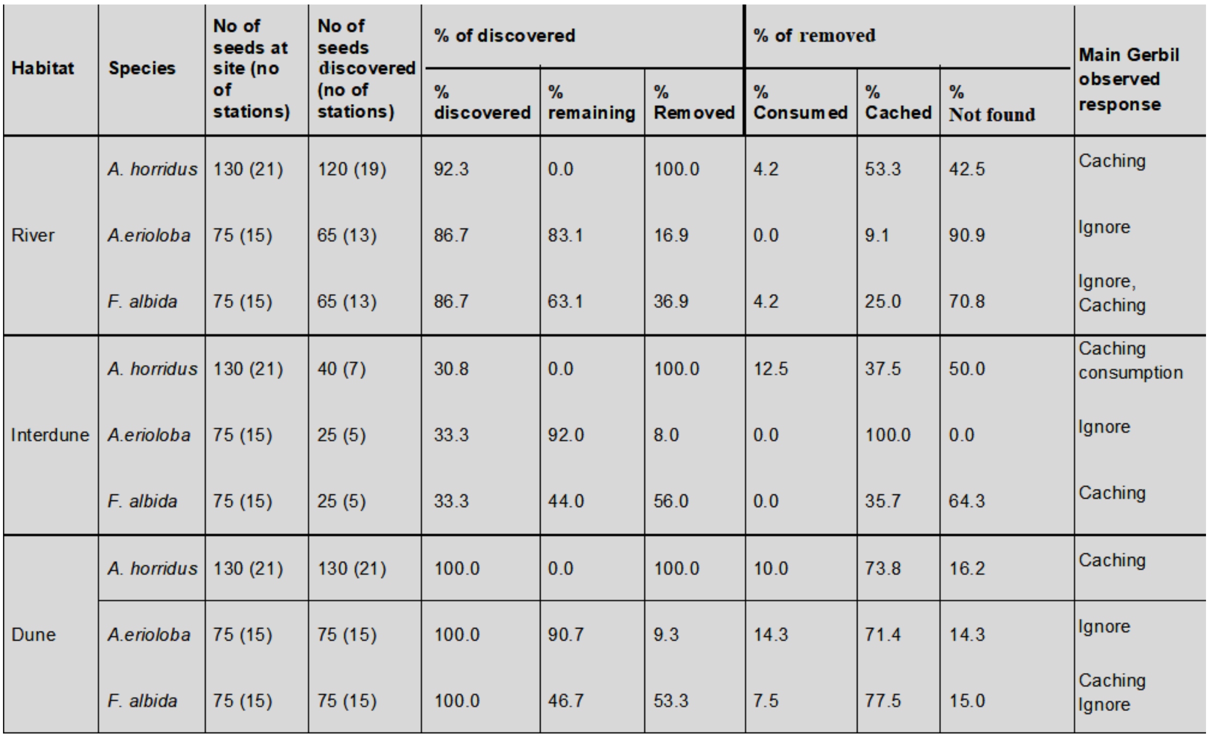
| **Species** | **Number of visits** | **Number of fruit removals** |
|---|---|---|
| Black-backed Jackal | 508 | 142 |
| Cape Fox | 3 | 0 |
| Small-spotted Genet | 7 | 0 |
| Slender Mongoose | 3 | 0 |
| African Polecat | 1 | 0 |
| Hare | 2 | 0 |
| Birds | 21 | 0 |

**Appendix 1.2:** Results from a generalised linear model to test if there is a significant difference in the proportion of seeds germinated from jackal scat (n=141) and seeds extracted from melons (n=141) at 95% confidence interval.

| | **Proportion of seeds germinated** | | | | |
|---|---|---|---|---|---|
| *Predictors* | *Odds Ratios* | *Std. Error* | *CI* | *Statistic* | *p* |
| (Intercept) | 1.01 | 0.17 | 0.73 – 1.41 | 0.08 | 0.933 |
| treatment [melon] | 0.51 | 0.24 | 0.31 – 0.82 | -2.76 | **0.006** |
| Observations | 36 | | | | |
| $R^2$ Tjur | 0.020 | | | | |

# Appendix 2

**Appendix 2.1**: Summary of seed fates (percentage discovered by gerbils, percentage removed and of this percentage consumed, buried, and not found) and the main gerbil responses observed on camera trap videos to local seeds in three habitats in the central Namib Desert.



| Habitat | Species | No of seeds at site (no of stations) | No of seeds discovered (no of stations) | % of discovered | | | % of removed | | | Main Gerbil observed response |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | % discovered | % remaining | % Removed | % Consumed | % Cached | % Not found | |
| River | *A. horridus* | 130 (21) | 120 (19) | 92.3 | 0.0 | 100.0 | 4.2 | 53.3 | 42.5 | Caching |
| | *A.erioloba* | 75 (15) | 65 (13) | 86.7 | 83.1 | 16.9 | 0.0 | 9.1 | 90.9 | Ignore |
| | *F. albida* | 75 (15) | 65 (13) | 86.7 | 63.1 | 36.9 | 4.2 | 25.0 | 70.8 | Ignore,<br>Caching |
| Interdune | *A. horridus* | 130 (21) | 40 (7) | 30.8 | 0.0 | 100.0 | 12.5 | 37.5 | 50.0 | Caching<br>consumption |
| | *A.erioloba* | 75 (15) | 25 (5) | 33.3 | 92.0 | 8.0 | 0.0 | 100.0 | 0.0 | Ignore |
| | *F. albida* | 75 (15) | 25 (5) | 33.3 | 44.0 | 56.0 | 0.0 | 35.7 | 64.3 | Caching |
| Dune | *A. horridus* | 130 (21) | 130 (21) | 100.0 | 0.0 | 100.0 | 10.0 | 73.8 | 16.2 | Caching |
| | *A.erioloba* | 75 (15) | 75 (15) | 100.0 | 90.7 | 9.3 | 14.3 | 71.4 | 14.3 | Ignore |
| | *F. albida* | 75 (15) | 75 (15) | 100.0 | 46.7 | 53.3 | 7.5 | 77.5 | 15.0 | Caching<br>Ignore |

**Appendix 2.2:** Results from a generalised linear model testing if the proportion seed that was removed by hairy-footed gerbils differs between species (n=3) at 95% confidence interval. !Nara=290 seeds, Camelthorn=165, Ana tree=165). See appendix 2.16 for the AIC Number that was used to select the best fit model.

| *Predictors* | *Odds Ratios* | *std. Error* | *Statistic* | *p* |
|---|---|---|---|---|
| (Intercept) | 950018659.27 | 1642.32 | 0.01 | 0.990 |
| Species [Camelthorn] | 0.00 | 1642.32 | -0.01 | 0.989 |
| Species [Ana] | 0.00 | 1642.32 | -0.01 | 0.990 |
| Observations | 115 | | | |
| $R^2$ Tjur | 0.135 | | | |

**Appendix 2.3:** Results from the post hoc test for Kruskal Wallis. This shows the significant difference between different species and different habitats for seeds that were removed.

| Comparisons | obs.dif | critical.dif | difference |
|---|---|---|---|
| !nara - Ana | 39.97059 | 17.97057 | TRUE |
| !nara - Camelthorn | 58.11765 | 17.97057 | TRUE |
| Ana - Camelthorn | 18.14706 | 19.35902 | FALSE |
| sand dunes - interdunes | 4.731335 | 22.30007 | FALSE |
| sand dunes - river | 2.506271 | 16.15624 | FALSE |
| interdunes - river | 2.225064 | 22.65555 | FALSE |

**Appendix 2.4:** Results from a generalised linear mixed effect model testing if the proportion of seed cached by gerbils differ between species (n=3) and habitat (n=3) at 95% confidence interval. The total number of seeds cached is n=225 (!nara=175 cached seeds, Camelthorn=8, Ana tree=42). See Appendix 2.9 for the AIC Number that was used to select the best fit model.

| *Predictors* | *Odds Ratios* | *std. Error* | *CI* | *Statistic* | *p* |
|---|---|---|---|---|---|
| (Intercept) | 4.54 | 0.21 | 2.99 – 6.88 | 7.12 | **<0.001** |
| Species [Camelthorn] | 0.01 | 0.19 | 0.01 – 0.02 | -23.47 | **<0.001** |
| Species [Ana] | 0.09 | 0.12 | 0.07 – 0.11 | -20.34 | **<0.001** |
| Habitat [interdune] | 0.12 | 0.18 | 0.08 – 0.16 | -12.23 | **<0.001** |
| Habitat [river] | 0.28 | 0.10 | 0.23 – 0.34 | -12.91 | **<0.001** |
| **Random Effects** | | | | | |
| $\sigma^2$ | 3.29 | | | | |
| $\tau_{00\ \text{Station}}$ | 0.79 | | | | |
| ICC | 0.19 | | | | |
| $N_{\text{Station}}$ | 21 | | | | |
| Observations | 115 | | | | |
| Marginal $R^2$/ Conditional $R^2$ | 0.505 / 0.602 | | | | |

**Appendix 2.5:** Results from the Tukey HSD post hoc test for the model in appendix 2.4. This shows the significant difference between different species and different habitats for seeds that that were cached.

| contrast | estimate | SE | df | z.ratio | p.value |
|---|---|---|---|---|---|
| !nara - Ana | 2.4 | 0.118 | Inf | 20.340 | <.0001 |
| !nara - Camelthorn | 4.5 | 0.192 | Inf | 23.471 | <.0001 |
| Ana - Camelthorn | 2.1 | 0.189 | Inf | 11.094 | <.0001 |
| sand dunes - interdunes | 2.157 | 0.1763 | Inf | 12.235 | <.0001 |
| sand dunes - river | 1.271 | 0.0985 | Inf | 12.906 | <.0001 |
| interdunes - river | -0.886 | 0.1702 | Inf | -5.207 | <.0001 |

**Appendix 2.6:** Results from a generalised linear mixed effect model testing if proportion seed consumed differs among species (n=3) and habitat (n=3) at 95% confidence interval. The total number of seeds consumed is n=31. See Appendix 2.9 for the AIC Number that was used to select the best fit model.

| *Predictors* | *Odds Ratios* | *std. Error* | *Statistic* | *p* |
|---|---|---|---|---|
| (Intercept) | 0.02 | 0.62 | -6.30 | **<0.001** |
| Species [Camelthorn] | 0.27 | 0.35 | -3.84 | **<0.001** |
| Species [Ana] | 0.55 | 0.26 | -2.30 | **0.021** |
| Habitat [interdune] | 7.18 | 0.39 | 5.06 | **<0.001** |
| Habitat [river] | 0.36 | 0.20 | -5.07 | **<0.001** |
| **Random Effects** | | | | |
| $\sigma^2$ | 3.29 | | | |
| $\tau_{00\ \text{Station}}$ | 4.97 | | | |
| ICC | 0.60 | | | |
| $N_{\text{Station}}$ | 21 | | | |
| Observations | 115 | | | |
| Marginal $R^2$ / Conditional $R^2$ | 0.135 / 0.655 | | | |

**Appendix 2.7:** Results from the Tukey HSD post hoc test for the model in appendix 2.6. This shows the significant difference between different species and different habitats for seeds that that were consumed.

| contrast | estimate | SE | d.f | z.ratio | p.value |
|---|---|---|---|---|---|
| !nara - Ana | 574.2 | 2428851 | Inf | 0.000 | 1.0000 |
| !nara - Camelthorn | 31.9 | 2260621 | Inf | 0.000 | 1.0000 |
| Ana - Camelthorn | -542.3 | 3165314 | Inf | 0.000 | 1.0000 |
| sand dunes- interdunes | -1.66 | 6.00e-01 | Inf | -2.604 | 0.0250 |
| sand dunes – river | 115.50 | 1.88e+06 | Inf | 0.000 | 1.0000 |
| interdunes - river | 117.16 | 1.88e+06 | Inf | 0.000 | 1.0000 |

**Appendix 2.8:** Results from a linear mixed effect model to test if habitat affects dispersal distance of !nara seeds, with seed stations as a random effect at 95% confidence interval. The total number of seed dispersal distances is n=175 (dune =96, interdune =15, river=64). See Appendix 2.9 for the AIC Number that was used to select the best fit model.

| *Predictors* | *Estimates* | *Std. Error* | *CI* | *Statistic* | *p* |
|---|---|---|---|---|---|
| (Intercept) | 30.82 | 2.35 | 26.21 -35.44 | 13.09 | **<0.001** |
| Habitat [interdune] | -16.29 | 4.72 | -25.54 -7.03 | -3.45 | **0.001** |
| Habitat [river] | -1.22 | 2.60 | -6.30 -3.86 | -0.47 | 0.639 |
| **Random Effects** | | | | | |
| $\sigma^2$ | 236.96 | | | | |
| $\tau_{00\ \text{Station}}$ | 56.89 | | | | |
| $N_{\text{Station}}$ | 21 | | | | |
| Observations | 175 | | | | |

**Appendix 2.9:** Results from the Tukey HSD post hoc test for the model in appendix 2.8. This shows the significant difference between the distance at which !nara seeds were moved in different habitats.

| contrast | estimate | SE | d.f | t.ratio | p.value |
|---|---|---|---|---|---|
| sand dunes - interdunes | 16.29 | 4.72 | 152 | 3.447 | 0.0021 |
| sand dunes - river | 1.22 | 2.60 | 152 | 0.470 | 0.8854 |
| interdunes - river | -15.07 | 4.85 | 152 | -3.103 | 0.0064 |

**Appendix 2.10:** Results of a linear mixed effect model to test the effect of habitat on burial depth of !nara seeds, with stations as a random effect at 95% confidence interval. The total number of seed burials are n=175 (dune =96, interdune =15, river=64). See Appendix 2.9 for the AIC Number that was used to select the best fit model.

| *Predictors* | *Estimates* | *Std. Error* | *CI* | *Statistic* | *p* |
|---|---|---|---|---|---|
| (Intercept) | 3.63 | 0.18 | 3.28 – 3.99 | 19.89 | **<0.001** |
| Habitat [interdune] | 0.68 | 0.41 | -0.12 – 1.48 | 1.67 | 0.096 |
| Habitat [river] | 0.07 | 0.23 | -0.38 – 0.51 | 0.30 | 0.767 |
| **Random Effects** | | | | | |
| $\sigma^2$ | 1.83 | | | | |
| $\tau_{00\ \text{Station}}$ | 0.26 | | | | |
| ICC | 0.12 | | | | |
| $N_{\text{Station}}$ | 21 | | | | |
| Observations | 175 | | | | |
| Marginal $R^2$ / Conditional $R^2$ | 0.016 / 0.137 | | | | |

**Appendix 2.11:** Results from the Tukey HSD post hoc test for the model in appendix 2.10. This shows the significant difference between the depth at which !nara seeds were buried in different habitats.

| contrast | estimate | SE | d.f | t.ratio | p.value |
|---|---|---|---|---|---|
| sand dunes - interdunes | -0.6823 | 0.408 | 152 | -1.674 | 0.2184 |
| sand dunes – river | -0.0671 | 0.226 | 152 | -0.297 | 0.9526 |
| interdunes - river | 0.6152 | 0.419 | 152 | 1.467 | 0.3095 |

**Appendix 2.12:** Results from a generalised linear model to test if the size of !nara seed caches (number of seeds per cache) is related to habitat at 95% confidence interval. See Appendix 2.9 for the AIC Number that was used to select the best fit model.

| *Predictors* | *Incidence Rate Ratios* | *Std. Error* | *CI* | *Statistic* | *p* |
|---|---|---|---|---|---|
| (Intercept) | 1.80 | 0.08 | 1.55 – 2.09 | 7.75 | **<0.001** |
| Habitat [interdune] | 0.70 | 0.24 | 0.44 – 1.13 | -1.46 | 0.145 |
| Habitat [river] | 1.08 | 0.12 | 0.86 – 1.36 | 0.69 | 0.493 |
| Observations | 175 | | | | |
| $R^2$ Nagelkerke | 0.066 | | | | |

**Appendix 2.13:** Results from the Tukey HSD post hoc test for the model in appendix 2.12. This shows the significant difference between !nara cache sizes in different habitats.

| contrast | estimate | SE | d.f | z.ratio | p.value |
|---|---|---|---|---|---|
| sand dunes - interdunes | 0.3526 | 0.242 | Inf | 1.459 | 0.3110 |
| Sand dunes - river | -0.0805 | 0.117 | Inf | -0.686 | 0.7719 |
| interdunes - river | -0.4330 | 0.246 | Inf | -1.759 | 0.1837 |

**Appendix 2.14:** Results from a general linear model used to test recovery rate of seeds between the three habitats at 95% confident interval. See Appendix 2.9 for the AIC Number that was used to select the best fit model.

| | | days | | | |
|---|---|---|---|---|---|
| *Predictors* | *Incidence Rate Ratios* | *Std. Error* | *CI* | *Statistic* | *p* |
| (Intercept) | 2.96 | 0.14 | 2.26 – 3.88 | 7.91 | **<0.001** |
| Habita [interdune] | 1.80 | 0.29 | 1.01 – 3.21 | 2.02 | **0.045** |
| Habitat [river] | 0.86 | 0.23 | 0.55 – 1.34 | -0.69 | 0.494 |
| Observations | 175 | | | | |
| $R^2$ Nagelkerke | 0.158 | | | | |

**Appendix 2.15:** Results from the Tukey HSD post hoc test for the model in appendix 2.14. This shows the significant difference between the !nara cache recovery rate different habitats.

| contrast | estimate | SE | df | z.ratio | p.value |
|---|---|---|---|---|---|
| sand dunes - interdunes | -0.589 | 0.1266 | Inf | -4.656 | <.0001 |
| sand dunes - river | 0.156 | 0.0985 | Inf | 1.584 | 0.2527 |
| interdunes - river | 0.745 | 0.1366 | Inf | 5.454 | <.0001 |

**Appendix 2.16**: The AIC numbers for the nested models. The model that best fits the data was selected for each parameter (small AIC number).

| | **Model** | **AIC numbers** |
|---|---|---|
| **Seeds cached (Appendix 2.3)** | Proportion cached ~ species + habitat | 1724 |
| | Proportion cached ~ habitat | 1736 |
| | Proportion cached ~ species | 3050 |
| | Proportion cached ~1 | 3172 |
| **Seeds consumed (Appendix 2.4)** | Proportion consumed ~ species + habitat | 582 |
| | Proportion consumed ~ species | 651 |
| | Proportion consumed ~ habitat | 600 |
| | Proportion consumed ~1 | 681 |
| **Distances (Appendix 2.5)** | Distance ~ habitat | 1485 |
| | Distance ~1 | 1492 |
| **Depth (Appendix 2 .6** | Depth ~ habitat | 627 |
| | Depth ~1 | 626 |
| **Cache size** | Number of seeds per cache ~ habitat | 1491 |

| | | |
|---|---|---|
| **(Appendix 2.7)** | Number of seeds per cache ~ 1 | 1490 |
| **Recovery rate**<br>**(Appendix 2.8)** | Number of days ~ habitat | 884 |
| | Number of days ~ 1 | 907 |