

The potential for failure: Low reproductive success and high skew in female Cape
ground squirrels (*Xerus inauris*)

by

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Thesis Abstract

Life-history theory states that organisms must navigate trade-offs in resource allocation among growth, maintenance, and reproduction, with the cost of reproduction being the most prominent. These trade-offs differ among species, with long-lived organisms often balancing current and future reproduction, while short-lived species trade off between current reproduction and survival. Both intrinsic factors, such as heterozygosity, age, and experience, and extrinsic factors, including conspecific interactions, predation, and climate, shape reproductive success. Social living can confer anti-predator benefits and cooperative breeding advantages, but also incurs costs from increased competition and parasitism, generating complex patterns of reproductive skew within groups. Rodents provide an ideal system to study these dynamics due to their diversity in social structures and wide ecological distribution. The Cape ground squirrel (*Xerus inauris*), a plural breeder inhabiting the arid regions of southern Africa, exemplifies many traits of a slow life-history strategy, including small litter sizes, relatively long lifespans, and investment in survival. Unlike many cooperative breeders, this species is characterized by an egalitarian social structure with low aggression and no apparent dominance hierarchy, though reproductive skew remains high among females. Factors influencing female reproductive success in this species include age, body condition, parasitism, and environmental conditions, particularly rainfall and temperature. This thesis investigates the intrinsic and extrinsic factors influencing reproductive success and skew in female Cape ground squirrels, integrating behavioral, genetic, and ecological data. Contrary to predictions for a promiscuous, plural breeding species, sexual selection in Cape ground squirrels exhibited high reproductive skew and fluctuating intensities between sexes, with male sexual selection often exceeding that of females. The study also demonstrated that female reproductive success was strongly influenced by

maternal body condition, age, and environmental variability. Moreover, the presence of non-dispersed related males within social groups altered alloparental care patterns, reducing female alloparental investment while enhancing juvenile condition, supporting a load-lightening effect affected by the age and composition of helpers. These findings highlight the unique life-history trade-offs, cooperative breeding dynamics, social structure, and environmental pressures that can shape the reproductive strategies of arid-adapted mammals. Altogether, these findings enhance our understanding of how life-history strategies, cooperative dynamics, and environmental pressures shape reproductive success among social, arid-adapted species.

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Table of Contents

Thesis abstract	1
Acknowledgements	3
Table of contents	4
List of Tables	6
List of Figures	7
List of Supplementary Information.....	11
Chapter 1: General introduction	12
Thesis objectives.....	18
Literature cited	19
Chapter 2: Sexual selection and reproductive skew in a promiscuous cooperative breeder	28
Abstract	29
Introduction	30
Methods	37
Results	42
Discussion	43
Acknowledgements	46
Literature cited	47
Chapter 3: Reproduction in an arid-adapted African ground squirrel: Impact of extrinsic and intrinsic factors.....	62
Abstract	63
Introduction	65
Methods	71

Results	77
Discussion	81
Acknowledgements	86
Literature cited	87
Chapter 4: Who Cares? Alloparental care and its effects on juvenile Cape ground squirrels	108
Abstract	109
Introduction	110
Methods	115
Results	121
Discussion	124
Acknowledgements	128
Literature cited	129
Chapter 5: Helping and load-lightening in an African ground squirrel	146
Abstract	147
Introduction	148
Methods	152
Results	157
Discussion	160
Acknowledgements	163
Literature cited	164
Chapter 6: General discussion.....	178
Literature cited	182

List of Tables

Table 2.1: Total number of breeding Cape ground squirrel females and litters with paternity.

Table 2.2 Total number of breeding Cape ground squirrel males and the number of pups sired per year.

Table 2.3 Annual adult sex ratios of Cape ground squirrels in South Africa.

Table 2.4 Cape ground squirrel quantitative measures of sexual selection and selection for overall reproduction and mating success and adjusted reproduction and mating success that accounts for the number of years as a mature adult.

Table 3.1 Hypotheses and predictions of the factors influencing reproductive success in female Cape ground squirrel (*Xerus inauris*).

Table 3.2 Effects of rainfall, temperature, and group size on female reproductive traits in Cape ground squirrels (*Xerus inauris*). Statistical analyses included GLMMs for continuous outcomes (pre-pregnancy body condition) and negative binomial mixed models for count data (number of weaned offspring). Interactions between rainfall and temperature were also examined. Significant variables are bolded.

Table 3.3 Female Cape ground squirrel (*Xerus inauris*) reproductive success variables. Statistical analyses included logistic regression for binary outcomes (e.g., pregnancy failure, litter loss). Significant values are bolded.

Table 4.1 Hypotheses and predictions for alloparental care and juvenile benefits in Cape ground squirrels (*Xerus inauris*).

Table 4.2 Behavioral description of Cape ground squirrel (*Xerus inauris*) behaviors used in alloparental care.

Table 5.1 Behavioral description of Cape ground squirrel (*Xerus inauris*) behaviors used in alloparental care.

List of Figures

Figure 2.1 Cape ground squirrel total number of offspring from 2011-2019 on S.A. Lombard Nature Reserve, South Africa, determined by genotyped offspring. Male (*a*, $n = 369$) and female (*b*, $n = 342$).

Figure 2.2 Total reproductive success *a*) and total mating success *b*) of female and male Cape ground squirrel from 2011-2019. Reproductive and mating success were determined by genotyping offspring (male $n = 369$ and female $n = 342$).

Figure 2.3 Adjusted female and male Cape ground squirrel reproductive *a*) and mating *b*) success determined by genotyping offspring from 2011-2019 (male $n = 369$ and female $n = 342$).

Figure 2.4 Annual differences in the direction of selection in Cape ground squirrels. $I = \sigma^2_{\text{reproductive success}} / \bar{X}^2_{\text{reproductive success}}$ was used to calculate I . $\Delta I = I_{\text{male}} - I_{\text{female}}$ gave the direction of the selection in which males had a higher opportunity of sexual selection than females in most years.

Figure 3.1 Effects of annual rainfall on the number of offspring weaned by Cape ground squirrels (*Xerus inauris*) from 2002–2006 and 2011–2019. A negative binomial GLMM revealed with quadratic effects using random intercepts for Squirrel ID accounted for repeated measures across 227 individuals ($n = 536$ observations) to determine the relationships between rainfall and number of offspring produced.

Figure 3.2 Flooding per month on the successful number of Cape ground squirrels (*Xerus inauris*) offspring weaned 2002-2006 and 2011-2019 (0 floods $n = 145$ months, 1 flood $n = 32$ months, 2 floods $n = 3$ months).

Figure 3.3 Minimum *a*) and maximum *b*) temperatures on female Cape ground squirrel (*Xerus inauris*) pre-pregnancy body condition from 2012-2019 ($n = 35$).

Figure 3.4 Interaction effect of group size and minimum temperature on pre-pregnancy body condition in female Cape ground squirrels (*Xerus inauris*), 2012–2019. The relationship was

assessed using a linear model including group size, minimum temperature, and their interaction ($n = 44$).

Figure 3.5 Effect of maximum temperature on the number of juvenile Cape ground squirrels (*Xerus inauris*) weaned from 2002–2006 and 2011–2019. Points represent observed counts of juveniles. The solid line shows model-predicted values from a zero-inflated negative binomial GLMM with a log link for maximum temperature. Shaded areas represent 95% confidence intervals for the predicted mean number of juveniles ($n = 20$).

Figure 3.6 Pre-pregnancy body condition of Cape ground squirrels (*Xerus inauris*) in relation to minimum temperatures and annual rainfall from 2012–2019. Points represent individual observations, with each break point containing roughly equal observations. The fitted linear model included minimum temperature, annual rainfall, and their interaction ($n = 40$).

Figure 3.7 Interaction of maximum temperatures and annual rainfall on pre-pregnancy body condition of Cape ground squirrels (*Xerus inauris*) from 2012–2019. Points represent individual observations. A linear model was fitted to examine the effects of maximum temperature, annual rainfall, and their interaction on female pre-pregnancy body condition ($n = 40$).

Figure 3.8 Relationship between female age and pre-pregnancy body condition (size–mass residuals, $n = 176$) in Cape ground squirrels (*Xerus inauris*) from 2012–2019. Body condition was modeled using a GLMM with age, age², and a random intercept for individual (Squirrel ID) with a curvilinear relationship. A fitted quadratic curve with 95% confidence intervals is shown.

Figure 3.9 A linear relationship between pre-pregnancy body condition and the decrease in post-pregnancy mass in female Cape ground squirrels (*Xerus inauris*) ($n = 22$).

Figure 3.10 Effect of pre-pregnancy body condition on pregnancy failure in female Cape ground squirrels (*Xerus inauris*) from 2012–2019. The logistic regression model explained variation in pregnancy and the plotted line shows the predicted probability of pregnancy failure across the observed range of body condition, with 95% confidence bands ($n = 31$).

Figure 3.11 Quadratic relationship between female age and the number of offspring weaned in Cape ground squirrels (*Xerus inauris*) from 2002–2006 and 2011–2019. A negative binomial GLMM revealed a strong quadratic effect of age on annual reproductive success. The model

predicts a peak in offspring production at approximately 4 years of age. Random intercepts for Squirrel ID accounted for repeated measures across 227 individuals (n = 536 observations).

Figure 4.1 The rate (acts /hours) of alloparental and maternal care to juvenile Cape ground squirrels (*Xerus inauris*) in South Africa. *a*) The rate of alloparental and maternal care in the absence of natal males within a group. *b*) The rate of alloparental and maternal care given in the presence of natal males within a group.

Figure 4.2 Total alloparental care (acts/hour) directed toward juveniles in family groups with and without natal males in South Africa (2018–2019).

Figure 4.3 Age (in years) of Cape ground squirrel (*Xerus inauris*) alloparental caregivers and the rate of care individual juveniles are receiving in South Africa. Care was quantified per caregiver per juvenile. *a*) Natal male care (per juvenile) age in years; age 1 n = 34 natal males, age 2 n = 8, and 3+ n = 3; *b*) female alloparent care (per juvenile) age in years; age 1 n = 48 females, age 2 n = 31, age 3 n = 23, age 4 n = 19, age 5 n = 2, age 6 n = 4).

Figure 4.4 *a*) Natal male (n = 26 natal males) and *b*) adult female alloparent (n = 51 female alloparents) Cape ground squirrel (*Xerus inauris*) alloparenting effect on juvenile (n = 41) growth (mass final-mass start/ number of days between).

Figure 4.5 The absence (0, n = 21 juveniles) or presence (1, n = 27 juveniles) of natal males within a family group on juvenile body condition (residuals of a linear regression of mass on spine length) of Cape ground squirrels (*Xerus inauris*).

Figure 4.6 Percent of time that juvenile Cape ground squirrel spent foraging in the absence (n = 21 juveniles) and presence (n = 27 juveniles) of natal male caregivers.

Figure 4.7 Percent of time natal male *a*) (n = 28) and female *b*) (n = 106) Cape ground squirrels (*Xerus inauris*) caregivers are present on juvenile foraging.

Figure 4.8 Female and natal male Cape ground squirrels (*Xerus inauris*) alloparental care on juvenile survival.

Figure 4.9 The absence (Natal male = 0, n = 20) and presence (Natal male = 1, n = 26) of natal male Cape ground squirrels (*Xerus inauris*) on juvenile survival probability.

Figure 5.1 The absence (n = 2 groups)/presence (n = 8 groups) of natal male Cape ground squirrels on the amount of maternal care (acts/hour) in South Africa.

Figure 5.2 The absence (n = 2 groups)/presence (n = 8 groups) of natal male Cape ground squirrels on the amount of alloparental care (acts/hour) a mother gave to other offspring in their group.

Figure 5.3 The absence (n = 5 groups)/ presence (n = 2 groups) of natal male Cape ground squirrels on the amount of alloparental care by non-mothers in South Africa.

Figure 5.4 The amount of alloparental care (acts/hour) given by *a*) natal males (n = 14 natal males and *b*) female alloparents (non-mothers, n = 8) on the amount of maternal care (acts/hour) given in South Africa.

Figure 5.5 Vigilance (percent of time) of mothers (n = 32) and non-mothers (n = 11) in the presence of juveniles.

Figure 5.6 Mother vigilance (percent of time, n = 32) *a*) and non-mother vigilance (n = 11) *b*) in the absence/presence of natal male Cape ground squirrels.

Figure 5.7 The absence and presence of natal male Cape ground squirrels on *a*) mother (n = 32) and *b*) non-mother (n = 11) female foraging time.

Figure 5.8 The absence and presence of natal male Cape ground squirrel age on *a*) mother (n = 32) and *b*) non-mother (n = 11) body condition.

Figure 5.9 The absence/presence of natal males Cape ground on successful pregnancies for *a*) mothers (n = 32) and *b*) non-mothers (n = 11).

List of Supplementary Information

Figure S4.1 Amount of allogrooming from family group natal males *a)* and adult females *b)* on the abundance of ectoparasites on juvenile Cape ground squirrels.

Chapter 1 General Introduction

Life-history theory provides a framework for understanding how organisms allocate limited resources among competing demands of survival, growth, and reproduction (Stearns 1992). These allocations are shaped by trade-offs that influence fitness across an individual's lifespan. Female mammals, in particular, face high energetic demands from gestation and lactation (Williams et al. 2017), forcing trade-offs between current reproduction, future reproduction, and survival, a concept formalized as the “cost of reproduction” (Rödel et al. 2016, Viblanc et al. 2018). Small mammals, with high metabolic rates, must balance energy allocation to maximize both their own survival and fitness (Culina et al. 2019). Variation among females in body condition, age, and reproductive experience further modulates these trade-offs, while reproductive performance generally follows a non-linear trajectory with age, peaking in mid-life and declining due to senescence (Cockburn et al. 2008, Culina et al. 2019, Lemaître et al. 2020, Roper et al. 2021).

Sexual selection closely interacts with life-history trade-offs, influencing how individuals allocate resources to maximize reproductive success. Sexual selection favors traits that increase mating success, either through mate attraction or competition with rivals, even when these traits do not directly enhance survival (Darwin 1871, Barber 1995). Differences in reproductive investment between males and females generate variation in reproductive success, with males typically exhibiting higher variance due to mate competition, while female reproductive success is constrained by the number and quality of offspring they can produce and rear (Batman 1948, Arnold and Duvall 1994, Wade and Shuster 2005). Multiple mating by females can modify these dynamics by increasing litter size, enhancing genetic diversity, or reducing infanticide risk (Hrdy 1999, Jones et al. 2002, Kvarnemo et al. 2013). Reproductive skew theory formalizes the

unequal distribution of reproductive success within groups, which varies from low, where opportunities are evenly distributed, to high, where dominants monopolize reproduction (Faulkes et al. 1997, Clutton-Brock et al. 2001, Schneider and Kappeler 2014, Wcislo and Fewell 2017). The structure of a population's mating system strongly shapes both sexual selection and skew: monogamous species often exhibit low variance and similar selective pressures across sexes, while polygynous and promiscuous systems can produce high variance and skew, particularly under multiple mating or high female fecundity (Arnold and Duvall 1994, Bergeron et al. 2012, Jones et al. 2012). Social and ecological factors, including mate availability, population density, and female mating strategies, also modulate these patterns (Hauber and Lacey 2005, Shuster 2009, Krakauer et al. 2011).

Rising temperatures can reduce foraging opportunities, body mass, and survival, particularly in arid-adapted species (Mason et al. 2014, Van de Ven et al. 2020, Thorley et al. 2025b, a). Although these species are adapted to high ambient temperatures, they typically live near the upper limits of their thermal tolerance and rely on behavioral strategies, such as restricting activity to cooler times of day, to cope with heat stress (Mason et al. 2014, Van de Ven et al. 2020, Thorley et al. 2025b, a). When temperatures rise further, these strategies increasingly constrain foraging time and food intake, leading to declines in body condition and survival. Thus, even small shifts in climate can have disproportionately large effects on arid-zone mammals, with cascading impacts on reproduction.

Resource availability is a central ecological constraint shaping reproductive strategies. Most small mammals are income breeders, relying on current environmental resources rather than stored reserves to fuel reproduction and survival (Stearns 1989, Rödel et al. 2016). In arid and semi-arid ecosystems, rainfall has a strong influence on primary productivity and food

availability, resulting in pronounced variability in resources (Neal 1984, Happold and Happold 1992). Many southern African mammals, including tropical ground squirrels and sengis, adjust reproductive schedules to track seasonal or pulsed resource availability, with reproductive peaks typically occurring during warm, wet periods and minimal activity during colder, drier months (Delany 1972, Emmons 1979, Viljoen and Du Toit 1985, Parrin 1986, Neal 1995, Bernard et al. 1996, Medger et al. 2012). Fluctuations in rainfall and temperature directly influence life-history traits, including reproductive timing, litter size, and maternal investment. Reduced rainfall can delay reproduction and lower fecundity, while extreme rainfall events or flooding can inundate dens and increase juvenile mortality, as observed in banded mongooses (*Mungos mungo*), Brandt's voles (*Lasiopodomys brandtii*), Richardson's ground squirrels (*Urocitellus richardsonii*), European ground squirrels (*Spermophilus citellus*), and Natterer's bats (*Myotis nattereri*; Proulx 2012, Kachamakova et al. 2022, Stapelfeldt et al. 2022, Li et al. 2023, Khera et al. 2025).

Maternal investment mediates the effects of environmental variability on reproductive success. Maternal body condition, age, and experience influence litter size, offspring survival, and future fitness (Lee et al. 1991, Broussard et al. 2008, Hamel et al. 2010, Taillon et al. 2012, Michel et al. 2019). Peak litter loss occurs during late gestation and early lactation, when energetic demands are highest (Marshall et al. 2016, Rödel et al. 2016, Culina et al. 2019). When food resources are critically low, females may cease breeding, abandon litters, or wean offspring early, increasing juvenile mortality (Bronson and Marsteller 1985, Lee et al. 1991, Thorley et al. 2025a). Beyond maternal investment, social dynamics can buffer or exacerbate environmental constraints, further shaping female reproductive strategies.

Social dynamics further shape reproductive strategies. Cooperative breeding species, defined by the occurrence of alloparental care, exhibit complex social systems that can include female-female competition, reproductive suppression, and delayed dispersal (Lacey and Sherman 1997, Hauber and Lacey 2005, Apakupakul and Rubenstein 2015). Helpers perform behaviors such as provisioning, babysitting, and predator defense, which can improve juvenile survival, growth, and condition (Russell et al. 2003, Rosenbaum and Gettler 2018, Vitikainen et al. 2019). These contributions reduce maternal investment costs through a “load-lightening effect,” allowing breeders to conserve energy for future reproduction and increase fecundity or shorten interbirth intervals (Crick 1992, Russell et al. 2003, Cockburn et al. 2008, Meade and Hatchwell 2010, Paquet et al. 2013).

Cooperative breeding interacts with sexual selection, reproductive skew, and environmental constraints. Dominant females often monopolize resources and helper care, enhancing reproductive success, while subordinates may experience suppression or delayed reproduction (Clutton-Brock et al. 2001, Young and Clutton-Brock 2006). In singular-breeder systems, high reproductive skew can intensify sexual selection, whereas plural-breeder groups typically exhibit lower skew and weaker sexual selection pressures (Hauber and Lacey 2005). Although kin selection under monogamy is a primary driver of helping behaviors in most species, cooperative breeding also occurs in species with variable mating systems and high promiscuity, demonstrating the complex interplay between social structure, mating system, and reproductive strategies (Cornwallis et al. 2010, Leggett et al. 2012). These social dynamics can buffer individuals against ecological challenges by distributing the costs of reproduction and enhancing offspring care. Alloparental care can buffer environmental stressors, but extreme conditions, such as high temperatures or drought, may override these benefits, reducing offspring

mass, female body condition, reproduction, and survival (Van de Ven et al. 2020, Groenewoud and Clutton-Brock 2021, Thorley et al. 2025a).

Overall, life-history trade-offs, sexual selection, ecological constraints, and social dynamics interact to influence variation in reproductive success across social species. By integrating these factors, we gain a more complete understanding of how organisms adaptively allocate resources and shape their reproductive strategies in complex and dynamic environments. To investigate these processes, rodents provide an ideal study system. They span a continuum of social organizations and occupy diverse environmental conditions, allowing comparative studies among closely related species experiencing different selective pressures (Wolff and Sherman 2007). For example, diurnal, semifossorial rodents living in arid environments are often highly social. This sociality is thought to reduce predation risk, enhance vigilance, and facilitate cooperative behaviors such as thermoregulation and offspring care (Hoogland 1999, Clutton-Brock et al. 2002, Randall and Rogovin 2002). In contrast, semifossorial temperate ground squirrels, such as marmots (tribe Marmotini), exhibit social variation, from solitary individuals to social cooperative breeders, which are influenced by multiple ecological and life-history factors, including predation pressure, short growing seasons, food availability, and body size (Armitage 1981, Michener 1983, Blumstein and Armitage 1997). African ground squirrels (tribe Xerini) inhabit arid and semi-arid regions and experience distinct ecological pressures, including low and unpredictable rainfall, high temperatures, and seasonal variation in food availability. These conditions may favor social strategies that buffer environmental uncertainty, such as group vigilance, alloparental care, and group foraging, rather than responses to a short growing season (Waterman 1995, Schradin et al. 2012, Shenbrot 2014). Understanding how these different

selective pressures shape social organization across closely related species provides a strong framework for investigating the evolution of sociality and reproductive strategies.

Cape ground squirrels (*Xerus inauris*) are well-suited to test hypotheses drawn from life-history theory, as they have many traits that are indicative of a slow life history and a high reproductive skew in females, both of which are rare in small mammals (Waterman 1996, Pettitt et al. 2008, Culina et al. 2019). Females of this species are philopatric, while males typically disperse (Waterman 1995), resulting in groups composed of related females, their subadult offspring, and adult male offspring that have delayed dispersal (natal males; Waterman 1995, Scantlebury et al. 2007, Manjerovic and Waterman 2015). These groups appear to be egalitarian in nature with no obvious dominance hierarchy and, unlike most mammalian social systems, aggression is rare (Waterman 1995, Pettitt and Waterman 2011). It has also been suggested that they are facultative cooperative breeders, but this hypothesis has never been tested (Waterman 1995, Pettitt and Waterman 2011, Manjerovic and Waterman 2015). They are also promiscuous breeders, as both males and females mate with multiple partners in a single breeding event (Waterman 1998, Manjerovic and Waterman 2015). Dispersed adult males live independently of females, in non-kin roving bands that are non-aggressive and lack dominance hierarchies (Waterman 1996, 2002, Manjerovic et al. 2022).

Female Cape ground squirrels can breed throughout the year, and they give birth in an isolated burrow away from the family burrow but within their group's feeding range (Waterman 1996). Females return to the family group after the litter is weaned or is lost (Waterman 1996). Reproductive failure is common, and 70% of reproductive events fail before weaning (Waterman 1996, Pettitt et al. 2008). Although experienced females are more successful in weaning offspring compared to first-time breeders, the mass of weaned offspring and litter size do not

differ between these groups (Pettitt et al. 2008). Parasites can reduce reproductive success in Cape ground squirrels, as when parasites were removed, there was a fourfold increase in reproductive success (Hillegass et al. 2010). However, reproductive success varies significantly among females, even within experienced females or females without parasites (Pettitt et al. 2008, Hillegass et al. 2010). The cause of this high reproductive skew in females in this egalitarian species is unknown.

The objective of this thesis is to explore fundamental questions in life history theory, reproductive ecology, and social behavior of Cape ground squirrels. In **Chapter 2**, I examine how the intensity of sexual selection and the degree of reproductive skew differ between males and females. In **Chapter 3**, I examine whether resource availability limits reproductive success and survival, testing the effects of extrinsic and intrinsic factors on female reproduction. In **Chapter 4**, I evaluate how alloparental care affects offspring's well-being and survival and if differences in alloparent sex and age affect the amount of care given. In **Chapter 5**, I assess whether helpers provide a load-lightening effect on breeding females. By integrating ecological, physiological, and social factors, this work addresses the trade-offs and constraints that shape reproduction and survival in an arid-adapted mammal with a unique social organization.

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Chapter 2 Sexual selection and reproductive skew in a promiscuous cooperative breeder

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Abstract

Sex differences in life history and reproduction lead to distinct sex differences in reproductive skew and sexual selection. In promiscuous species, sexual selection is expected to be similar in both sexes, whereas in cooperative species, female sexual selection is expected to be stronger due to female-female competition. Cape ground squirrels (*Xerus inauris*) are a promiscuous, plural-breeding species with cooperative breeding. Adult males and females live independently, as males disperse and join all-male roving bands, and females live in family groups. Sexual selection is predicted to follow promiscuous indicators in this species, meaning females and males will have a similar strength of sexual selection, and both males and females are expected to have high reproductive skew. Individual squirrels were live-trapped and monitored for copulations, lactation, pregnancy, and weaning of offspring from 2011-2019. The number of copulations (genetics) and the number of offspring were used to examine the differences in intensity of selection and sexual selection in females and males. Our results support high reproductive skew, and sexual selection acts differently on male and female Cape ground squirrels, contrary to what is predicted for a promiscuous species. While both females and males exhibited high reproductive skew, the intensity of sexual selection fluctuated yearly, with male sexual selection often higher than female sexual selection. Differences in male and female social organization, combined with cooperative breeding, shaped the distribution of reproductive success and the strength of sexual selection in Cape ground squirrels. Examining these dynamics, along with the impact of sexual selection, will enhance our understanding of life history strategies and how they influence reproductive outcomes.

Keywords: Cape Ground Squirrels, Plural Breeder, Promiscuous, Reproductive Skew, Sexual Selection, *Xerus*

Introduction

Sexual selection is defined as the evolution of traits that increase mating success by either attracting mates or intimidating rivals (Darwin 1871, Barber 1995). Although these traits may not directly enhance survival, they contribute to increased mating and reproductive success. The "currency" of sexual selection can be measured in terms of the number of mates attained (mating success) or the number of offspring sired (reproductive success; Jones et al. 2005), typically assessed through metrics such as offspring production within a sex or the variance in the number of copulations (Drury 2010).

One way to measure sexual selection is a variance-based approach that includes the opportunity for sexual selection and the opportunity for selection (Krakauer et al. 2011). The opportunity for sexual selection is calculated using variance in mating success (i.e., the number of copulations), while the opportunity for selection is calculated using variance in reproductive success (i.e., the number of offspring produced; Wade 1979, Arnold and Duvall 1994, Krakauer et al. 2011). Both the opportunity for sexual selection and selection are measures of the maximum potential strength of sexual selection (Jones et al. 2005, Jones 2009, Krakauer et al. 2011).

The sex with the larger variance or skew in reproductive and mating success is the sex with greater strength of sexual selection (Krakauer et al. 2011). Because of anisogamy, female reproductive success is often constrained by the number of offspring they can invest in, which lowers their selection potential (the upper boundary of selection; Bateman 1948, Trivers 1972, Wade 1979). Multiple mating can influence female reproductive success in several ways, both directly and indirectly. In some species, females who mate with multiple males produce larger litters, thereby increasing their reproductive success (Jones et al. 2002, Calsbeek et al. 2007). Mating with multiple partners may also reduce male harassment and improve offspring survival

by providing greater access to resources or decreasing the risk of infanticide (Hrdy 1999). Additionally, females may benefit indirectly by increasing genetic diversity within a litter, which can enhance offspring quality (Kvarnemo and Simmons 2013). Male reproductive success is largely determined by access to mates (Bateman 1948, Trivers 1972). While multiple mating by females can benefit males, high rates of multiple paternity tend to reduce male reproductive skew, leading to a more equal distribution of mating success among males (Jones et al. 2012).

Multiple paternity, in which more than one male sires offspring from a single reproductive event, is a common occurrence among mammalian species. However, the frequency of multiple paternity varies considerably both between species and across populations within a species (Dean et al. 2006). Variation in multiple paternity is thought to be influenced by a combination of environmental and social factors that affect mating behavior and reproductive strategies (Solomon and Keane 2007). Among these, the degree of sociality, the extent to which individuals associate and interact within a group, has emerged as a potential predictor. Research suggests that species with higher levels of social organization often exhibit lower rates of multiple paternity, possibly due to increased male monopolization of mates or stronger social bonds that limit opportunities for multiple mating (Munroe and Koprowski 2011).

Reproductive skew theory seeks to explain the unequal distribution of reproductive success in animal societies. In social animals, reproductive skew may be variable within a species, between populations, or between sexes. Reproductive skew exists along a continuum, from low skew, where all mature individuals have similar chances to reproduce, to high skew, where reproductive opportunities are highly unequal. High skew is particularly evident in eusocial species, such as honeybees (*Apis* spp.) and naked mole rats (*Heterocephalus glaber*), as well as in cooperative breeding species where reproduction is monopolized by dominant

individuals, such as suricates (*Suricata suricatta*) and dwarf mongooses (*Helogale parvula*) (Faulkes et al. 1997, Clutton-Brock et al. 2001, Schneider and Kappeler 2014, Wcislo and Fewell 2017).

Mating systems, mating strategies, and even the number of mates available in populations may change reproductive skew and the within-sex variance in mating and reproductive success, causing the strength of sexual selection to vary, within genera to differences between sexes of a single population (Jones et al. 2002, Hauber and Lacey 2005, Shuster 2009, Krakauer et al. 2011). In monogamous species, both males and females usually have low reproductive skew, and sexual selection is equal, as both have low but equivalent reproductive and mating success (Lack 1968, Parker and Tang-Martinez 2005). In polygynous species, males compete for access to mates and are, therefore, under stronger sexual selection pressures compared to females (Wade and Shuster 2004, Jones et al. 2012). In promiscuous mating systems, both males and females are expected to have large variances in mating and reproductive success (Arnold and Duvall 1994, Munroe and Koprowski 2011, Bergeron et al. 2012, Jones et al. 2012). Thus, the sexes in promiscuous species may be under similar selective pressures, especially when females have large litter sizes with multiple paternity (Arnold and Duvall 1994, Munroe and Koprowski 2011, Bergeron et al. 2012, Jones et al. 2012). However, the degree of promiscuity may vary between the sexes, which would create different selective pressures. In systems where females may compete for mates, such as polyandrous, promiscuous, and cooperative breeding species, females may also experience strong sexual selection pressures (Hauber and Lacey 2005, Jones et al. 2005, Apakupakul and Rubenstein 2015).

Social systems may also affect the intensity of sexual selection, especially in cooperative breeding species. A main characteristic of cooperative breeding is alloparental care (care by non-

parents), but cooperative breeding species may also have strong female-female competition, reproductive suppression, and delayed dispersal (Lacey and Sherman 1997). These cooperative breeding characteristics significantly impact variance in reproductive success (Hauber and Lacey 2005, Apakupakul and Rubenstein 2015). Especially in cooperative breeders with dominance hierarchies, selective pressures on females will increase through within-group female-female competition (Hauber and Lacey 2005, Apakupakul and Rubenstein 2015). Female and male reproductive and mating success would also be affected in singular breeder cooperative societies (a single pair in the group reproduces) vs plural breeders (all mature adults can reproduce). High skew and high sexual selection are expected for singular breeders, while plural breeders are expected to have low skew and low sexual selection (Hauber and Lacey 2005).

Monogamy and low levels of promiscuity may be prerequisites to the evolution of cooperative breeding, as such groups are highly related, and kin selection is the most supported hypothesis for the evolution of helping (Leggett et al. 2012). However, cooperative breeding occurs in species with various mating systems (Cornwallis et al. 2010). Some of the highest rates of promiscuity have been described in cooperative breeders, e.g., superb fairywren (*Malurus cyaneus*; Cornwallis et al. 2010). Whether promiscuity and/or cooperative breeding systems work in situ or separately to affect the intensity of sexual selection within a species has been understudied. There is also very little literature on differences in rates of promiscuity between males and females and how these rates of promiscuity affect sexual selection.

Squirrels are an apt group for investigating the relationship between social and mating systems and the intensity of sexual selection. Ground-dwelling squirrels exhibit a diverse range of mating and social systems (Armitage 1981, Blumstein and Armitage 1997, Munroe and Koprowski 2011, Bergeron et al. 2012, Jones et al. 2012), from solitary individuals that are

highly promiscuous (e.g., golden-mantled ground squirrels, *Callospermophilus lateralis*; Wells et al. 2017) to cooperative singular breeders (e.g., *Marmota marmota*; Allainé 2000). Cape ground squirrels (*Xerus inauris*) are cooperative breeders that are highly promiscuous, which makes them a good species to examine sex differences in reproductive skew (Waterman 1995, Manjerovic et al. 2022). Female Cape ground squirrels live in matrilineal groups composed of related females and their subadult offspring (Waterman 1995, 2002). These family groups appear egalitarian with no obvious dominance hierarchy (Waterman 1995, Pettitt et al. 2008). Every female can breed once reaching maturity, but the number of litters weaned varies greatly, and 70% of reproductive attempts fail (Waterman 1996, Pettitt and Waterman 2011). Immature females can be reproductively suppressed through delayed maturity, but there has been no evidence of suppression in mature females within a group (Waterman 2002, Pettitt and Waterman 2011).

Adult male Cape ground squirrels have two alternative reproductive tactics (Manjerovic and Waterman 2015). Males that have dispersed from their families live in non-kin roving bands (hereafter referred to as band males) that constantly change in composition and size (Waterman 1996, 1997). Roving male bands overlap with multiple female groups, where males continually search for estrous females (Waterman 1995, Manjerovic et al. 2022, Warrington et al. 2024). The alternative tactic is males delay dispersal to remain with their natal group after reaching maturity, known as natal males (Waterman 1995, Manjerovic et al. 2022, Warrington et al. 2024). Multiple males can mate with a single female during their short estrus (approximately 3 hrs; Waterman 1996, Pettitt et al. 2008), and females mate with up to six males during this time (Manjerovic et al. 2022). However, the likelihood of multiple paternity is very low in this species because of their small litter size (1-2 offspring; Waterman 1996, Manjerovic and Waterman 2015). While

most litters are composed of a single offspring, in litters of two offspring, almost every litter has two sires. In addition, only 28% of males successfully sire offspring in any given year (Manjerovic and Waterman 2015). Precopulatory sexual selection in male bands may change with the distribution of females in different habitats. In a population in Namibia, males establish a dominance hierarchy, whereas in South Africa, body condition and sperm competition appear to be pivotal factors in higher mating success (Manjerovic et al. 2022). In the South African population, males appear to compete for mating opportunities via scramble competition, mate guarding, and sperm competition (Manjerovic et al. 2008, 2022). Whether the mating system or the social system in either sex will have a more or equal impact on reproductive skew and the intensity of sexual selection in Cape ground squirrels is unknown.

We used long-term data from a study of Cape ground squirrels in South Africa to examine the strength of sexual selection and reproductive skew in males and females. We hypothesized that promiscuity would affect sexual selection. Cape ground squirrels are highly promiscuous with a high operational sex ratio of 11 males to 1 female during estrus (Manjerovic et al. 2022). While females mate with an average of 3.24 mates per estrus, males mate with an average of 5 females annually (Waterman 1996, 1998, Manjerovic et al. 2022). We hypothesized that: 1) promiscuity equalizes sexual selection across sexes, as both sexes have high promiscuity in their mating system, we predicted similar sexual selection strengths for males and females (Munroe and Koprowski 2011, Bergeron et al. 2012). 2) If male-male competition increases male intensity of sexual selection, then the social structure and within-group competition differences between the sexes would affect the intensity of sexual selection. Thus, we predicted that intragroup competition would be higher in male groups, leading to a higher intensity of sexual selection for males relative to females, as males tend to mate guard and the male bands tend to be larger than

female family groups (Manjerovic et al. 2022). We also predicted that multiple paternity would be low, as Cape ground squirrels have small litter sizes and are highly social. Multiple paternity is not common in species with higher levels of sociality (Munroe and Koprowski 2011, Schwanz et al. 2016). 3) We also hypothesized that female competition increases female intensity of sexual selection. While neither male nor female groups have dominance hierarchies, there is evidence of female-female competition (Waterman 1995, 2002, Manjerovic et al. 2022). Female-female competition would increase the intensity of sexual selection among females and subsequently diminish the intensity of sexual selection among males (Wade 1979). Evidence to support female-female competition for reproductive positions in Cape ground squirrels includes family group splitting, where larger groups split (Waterman 2002). In addition, group splitting is usually along kinship lines, with an older female usually moving with her offspring (usually a daughter) to form a new family group (Lem 2020). Sub-adult females have delayed maturity when too many mature adult females are present within the family group, suggesting young females split to avoid delaying reproduction (Waterman 2002, Lem 2020). Given this potential for competition for reproductive positions, we predicted that females would have a stronger intensity of sexual selection than males, resulting from large variances in mating and reproductive success (Hauber and Lacey 2005, Rubenstein and Lovette 2009, Apakupakul and Rubenstein 2015). However, given the life history of this species and the perceived lack of dominance and aggression among females and males within and between groups, 4) reproductive skew should not differ between males and females. This latter hypothesis also predicts they should have low reproductive skew, as every member of the group can breed once reaching maturity, a prediction that is contrary to what is already known about the species and that should be eliminated.

Methods

Study species

Cape ground squirrels are long-lived rodents with a slow life history endemic to the arid regions of southern Africa (Herzig-Straschil 1978, Waterman 1995, 1996). Their social structure is highly sex-segregated, with females living in family groups and adult males forming roving bands.

Females are philopatric, meaning they remain in their natal area, living with their relatives (Waterman 1995). Each family group occupies a separate underground aggregation of burrows, known as a burrow cluster, with only one group residing in a cluster (Herzig-Straschil 1978, Waterman 1995). Over time, some burrow clusters are abandoned, creating vacant burrows across the landscape (Waterman 1995). Females are spontaneous ovulators and breed year-round (Waterman 1995, Bouchie et al. 2006). However, reproduction within and among family groups is highly asynchronous (Bouchie et al. 2006, Manjerovic and Waterman 2015). Gestation lasts approximately 50 days, followed by a 47.5-day lactation period (Pettitt et al. 2008). Females give birth in isolated, small burrows they dig, away from their family group, and only return to their group after weaning their offspring or losing their litter. Both sexes reach reproductive maturity at around eight to ten months, though subadult females may experience delayed maturity due to reproductive suppression (Waterman 2002, Pettitt and Waterman 2011).

In contrast to females, males disperse from their natal groups at maturity and form all-male roving bands (Waterman 1995, 1997). These bands use vacant burrow clusters as temporary shelters while travelling in search of estrous females (Waterman 1995, 1997, 1998). Despite their high operational sex ratio, sexual dimorphism is relatively slight, as males are only 1.1 times larger than females (LaFlèche and Waterman 2020). Band male home ranges are extensive,

encompassing multiple female groups (Waterman 1998, Manjerovic et al. 2022). However, not all males disperse when reaching maturity. Natal males, mature males that delay dispersal, can remain in their family group for up to five years (Waterman 1995, Manjerovic and Waterman 2015, O'Brien et al. 2021). Though natal males temporarily leave to mate with females from neighboring groups, they rarely sire offspring in their own family group, with only one instance of inbreeding out of 93 juveniles (Manjerovic and Waterman 2015). The average dispersal age for natal males is 3.5 years (O'Brien et al. 2021).

Study area and trapping

Fieldwork was conducted at S.A. Lombard Nature Reserve in South Africa (27° 35'S, 25° 35'E) from 2011 to 2019. This study site averages 509 mm of rainfall (1952-2019), varying annually from 202.6 to 934.3 mm. The squirrel sex ratio at this site is 1:1, with approximately 6 adult females and 6 adult males per hectare (Manjerovic et al. 2022).

Squirrels were captured using live traps (Tomahawk Live Trap Inc., WI, USA; 15x15x50 cm) baited with peanut butter and bird seed (Waterman 1995). Upon their first capture, every squirrel was injected with a passive integrated transponder (PIT tag; AVID Inc., Folsom, LA or Shenzhen XCC RFID Technology Co., Ltd., China) for permanent identification, and a tail tip skin sample was taken for genetic analysis. For identification from a distance, all squirrels were given a unique dye mark on the dorsal pelage (Rodol D; Lowenstein and Sons, New York, NY, U.S.A.; Waterman 1996). We assessed the reproductive condition of all individuals at each handling to check for sexual maturity and reproductive condition. Females were reproductively mature if their nipples were elongated. Females' reproductive condition was assessed through the swelling of the vulva (indicating estrus), and we palpated the abdomen to detect pregnancy. Sexual maturity in males was indicated by descended scrotums. All procedures were approved by

the University of Manitoba's Animal Care and Use Protocol (#F10-030, #F14-032, and #F18-039).

Observations

As only one family group sleeps in a burrow cluster and they share a communal sleeping burrow, we observed who emerged together from a burrow in the morning or immersed together into the burrow in the evening to determine family group membership (Waterman 1995). In addition to trapping and genetics, the maternity of offspring was determined by observations of female isolation and their subsequent return to their family with their newly weaned offspring, and if those offspring were subsequently trapped. We observed squirrels from towers or blinds mounted on vehicles using 10 x 50 binoculars and 20 x 60 spotting scopes (Phillips and Waterman 2013).

DNA Extraction and Genotyping

To determine parentage, we used E.Z.N.A. Tissue DNA kits (Omega Bio-Tek Inc., Norcross, U.S.A.) and High Pure PCR Template Preparation kits (Roche Diagnostics, Mannheim, Germany) to extract the DNA from tail tissue samples. DNA from 2011-2019 was amplified using the polymerase chain reactions (PCR) with GoTaq® Flexi DNA Polymerase (Promega Corporation, Madison, U.S.A.; Manjerovic and Waterman 2015, Shave and Waterman 2017). Nineteen fluorescently labeled forward primers and unlabeled reverse microsatellite primer sets were developed for this species and were used for PCR (Abercrombie et al. 2009, Manjerovic and Waterman 2015). We visualized the PCR product on a 2% agarose gel to check PCR success. Post-PCR products were pooled, diluted, and sent to the Centre for Applied Genomics (Hospital for Sick Children, Toronto, Canada) for genotyping. Genotypes were scored through GeneMarker v. 2.6.0 and then manually to reduce errors.

Parentage Analysis

The genotyping results were put through Genecap v.1.4. and COANCESTRY v.1.0.1.10 (Wang 2011) to eliminate duplicate samples. We genotyped 1521 individuals, with 77.4% genotyped at 15 or more loci (19 total loci). The 344 individuals that had fewer than 15 genotyped loci were removed from the analysis.

We used MasterBayes v.2.57 R package, which is a Bayesian approach incorporating genetic (microsatellite loci) and non-genetic data (distance, area, colony, year, age; Hadfield et al. 2006) and CERVUS v. 3.0.7, which uses just genetic data (Kalinowski et al. 2007) to genetically assign parentage at an 80% confidence level. All potential parents assigned under the 80% match to the offspring were dropped (46 potential mothers and 99 potential fathers). Individuals at or above 80% confidence were checked for accuracy using a 4-step process that included observation and trapping data to verify parentage. For mothers, the 4-step check was as follows: 1) Potential mothers were checked for age versus offspring emergence date. If potential mothers were not sexually mature when the offspring would have been in utero, these individuals were eliminated as potential mothers. 2) Potential mothers were then checked to see if they were in the same group as the offspring recorded in our group observation data. If potential mothers were in another group, these females were eliminated as potential mothers. 3) If the candidate's mother was not observed with any group, they were checked for timing of trapping to determine whether they were alive at the time of offspring birth. 4) If candidate mothers were alive, we checked if the potential mother was in the same area as the offspring using our trapping data. If potential mothers passed all these criteria, they were determined to be the mother. For potential fathers, we checked if the father was 1) sexually mature and 2) if the father was trapped or alive when the offspring were conceived. Individuals who failed this process were not identified as

parents. Twenty-six potential mothers and 20 potential fathers out of 628 offspring failed the assignment check and were removed from the dataset. In total, 519 offspring were assigned to females and 428 to males, with some individuals represented in both groups.

Statistical Analysis

We calculated total and adjusted reproductive success (RS) and mating success (MS) based on the number of individuals genetically assigned through parentage analysis (Jones 2009). Total reproductive success was the total number of offspring an individual produced on-site between 2011-2019, whereas adjusted reproductive success was the total number of offspring produced divided by the number of years an animal was sexually mature within our trapping area (RS/years sexually mature). Similarly, mating success was the total number of mates over the total of the individual's mating success (MS), while adjusted reproductive success (RS) was the total number of mates divided by the number of years an animal was sexually mature. Reproductive individuals with no assigned offspring received a 0 score for reproductive success and mating success unless an individual was not reproductively mature or considered deceased. Individuals were considered deceased when not trapped in the current year or the following year (Warrington et al. 2025). The opportunity for selection (I) and sexual selection (I_s) were calculated following Bergeron (2012). The opportunity for selection (I) is the variance in reproductive success divided by its mean squared, while the opportunity for sexual selection (I_s) is the variance in mating success divided by its mean squared (i.e. $I = \sigma_{rs}^2 / (\bar{X}_{rs}^2)$ and $I_s = \sigma_{ms}^2 / (\bar{X}_{ms}^2)$; Arnold and Duvall 1994, Krakauer et al. 2011, Bergeron et al. 2012). The opportunity of selection and sexual selection are unitless metrics that are sufficient for measuring the potential for selection and whether sexual selection is operating, and these measures can be compared between sexes and populations (Krakauer et al. 2011). I and I_s can be used to

investigate sex differences in patterns of sexual selection and investigate whether ecological or social factors can shape these sexual selection patterns (Krakauer et al. 2011). I and I_s are calculated for males and females separately, which allows for a comparison between the sexes (Krakauer et al. 2011). We calculated I and I_s for total reproduction and mating, as well as the adjusted values. For adjusted values, we divided reproduction and mating by the number of years an individual was a mature adult (able to reproduce) on the study site. To determine sex differences in sexual selection, we ran a t-test assuming unequal variances for σ_{rs} , σ_{ms} , $\bar{X}_{rs}$, and $\bar{X}_{ms}$ and a Mann-Whitney U test for mating and reproductive success using R version 2023.09.1 (R Core Team 2021). Delta I (ΔI) is the difference in reproduction of males minus the difference in reproduction in females. We used $\Delta I = I_{\text{males}} - I_{\text{females}}$ to identify the direction of selection towards either male or female in a given year (mating) (Krakauer et al. 2011, Apakupakul and Rubenstein 2015).

Results

From 2011 to 2019, we captured and genotyped 1131 individuals, of which 345 were mature females, 369 were mature males, and 628 were offspring. Only 320 females ($\bar{X} \pm \text{SE}$; $39.1 \pm 5.0\%$ per year; range 12% to 55%; Table 2.1, Figure 2.1) and 290 males ($35.8 \pm 3.1\%$ per year; range 27.1% to 55.9%; Table 2.2, Figure 2.1) successfully weaned offspring. A total of 404 litters were weaned, with 287 litters of one (71%), 116 litters of two (28.7%), and one litter of three (0.25%). Of the litters of two, 80% (93/116) had multiple paternity, with 5 (4.3%) litters of unknown paternity being excluded. In addition, the single litter of three had different fathers identified for each offspring. Overall, adult sex ratios did not differ ($t = -0.29$, $df = 14.11$, $p = 0.78$); therefore, adult Cape ground squirrels had a 1:1 sex ratio (Table 2.3).

We found sex differences in total reproductive success and total mating success using a Mann-Whitney U test based on the number of total mates and reproductive success per individual. Mating success differed significantly between sexes ($w = 71366$, $p = 0.002$, n female = 342, n male = 369, Figure 2.2), as did reproductive success ($w = 72068$, $p \geq 0.0001$, n female = 342, n male = 369, Figure 2.2). We also examined adjusted mating and reproductive success, which differed significantly between the sexes (adjusted reproductive success: $w = 689886$, $p = 0.016$; adjusted mating success: $w = 68905$, $p = 0.023$; n female = 342, n male = 369, Figure 2.3). Males had a higher total opportunity for selection ($I = 2.66$) and higher total opportunity for sexual selection ($I_s = 2.86$) than females ($I = 2.34$, $I_s = 2.17$; Table 2.4). The adjusted opportunity for sexual selection and selection was also higher in males compared to females (Table 2.4). The total difference in the annual variance of reproduction between the sexes, $\Delta I = (I_{male} - I_{female})$, was 0.33. The opportunity for selection ΔI on average was greater than zero, indicating that males tended to have a greater opportunity in sexual selection, although in three out of nine years (2011–2012, 2014), females showed higher sexual selection intensity than males (Figure 2.4).

Discussion

Our results demonstrate that sexual selection operates differently between male and female Cape ground squirrels, contrary to our initial prediction for this promiscuous species (Arnold and Duvall 1994, Bergeron et al. 2012). While we expected high promiscuity to lead to similar sexual selection in both sexes, mating success and reproductive success differed. Females experienced strong sexual selection, consistent with predictions based on high variance in reproductive success and potential within-group competition. Nevertheless, males generally exhibited higher opportunities for selection (ΔI), than females in most years. These annual fluctuations resemble patterns reported in other cooperative breeders, such as the superb starling

(*Lamprotornis superbus*; Apakupakul and Rubenstein 2015). Despite this, the overall pattern indicates that males have a higher potential for sexual selection, likely influenced by male dispersal around 3.5 years of age (O'Brien et al., 2021). While some males may have reproduced outside our study area prior to dispersal, young males in roving bands or natal groups rarely sire offspring (Warrington et al., 2024), suggesting our measures of male reproductive success are reasonably accurate. Although we hypothesized low reproductive skew due to the potential for all mature individuals to breed, our findings instead revealed high reproductive skew in both male and female Cape ground squirrels, aligning with previous knowledge of the species (Waterman 1996, Manjerovic et al. 2022).

Male reproductive success in Cape ground squirrels is influenced by pre- and post-copulatory mechanisms. Pre-copulatory competition occurs through scramble competition and competitive searching, where only 35–36% of males successfully copulate or sire offspring (Waterman 1998, Manjerovic et al. 2022). Males also exhibited post-copulatory competition through sperm competition and multiple paternity, which is especially pronounced in litters of two or more offspring, where over 80% were multiply sired. Additional mechanisms include female mate choice, where older and more docile males have higher reproductive success (Warrington et al., 2024), as well as population-specific behaviors such as copulatory plug formation in South African populations that limit female remating (Manjerovic et al. 2022). Males may also differ in mating strategies depending on local social structure: in Namibia, dominance hierarchies influence access to mates, whereas in South Africa, males rely on mate guarding rather than physical aggression (Waterman 1995, 1997, Manjerovic et al. 2022). These factors, combined with cryptic female choice and experience in locating mates, generate high variance in male reproductive success: 65% of males never sired offspring, and among

successful sires, only 30% fathered more than one offspring over the study period. Collectively, pre-copulatory competition, post-copulatory mechanisms, and female mate choice interact to drive the high intensity of sexual selection observed in males ($I_s = 2.86$), a value more comparable to polygynous than promiscuous species (Arnold and Duvall 1994, Krakauer et al. 2011).

Female reproductive success was influenced by social and ecological factors. Unlike many mammals, female Cape ground squirrels lack dominance hierarchies within family groups (Waterman 1995), yet they exhibited substantial reproductive skew, with 60% never reproducing and over 70% of breeding events failing (this study; Waterman 1996, Pettitt et al. 2008). Group size affects the age at maturity of sub-adult females (Waterman 2002, Pettitt and Waterman 2011), and large female groups eventually fission to form new family units (Lem 2020). Elevated sexual selection indices ($I_s = 2.18$) and high reproductive failure suggest competition for resources even in the absence of overt aggression. Environmental constraints, such as ectoparasite load, also shape female reproductive outcomes; females from which parasites were removed weaned offspring at four times the rate of untreated females (Hillegass et al. 2010). These findings indicate that female reproductive success is determined by both subtle social interactions and ecological pressures.

Despite the expectation that promiscuity and egalitarian social systems reduce reproductive skew (Hauber and Lacey 2005, Munroe and Koprowski 2011, Apakupakul and Rubenstein 2015), Cape ground squirrels exhibited high skew in both sexes. Both males and females are capable of breeding upon maturity, with no evidence of adult reproductive suppression (Pettitt and Waterman 2011). Females form matrilineal, egalitarian groups, while males reside in overlapping roving bands, providing broad access to mates without

monopolization (Waterman 1997, 1998, 2006). Existing theoretical models of reproductive skew, including transactional, tug-of-war, and priority-of-access models, fail to fully explain observed patterns. Subtle reproductive suppression, cryptic dominance, physiological variation, or resource-based limitations likely contributed to the high skew, particularly among females (Altmann 1962, Cant 1998, Clutton-Brock 1998, Reeve and Keller 1998, Charpentier et al. 2005, Boesch et al. 2006, Port and Kappeler 2010, Nonacs and Hager 2011).

Overall, sexual selection in Cape ground squirrels is complex and sex-specific. Males experience intense competition driven by pre-copulatory searching, sperm competition, and female mate choice, whereas females face strong selection pressures associated with resource competition and environmental constraints. Despite the absence of dominance hierarchies or overt aggression in females, substantial variance in reproductive success indicates that subtle social and ecological factors shape outcomes. Our findings highlight that sexual selection and reproductive skew in this species are shaped by the combined influences of mating system dynamics, social organization, and ecological constraints.

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Tables

Table 2.1: Total number of breeding Cape ground squirrel females and litters with paternity.

Year	Total adult females	Breeding females	Non-breeding females	Litters	Litters with 1 pup	Litters with 2 pups	Litters with 3 pups	2 pup litters with 1 father	2 pup litters with 2 fathers	2 pup litters with unknown fathers
2011	50	26	24	31	23	6	0	2	4	0
2012	67	8	59	9	6	3	0	0	3	0
2013	80	34	46	41	32	9	0	2	7	0
2014	73	17	56	18	15	4	0	1	3	0
2015	75	24	51	29	19	10	0	0	9	1
2016	79	26	53	33	22	11	0	1	9	1
2017	89	48	41	69	51	18	0	1	15	2
2018	123	59	64	72	51	21	1	6	15	0
2019	141	78	63	102	68	34	0	5	28	1
Total	777	320	457	404	287	116	1	18	93	5

Table 2.2: Total number of breeding Cape ground squirrel males and the number of pups sired per year.

Year	Total adult males	Successful males	Unsuccessful males	Number of pups	Sired 1 offspring	Sired 2 offspring	Sired 3 offspring	Sired 4 offspring	Sired 5 offspring	Sired 6 offspring	Sired 7 offspring
2011	34	19	15	25	13	6	0	0	0	0	0
2012	48	13	35	15	11	2	0	0	0	0	0
2013	76	29	47	50	19	5	3	0	1	0	1
2014	70	20	50	27	15	4	0	1	0	0	0
2015	90	26	64	35	19	5	2	0	0	0	0
2016	84	25	59	34	18	5	2	0	0	0	0
2017	115	47	68	68	33	9	3	2	0	0	0
2018	149	47	102	75	30	11	3	2	0	1	0
2019	155	64	91	99	42	15	2	4	1	0	0
Total	821	290	531	428	200	62	15	9	2	1	1

Table 2.3: Annual adult sex ratios of Cape ground squirrels in South Africa

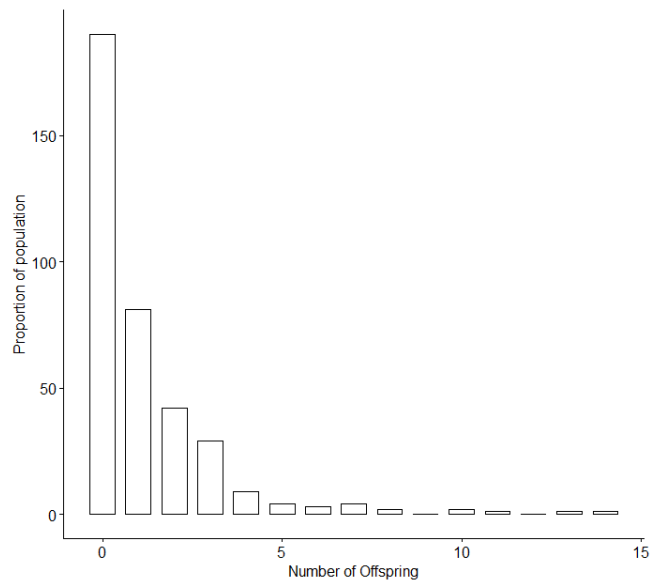
Year	Total adult females	Total adult males	Ratio (males to 1 female)
2011	50	34	0.68 : 1
2012	67	48	0.72 : 1
2013	80	76	0.95 : 1
2014	73	70	0.96 : 1
2015	75	90	1.20 : 1
2016	79	84	1.06 : 1
2017	89	115	1.29 : 1
2018	123	149	1.21 : 1
2019	141	155	1.10 : 1
Total	777	821	1.06 : 1

Table 2.4: Cape ground squirrel quantitative measures of sexual selection and selection for overall reproduction and mating success and adjusted reproduction and mating success that accounts for the number of years as a mature adult

Measures	Males (n=817)	Females (n=775)
Reproductive success (Total)		
$\bar{x}_{rs}$	0.49	0.68
σ^2_{rs}	0.64	1.08
I	2.67	2.34
Mating success (Total)		
$\bar{x}_{ms}$	0.52	0.63
σ^2_{ms}	0.77	0.86
I_s	2.86	2.18
Reproductive success (Adjusted)		
$\bar{x}_{rs}$	0.40	0.59
σ^2_{rs}	0.30	1.53
I	1.86	1.52
Mating success (Adjusted)		
$\bar{x}_{ms}$	0.38	0.59
σ^2_{ms}	0.26	0.53
I_s	1.80	1.53

Figures

a



b

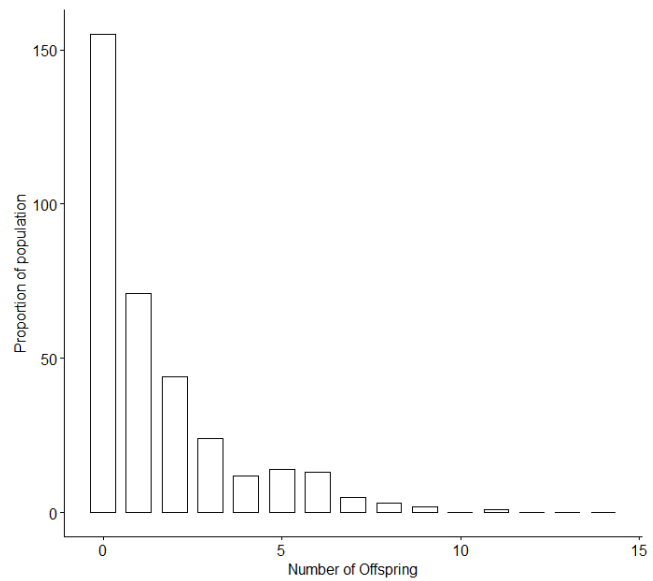
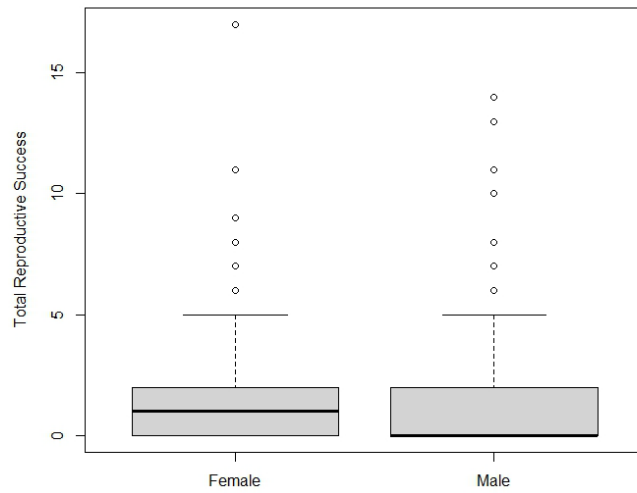


Figure 2.1: Cape ground squirrel total number of offspring from 2011-2019 on S.A. Lombard Nature Reserve, South Africa, determined by genotyped offspring. Male (*a*, $n = 369$) and female (*b*, $n = 342$).

a



b

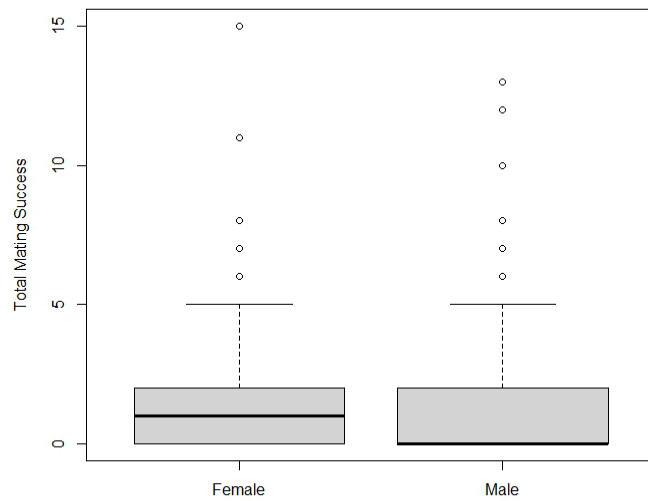
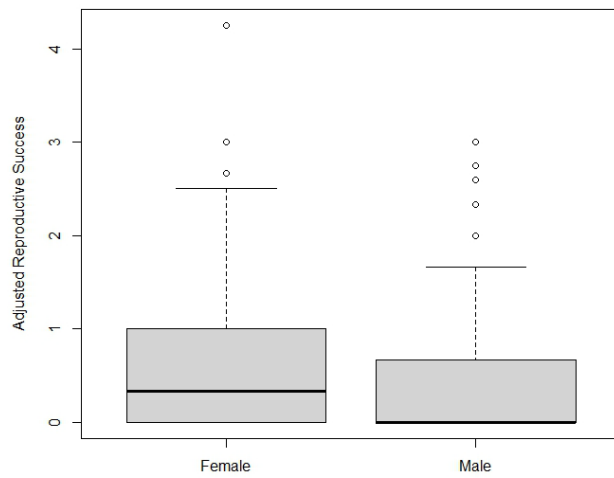


Figure 2.2: Total reproductive success *a*) and total mating success *b*) of female and male Cape ground squirrel from 2011-2019. Reproductive and mating success were determined by genotyping offspring (male $n = 369$ and female $n = 342$).

a



b

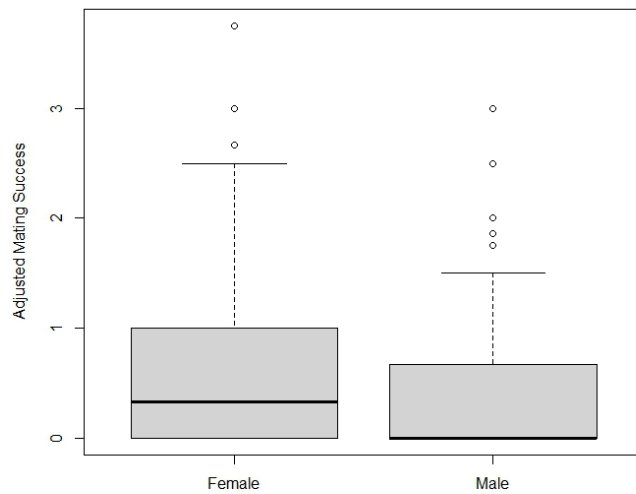


Figure 2.3: Adjusted female and male Cape ground squirrel reproductive *a*) and mating *b*) success determined by genotyping offspring from 2011-2019 (male $n = 369$ and female $n = 342$).

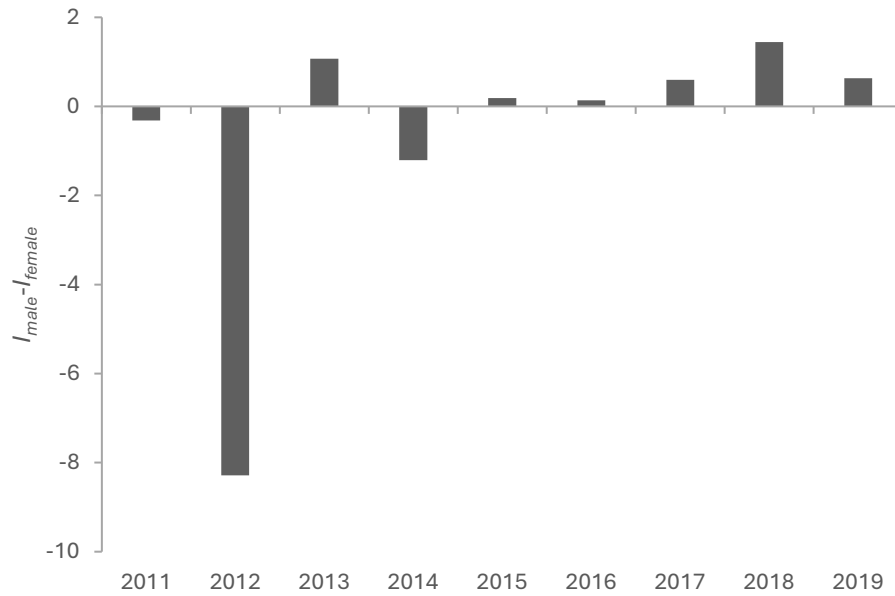


Figure 2.4: Annual differences in the direction of selection in Cape ground squirrels. $I = \sigma^2_{\text{reproductive success}} / \bar{X}^2_{\text{reproductive success}}$ was used to calculate I . $\Delta I = I_{male} - I_{female}$ gave the direction of the selection in which males had a higher opportunity of sexual selection than females in most years.

Chapter 3 Reproduction in an arid-adapted African ground squirrel: Impact of extrinsic and intrinsic factors

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Abstract

Life history theory examines the trade-offs in resource allocation between maintenance, growth, and reproduction. In arid regions, where temperature extremes and fluctuating rainfall create substantial ecological constraints, such trade-offs can significantly influence reproductive success. Climate influences these investments by affecting thermoregulation costs and food availability, and rainfall is often used as a proxy for resources in many desert-adapted mammals. Temperature and rainfall are related to maternal quality and reproductive investment measures in many mammals, driving variation in reproductive success. Cape ground squirrels are an arid-adapted social species with small litter sizes (1-2 offspring) and high rates of reproductive failure. They produce most of their offspring during the dry winter months. The objective of this study was to determine the major factors that affect reproduction in Cape ground squirrels. We live-trapped squirrels in central South Africa and collected data on the effects of environmental factors (rainfall, temperature), female age, and body condition (size-mass residuals) on reproductive success (reproductive failure, number of reproductive attempts and litter size). Female reproductive success was affected by both rainfall and temperature, as well as pre-pregnancy body condition and age. Increased rainfall decreased litter size. Increased maximum temperatures negatively impacted litter size, and higher minimum temperatures decreased female body condition. Females with a lower body condition before pregnancy were more likely to lose their litters during pregnancy. Reproductive success in females peaked at the age of four, and no offspring were successfully weaned after the age of seven. Elevated temperatures, coupled with increasing frequency and intensity of droughts, are both predicted to occur in Africa and will substantially affect primary productivity. As climate change increases the variability of

temperature and rainfall, it may reduce the reproductive success and body condition of this arid-adapted ground squirrel.

Keywords: Arid adapted, Cape ground squirrel, Cooperative Breeder, Female, Rainfall, Reproduction, Temperature, *Xerus inauris*

Introduction

Life history theory provides a framework for understanding how organisms allocate limited resources among competing demands such as growth, maintenance, and reproduction (Stearns 1992). These trade-offs are particularly pronounced in environments where resource availability is highly variable and unpredictable. Arid and semi-arid systems exemplify such environments, where fluctuations in rainfall and temperature impose substantial energetic constraints on survival and reproduction (Hulme et al. 2001, Dore 2005, Heffelfinger et al. 2018). Ongoing climate change further intensifies these pressures, with an increased frequency of droughts, floods, and extreme heat events (Sithole and Murewi 2009).

Environmental variability, particularly fluctuations in rainfall and temperature, directly shapes life history traits such as reproductive timing, litter size, and offspring investment. Reduced rainfall can delay reproduction and lower fecundity in large herbivores (Ogutu and Owen-Smith 2005, Ogutu et al. 2013), while rising temperatures restrict foraging opportunities and food availability, leading to declines in body mass across species (Mason et al. 2014), including small mammals such as suricates (*Suricata suricatta*, Thorley et al. 2025a). In tropical and subtropical latitudes, reproduction is closely tied to rainfall, which enhances food availability, and many southern African mammals show corresponding peaks in reproductive activity during the warm, wet spring and summer months when resources are most abundant, remaining largely inactive during the colder, drier period (Neal 1995, Bernard et al. 1996, Tinney et al. 2001, Medger et al. 2012, Thorley et al. 2025b). Even species capable of breeding year-round, such as sengis and tropical squirrels, adjust reproductive schedules to track pulses of food availability (Delany 1972, Emmons 1979, Viljoen and Du Toit 1985, Parrin 1986). Alignment of reproduction with rainfall-driven resource pulses is mirrored in larger mammals such as African

buffalo (*Syncerus caffer*), further underscoring the central role of rainfall and temperature as environmental drivers of reproductive strategies (Brown et al. 1991).

Increased rainfall is not always beneficial. Flooding that occurs during years of high rainfall can substantially reduce offspring survival. In banded mongooses (*Mungos mungo*), for example, heavy rain can inundate birthing dens, while high temperatures further lower pup survival (Khera et al. 2025). Similar patterns are observed in a range of small mammals, where extreme rainfall events directly increase mortality. Brandt's voles (*Lasiopodomys brandtii*) have been found dead following intense storms (Li et al. 2023), hibernating Richardson's ground squirrels (*Urocitellus richardsonii*) experience higher mortality when dens flood (Proulx 2012), and European ground squirrels (*Spermophilus citellus*) suffer greater losses during years of excessive rainfall and snowmelt (Kachamakova et al. 2022). Even in non-rodents, such as Natterer's bats (*Myotis nattereri*), elevated spring rainfall is associated with reduced reproductive success (Stapelfeldt et al. 2022).

Unpredictable rainfall can create resource unpredictability, which exacerbates life history trade-offs, especially in arid-adapted species (Williams 1966, O'Brien et al. 2021, Culina et al. 2019). When conditions are poor, individuals may reduce or forego reproduction in favor of survival (Waterman and Fenton 2000, Thorley et al. 2025b). Maternal investment, a key component of reproductive strategy, reflects these trade-offs, as the energy and resources allocated to offspring can directly affect both offspring fitness and the mother's future reproductive potential (Lee et al. 1991, Broussard et al. 2008). Maternal traits, such as body condition and age, significantly influence offspring survival, especially during late gestation and early lactation when energetic demands peak (Taillon et al. 2012). Maternal energetic condition strongly governs litter survival, with most losses occurring in late gestation and early lactation.

When resources are low, females may abandon litters, cease lactation, or forgo breeding entirely (Bronson and Marsteller 1985, Lee et al. 1991, Hamel et al. 2010, Marshall et al. 2016, Rödel et al. 2016, Richard et al. 2017, Culina et al. 2019, Michel et al. 2019, Thorley et al. 2025b).

Adequate food resources before and during lactation affect the amount of nutrients in the milk, and low resources can increase weaning time and reduce the probability of having a subsequent litter (Bronson and Marsteller 1985, Lee et al. 1991, Heldstab et al. 2017).

Variance in female condition and quality can affect energetic trade-offs as long-lived mammals maximize their survival by decreasing immediate reproductive effort (Trivers 1972, Stearns 1989, McLean et al. 2019). Reproductive performance in female mammals often follows a non-linear trajectory with age (Wang and Novak 1994, Forslund and Pärt 1995). The ability to have a successful pregnancy also increases with age, and older females tend to have larger and heavier litters with higher survivorship (Culina et al. 2019). These differences may reflect age-related variation in foraging efficiency, dominance, or home range quality (Cockburn et al. 2008, Hamel et al. 2010). Early in life, primiparous females typically have lower reproductive success compared to multiparous females, as reproductive experience and maternal efficiency improve with age (Culina et al. 2019, Cockburn et al. 2008). However, reproductive senescence (age-related decline in reproductive output) has been observed across many mammalian species (Lemaître et al. 2020). Reproductive decline may reflect cumulative physiological deterioration or age-specific trade-offs among reproduction, such as fertility, parental investment, or offspring survival, that increasingly prioritize maintenance or survival over continued reproductive output (Stearns 1989, Roper et al. 2002). In socially complex or cooperative species, where caregiving roles are shared, the onset and severity of senescence may be buffered, but age-related declines in fertility and maternal performance are still expected (Vágási et al. 2020).

In cooperative breeders, social dynamics may modulate life history trade-offs. Alloparental care can reduce the costs of reproduction and buffer against environmental variability (Jetz and Rubenstein 2011, Groenewoud and Clutton-Brock 2021), though such buffering may fail under increasingly extreme climates (Van de Ven et al. 2020, Thorley et al. 2025a). Together, these patterns emphasize the importance of life history theory in explaining how organisms adaptively respond to ecological constraints. However, cooperative breeding can also be an insufficient buffer when temperatures keep climbing. In suricates, increasing temperatures are associated with declining pup mass (Van de Ven et al. 2020) and also decreased reproduction, body mass, and survival in adults (Thorley et al. 2025a).

Female reproductive success is also often closely tied to dominance status in cooperative breeders. Life-history theory predicts that when reproduction is costly and resources are limited, dominant individuals will monopolize breeding opportunities (Stearns 1992). High-ranking females often gain priority access to food, secure the majority of alloparental care, and are more likely to breed successfully, while subordinates may experience reproductive suppression or delay reproduction entirely (Clutton-Brock et al. 2001). In species like suricates and African wild dogs (*Lycaon pictus*), dominant females not only reproduce more frequently but also have higher offspring survival due to greater access to helpers and superior territories (Creel and Creel 1991, Russell et al. 2002). These patterns reflect a strategic allocation of reproductive effort that is shaped by social structure and environmental constraints, reinforcing the significance of social rank as a mediator of life history trade-offs in cooperative systems. Although dominance has been well studied, our understanding of the variance in reproductive success of species without classic dominance hierarchies and aggression is lacking.

This study examines both extrinsic and intrinsic factors influencing reproduction in female Cape ground squirrels (*Xerus inauris*), a cooperative breeding species characterized by an egalitarian social structure with low aggression and no dominance hierarchies (Waterman 1996). Adapted to arid environments where droughts are frequent (Herzig-Straschil 1978, Waterman and Fenton 2000, Skurski and Waterman 2005), Cape ground squirrels remain active and capable of breeding year-round. However, a distinct peak in breeding occurs during winter, which does not align with periods of peak resource availability (Waterman 1996, 2002, Pettitt et al. 2008). This winter breeding peak may be influenced by residual resources from the preceding summer rainy season (Pettitt et al. 2008). In years with low rainfall, few if any offspring are successfully weaned (Waterman 1996, Waterman & Fenton 2000). Temperature also affects this arid-adapted species, as high ambient temperatures are thermally challenging for Cape ground squirrels and they use their burrows as thermal refuges to offload heat (Scantlebury et al. 2012). Body temperature in Cape ground squirrels decreases with larger group sizes, suggesting less energy expenditure in larger groups (Scantlebury et al. 2012).

Cape ground squirrels are long-lived, with lifespans of up to 13 years, and typically produce small litters of one to two offspring (Waterman 1996, Skurski and Waterman 2005, Chapter 2). These squirrels are promiscuous communal breeders, with females residing in matrilineal groups ranging from 2 to 20 individuals (Pettitt and Waterman 2011). As reproductive success in males increases with age (Warrington et al. 2024), similar patterns are predicted for females. Despite this prediction, reproductive failure is common in adult females; approximately 70% of breeding events fail to produce weaned young (Waterman 1996, Waterman and Fenton 2000, Pettitt et al. 2008). This high rate of litter loss contributes to significant reproductive skew, with only 39% of females successfully producing surviving

offspring during their lifetimes (Chapter 2). While female parity influences reproductive success, female body condition appears to be unrelated to offspring survival (Pettitt et al. 2008) and the factors leading to this high reproductive skew have not been identified.

We used a long-term data set of a South African population of Cape ground squirrels to investigate how extrinsic and intrinsic factors affect female reproductive success (Table 3.1). We hypothesized that the extrinsic factors of rainfall and temperature would affect female reproductive success. Environmental unpredictability could intensify trade-offs between reproduction and survival. Therefore, we predicted that increased rainfall would improve environmental resource availability and female condition, reducing pregnancy failure. However, we also expected that heavy rainfall (rainfall ≥ 40 mm within 48 hours) may increase lactation failure due to the flooding of burrows, while increased annual rainfall will reduce pregnancy failure by increasing resources. We also predicted that both high maximum and low minimum monthly temperatures would negatively impact female reproductive success by lowering body condition, increasing reproductive failure, and decreasing litter size.

In line with life history theory's prediction that social structure can modulate life history trade-offs, we hypothesized that larger group sizes would buffer females from the negative effects of environmental stress. Specifically, we expected that group living may mitigate the effects of temperature extremes (particularly cold stress) by enhancing thermoregulation in large groups, thereby improving reproductive success.

We hypothesized that two key intrinsic factors, body condition and age, would strongly influence reproductive success. Following life history theory, which predicts that organisms in better condition should be more capable of investing in reproduction, we expected that females in better pre-pregnancy condition would have greater reproductive success, larger litter sizes, and

improved post-isolation condition. Poor pre-pregnancy condition is expected to increase the likelihood of pregnancy and lactation failure, potentially prompting repeated breeding attempts as females attempt to offset early losses.

Age is also expected to influence reproductive performance. Older females may exhibit higher reproductive success due to accumulated maternal experience and/or improved foraging efficiency. Thus, we predicted that older females would have larger litters, more total offspring, and fewer reproductive failures. However, consistent with the theory of reproductive senescence, we also expected that reproductive performance would decline in the oldest females.

Methods

Study species

Cape ground squirrel groups occupy aggregations of burrows, known as burrow clusters, with only one female family group residing in a cluster (Herzig-Straschil 1978, Waterman 1995). Upon maturity (descended scrotum), many Cape ground squirrel males disperse and join all-male bands (hereafter band males; Waterman 1995, Waterman 1997, Manjerovic and Waterman 2015). Females live in cooperative breeding family groups consisting of up to seven females, subadults of either sex and undispersed related adult males (hereafter, natal males; Waterman 1995, Scantlebury et al. 2007, Hillegass et al. 2010, Manjerovic and Waterman 2015, Chapter 4). Family groups do not have dominance hierarchies, nor does reproductive suppression occur in mature adult females, but the maturity of sub-adult females can be delayed, especially when groups include three or more adult females and/or any natal males (Waterman 1995, Pettitt and Waterman 2011). When not reproductively suppressed, females reach sexual maturity at around 8 months and can breed up to four times annually (Waterman 1995, Pettitt et al. 2008). Gestation lasts approximately 50 days. When ready to give birth, females temporarily isolate themselves

from their group and dig a small natal burrow for parturition (Waterman 1996, Pettitt et al. 2008). Cape ground squirrel neonates are altricial, born blind and naked, and mothers nurse their young over a lactation period of roughly 48 days (Herzig-Straschil 1978, Waterman 1996, Skinner and Chimimba 2005, Pettitt et al. 2008). After weaning, both the mother and her offspring reintegrate into the family group.

Study area and trapping

We conducted fieldwork at S.A. Lombard Nature Reserve in South Africa (27 ° 35'S, 25 ° 35'E) from 2002-2019, with a gap from 2008-2010. The reserve averages 509 mm of rainfall (1952-2019), with rainfall varying annually from 202.6 to 934.3 mm. Nature reserve personnel measured on-site rainfall using a rain gauge after each rainfall event (LaFlèche and Waterman 2020). We used the rainfall from November (start of the rainy season), prior to the field season, to October (end of the dry season) to investigate the effects of rainfall on reproduction during the winter. We trap on the eastern part of the reserve, which is characterized by Cymbopogon-Themeda veld and black soil turf veld, including a dry watercourse (a flood plain and our main study area) that floods during high rainfall (van Zyl 1965, Skead 1974). We calculated flooding as occurring when ≥ 40 mm of rainfall fell within 48 hours, as runoff from surrounding areas several kilometers away drains into the flood plain (van Zyl 1965, Skead 1974). In other nearby areas, between 40 and 60 mm is considered significant rainfall or heavy rainfall (McBride et al. 2022); thus, we considered > 40 mm as a measure of flooding in this flood plain as a conservative estimate. Flooding was recorded during our fieldwork (2002–2006 and 2011–2019), but flooding did not occur annually.

We captured squirrels using live traps (Tomahawk Live Trap Inc., WI, USA; 15X15X50 cm) baited with peanut butter and bird seed (Waterman 1995). Upon the first capture, we

injected every squirrel with a passive integrated transponder (PIT tag; AVID Inc., Folsom, LA or Shenzhen XCC RFID Technology Co., Ltd., China) for permanent identification, and a tail tip skin sample was taken for genetic analysis (see below). For identification from a distance, all squirrels were given a unique dye mark on the dorsal pelage (Rodol D; Lowenstein and Sons, New York, 235 NY, U.S.A.; Waterman 1996). We weighed individuals with spring scales (Pesola, Switzerland) and measured their spine length from the base of the skull to the base of the caudal vertebrae using a flexible polypropylene tape measure (only post-2011; prior to 2012, spine length was not taken; Schulte-Hostedde et al. 2005). Female sexual maturity was determined by mass ($> 500\text{g}$) and nipple length, as nipples permanently swell after the first estrus (Pettitt et al. 2008). We classified females as subadults (immature) if they were greater than 500g and had non-elongated nipples. Squirrels were classified as juveniles if they had tiny nipples and weighed $< 500\text{ g}$ (Pettitt et al. 2008). During each capture, all adult females had their reproductive condition assessed by checking for the swelling and opening of the vulva (indicating estrus), abdominal palpation for pregnancy and a light manual palpation of an elongated nipple to detect milk, indicating lactation (Waterman 1996, Pettitt et al. 2008, Skibieli and Hood 2015). Pre-pregnancy body condition was measured when an individual was not pregnant. All procedures were approved by Animal Ethics committees at the University of Manitoba (Protocols #F10-030, #F14-032, and #F18-039), the University of Central Florida (Protocols #01-11W, #04-33W, #07-43W) and by the University of Pretoria (Protocol # EC020514-006).

Observations

We observed squirrels to determine family group membership and maternity from towers or blinds mounted on vehicles using 10 x 50 binoculars and 20 x 60 spotting scopes (Phillips and

Waterman 2013). As only a single family group inhabits a burrow cluster and shares a sleeping burrow, we recorded who emerged or immersed together, in the morning and evening, respectively, from the same burrow to determine family group membership (Waterman 1995).

DNA Extraction, Genotyping and Parentage

We used E.Z.N.A. Tissue DNA kits (Omega Bio-tek Inc., Norcross, U.S.A.) and High Pure PCR Template Preparation kits (Roche Diagnostics, Mannheim, Germany) to extract the DNA from tail tissue samples. DNA was amplified using the polymerase chain reactions (PCR) with GoTaq® Flexi DNA Polymerase (Promega Corporation, Madison, U.S.A.; Manjerovic and Waterman 2015, Shave and Waterman 2017). Nineteen fluorescently labeled forward primers and unlabeled reverse microsatellite primer sets were developed for this species and were used for PCR (Abercrombie et al. 2009, Manjerovic and Waterman 2015, Shave and Waterman 2017). We visualized the PCR product on a 2% agarose gel to check PCR success. Post-PCR products were pooled, diluted, and sent to the Centre for Applied Genomics (Hospital for Sick Children, Toronto, Canada) for genotyping. Genotypes were scored through GeneMarker v. 2.6.0 and then scored manually to reduce errors.

Genotyping results were first screened using Genecap v.1.4 and COANCESTRY v.1.0.1.10 (Wang 2011) to identify and remove genetic matches. Parentage was then assigned using two complementary approaches: (1) MasterBayes v.2.57, a Bayesian method that integrates microsatellite genotypes with non-genetic information (distance, area, colony, year, and age; Hadfield et al. 2006), and (2) CERVUS v.3.0.7, which applies likelihood-based parentage assignment (Kalinowski et al. 2007) at an 80% confidence threshold. Potential parents assigned below 80% confidence were excluded from further consideration. Assignments meeting or exceeding the 80% threshold were subsequently validated using a four-step procedure

incorporating observational and trapping data. For maternal assignments, we confirmed: (1) that the candidate's age was consistent with the offspring's gestation period; (2) that the candidate belonged to the same family group as the offspring; (3) that ungrouped candidates were alive at the estimated time of birth; and (4) that the candidate overlapped spatially with the offspring based on trapping records. Candidates passing all criteria were accepted as mothers.

Statistical Analyses

We used females that were trapped a minimum of four times between May and August each year in our analysis. A reproductive attempt was defined as a period of female reproductive activity identified between May and August, based on indicators such as estrus, pregnancy, changes in body mass, signs of lactation, and temporary isolation from the family group. Each attempt was classified as successful, failed, or undetermined depending on whether isolation or lactation occurred, whether isolation ended prematurely, and whether offspring successfully returned to the family group burrow. We used multiple methods to determine estrus, including observations of mate chasing and visually observing copulations (Waterman 1996, 1998). Another way we determined estrus was by trapping females and either collecting copulatory plugs or seeing an enlarged or nearly enlarged vulva (Manjerovic et al. 2022). Finally, we calculated the date of estrus by backdating from the date of female isolation (Waterman 1996). Pregnancy detection via palpation allowed us to assess whether a female had conceived and whether the pregnancy was ultimately successful. We confirmed parturition by recording the date a female isolated from their family group, when they moved to give birth in natal burrows (Waterman 1996). The onset of lactation (elongated nipples secreting milk) indicated that the offspring had either been born or were close to parturition. If litters died, we used the cessation of lactation and/or the date of a breeding female returning to their family group to pinpoint the

timing of loss. Females typically rejoin their families within a day after weaning their offspring or following litter loss (Waterman 1996). Tracking changes in body mass over time, before pregnancy, during isolation (lactation), and post-isolation (return to group), also allowed us to identify if and when a litter loss occurred. To assess the occurrence of flooding events, we used rainfall data from S. A. Lombard Nature Reserve and identified any instance where rainfall ≥ 40 mm within a 48-hour period as a flood. For each month that we were on site, we recorded the number of flood events and matched these to the number of offspring weaned during that same month.

We conducted all analyses using R v 4.2.2 (R Core Team 2021). To estimate annual female reproductive success, we used long-term data from all females trapped as juveniles or subadults from 2002-2007 and 2011-2019, as well as genetically assigned offspring. To examine how rainfall and temperature affect female reproduction and body condition, we used generalized linear mixed models (GLMMs) in the *glmmTMB* package (v. 1.1.5). We included Squirrel ID and Group ID as random intercepts to account for repeated measures of the same individuals across years and the non-independence of individuals within social groups. DHARMA package v 0.4.6. was used to perform residual diagnostics on GLMMs, which were checked for standardized residuals, collinearity, zero inflation, and uniformity. To examine flooding on offspring emergence (number of offspring weaned), we used a Kruskal-Wallis test. To assess female body condition before pregnancy, we used the residuals from an ordinary least squares (OLS) regression of log body mass on log spine length to estimate body condition (Schulte-Hostedde et al. 2005, O'Brien et al. 2021). GLMMs with quadratic effects using package *glmmTMB* v.1.1.8 were used to examine the relationship between female age and reproductive success, anticipating a non-linear pattern where success increases with early-life experience and declines with

senescence. We used logistic regression models implemented in the *aod* package (v. 1.3.3) to account for overdispersion in the pregnancy loss, lactation loss, and litter-size data. We used a significance threshold of $p < 0.05$ and report results as mean $\pm$ standard error (SE).

Results

We trapped 295 adult females and observed 364 reproductive attempts of Cape ground squirrels across two study periods: 2002–2006 and 2011–2019. Females that were not captured at least four times from May to August, and data from 2016 - 2017 were excluded due to insufficient trapping effort to reliably assess female reproduction and reproductive attempts. Of these 295 females, we detected no reproductive attempts during the winter season (May–Aug) in 53 (18.0%), one attempt in 179 (60.7%), two attempts in 55 (18.6%), and three attempts in 7 (2.4%). During this time, 115 reproductive attempts failed (31.5%), 130 were successful (35.6%), and 120 were undetermined (32.9%). During these same years, we captured 416 juveniles and successfully matched all of them to their mothers. Of these, 141 juveniles were directly linked to observed reproductive attempts during the May through August trapping season.

Rainfall

Annual rainfall was not associated with female body condition or pre-pregnancy mass (Table 3.2). Linear mixed-effects models indicated that individual ID accounted for the majority of variation in both response variables. For body condition, the random effect of tag explained 75.4% of the total variance (variance = 0.00512), with residual variance accounting for 24.6% (variance = 0.00167; ICC $\approx$ 0.75). Similarly, for pre-pregnancy mass, tag explained 71.9% of the total variance (variance = 2054.0), while residual variance contributed 28.1% (variance = 801.4; ICC $\approx$ 0.72). A generalized linear mixed-effects model with a negative binomial error distribution

showed that annual rainfall had a significant quadratic relationship with offspring number. Higher annual rainfall was associated with an increase in the number of offspring born ($\beta = 0.0318 \pm 0.0089, p < 0.001$), with reproductive output peaking at approximately 480 mm of rainfall before declining at higher rainfall levels (rainfall²: $\beta = -0.0000319 \pm 0.0000087, p < 0.001$; Figure 3.1). Individual ID accounted for substantial variation in reproductive output (variance = 0.336, $SD = 0.579$), indicating consistent differences among females. Litter size did not vary with increasing annual rainfall (Table 3.2), a pattern supported by results from a linear mixed-effects model. Individual ID explained a modest proportion of the variance in litter size (variance = 0.0329, $SD = 0.181$), while residual variance accounted for the majority of variation (variance = 0.2106, $SD = 0.459$), suggesting substantial within-individual variability and indicating that annual rainfall was not a significant predictor of litter size. Flooding was associated with a decrease in the number of offspring that emerged (Kruskal-Wallis: $\chi^2 = 7.081, p = 0.029, df = 2$, Figure 3.2).

Temperature (Maximum/Minimum)

Maximum and minimum temperatures were also related to breeding in females. A linear mixed-effects model showed that higher minimum temperatures were associated with lower pre-pregnancy body condition (Table 3.2, Figure 3.3), although this effect was moderated by group size, with females in larger groups less affected (Table 3.2, Figure 3.4). A generalized linear mixed-effects model with a binomial error distribution indicated that higher maximum temperatures were negatively associated with female reproductive success, as females weaned fewer juveniles under warmer conditions (Table 3.2, Figure 3.5). Individual ID accounted for a substantial proportion of the variance in body condition (variance = 0.00530, $SD = 0.0728$), with lower residual variance (variance = 0.00058, $SD = 0.0241$), indicating that most variation

occurred among individuals. Similarly, there was considerable variation among individuals in the probability of litters being weaned (variance = 1493, $SD = 38.64$), although this estimate is likely inflated given the small sample size. Interactions between annual rainfall and temperature significantly influenced female reproductive traits (Table 3.2). Pre-pregnancy body condition declined with increasing minimum temperatures in wetter years (Table 3.2, Figure 3.6), while maximum temperatures did not significantly interact with rainfall to affect condition (Table 3.2, Figure 3.7). A linear mixed-effects model indicated that maximum temperature was a significant positive predictor of litter size, whereas minimum temperature was not significant. However, individual ID explained a moderate proportion of the variance in litter size (variance = 0.0316, $SD = 0.178$), with lower residual variance (variance = 0.0101, $SD = 0.100$; Table 3.2).

Female condition and reproductive success

Female pre-pregnancy body condition varied quadratically with age, peaking at ~ 5.1 years (LMM with random intercept for Squirrel ID; age: $\beta = 0.069 \pm 0.019$, $z = 3.61$, $p < 0.001$; age²: $\beta = -0.0067 \pm 0.0019$, $z = -3.60$, $p < 0.001$; $n = 176$; Figure 3.8). A linear mixed-effects model (Gaussian, identity link) showed that pre-pregnancy body condition was positively correlated with the mass of females during lactation ($\beta = 660.79 \pm 122.95$ SE, $z = 5.37$, $p < 0.001$, $n = 22$; Figure 3.9). Individual ID explained negligible variation in post-pregnancy mass (variance = 0.00029, $SD = 0.017$), while residual variance was substantially higher (variance = 1755, $SD = 41.89$), indicating that most variation occurred within individuals.

Pre-pregnancy body condition was not associated with the number of reproductive attempts from May to August (Table 3.3). Pre-pregnancy body condition did influence the likelihood of pregnancy failure, with higher body condition associated with fewer failures (logistic regression: body condition $\beta = 18.10 \pm 7.71$ SE, $z = 2.35$, $p = 0.019$; $n = 31$; Figure

3.10). However, individual ID was included as a random effect, which accounted for substantial variation in litter failure (variance = 1572, $SD = 39.65$) and indicated large differences among individuals. These results suggest that the apparent effect of body condition on litter failure may be driven by among-individual variation. Pre-pregnancy body condition was not related to whether females failed to wean offspring during their reproductive attempt, and any apparent relationship with litter failure was weak and largely driven by among-individual variation (Table 3.3).

Successful reproduction did not affect a female's number of reproductive attempts ($\beta = -0.03 \pm 0.16$ SE, $z = -0.20$, $p = 0.842$, $n = 245$). Litter loss during pregnancy resulted in at least one additional reproductive attempt during the study period (May – August; $z = 3.634$, $p < 0.001$). However, reproductive failure during lactation did not affect the number of subsequent reproductive attempts within the same period (Table 3.3). Individual ID explained negligible variation in litter number (variance ≈ 0 , $SD \approx 0$), while residual variance accounted for nearly all of the variation (variance = 0.193, $SD = 0.439$), indicating little consistent difference among individuals. Post-pregnancy (lactation) mass of females did not affect their likelihood of litter loss during lactation. Individual identity (tag) accounted for moderate variation in the probability of litter failure (variance = 1.058, $SD = 1.029$), indicating some differences among females (Table 3.3).

The number of weaned offspring varied quadratically with female age, peaking at ~4 years and declining to zero by age 8 (negative binomial mixed model with random intercept for Squirrel ID; Age: $\beta = 0.906 \pm 0.143$ SE, $z = 6.36$, $p < 0.0001$; Age²: $\beta = -0.118 \pm 0.022$ SE, $z = -5.31$, $p < 0.0001$, $n = 536$; Figure 3.11). We monitored 18 females who were 8 years or older (either of known age or observed on site for ≥ 8 years). Of these, 17 (94%) went into estrus. A

total of 3 females (17%) became pregnant, but two (11%) experienced pregnancy failure, and one (6%) carried to term but lost the offspring immediately after birth. Overall, no female over the age of 7 produced a surviving offspring.

Discussion

Both extrinsic and intrinsic factors influenced reproductive success in female Cape ground squirrels. Higher annual rainfall increased the number of offspring born, whereas flooding and higher maximum temperatures reduced reproductive success. Higher minimum temperatures were associated with lower pre-pregnancy body condition, but this negative effect was mitigated in larger groups, where females maintained better condition under warmer minimum temperatures. Among intrinsic factors, higher pre-pregnancy body condition and intermediate female age increased annual reproductive success, while old age decreased it.

The extrinsic factors of rainfall and temperature influenced reproductive success. Our results indicated that reproductive success was highest at intermediate levels of rainfall (~480 mm) but declined at both lower and higher extremes. High rainfall over short periods may reduce reproductive success through flooding of burrow systems, which is likely to increase offspring mortality, particularly early in lactation. Similar nonlinear rainfall effects have been observed in banded mongooses, where both very wet and very dry conditions reduce reproductive success (Nichols et al. 2012). Suricates showed strong rainfall effects on body condition, with rainfall during the previous 9–12 months positively affecting condition (Hodge 2005). Female suricates' reproductive success is closely tied to annual variation; in years of low rainfall, suricates experience lower reproductive success and smaller group sizes (Thorley et al. 2025a). Extreme rainfall events also negatively affected both offspring survival (weaning) and reproductive success in Cape ground squirrels.

Temperature exerts additional constraints on reproduction, with average minimum and maximum temperatures affecting different aspects of the reproductive success of female Cape ground squirrels. Minimum temperatures reduced female body condition, but this effect was buffered by group size. Female Cape ground squirrels in larger groups exhibit lower and more stable body temperatures (Scantlebury et al. 2007), suggesting that social thermoregulation, through mechanisms like huddling, helps conserve energy and maintain body condition during periods of cold stress. Similar patterns have been observed in other species. For instance, in Damaraland (*Cryptomys damarensis*) and Natal mole-rats (*Cryptomys hottentotus natalensis*), huddling behavior in larger groups results in significant energy savings (Kotze et al. 2008).

By contrast, maximum temperatures negatively affect many species. The southern pied babbler (*Turdoides bicolor*), a subtropical arid-adapted species, experiences decreased body condition and reduced foraging efficiency in high temperatures (du Plessis et al. 2012). Likewise, in the arid-adapted suricates, reproduction and juvenile survival are reduced in high ambient temperatures (Thorley 2025a). Cape ground squirrels were also affected by high temperatures, which reduced both litter size and the number of offspring surviving to weaning. While group living can buffer individuals against cold stress, it does not fully mitigate the challenges of heat stress. These results are consistent with Scantlebury et al. (2012), who found that high ambient temperatures were thermally challenging for Cape ground squirrels and that they use their burrows as refuges to offload heat. Rising maximum temperatures are therefore likely to pose increasing risks under climate change. Additionally, morphological changes observed in Cape ground squirrels have been linked to increasing temperatures, reflecting longer-term adaptations to warming (Warrington et al. 2022)

Extrinsic effects affect the body condition of suricates (Hodge 2005, Thorley et al. 2025a), but only minimum temperature significantly influenced female body condition in Cape ground squirrels. Maternal body condition is one of the most widely used indicators of reproductive potential in mammals (Hamel et al. 2010, Barnett et al. 2015, Michel et al. 2019). Body condition has been linked to pregnancy success across a wide range of mammals, with females in better condition generally more likely to conceive and successfully carry offspring to term (Clutton-Brock 1989, Dobson and Michener 1995, Festa-Bianchet and Jorgenson 1998, Descamps et al. 2008, Hamel et al. 2010). In Cape ground squirrels, pre-pregnancy body condition was strongly related to pregnancy success, but not to litter size or the number of offspring weaned. In other ground squirrels, maternal feeding rates can be a more important determinant of juvenile body mass than maternal mass itself (Bachman 1993), highlighting the variability in how maternal and environmental factors shape reproductive outcomes. For Cape ground squirrels, body condition was a key driver of pregnancy success, but their condition appeared to be relatively buffered from most extrinsic effects.

Reproductive investment strategies are shaped by life-history trade-offs and the timing of reproductive failure. In many long-lived species, individuals must weigh the costs of investing in current offspring against conserving resources for future attempts, with this trade-off shaped by changes in body condition during gestation and lactation (Hamel et al. 2010, Testa and Adams 1998, Kroeger et al. 2018). These dynamics are also influenced by life history tactics: species with “fast” life histories often maximize current reproductive output at the expense of survival, whereas species with “slow” life histories, such as Cape ground squirrels, tend to prioritize survival and future opportunities to reproduce (Stearns 1992).

The number of reproductive attempts in winter increased when females experienced failure during pregnancy, but not when failure occurred during lactation. This pattern aligns with observations from Namibia, where inter-estrus intervals were shorter following pregnancy failure and longer after litter loss during lactation or success in weaning offspring (Waterman 1996). Such patterns suggest that females adjust reproductive investment depending on the stage of failure and the energetic costs already incurred. Across mammals, females tend to rebreed quickly after early failure but delay or reduce maternal investment following late failure. For example, in olive baboons (*Papio cynocephalus anubis*), inter-birth intervals were shorter for females that lost infants compared to those whose offspring survived (Smuts and Nicolson 1989). Similarly, in red deer (*Cervus elaphus*), the energetic cost of gestation is substantially lower than that of lactation, which may explain why reproductive delays are more pronounced after losses during the energetically expensive nursing period (Clutton-Brock et al. 1989).

The differing effects of reproductive failure during pregnancy versus lactation may reflect both timing constraints and changes in female body condition. Females who lose their litters during pregnancy have sufficient time to attempt another breeding before the onset of the rainy season. In contrast, females who experience failure during lactation may not have adequate body reserves for a subsequent attempt, especially given the lack of plant growth during the austral dry winter season. While our data did not allow us to directly test this hypothesis, it is consistent with patterns seen in Nearctic ground squirrels, where gestation is constrained by the body mass of females and influenced by the length of the active season (Armitage 1981). Notably, Cape ground squirrels breed year-round, and they can attempt reproduction in any season (Waterman 1996). This strategy contrasts with North American ground squirrels, which are constrained to a short breeding season and compensate by producing large litters (Armitage

1981). In contrast, Cape ground squirrels produce smaller litters, potentially multiple times in a year, thereby reducing the cost of any single reproductive attempt.

Female age further modifies reproductive strategies, with evidence for reproductive senescence. Among Cape ground squirrel females 8 years and older, nearly all attempted to breed, but none produced surviving offspring. In red deer, very few individuals breed past age seventeen, but the onset of reproductive senescence occurs around nine years of age (Nussey et al. 2006). This decline is consistent with reproductive senescence, a pattern widely documented in other long-lived mammals, where advancing age reduces both reproductive output and offspring survival (Clutton-Brock et al. 1999, Sharp and Clutton-Brock 2010, Nussey et al. 2013). In suricates, reproductive output peaks around 4 years of age and then sharply declines (Sharp and Clutton-Brock 2010). Reproductive success in female Cape ground squirrels also peaked around 4 years of age, with a subsequent decline in litter size, while males show different senescence patterns, as seen in earlier studies (Warrington et al. 2024, 2025). Thus, Cape ground squirrels appear to balance reproductive decisions based not only on immediate condition and failure during gestation or lactation, but also on age-related declines in reproductive performance.

In conclusion, both extrinsic and intrinsic factors interact to shape reproductive success in Cape ground squirrels. Extrinsic factors such as rainfall and temperature constrain reproductive attempts, while intrinsic traits such as body condition and age determine pregnancy success and investment strategies. Overall, reproductive success was greatest under moderate rainfall and temperature conditions, and females exhibited flexibility in reproductive decisions depending on when reproductive failure occurred and seasonal constraints. However, age-related senescence limits reproductive success in older females. These findings underscore how Cape ground

squirrels balance immediate reproductive investment against long-term survival, and they highlight the vulnerability of this system to ongoing climate change, particularly increases in temperature extremes and variability in rainfall.

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Tables

Table 3.1: Hypotheses and predictions of the factors influencing reproductive success in female Cape ground squirrels (*Xerus inauris*).

	Hypothesis	Prediction	Supported
Extrinsic factors (Rainfall, Temperature (max/min), and season) affect reproductive success.	Rainfall affects reproduction	Higher annual rainfall increases pre-pregnancy body condition	No
		Annual rainfall will increase lactation failure and decrease pregnancy failure	No
	Temperature (max/min) affects female reproduction	Maximum temperature decreases female body condition	No
		Minimum temperature decreases female body condition	Yes
		Maximum temperature increases reproductive failure	No
		Minimum temperature increases reproductive failure	No
		Maximum temperature decreases litter size	Yes
		Minimum temperature decreases litter size	No
Intrinsic factors (pre-pregnancy body condition, and age) affect reproductive success	Reproductive failure is affected by body condition during reproduction	Increased group size decreases temperature effects	Yes
		Decreased pre-pregnancy body condition increases pregnancy failure rate	Yes
	Pre-pregnancy body condition affects reproduction	Decreased pre-pregnancy body condition increased lactation failure rate	No
		Pre-pregnancy body condition affects the number of reproductive attempts	No

Age affects female reproduction	Increased number of reproductive failures increases reproductive attempts	Yes Pregnancy failure/ No lactation failure
	Increased pre-pregnancy body condition increases litter size	No
	Older females have more offspring	Yes
	Older females have larger litters	No
	Older females have fewer reproductive failures	No

Table 3.2: Effects of rainfall, temperature, and group size on female reproductive traits in Cape ground squirrels (*Xerus inauris*). Statistical analyses included GLMMs for continuous outcomes (pre-pregnancy body condition) and negative binomial mixed models for count data (number of weaned offspring). Interactions between rainfall and temperature were also examined. Significant variables are bolded.

Independent Variable	Dependent Variable	Estimate	SE	z-value	p-value	n
Annual rainfall						
	Pre-pregnancy body condition	<-0.001	0.048	-1.31	0.191	49
	Litter size	<-0.001	<0.001	-0.925	0.357	90
Minimum temperature						
	Pre-pregnancy body condition	-0.005	0.002	-1.911	0.056	35
	Juveniles weaned	-0.015	0.031	-0.492	0.622	20
	Litter size	0.056	0.045	1.246	0.213	10
Maximum temperature						
	Pre-pregnancy body condition	0.001	0.004	0.337	0.736	35
	Juveniles weaned	-0.103	0.14	-2.815	0.005	20
	Litter size	0.148	0.05	3.01	0.003	10
Interactions						
Annual rainfall and Minimum temperature	Pre-pregnancy body condition	<0.001	<0.001	-1.996	0.046	44
	Litter size	<0.001	<0.001	0.427	0.669	40
Annual rainfall and Maximum temperature	Pre-pregnancy body condition	<0.001	<0.001	0.791	0.429	44
	Litter size	<0.001	<0.001	0.432	0.666	40
Minimum temperature and Group size	Pre-pregnancy body condition	<0.001	<0.001	-2.008	0.045	40
Maximum temperature and Group size	Pre-pregnancy body condition	<0.001	<0.001	1.168	0.243	40

Table 3.3: Female Cape ground squirrel (*Xerus inauris*) reproductive success variables. Statistical analyses included logistic regression for binary outcomes (e.g., pregnancy failure, litter loss). Significant values are bolded.

Independent Variable	Dependent Variable	Estimate	SE	z-value	p-value	n
Pre-pregnancy body condition	Reproductive attempts	1.643	1.074	1.529	0.126	62
	Failed to wean offspring	12.808	7.244	1.768	0.077	21
	Failed pregnancy	18.098	7.706	2.349	0.019	28
Reproductive attempts	Pregnancy litter loss	0.352	0.097	3.634	<0.001	75
	Lactation litter loss	0.083	0.086	0.967	0.333	88
	Successful Weaning	-0.036	0.097	-0.528	0.597	130
Reproductive success	Female Age	0.205	0.043	4.794	<0.001	543
Pregnancy mass (g)	Lactation litter loss	0.007	0.004	1.89	0.073	114
Post-pregnancy mass (g)	Lactation litter loss	0.002	0.004	0.572	0.567	66

Figures

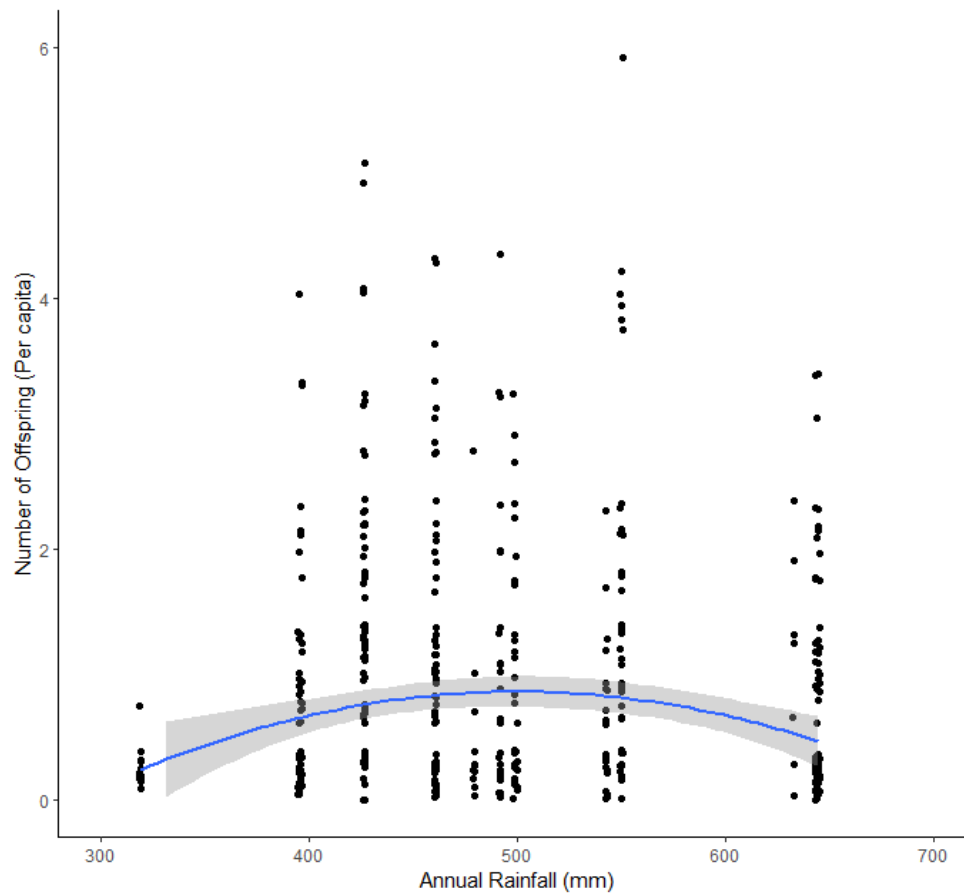


Figure 3.1: Effects of annual rainfall on the number of offspring weaned by Cape ground squirrels (*Xerus inauris*) from 2002–2006 and 2011–2019. A negative binomial GLMM revealed with quadratic effects using random intercepts for Squirrel ID accounted for repeated measures across 227 individuals ($n = 536$ observations) to determine the relationships between rainfall and number of offspring produced.

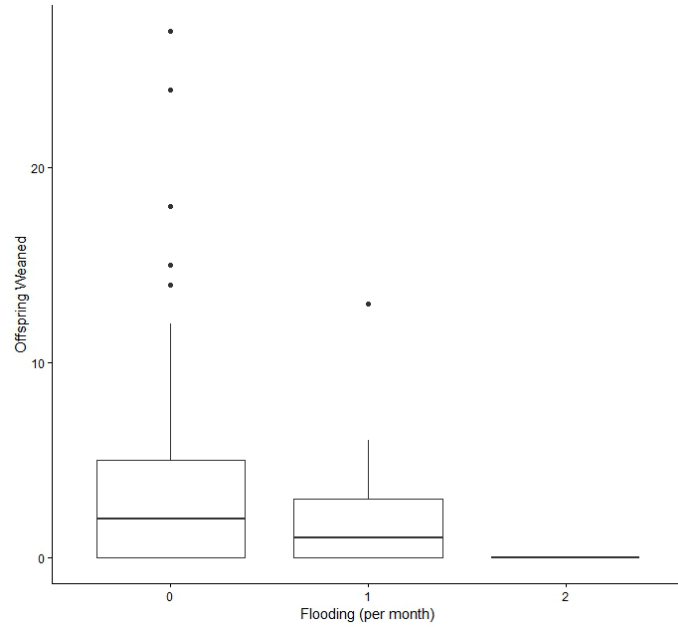


Figure 3.2: Flooding per month on the successful number of Cape ground squirrels (*Xerus inauris*) offspring weaned 2002-2006 and 2011-2019 (0 floods n = 145 months, 1 flood n = 32 months, 2 floods n = 3 months).

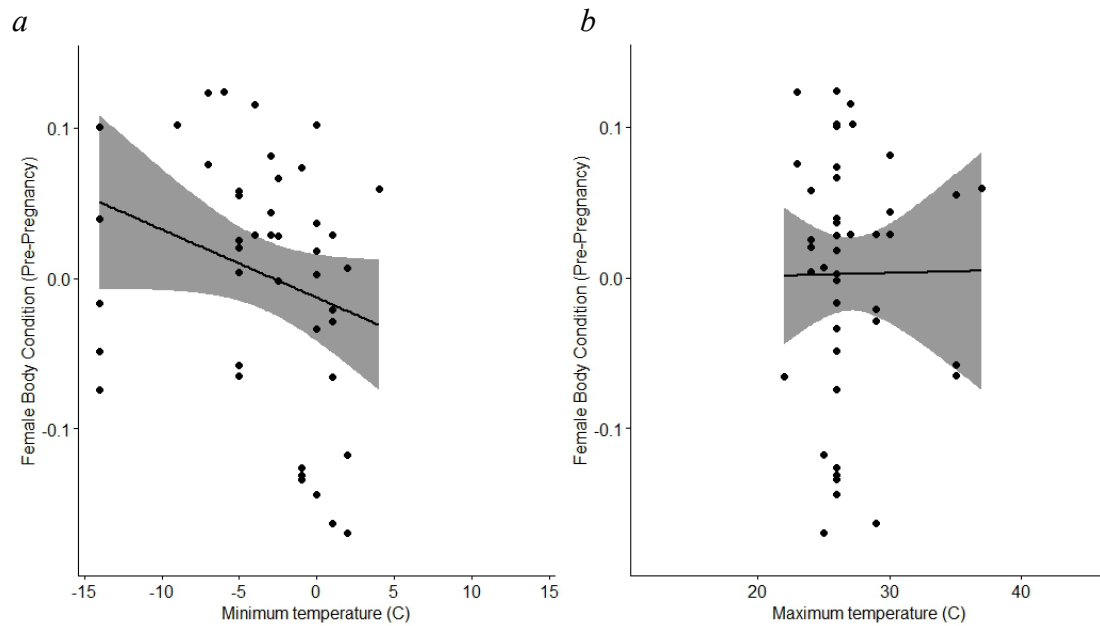


Figure 3.3: Minimum *a*) and maximum *b*) temperatures on female Cape ground squirrel (*Xerus inauris*) pre-pregnancy body condition from 2012-2019 (n = 35).

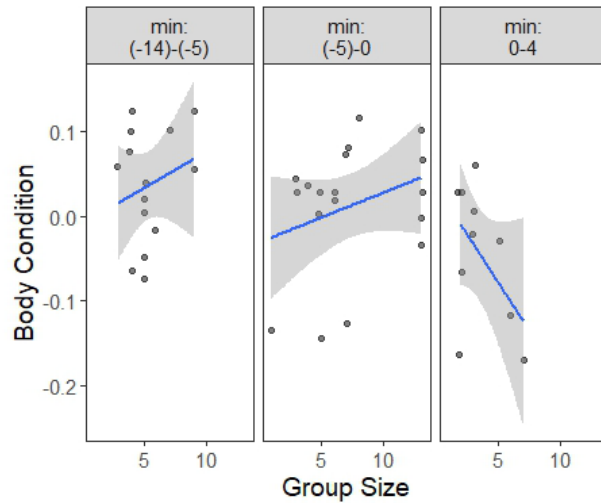


Figure 3.4: Interaction effect of group size and minimum temperature on pre-pregnancy body condition in female Cape ground squirrels (*Xerus inauris*), 2012–2019. The relationship was assessed using a linear model including group size, minimum temperature, and their interaction ($n = 44$).

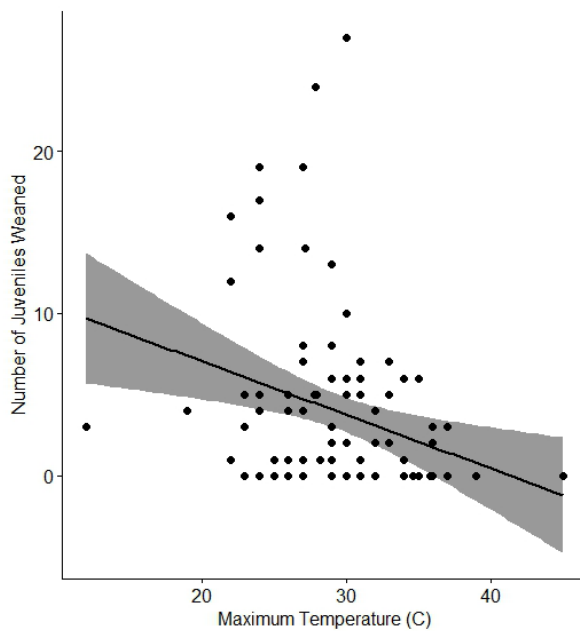


Figure 3.5: Effect of maximum temperature on the number of juvenile Cape ground squirrels (*Xerus inauris*) weaned from 2002–2006 and 2011–2019. Points represent observed counts of juveniles. The solid line shows model-predicted values from a zero-inflated negative binomial GLMM with a log link for maximum temperature. Shaded areas represent 95% confidence intervals for the predicted mean number of juveniles ($n = 20$).

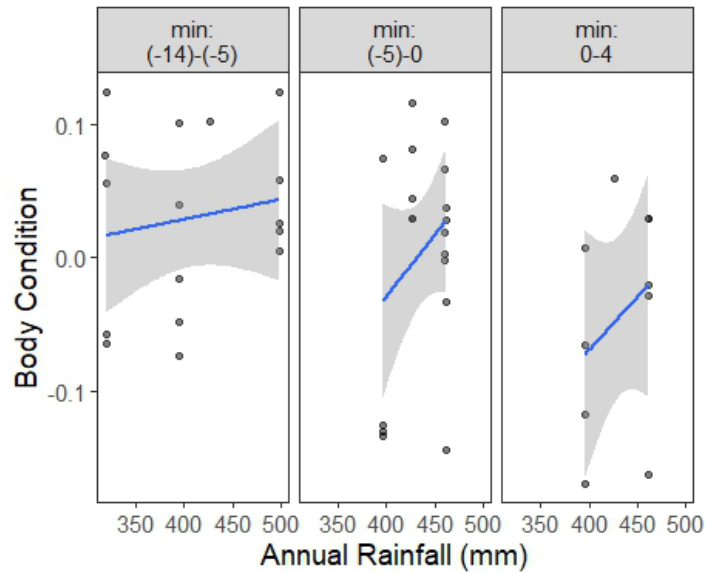


Figure 3.6: Pre-pregnancy body condition of Cape ground squirrels (*Xerus inauris*) in relation to minimum temperatures and annual rainfall from 2012–2019. Points represent individual observations, with each break point containing roughly equal observations. The fitted linear model included minimum temperature, annual rainfall, and their interaction ($n = 40$).

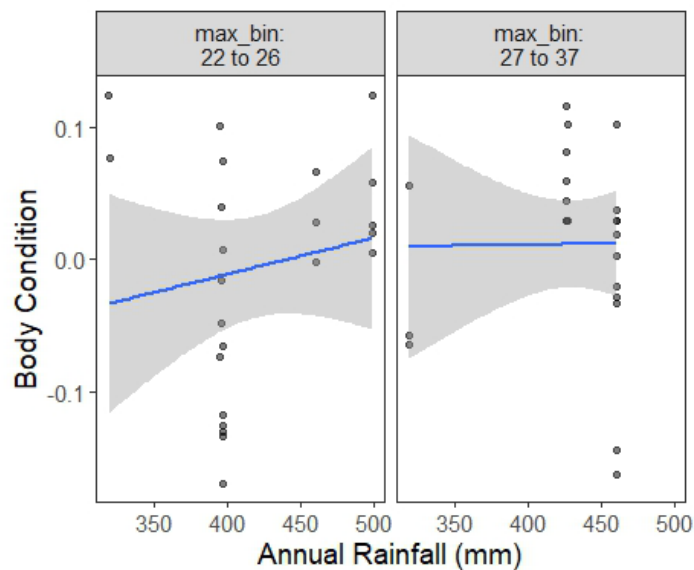


Figure 3.7: Interaction of maximum temperatures and annual rainfall on pre-pregnancy body condition of Cape ground squirrels (*Xerus inauris*) from 2012–2019. Points represent individual observations. A linear model was fitted to examine the effects of maximum temperature, annual rainfall, and their interaction on female pre-pregnancy body condition ($n = 40$).

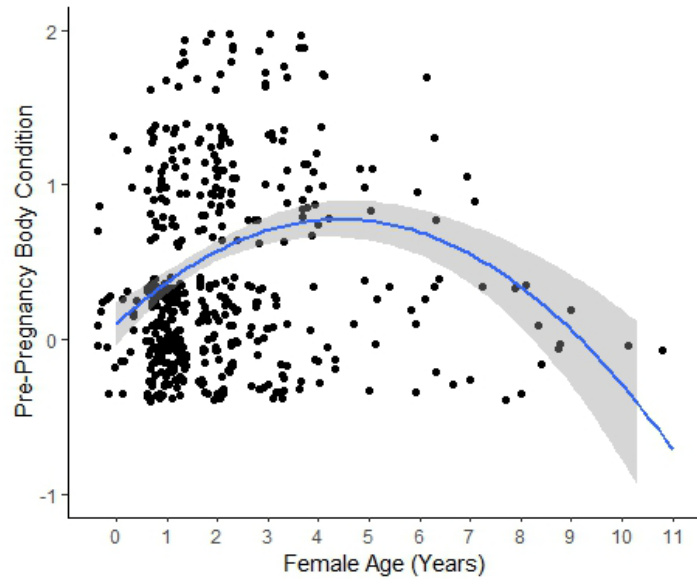


Figure 3.8: Relationship between female age and pre-pregnancy body condition (size–mass residuals, $n = 176$) in Cape ground squirrels (*Xerus inauris*) from 2012–2019. Body condition was modeled using a GLMM with age, age², and a random intercept for individual (Squirrel ID) with a curvilinear relationship. A fitted quadratic curve with 95% confidence intervals is shown.

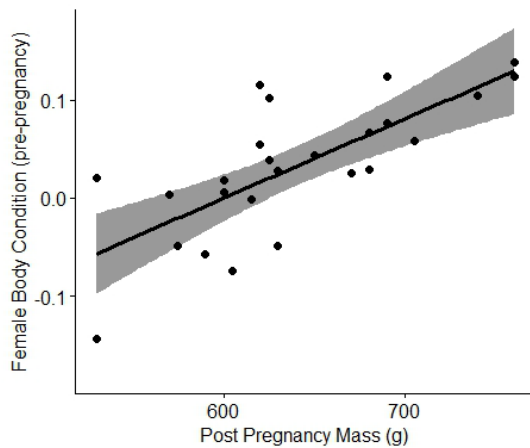


Figure 3.9: A linear relationship between pre-pregnancy body condition and the decrease in post-pregnancy mass in female Cape ground squirrels (*Xerus inauris*) ($n = 22$)

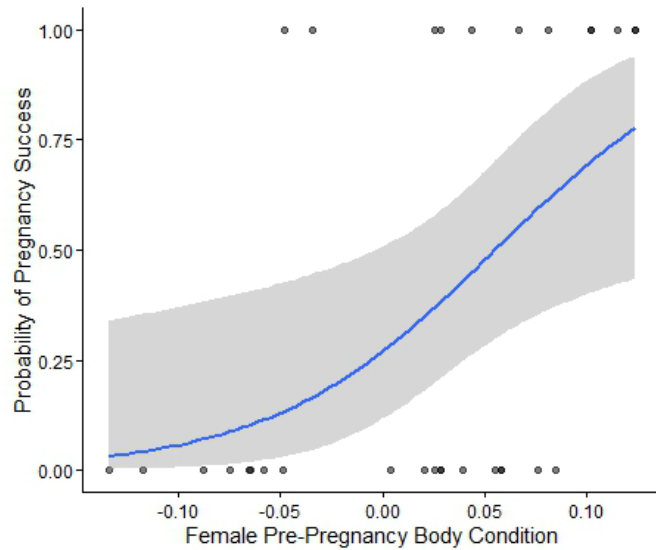


Figure 3.10: Effect of pre-pregnancy body condition on pregnancy failure in female Cape ground squirrels (*Xerus inauris*) from 2012–2019. The logistic regression model explained variation in pregnancy and the plotted line shows the predicted probability of pregnancy failure across the observed range of body condition, with 95% confidence bands (n = 31).

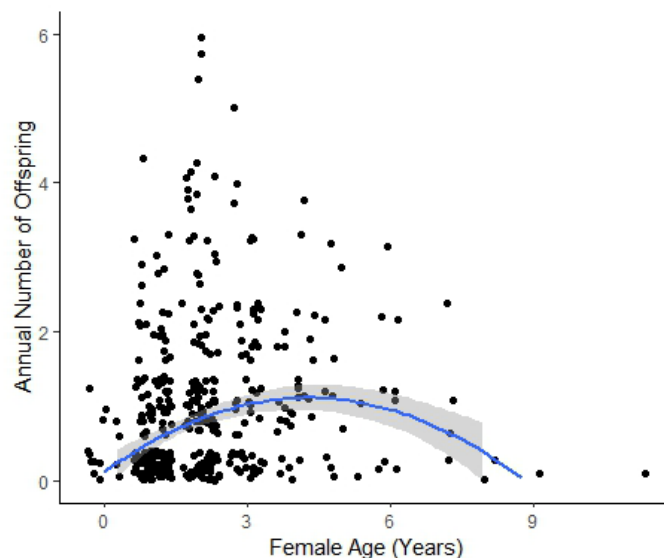


Figure 3.11: Quadratic relationship between female age and the number of offspring weaned in Cape ground squirrels (*Xerus inauris*) from 2002–2006 and 2011–2019. A negative binomial GLMM revealed a strong quadratic effect of age on annual reproductive success. The model predicts a peak in offspring production at approximately 4 years of age. Random intercepts for Squirrel ID accounted for repeated measures across 227 individuals (n = 536 observations).

Chapter 4 Who Cares? Alloparental care and its effects on juvenile Cape ground squirrels

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Author contributions Conceptualization (J. Waterman, L. LaFléche, K. Shofstall); methodology (J. Waterman, L. LaFléche, K. Shofstall); investigation (J. Waterman, L. LaFléche, K. Shofstall); formal analysis (M. Warrington, K. Shofstall); data curation (K. Shofstall); supervision (J. Waterman); visualization (K. Shofstall); writing – original draft (K. Shofstall); writing – review & editing (J. Waterman, K. Shofstall).

Abstract

Care by non-parent individuals is a vital part of cooperative breeding, and variation in alloparental care among group members in cooperative breeding societies can be considerable. Alloparental care can benefit offspring through increased body condition and survival, but the benefits can depend on the identity and characteristics of the caregivers. Cape ground squirrels (*Xerus inauris*) are promiscuous plural breeders living in family groups, but the occurrence of alloparental care has never been examined. We trapped squirrels at a site in central South Africa, collecting data on genetics, reproductive status, body condition, parasites, and behavioral data on possible care behavior. We found that adult group members, regardless of sex, provided alloparental care. Group composition influenced both the caregivers and the extent of alloparental care. The presence of related males in a group reduced the amount of alloparental care provided by adult females, but overall increased the care received by offspring. Offspring benefited from care, with increased foraging and body condition but not increased survival. These results contribute to our understanding of alloparental care in a system where every adult member has the potential to breed and provide care, particularly in a promiscuous system with highly variable group size and composition, both factors that contribute to variation in caregiving behavior.

Keywords: Alloparental Care, Cape Ground Squirrels, Cooperative Breeder, Plural Breeder, Promiscuous, Quality, Quantity, *Xerus*

Introduction

Cooperative breeding has been reported in numerous vertebrate and invertebrate species (Lacey and Sherman 1997, Solomon and French 1997, Cockburn 1998, Josi et al. 2020, Mocha et al. 2023) and is considered to be the most complex social system (Blumstein and Armitage 1997, Cornwallis et al. 2010, Clutton-Brock and Lukas 2012, Kramer and Russell 2014, Apakupakul and Rubenstein 2015, Koenig and Dickinson 2016). Given the wide range of species and the complexity of their social systems, definitions of cooperative breeding vary across the literature (Mocha et al. 2023). Some definitions are restrictive, where groups must have mature non-breeding individuals providing care to offspring, while other definitions are more inclusive, where care is provided by breeding or non-breeding individuals, and cooperative breeding is a continuum (Mocha et al. 2023). Some definitions also include delayed maturity and/or delayed dispersal (Russell 2009). One essential part of all definitions of cooperative breeding is the caring of offspring by non-parent individuals (Cockburn 1998, Clutton-Brock 2025). Alloparental care also varies greatly, not only in terms of care but also in the type of behaviors among and within species. These behaviors can include cooperative defense of offspring, predator surveillance, food acquisition, following behaviors, affiliative care, escorting, and allogrooming (Russell 2009).

Young animals that receive alloparental care often have better body condition, faster growth, and larger adult body size compared to those without such support (Russell et al. 2003, Rosenbaum and Gettler 2018, Vitikainen et al. 2019). Spending more time with adults can also reduce predation risk (Powell and Fried 1992, Silk 2007), and the presence of caregivers has been linked to higher feeding rates and better chances of survival in offspring in mammals (Russell et al. 2003, Rosenbaum and Gettler 2018).

Providing care to offspring can be costly for the caregivers, and the cost of energy investment can vary with the sex, age, and body condition of the caregiver (Heinsohn and Legge 1999, Clutton-Brock et al. 2000, Cant 2003, Heldstab et al. 2017, Josi et al. 2020). Caregivers can have reduced foraging time, delayed reproduction, decreased body condition and reduced survival (Heinsohn and Legge 1999, Meade and Hatchwell 2010, Klauke et al. 2013). So why do alloparental caregivers give care when it is so costly? Individuals provide alloparental care for a myriad of reasons and can gain direct and indirect benefits, which can outweigh the potential costs of caregiving (Russell 2009, Meade and Hatchwell 2010).

The theory of inclusive fitness stipulates that caregivers gain indirect benefits when taking care of the offspring of closely related individuals (Cornwallis et al. 2009, Abbot et al. 2011). Evidence that alloparents benefit via inclusive fitness is strongly supported and is invoked in how alloparental care evolved, especially in monogamous species where the group members are closely related (Boomsma 2007, Clutton-Brock and Lukas 2012). In mammals, most cooperative breeding species are monogamous (Lukas and Clutton-Brock 2012). However, the mating systems of cooperative breeders can range from eusociality with strict monogamy to group-living promiscuous species where within-group relatedness varies (Boomsma 2007, Cornwallis et al. 2010, Leggett et al. 2012). Other explanations focused on direct benefits have also been proposed for giving alloparental care, such as increased reproductive success through practicing providing care as an alloparent (Gilchrist et al. 2008). Individual alloparental caregivers also can gain benefits from remaining in the group for longer through group augmentation, including increased survival, better body condition and getting old enough to compete for territory, or simply waiting to inherit a territory, all of which increase future breeding success (Heinsohn and Legge 1999, Leggett et al. 2012).

There is still much to learn about how group composition influences alloparental care and how factors like age and sex shape the care given. Cape ground squirrels (*Xerus inauris*) provide a unique opportunity to test hypotheses on alloparental care in a cooperative and highly promiscuous species (Waterman 1995). Female Cape ground squirrels live in family groups consisting of 1-6 related adult females, 0-9 subadults of either sex and 0-4 non-dispersed related adult males (natal males; Hillegass et al. 2008, Manjerovic and Waterman 2015, O'Brien et al. 2021). These family groups lack dominance hierarchies and territoriality, and aggression is rare (Waterman 1995). Cape ground squirrels have high variance in relatedness within their family groups (Waterman and Archibald 2019), which contrasts with other cooperative breeding monogamous species (Solomon and French 1997, Cockburn 1998, Clutton-Brock et al. 2001).

In Cape ground squirrels, every group member has the potential to be an alloparent, but adult females can breed and also be alloparents (communal breeders; Waterman 1995). Both group size and group composition, i.e., the number and presence of sub-adults, natal males, and adult females, can change over time (Waterman 1995). Young maturing females may have delayed maturity (suppression) if there are ≥ 2 adult females in their family group (Pettitt and Waterman 2011). However, females are not reproductively suppressed once they reach maturity, and they can breed year-round (Waterman 2002, Bouchie et al. 2006, Pettitt and Waterman 2011). At the age of maturity (8-10 months), young maturing males either disperse to join all-male groups of dispersed, mature males (band males) or remain with their family group as natal males (Waterman 1995, Manjerovic and Waterman 2015). In South Africa, over 60% of males delay dispersal at maturity, remaining with their family for up to 5 years as natal males (Scantlebury et al. 2007, Manjerovic and Waterman 2015). The average age of dispersal is approximately 3 years (O'Brien et al. 2021). There may be costs to females in allowing natal

males to remain in the family group after maturity, as the presence of 2 or more natal males delays the maturity of sub-adult females (Pettitt and Waterman 2011). Natal males also have more ectoparasites than females, and parasites are known to reduce female reproductive success (Hillegass et al. 2008, 2010). It is not currently understood why females tolerate these costs or if there are benefits to having these natal males remain in the group.

There are also costs and benefits to natal males delaying dispersal. Relative to band males, natal males have smaller home range sizes, which means they overlap with fewer potential mates (Manjerovic and Waterman 2015). Natal males rarely mate with females in their own family group (only 1 out of 93 males was recorded siring offspring within his group in Manjerovic and Waterman 2015), but they will attempt to mate with females in neighboring groups. Thus, natal males have fewer mating opportunities and only sire around 17% of offspring relative to band males (Manjerovic and Waterman 2015).

However, natal males may gain benefits through philopatry, as they have better body condition in low rainfall years compared to band males (O'Brien et al. 2021). Older natal males that disperse have better body condition than young band males (O'Brien et al. 2021). This better body condition may increase their lifetime reproductive success as older males are more successful at securing copulations with females and have higher reproductive success than young males (Manjerovic et al. 2022, Warrington et al. 2024). Natal males also have reduced parasite infections relative to dispersed males in years of low rainfall, thereby increasing their energy resources (Scantlebury et al. 2008, O'Brien et al. 2021). Additionally, natal males allocate more time to foraging and less time to roving around their smaller home ranges compared to band males (Scantlebury et al. 2008, Manjerovic and Waterman 2015, Manjerovic et al. 2022). Natal

males might also gain some inclusive fitness benefits by providing alloparental care to offspring in their family group (Pettitt and Waterman 2015).

The objective of this study was to examine whether alloparental care occurs in Cape ground squirrels and how it influences offspring success. We define cooperative breeding as at least one individual per group providing care for offspring that are not their own, i.e. providing alloparental care (Russell 2009). We define alloparental care similarly to Russell (2009), as individuals giving care to offspring that are not their own, at a potential cost to themselves, and with the potential for either or both parents and/ or offspring to gain benefits. We tested three main hypotheses related to alloparental care (Table 4.1).

(1) The offspring of Cape ground squirrels receive care from non-mothers in their family groups. Previous studies found that juvenile care from mothers does not differ from other females in the family group (Waterman 1995) and behaviors defined as alloparental care (such as affiliative, escorting, and allogrooming) in other studies (Clutton-Brock et al. 2000, Cant 2003, Gilchrist and Russell 2007, Müller and Manser 2008, Schubert et al. 2009) have been documented in this species (Waterman 1995, Pettitt and Waterman 2011). (2) Group composition influences who provides alloparental care. We predict that sex and age would influence the amount of care provided. This hypothesis is based on the idea that not all individuals are equally likely or able to give care. In Cape ground squirrels, groups with more non-parent individuals, such as natal males, may provide higher levels of alloparental care, as these individuals are not constrained by the energetic demands of raising their own offspring. Unlike adult females, who have the opportunity to reproduce and must allocate resources toward their own young, natal males are unlikely to achieve immediate reproductive success, as younger males rarely sire offspring in neighboring groups (Manjerovic et al. 2022, Warrington et al. 2024). We therefore

predicted that natal males would contribute more care than females. By contrast, adult females may be more selective in providing care, as they must balance investment in others' offspring with their own reproductive potential. We also predicted that younger natal males (1 year) would provide more care as they wait for their time to disperse, compared to older natal males (3-4 years) that are about to disperse (Waterman 1995, 1997). (3) We also predict that alloparental care translates into measurable benefits for offspring, including increased forage time, offspring growth, survival, and decreased parasite infection. These predictions are based on the idea that additional care, beyond maternal investment, may benefit offspring during ecological and developmental challenges. In Cape ground squirrels, where group living is common, and offspring are exposed to high predation risk and variable environmental conditions (Unck et al. 2009, Warrington et al. 2024), extra care from non-parent individuals may play a critical role in enhancing offspring persistence. For example, when helpers contribute to escorting behavior, offspring may be able to spend more time foraging rather than scanning for predators, which can promote faster growth and better condition. Similarly, grooming from alloparents is predicted to reduce ectoparasite loads, directly impacting health and survival.

Methods

Study System

Male and female groups differ in structure and complexity (Waterman 1995, Pettitt et al. 2008, Pettitt and Waterman 2011, Manjerovic et al. 2022). Dispersed males live independently of female family groups in all-male groups or bands (Waterman 1995, 1997). These year-round bands are comprised of non-kin males with membership changing frequently (Manjerovic et al. 2022). Band males interact non-aggressively, and there is no dominance hierarchy, nor are they territorial (Waterman 1995, 1997, Manjerovic et al. 2022).

Family groups live in burrow clusters or an aggregation of burrow systems, which are separated from other clusters by areas with no burrows (Waterman 1995). Only a single-family group inhabits a burrow cluster (Waterman 1995). While the age of maturity of sub-adult females can be delayed, once reaching maturity, each adult female can potentially breed up to 4 times a year, with peak breeding occurring during winter (Herzig-Straschil 1978, Waterman 1996). However, females have a high rate of litter loss, and around 70% of reproductive events fail to produce weaned offspring (Waterman 1996, Pettitt et al. 2008). Multiparous females have higher reproductive success than primiparous females (Pettitt et al. 2008). When females are about to give birth, they isolate from their family group by digging out a small natal burrow away from the family burrow cluster (Waterman 1996). Females have small litter sizes (1 or 2 offspring), and after a lactation of 47 days, females return to their family group with their weaned offspring (Waterman 1996). Parasites can impact reproductive success, as females with parasites experimentally removed were four times more successful in weaning offspring (Hillegass et al. 2010). The species of ectoparasites found on Cape ground squirrels include *Ctenocephallae connatus*, *Echidnophaga bradyta*, *Echidnophaga gallinacea*, *Neohaematopinus faurei*, *Synosternus caffer*, *Rhipicephalus theileri*, and *Xeroxyuris parallela*, which is exclusive to this species (Herzig-Straschil 1975, Hugot 1995, Waterman 2002, Scantlebury et al. 2007).

We conducted fieldwork at S.A. Lombard Nature Reserve in South Africa (27° 35'S, 25° 35'E) during the winter months of May to August in 2018 and May to October in 2019. The study site averages 509 mm of rainfall (1952-2019), with a 5-month wet season (November to March). The dry season (winter) occurs from April through October (Pettitt et al. 2008). The site experiences a wide range of annual temperatures from below freezing in the winter to above 40°C in the summer (Mbokodo et al. 2023).

Data Collection

We captured squirrels using live traps (Tomahawk Live Trap Inc., WI, USA; 15X15X50 cm) baited with peanut butter and bird seed (Waterman 1995). Every individual was injected with a passive integrated transponder (PIT tags; 134.2 kHz, Shenzhen XCC RFID Technology Co., Ltd., China) during their first capture for permanent identification. All individuals were assessed for sex, age, and reproductive status, and their body mass (g), spine length (cm; from occipitals to last lumbar vertebrae), and ectoparasite abundance were recorded. Juvenile growth was calculated from the body mass of the last capture minus the body mass of the first capture, divided by the number of days between. Ectoparasites were quantified using a flea comb, combing from head to tail 3 times and by examining the stomach and groin areas (Hillegass et al. 2008, O'Brien et al. 2021). Squirrels were classified as juveniles, sub-adults, and adults. Juveniles were less than six months of age and weighed less than 500g; sub-adults were adult size (> 500g) but were non-reproductive (not sexually mature; Pettitt and Waterman 2011). Males were considered adults once their scrotum descended (Waterman 1996). Females were considered adults based on the presence or absence of elongated nipples (Waterman 1996). Nipples elongate and swell after a female's first estrus (usually at around 8 months unless their maturity is delayed), and during her first pregnancy, and the nipples remain elongated indefinitely, although they are even more distended during lactation (Waterman 1996, Pettitt and Waterman 2011). Temporary unique dye marks were painted on a squirrel's dorsal fur (Rodol D; Lowenstein and Sons, New York, 235 NY, U.S.A.) for identification at a distance, and squirrels were released at their site of capture (Waterman 1995). All procedures were approved by the University of Manitoba's Animal Care and Use Protocol (#F10-030, #F14-032, and #F18-039).

Observations

Observations were used to determine family group composition and collect interaction data. We observed squirrels from towers or blinds mounted on vehicles using 10x50 binoculars and 20x60 spotting scopes from a distance greater than 50m (Phillips and Waterman 2013). A squirrel's location was determined via a grid of painted stones placed on the burrow clusters and surrounding areas (Waterman 1995). On a burrow cluster, the stones were placed in a 10 x 10m grid, while in the surrounding area, we used a 20 x 20m grid (Waterman 1995). Family groups emerge together in the mornings and immerge in the evenings in the same burrow cluster, allowing us to identify all members and record interactions. We used all-occurrence sampling to record all social interactions, and scan sampling at 10-minute intervals was used to record the frequency of behaviors and the location of squirrels throughout the observation time (Waterman 1995). Allogrooming, affiliative contact, and escorting have been documented as forms of alloparental behavior in other cooperative breeders and also occurred in our study species (Gilchrist and Russell 2007, Müller and Manser 2008, Schubert et al. 2009). We recorded these alloparental behaviors (allogrooming, affiliative interactions, and escorting) toward weaned offspring using all-occurrence sampling and calculated total acts/hour (Waterman 1995, Pettitt and Waterman 2011, defined in Table 4.2). All behaviors were recorded using an Android™ app (CGSapp) on Android™ devices, designed specifically for this research.

DNA Extraction and Genotyping

During an individual's first trapping, a 3mm piece of the tail tip tissue was taken and immediately placed in 96% ethanol. The DNA was extracted using High Pure PCR Template Preparation kits (Roche Diagnostics, Mannheim, Germany) in 2018 and E.Z.N.A. Tissue DNA kits (Omega Bio-Tek Inc., Norcross, U.S.A.) in 2019. We used polymerase chain reactions (PCR)

with GoTaq® Flexi DNA Polymerase to amplify our DNA (Promega Corporation, Madison, U.S.A.; Manjerovic and Waterman 2015, Shave and Waterman 2017). For PCR, we used nineteen fluorescently labeled forward primers and unlabeled reverse primer sets developed for this species' microsatellite loci (Abercrombie et al. 2009, Manjerovic and Waterman 2015). We used 2% agarose gel to visualize the PCR product. After checking the PCR product, successfully amplified PCR products were pooled, diluted, and sent to the Center for Applied Genomics (Hospital for Sick Children, Toronto, Canada) for genotyping. Genotypes were scored manually and through GeneMarker v. 2.6.0 to reduce errors.

Parentage Analysis

We first screened genotypes using Genecap v.1.4 and COANCESTRY v.1.0.1.10 (Wang 2011) to eliminate genetic matches. Parentage was then inferred using MasterBayes v.2.57, a Bayesian method incorporating microsatellite genotypes and non-genetic variables (distance, area, colony, year, and age; Hadfield et al. 2006), and CERVUS v.3.0.7, which assigns parentage through likelihood calculations (Kalinowski et al. 2007) at an 80% confidence level. Individuals assigned below 80% confidence were removed from consideration. Assignments at or above 80% were subjected to a four-step verification using observational and trapping data. Maternal checks consisted of confirming: (1) age compatibility with the offspring's gestation period; (2) membership in the same family group; (3) survival at the time of birth; and (4) spatial proximity. Candidates meeting all criteria were accepted as mothers.

Statistical Analysis

All statistical analyses were conducted using R version 4.2.2 (R Core Team 2021). Generalized linear mixed models (GLMMs) were implemented using the *glmmTMB* package (version 1.1.5). Fixed effects were category of caregiver (mother, female alloparent, and natal

male), group composition (presence and absence of the natal male and subadults), caregiver characteristics (sex, age in years), other variables (group size, number of juveniles within group), while response variables included juvenile characteristics (body condition, growth rate, juvenile foraging time (percent time), caregiving behaviors/amount (care given by each group member, overall frequency of care behaviors, affiliative care, escorting, allogrooming, percent time present, mean distance (meters) of caregivers to juveniles), ectoparasites (total parasite count on juveniles, flea, lice). We fitted a separate GLMM for each fixed effect, using a Gaussian distribution when the data were normally distributed, and a negative binomial distribution if the data were non-normal. If the data were zero-weighted, we used a negative binomial zero-inflated model. Residual diagnostics for all GLMMs were conducted using the DHARMA package (v. 0.4.6), which enabled evaluation of standardized residuals, collinearity, zero inflation, and uniformity. Interaction effects tested included sex and caregiver age in years, as well as sex and age category. Random intercepts for squirrel ID and family group were included to account for repeated measures of individuals and the non-independence of observations within social groups. Preliminary GLMM was used to test for differences between the 2018 and 2019 datasets; year had no significant effect on the amount of total alloparental care (acts/hour; $\beta = -1.025 \pm 0.791$, $z = -1.30$, $p = 0.195$, $n = 244$), so data from both years were pooled for all subsequent analyses. GLMMs were then used to assess variation in alloparental care and to evaluate the influence of group-level factors on juvenile outcomes. To investigate variation in alloparental care, we examined the relationship between the rates of care-related behaviors performed by caregivers (including allogrooming, affiliative behaviors, and escorting; see Table 4.2) that were directed toward juveniles, measured as acts per hour (acts/h).

Juvenile annual survival data were analyzed using Cox proportional hazards models in the *survival* package (version 3.4.0), as well as GLMMs incorporating Group as a random effect. Each survival model was assessed for multicollinearity using variance inflation factors (VIFs) and for the proportional hazards (PH) assumption to verify that the risk of mortality remained constant over time. Variables that violated the PH assumption or exhibited high multicollinearity were excluded from the final models. A significant level of $p < 0.05$ was applied throughout. Data is presented as means $\pm$ standard errors (SE).

Results

A total of 475 hours of behavioral observations were collected from 16 family groups of Cape ground squirrels, 10 groups observed in 2018 and 6 in 2019. Over the two years, 10 family groups had at least one natal male, and 6 family groups did not have any natal males. Mean family group size was 9.6 ± 1.37 (range 4 to 20) individuals, consisting of 3.06 ± 0.38 (range 1 to 7) adult females, 2.1 ± 0.24 (range 0 to 3) natal males, 0.47 ± 0.22 (range 0 to 4) sub-adult males, 0.47 ± 0.21 (range 0 to 3) sub-adult females, and 4.4 ± 0.72 (range 1 to 10) juveniles. We had 25 mothers and 47 female alloparental caregivers, of which 23 mothers were also alloparental caregivers. One juvenile out of 49 observed was sired by a natal male within the group during this study (2018-2019), but this year-old natal male dispersed at the beginning of the study and did not provide any offspring care. Most adults in a family group provided affiliative, escorting, and allogrooming behaviors to juveniles, but there was variation in the type and amount of care given based on age and sex (Figure 4.1). As subadult males (0.47 ± 0.22 acts/hr) and females (0.47 ± 0.21 acts/hr) gave little care and did not occur in many groups, they were not included in subsequent analyses.

The presence of natal males in a group decreased the amount of alloparental care adult female alloparents gave juveniles (GLMM: $\beta = -0.09 \pm 0.04$ SE, $z = -2.71$, $p = 0.007$, Figure 4.1), yet increased the total rate of care given to juveniles (GLMM: $\beta = 0.88 \pm 0.20$ SE, $z = 4.30$, $p < 0.001$, Figure 4.2). Group identity accounted for most of the variation in total care rates (variance = 0.990, $SD = 0.995$), while residual variance was lower (variance = 0.221, $SD = 0.470$), indicating that most variation occurred among groups ($ICC \approx 0.82$). Younger natal males (1 year of age) gave more care to juveniles than older natal males (2-4 years of age; GLMM: $\beta = 0.55 \pm 0.13$ SE, $z = 4.03$, $p \leq 0.001$, Figure 4.3), whereas, for adult females, age did not affect the amount of care they provided ($\beta = -0.01 \pm 0.03$ SE, $z = -0.56$, $p = 0.59$, Figure 4.3). Because older natal males provided minimal care, our analysis focused on the contributions of one-year-old natal males.

The average distance to juveniles by female alloparents did not change in groups without natal males compared to groups with natal males (female distance for groups with natal males 25.17 ± 19.59 m, without natal males 18.96 ± 20.46 m; GLMM: $z = 1.74$, $p = 0.08$, SE = 0.11). However, natal males stay closer to the juveniles than adult female caregivers (natal male 8.77 ± 2.03 m, female alloparent 11.21 ± 1.53 m; GLMM: $z = 23.1$, $p < 0.001$, SE = 0.13).

Juvenile growth also increased with higher rates of alloparental care from adult females (GLMM: $\beta = 1.73 \pm 0.73$ SE, $z = 2.59$, $p = 0.01$, Figure 4.4), but was not affected by care from natal males (GLMM: $\beta = -0.66 \pm 0.09$ SE, $z = 0.68$, $p = 0.49$, Figure 4.4). Juvenile body condition increased when natal males were present in the group vs when they were absent (GLMM: $\beta = 0.05 \pm 0.04$, $z = 1.91$, $p = 0.057$, Figure 4.5). Group identity accounted for a substantial proportion of the variation in juvenile body condition (variance = 0.114, $SD = 0.337$),

while residual variance was lower (variance = 0.0186, $SD = 0.137$), indicating that most variation occurred among groups ($ICC \approx 0.86$).

Juvenile following of caregivers did not affect juvenile mass (GLMM: natal males $\beta = 5.98 \pm 0.62 SE$, $z = 0.66$, $p = 0.51$; adult females $\beta = -0.54 \pm 4.54 SE$, $z = -0.12$, $p = 0.91$). However, juvenile foraging increased when natal males were present (GLMM: $\beta = 0.96 \pm 0.01 SE$, $z = 114.26$, $p < 0.001$, Figure 4.6), and when caregivers were more frequently present near juveniles (GLMM: $\beta = 0.28 \pm 0.06 SE$, $z = 4.41$, $p < 0.001$, Figure 4.7). Juvenile foraging was not affected by the average distance to caregivers (GLMM: $\beta = -0.00052 \pm 0.00063 SE$, $z = -0.83$, $p = 0.41$, Figure 4.7). Group identity explained a small proportion of the variation in foraging rates (variance = 0.0264, $SD = 0.163$), while individual identity (Tag) explained negligible variation (variance ≈ 0 , $SD \approx 0$), indicating that most variation occurred within individuals.

The number of ectoparasites on juveniles was not affected by the total amount of care received (GLMM: $\beta = -0.16 \pm 0.13 SE$, $z = -0.15$, $p = 0.66$). The number of ectoparasites on juveniles was not affected by allogrooming rates from natal males or adult female alloparents (GLMM: natal males $\beta = 0.13 \pm 0.14 SE$, $z = 0.94$, $p = 0.35$; adult females $\beta = -0.05 \pm 0.09 SE$, $z = -0.49$, $p = 0.63$; Figure S4.1). Group identity explained negligible variation in ectoparasite abundance (variance ≈ 0 , $SD \approx 0$), indicating little consistent difference among groups. Overall, weaned juveniles survived 111 ± 0.06 days out of 365 days. Female alloparental care did not affect the survival of juveniles (Cox Model: coef = $1.08 \pm 1.08 SE$, $z = 1.00$, $p = 0.23$, Figure 4.8), nor did care from natal males (Cox Model: coef = $-0.15 \pm 0.18 SE$, $z = -0.83$, $p = 0.41$, Figure 4.8). The presence/absence of natal males within the group also did not significantly affect juvenile survival (coef = $-0.34 \pm 0.88 SE$, $z = -0.39$, $p = 0.26$). Survival of juveniles with

($N_{\text{male}} = 1$) or without ($N_{\text{male}} = 0$) a natal male within the family group declined similarly over 365 days, with intermediate survival probabilities largely overlapping (e.g., 0.950 vs. 0.950 at day 25, 0.515 vs. 0.452 at day 140), indicating minimal effect of natal male presence on survival (Figure 4.9).

Discussion

The behaviors we observed in Cape ground squirrels are consistent with alloparental care and fit within the definitions of cooperative breeding (Mocha et al. 2023). Alloparental care involves investment in offspring by individuals other than the genetic parents, and our findings support that both adult females and natal males contribute to the care of non-offspring that enhances offspring condition but not their short-term survival. Juveniles gained significant benefits from the care they received, including improved body condition and enhanced foraging. Due to the positive impact of care on juvenile condition, individuals providing care in this species should be considered as helpers (Mitani and Watts 1997).

Alloparental care is care provided by individuals other than the genetic parents, and our study demonstrates that non-parental individuals (adult female alloparents and natal males) engage in behaviors that benefit juvenile condition. While traditional definitions of cooperative breeding require reproductively suppressed helpers or non-breeding individuals providing care (Cockburn 1998, Lukas and Clutton-Brock 2012), Mocha et al. (2023) proposed a continuum-based framework that accommodates systems like that of the Cape ground squirrel, where care is distributed among multiple potential breeders. Although adult females are not reproductively suppressed and can be both breeders and caregivers, natal males rarely sire offspring within their groups, yet they contribute to juvenile care. These interactions meet the criteria for cooperative breeding in a promiscuous and communal breeding system where relatedness may vary, and care may yield both direct and indirect fitness benefits.

Young natal males were the most effective helpers, providing the highest levels of alloparental care within a family group. Their presence influenced the behavior of other non-mothers, who reduced their own alloparental care. Overall, the total amount of alloparental care increased when a natal male was present, and young natal males stayed closer to juveniles than adult female alloparents. In contrast, older natal males contributed relatively little care. A similar pattern occurs in male meerkats, where caregiving declines as dispersal approaches (Clutton-Brock et al. 2002). As natal male Cape ground squirrels age, they may prioritize nearby mating opportunities or the onset of dispersal, leading to reduced caregiving and increased ranging distances. Comparable age-related patterns have also been documented in banded mongooses (*Mungos mungo*), also a communally breeding species, where pup escorting declines as male escorts grow older, and where males escort pups more frequently than females (Gilchrist and Russell 2007).

Adult female Cape ground squirrel caregivers are also key contributors to juvenile development, supporting greater mass gain during the first year. However, their involvement in alloparental care declines by over 50% when natal males are present, suggesting that males may take on caregiving roles and reduce the need for female investment. This shift indicates that females act as helpers primarily when natal males are absent, but redirect their energy when caregiving duties are shared. A similar “load-lightening” effect is observed in superb starlings (*Lamprotornis superbus*), where additional helpers allow breeding females to reduce parental effort and allocate more time to self-maintenance or future reproduction (Guindre-Parker and Rubenstein 2018).

Alloparental care enhanced juvenile condition and development in Cape ground squirrels, with both natal males and females contributing to improved juvenile condition. Cape ground

squirrel female alloparents also influenced juvenile growth. Natal males in particular increased juvenile foraging time, likely by acting as escorts. These behaviors allowed juveniles to devote more time to feeding, a pattern also observed in banded mongooses, where pup survival increases when helpers closely associate with young and facilitate safe foraging opportunities (Gilchrist 2004).

Across mammals and birds, cooperative care shows similar alloparental behaviors. In meerkats (*Suricata suricatta*), helpers enhance pup growth and survival through provisioning, babysitting, and sentinel behavior (Russell et al. 2002), whereas in primates and mole-rats, non-breeding helpers carry, feed, and thermoregulate young, providing direct energetic benefits (Solomon and French 1997, Clutton-Brock et al. 2002). In alpine marmots (*Marmota marmota*), helpers increase juvenile survival through thermal benefits of grooming, covering offspring, and producing heat (Allainé and Theuriau 2004). In Seychelles warblers (*Acrocephalus sechellensis*), helpers provision and defend nestlings, improving condition and survival (Komdeur 1994), while fairy-wrens show that helpers increase feeding rates and reduce breeder workload, particularly under harsh conditions. These patterns indicate that helper contributions vary with life history and ecology but consistently improve juvenile growth, survival, or condition. Similarly, in juvenile Cape ground squirrels, care from female alloparents positively influenced juvenile growth. Additionally, the presence of natal males was associated with increased juvenile foraging time and higher body condition.

Allogrooming is common in mammalian cooperative breeders, serving both as a social mechanism and as a means of parasite removal (Kutsukake and Clutton-Brock 2006, Eads et al. 2017, Dunbar 2018). In some species, such as black-tailed prairie dogs (*Cynomys ludovicianus*), allogrooming can reduce ectoparasite loads (Eads et al. 2017). In Cape ground squirrels,

however, parasite dynamics differ between the sexes. Males consistently carry higher ectoparasite loads than females (Hillegass et al. 2008), and females spend more time grooming and allogrooming, behaviors that are linked to parasite removal (Hillegass et al. 2008, 2010). A potential drawback of male caregiving, therefore, is that males may serve as sources of parasite transmission to the group, as has also been suggested in other systems (Perez-Orella and Schulte-Hostedde 2005). Parasites impose substantial energetic costs on hosts (Scantlebury et al. 2007), and these costs are particularly pronounced in smaller-bodied individuals (George-Nascimento and Oliva 2015). Juvenile Cape ground squirrels are especially vulnerable, as they both face high energetic demands of growth (Steyermark 2002) and their small size makes them susceptible to the costs of parasitism. Thus, the increased ectoparasite exposure juveniles may experience from interacting with natal males could potentially offset the benefits of additional care. Surprisingly, however, we found that the total amount of care received, whether from natal male or female alloparents, did not influence ectoparasite abundance in juveniles.

In many cooperative breeders, alloparental care directly enhances offspring survival. For example, banded mongoose pups experience higher survival when associated with an alloparent (Gilchrist 2004), while in Seychelles warblers, female helpers improve offspring survival through both feeding and defense (Komdeur 1994). In Cape ground squirrels, by contrast, alloparental care from female or male helpers did not increase juvenile survival. One possible explanation is that the presence of natal males was associated with higher ectoparasite abundance in juveniles, which may offset the potential advantages of their care. It is also possible that alloparental care effects extend beyond immediate survival, influencing long-term survival, body condition, or the timing of maturity, factors that were not directly tested in this study but remain important avenues for future research. Thus, Cape ground squirrels present a flexible and socially

complex model of cooperative breeding, characterized by alloparental care, and offer valuable insight into how group composition and individual traits influence cooperative behaviors.

In summary, our results demonstrate that Cape ground squirrels engage in alloparental care that fits within the continuum of cooperative breeding, with both adult females and natal males providing care that improves juvenile condition but not survival. Care patterns shifted with group composition, as young natal males contributed the most care and reduced investment by adult females, suggesting flexible strategies in how care is distributed within family groups. These findings highlight that the benefits of alloparental care may be more important for juvenile growth and development rather than short-term survival and emphasize the influence of age and sex on caregiving roles. This study adds to our understanding of cooperative breeding by showing how individual traits and group structure shape caregiving behavior in a communal and promiscuous mammal.

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Tables

Table 4.1: Hypotheses and predictions for alloparental care and juvenile benefits in Cape ground squirrels (*Xerus inauris*).

Hypothesis	Prediction	Supported
Care provided to juveniles	Adult natal males and females will provide care	Yes
Sex and age of the caregiver will affect the amount of care given	Females balance care with their own reproductive potential, and provide less alloparental care if they can.	Yes
	Natal males will provide more alloparental care than females because they are unlikely to reproduce	Yes
	Older natal males provide less care as they are about to disperse	Yes
	Females will have no age bias in care	Yes
Alloparental care affects juveniles	Juveniles will have more growth with care	Yes
	Juvenile time foraging will increase with care	Natal Males Yes
	Parasite infection will decrease with care	No
	Survival will increase with the amount of care	No

Table 4.2: Behavioral description of Cape ground squirrel (*Xerus inauris*) behaviors used in alloparental care.

Behavior Category	Behavior	Description
Allogroom	Allogroom	One individual grooms another, including licking, sniffing, and nibbling
Affiliative behavior	Approach	Individual walks to a second individual within 10 cm
	Greet	Individual places in the naso-oral area within 1 cm of the naso-oral area of a second individual
Escorting behavior	Follow	When individuals moved together with one individual less than 1m behind the leading individual

Figures

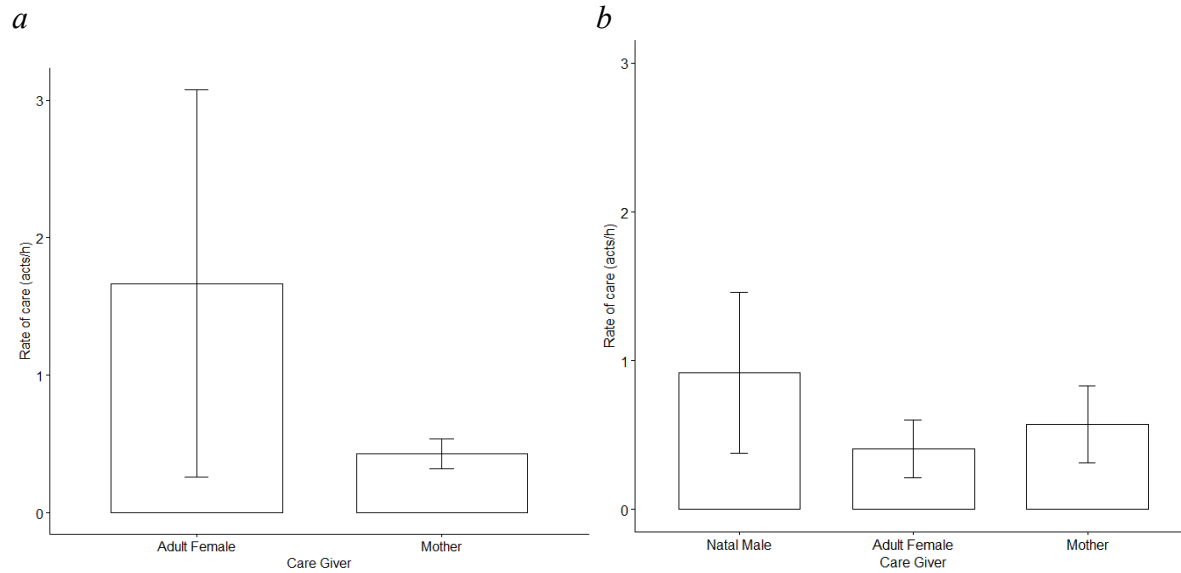


Figure 4.1: The rate (acts /hours) of alloparental and maternal care to juvenile Cape ground squirrels (*Xerus inauris*) in South Africa. *a*) The rate of alloparental and maternal care in the absence of natal males within a group. *b*) The rate of alloparental and maternal care given in the presence of natal males within a group.

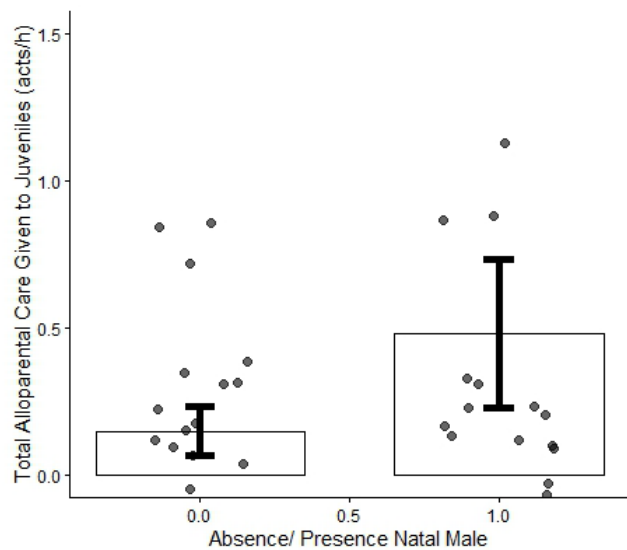


Figure 4.2: Total alloparental care (acts/hour) directed toward juveniles in family groups with and without natal males in South Africa (2018–2019).

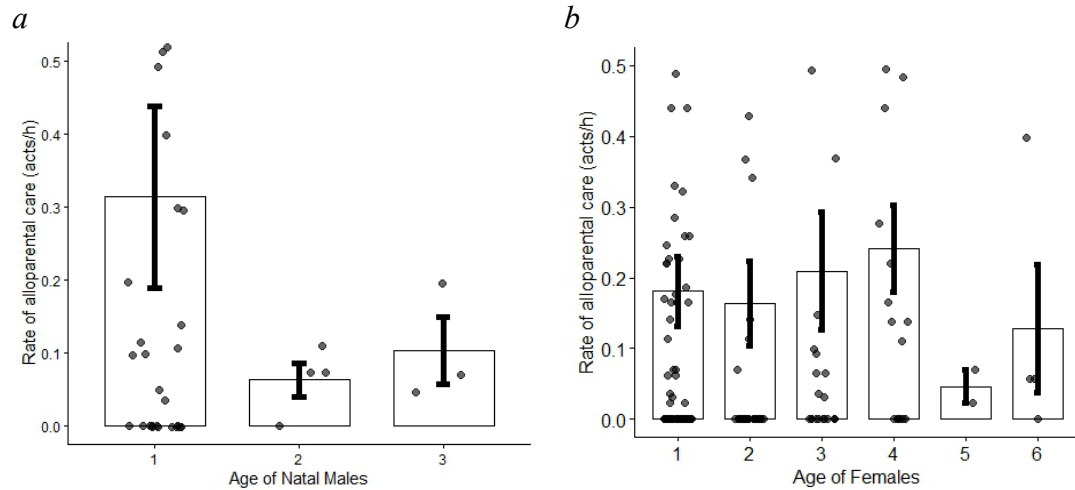


Figure 4.3: Age (in years) of Cape ground squirrel (*Xerus inauris*) alloparental caregivers and the rate of care individual juveniles are receiving in South Africa. Care was quantified per caregiver per juvenile. *a*) Natal male care (per juvenile) age in years; age 1 $n = 34$ natal males, age 2 $n = 8$, and 3+ $n = 3$; *b*) female alloparent care (per juvenile) age in years; age 1 $n = 48$ females, age 2 $n = 31$, age 3 $n = 23$, age 4 $n = 19$, age 5 $n = 2$, age 6 $n = 4$).

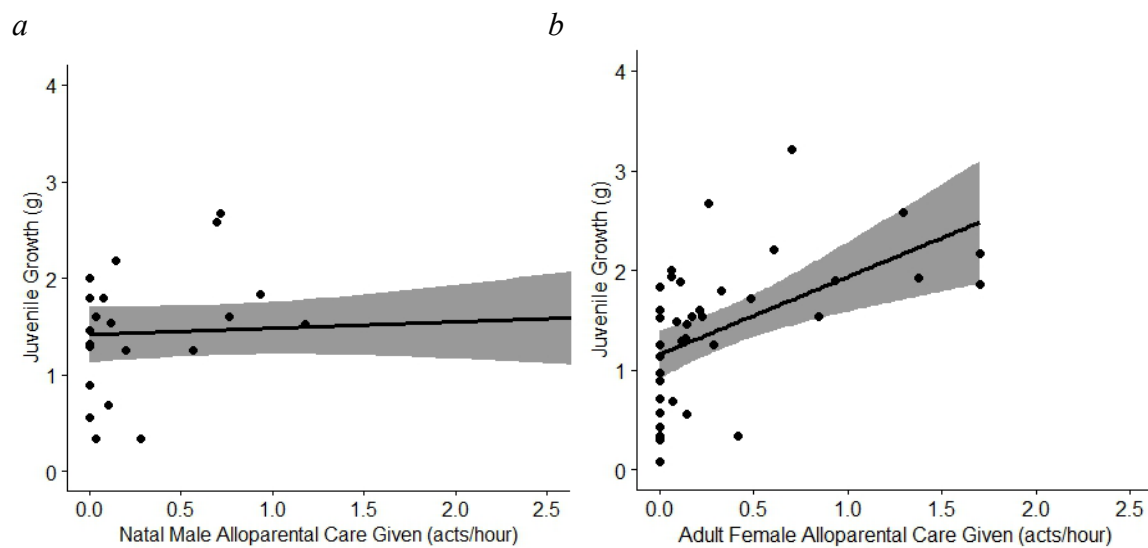


Figure 4.4: *a* Natal male ($n = 26$ natal males) and *b* adult female alloparent ($n = 51$ female alloporents) Cape ground squirrel (*Xerus inauris*) alloparenting effect on juvenile ($n = 41$) growth (mass final-mass start/ number of days between).

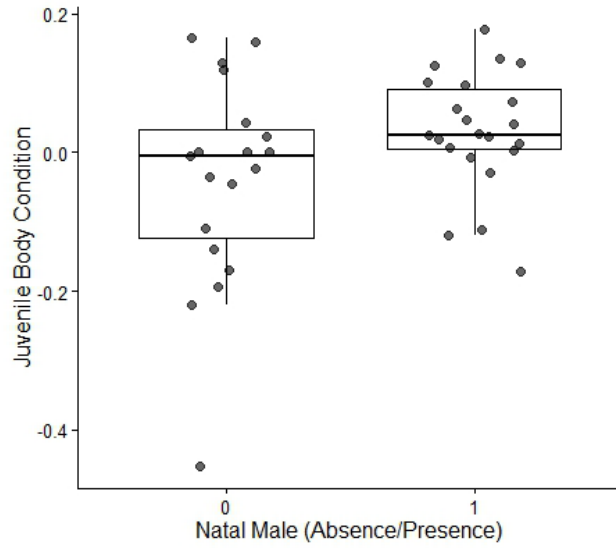


Figure 4.5: The absence (0, $n = 21$ juveniles) or presence (1, $n = 27$ juveniles) of natal males within a family group on juvenile body condition (residuals of a linear regression of mass on spine length) of Cape ground squirrels (*Xerus inauris*).

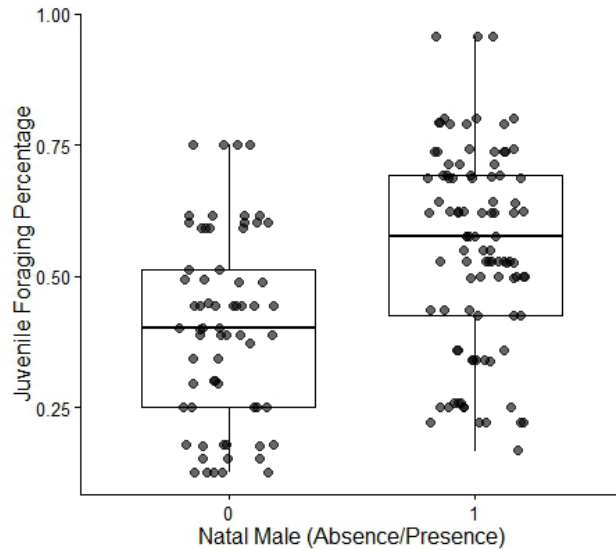


Figure 4.6: Percent of time that juvenile Cape ground squirrel spent foraging in the absence ($n = 21$ juveniles) and presence ($n = 27$ juveniles) of natal male caregivers.

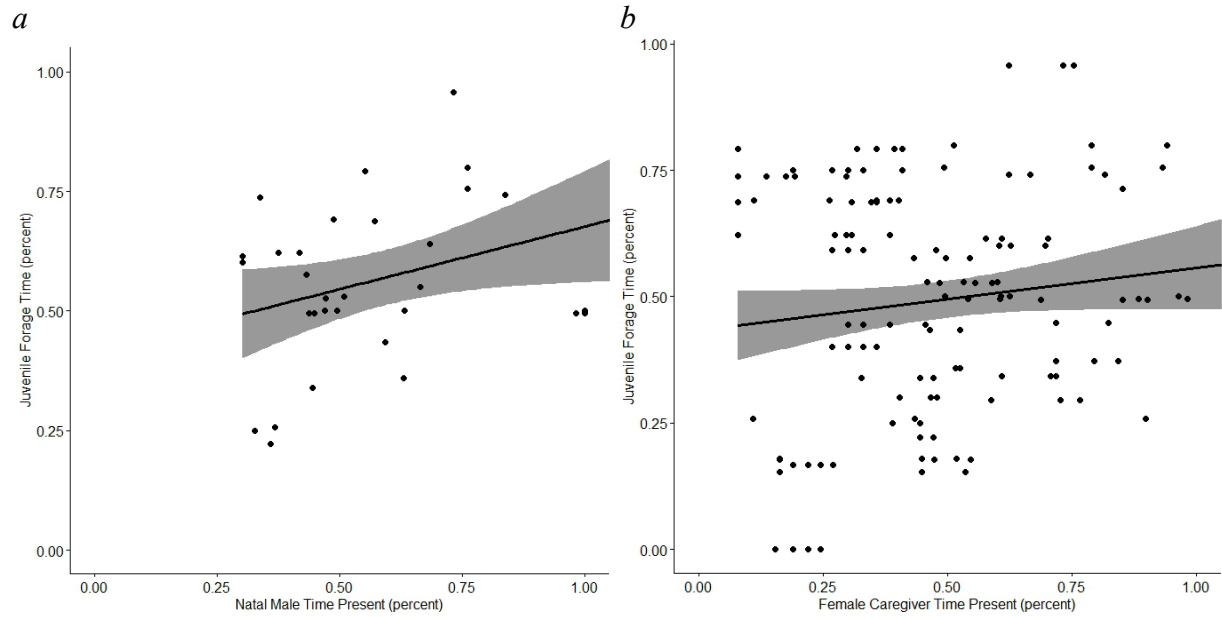


Figure 4.7: Percent of time natal male *a*) (n = 28) and female *b*) (n = 106) Cape ground squirrels (*Xerus inauris*) caregivers are present on juvenile foraging.

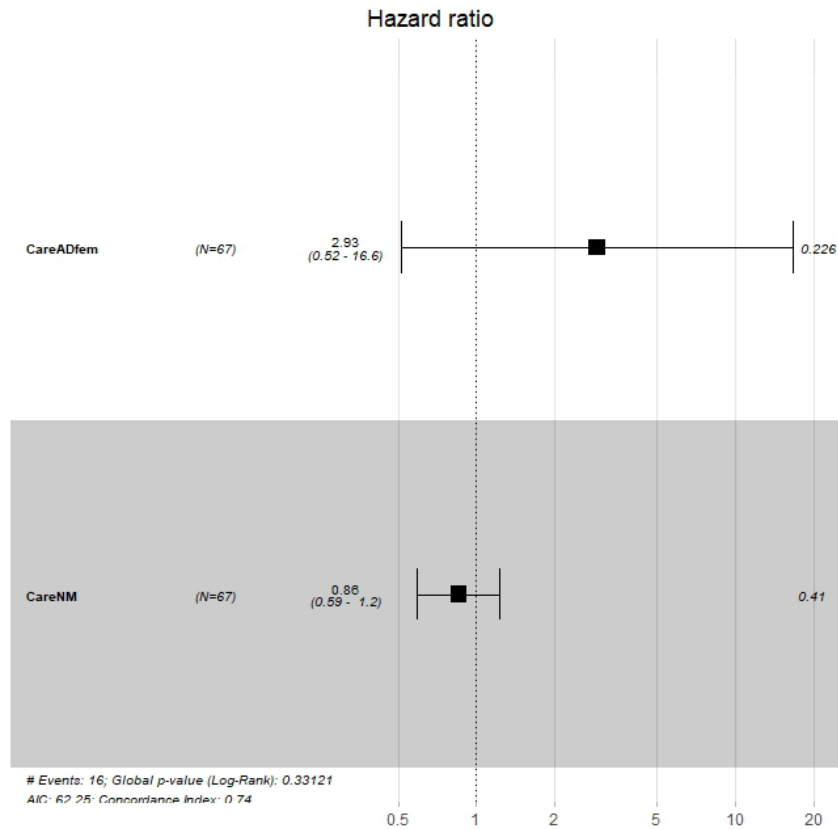


Figure 4.8: Female (CareADfem) and natal male (CareNM) Cape ground squirrels (*Xerus inauris*) alloparental care on juvenile survival.

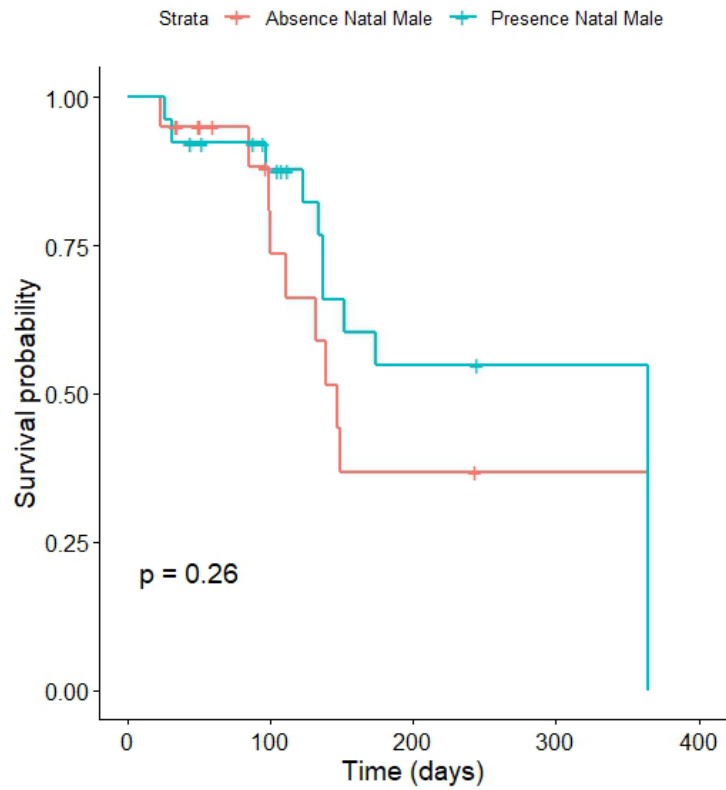


Figure 4.9: The absence ($n = 20$) and presence ($n = 26$) of natal male Cape ground squirrels (*Xerus inauris*) on juvenile survival probability.

Supplementary Figures

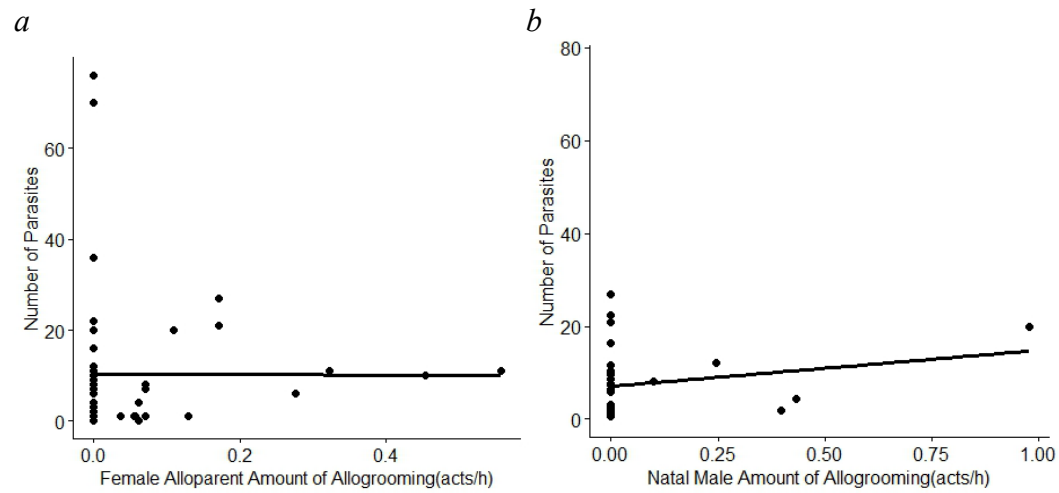


Figure S4.1: Amount of allogrooming from family group adult females *a*) and natal males *b*) on the abundance of ectoparasites on juvenile Cape ground squirrels.

Chapter 5 Helping and load-lightening in an African ground squirrel

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Author contributions Conceptualization (J. Waterman, L. LaFléche, K. Shofstall); methodology (J. Waterman, L. LaFléche, K. Shofstall); investigation (J. Waterman, L. LaFléche, K. Shofstall); formal analysis (M. Warrington, K. Shofstall); data curation (K. Shofstall); supervision (J. Waterman); visualization (K. Shofstall); writing – original draft (K. Shofstall); writing – review & editing (J. Waterman, K. Shofstall).

Abstract

Alloparental care, or care by non-parents, is a key feature of cooperative breeding systems and is often associated with improved reproductive success. In many cases, the presence of alloparents creates a “load-lightening effect,” allowing mothers to reduce their own investment while their offspring continue to receive adequate care. Cape ground squirrels (*Xerus inauris*) are plural breeders that exhibit alloparental care, most commonly provided by non-dispersed related males (natal males). Our study tested whether male alloparents provide a load-lightening effect for breeding females within a family group. We live-trapped squirrels in central South Africa to record reproductive status and body condition, and we collected 289 hours of behavioral observations on helping, foraging, and vigilance from 15 family groups. Natal males contributed care, but their presence did not alter the care, reproductive success, or survival of mothers. Instead, the presence of natal males reduced the amount of alloparental care provided by non-mothers and improved non-mothers’ body condition. Overall, our findings suggest that male helpers do not reduce maternal effort but may directly benefit other females in the group, broadening our understanding of how load-lightening effects may occur in cooperative systems.

Keywords: Alloparental Care, Cape Ground Squirrels, Cooperative Breeder, Helper, Male Care, Plural Breeder, Promiscuous, Load-lighting Hypothesis, *Xerus*

Introduction

Reproduction and parental care are energetically costly processes that demand significant investment by breeding individuals (McNab 2006, Speakman 2008, Heldstab et al. 2017). To manage these reproductive costs, species have evolved life-history trade-offs that balance current reproductive effort with future survival and fecundity (Stearns 1989). In some social species, individuals provide care to offspring that are not their own, known as alloparental care. By sharing the costs of offspring care, such cooperative behaviors reduce the investment required from breeders, allowing them to conserve energy for future reproductive efforts (Russell et al. 2003).

Much of the research on alloparental care focuses on cooperative breeding systems in monogamous species where group members are highly related (Boomsma 2007, Cockburn et al. 2008, Lukas and Clutton-Brock 2012). According to inclusive fitness theory, individuals can gain indirect fitness benefits by helping raise related offspring, thereby increasing the probability of shared genes being passed on (Cornwallis et al. 2009, Abbot et al. 2011). Strong empirical support exists for this model, particularly in monogamous systems where kinship is high (Boomsma 2007, Lukas and Clutton-Brock 2012). However, alloparental care also occurs in polygynous and promiscuous systems, where relatedness among group members is more variable (Cockburn et al. 2008). In these cases, alloparents may also gain direct benefits, such as increased survival through group augmentation, the acquisition of parental skills that enhance future reproductive success, or practice kin discrimination (Meade and Hatchwell 2010, Heldstab et al. 2017). Although providing care is energetically expensive, these indirect and direct benefits may offset the costs for alloparents (Meade and Hatchwell 2010, Klauke et al. 2013).

Most studies have focused on the benefits of alloparental care to alloparents and offspring, often overlooking how such care could affect breeding females (Rosenbaum and Gettler 2018). Breeding females must balance their own self-maintenance with the needs of their offspring, as high maternal investment can reduce the future survival and reproductive output of these females (Broussard et al. 2008, Paquet et al. 2013). Thus, the presence and number of helpers can influence maternal condition and reproductive outcomes (Russell et al. 2003, Heldstab et al. 2017). In harsh environments, helpers can improve maternal fecundity and buffer environmental stressors, enhancing female fitness (Groenewoud and Clutton-Brock 2021). By reducing the energetic demands of reproduction, alloparental care can allow mothers to shorten interbirth intervals, produce more offspring, and increase their own and/or their offspring's survival (Russell et al. 2003a, Rosenbaum and Gettler 2018).

This reduction in maternal investment when help is available is known as the “load-lightening” effect (Crick 1992, Russell and Lummaa 2009). Alloparents may participate in tasks such as nest building, incubation, babysitting, provisioning, and defense of young (Clutton-Brock et al. 2002, Santema and Clutton-Brock 2013, Koenig and Dickinson 2016) and may also engage in vigilance that protects the group and allows breeders to reduce their own effort (Cockburn et al. 2008). Helpers can augment groups, and larger groups may be more effective at acquiring food through cooperative hunting, foraging, or defending higher-quality territories, leading to better body condition and increased fecundity in dominant females (Dyble et al. 2019).

Alloparental care and load-lightening have been well documented in a variety of species. In superb starlings (*Lamprolornis superbus*), additional helpers allow breeding females to reduce provisioning effort and allocate more time to self-maintenance or future reproduction (Guindre-Parker and Rubenstein 2018). Similar patterns are observed in banded mongooses and other

cooperative breeders, where multiple caregivers allow mothers to maintain their body condition without compromising offspring development (Gilchrist et al. 2004). However, much remains unknown, particularly in plural breeding systems where multiple females reproduce simultaneously.

Cooperative breeding in polygamous and plural breeding species represents a continuum of cooperative breeding systems that are highly variable in social structure (Brown 1987, Koenig and Dickinson 2016). Plural breeders, in particular, are characterized by multiple male and/or female breeders within a single social group, which can lead to substantial variation in patterns of care among group members (Nyaguthii 2025). In these species, the distribution of care is often uneven, with some individuals investing heavily in offspring while others contribute little, and this variance is influenced by age, sex, dominance, and reproductive opportunities, as seen in banded mongoose (*Mungos mungo*) and vulturine guineafowl (*Acryllium vulturinum*; Gilchrist et al. 2008, Nyaguthii 2025). It is unclear how load-lightening is shared among breeders and non-breeders or whether some individuals consistently benefit more than others. Understanding how group composition, age, sex, and social hierarchy influence the magnitude and effectiveness of load-lightening is critical for evaluating the costs and benefits of cooperative care in these complex social systems.

We investigated whether the load-lightening effect occurs in Cape ground squirrels (*Xerus inauris*), a cooperative breeder that lives in matrilineal family groups composed of up to five breeding adult females and 1–9 subadults of either sex (Hillegass et al. 2010, Chapter 4). Female sexual maturity occurs around eight months, although maturity may be delayed due to reproductive suppression (Waterman 2002, Pettitt and Waterman 2011). Cape ground squirrels are plural breeders with no dominance hierarchy, and all adult females can reproduce (Waterman

2002, Pettitt and Waterman 2011). Breeding occurs year-round, but estruses in this species occur asynchronously both within and among family groups (Waterman 1995, Bouchie et al. 2006, Manjerovic and Waterman 2015). Despite what appears to be equal reproductive potential among mature females, reproductive skew is high due to substantial litter loss; 70% of reproductive attempts fail, and only 39% of females successfully produce surviving offspring in their lifetime (Waterman 1996, Pettitt et al. 2008, Chapter 2).

In a population in central South Africa, more than 60% of males delay dispersal, remaining with their family groups for up to five years (hereafter, natal males; Scantlebury et al. 2008, O'Brien et al. 2021). These natal males, particularly those aged 1–2 years, provide the bulk of alloparental care, including allogrooming, affiliative interactions, and escorting weaned offspring (LaFleche et al. in prep, Waterman 1995, Pettitt and Waterman 2011, Chapter 4). Natal males do not vary their care based on relatedness to offspring or breeding females (LaFleche et al., in prep., Chapter 4). Non-mother adult females within the group also provide care to offspring (Chapter 4).

This study investigated whether non-mother adult female alloparents (hereafter non-mothers) and natal male alloparents (collectively, “helpers”) provide a load-lightening effect to female Cape ground squirrels with offspring (mothers). We categorized load-lightening into four hypotheses: 1) Maternal effort: We predicted that mothers would decrease both offspring care and vigilance when helpers were present and that higher numbers of helpers would result in a greater reduction in maternal effort. 2) Self-maintenance: If helpers reduce offspring care demands, mothers will invest more in self-maintenance. We predicted that mothers would spend more time foraging and have improved body condition when receiving more care from the presence of helpers. 3) Reproductive success: Load-lightening is expected to enhance

reproduction in mothers. We predicted that mothers with helpers would experience less litter loss. 4) Survival: By reducing energetic costs, helpers may contribute to the increased survival of mothers. We predicted that the presence of helpers of either sex and the increased care provided would be positively associated with female survival.

Methods

Species description

The Cape ground squirrel (*Xerus inauris*) is a long-lived rodent with a slow life history, endemic to the arid regions of southern Africa (Herzig-Straschil 1978, Waterman 1995, 1996). Cape ground squirrels have a long gestation that lasts approximately 50 days, followed by a lactation period of 47.5 days (Pettitt et al. 2008). Litter sizes are small (1–2 offspring), and 71% of litters contain only a single pup (Chapter 2). Females give birth in isolated burrows away from their family group and only return to the group after litter loss or weaning.

Females are philopatric, remaining in their natal area and living in stable family groups (Waterman 1995). Each family group occupies a discrete underground aggregation of burrow systems, known as a burrow cluster. These clusters are separated by areas without burrows and are typically inhabited by a single family group (Herzig-Straschil 1978, Waterman 1995). The reproductive maturity of immature females is influenced by social context. Females experience delayed sexual maturation in groups with more than two adult females or the presence of two or more natal males (Pettitt and Waterman 2011), suggesting that social and reproductive dynamics within groups play a role in female development.

In contrast to females, dispersed adult males form mobile roving bands that travel widely in search of estrous females (Waterman 1998, Manjerovic et al. 2022). However, not all males disperse immediately upon reaching maturity. Although their chances of siring offspring are low,

natal males may benefit from remaining in their family groups by maintaining better body condition in poor rainfall years compared to band males (O'Brien et al. 2021). While natal males may temporarily leave to mate with females in neighboring groups, successful reproduction within their natal group is extremely rare, with only one such instance detected in 93 offspring (Manjerovic and Waterman 2015).

In Cape ground squirrels, all adults in a family group provide alloparental care, with natal males and non-mothers playing distinct roles (Chapter 4). Younger natal males (1-2 years of age) give the bulk of alloparental care, while older natal males (≥ 3 years of age) give little to no alloparental care (L. LaFleche, pers comm., Chapter 4). Younger natal male caregivers are associated with better offspring body condition and foraging, while non-mother care enhances offspring growth (Chapter 4).

Trapping and Observations

We conducted our study from May to August 2018 and from May to October 2019 at the S.A. Lombard Nature Reserve in South Africa (27°35'S, 25°35'E). The Cape ground squirrels at this site are part of a long-term study established in 2002. The reserve receives an average annual rainfall of 509 mm (range: 202.6–934.3 mm from 1952–2019).

Squirrels were captured using live traps (Tomahawk Live Trap Inc., WI, USA; 15 × 15 × 50 cm) baited with peanut butter and bird seed (Waterman 1995). Upon first capture, individuals were injected with a passive integrated transponder (PIT tag; 134.2 kHz, Shenzhen XCC RFID Technology Co., Ltd., China) for permanent identification. A tail tip skin sample was also collected for genetic analysis.

We categorized individuals into three age classes: juveniles, sub-adults, and adults. Juveniles were defined as individuals less than six months old and weighing under 500g. Sub-

adults were adult-sized ($\geq 500\text{g}$) but were not yet sexually mature (Pettitt and Waterman 2011). Males were considered reproductively mature when their scrotum descended around 8 months old (Waterman 1996), while females were considered adults based on the elongation of their nipples, which occurred after their first estrus and pregnancy around 10 months old (Waterman 1996, Pettitt and Waterman 2011). Once elongated, nipples remain enlarged indefinitely and become further distended during lactation (Waterman 1996, Pettitt and Waterman 2011). The reproductive condition of breeding females was also assessed via vulva swelling (indicating estrus) and pregnancy palpation. All individuals were measured for their body mass (g), spine length (cm; from occipitals to last lumbar vertebrae) and dyed with a temporary unique dye marks were painted on a squirrel's dorsal fur that can be observed from a distance (Rodol D; Lowenstein and Sons, New York, 235 NY, U.S.A.). All procedures were approved by the University of Manitoba Animal Care and Use Protocol (#F18-039).

We classified mothers as adult females with an offspring present in the social group (post-weaning). Non-mothers were defined as adult females without an offspring in the group, though they could be pregnant. Females that had isolated to give birth did not provide alloparental care and were therefore excluded from analyses. Offspring care was categorized as follows: care received from an individual's own mother was defined as maternal care; care provided by a mother to an offspring that was not her own was defined as mother alloparental care; and care provided by an adult female without an offspring (a non-mother) was defined as non-mother alloparental care.

We collected behavioral data to assess family group composition and alloparental care. Observations were conducted from elevated towers or blinds mounted on vehicles using 10×50 binoculars and 20×60 spotting scopes, maintaining a distance of at least 50 m (Phillips and

Waterman 2013). To determine the location of individuals during observations, we used a reference grid of painted stones laid out across burrow clusters and the surrounding habitat (Waterman 1995). The grid was arranged in 10×10 m squares over burrow clusters and 20×20 m squares in adjacent areas. Behavioral data were collected using two sampling methods, scan sampling at 10-minute intervals to record individual activities (e.g., foraging, vigilance) and identify individuals emerging together from burrows in the morning and evening to assess group membership (Waterman 1995; Table 5.1). Adult and juvenile vigilance behaviors are defined as vertical stretch (bipedal with legs extended and back straight), and vertical (bipedal without leg extension in a vertical back posture; Unck et al. 2009). All-occurrence sampling to record all social interactions, particularly those involving juveniles, including interactions with both mothers and other group members. All behaviors followed the ethogram outlined in Waterman (1995; Table 5.1) and were recorded using a custom Android™ application (CGSapp) developed for this study.

DNA Extraction and Genotyping

The tail tip tissues that we obtained during trapping were immediately placed in 96% ethanol. To extract the DNA, we used High Pure PCR Template Preparation kits (Roche Diagnostics, Mannheim, Germany) in 2018 and E.Z.N.A. Tissue DNA kits (Omega Bio-Tek Inc., Norcross, U.S.A.) in 2019. The DNA was amplified using the polymerase chain reactions (PCR) with GoTaq® Flexi DNA Polymerase (Promega Corporation, Madison U.S.A.;(Manjerovic and Waterman 2015, Shave and Waterman 2017) using nineteen fluorescently labeled forward primer and unlabeled reverse primer sets developed for this species microsatellites loci were used for PCR (Abercrombie et al. 2009, Manjerovic et al. 2009, Manjerovic and Waterman 2015). We visualized the PCR product on a 2% agarose gel to check the results. Post-PCR products were

pooled, diluted, and sent to the Center for Applied Genomics (Hospital for Sick Children, Toronto, Canada) for genotyping. Genotypes were scored manually and through GeneMarker v. 2.6.0 to reduce errors.

Parentage Analysis

After the manual checking of the genotyping, the results were run through Genecap v.1.4. and COANCESTRY v.1.0.1.10 (Wang 2011) to eliminate complete genetic matches. We used MasterBayes v.2.57 R package (Hadfield et al. 2006) and CERVUS v. 3.0.7 (Kalinowski et al. 2007) to genetically assign parentage at an 80% confidence level. All individuals assigned under 80% were dropped. Individuals at or above 80% confidence were checked for accuracy using a 4-step process to increase our confidence level using trapping and observational data. We first compared the MasterBayes and CERVUS data to make a streamlined list of potential mothers. The potential mothers were checked for age vs offspring emergence date. If potential mothers were sub-adults at the time the juvenile was in utero, then these individuals were dropped. Potential mothers were then checked to see if they were in the same social group using our group observation data. If potential mothers were living in another social group, then these individuals were dropped. If the mother was not observed with any social group, then they were checked for the timing of trapping to determine whether they were alive at the time of juvenile birth. If they were alive, then we checked if the potential mother was in the same area as the offspring using trapping data. If potential mothers met all these criteria, they were assigned as the mother.

Statistical Methods

To examine how helpers may create a load-lightening effect on females, we conducted our analyses in R v 4.2.2 (R Core Team 2021) using generalized linear mixed models (GLMMs) in the package glmmTMB v 1.1.5. Fixed effects were caregiver/group composition

(number/presence of natal males, number of mothers, number of non-mothers). Response variables included the amount of care given (acts per hour; maternal care and alloparental care by natal males, mothers and non-mothers). Family group and individual ID were included as random effects. For vigilance data, we used a logistic regression model implemented in the *aod* package (v. 1.3.3) to account for overdispersion.

Residual diagnostics for all GLMMs were performed using the DHARMA package (v. 0.4.6), allowing assessment of standardized residuals, collinearity, zero inflation, and uniformity. Female annual survival was analyzed using Cox proportional hazards models in the *survival* package (version 3.4.0). All models were evaluated for multicollinearity using variance inflation factors (VIFs) and for compliance with the proportional hazards (PH) assumption to ensure that mortality risk remained constant over time. Variables violating the PH assumption or showing high multicollinearity were excluded from the final analyses. Group size and the number of adult females in the group were highly correlated. The number of adult females was excluded from the survival analysis. Statistical significance was set at $p < 0.05$, and results are reported as means $\pm$ standard errors (SE).

Results

In 2018, we collected 217 observation hours from 10 family groups and in 2019, we collected 72 observation hours from 5 family groups, with 2 groups overlapping between the 2 years. In total, we observed a total of 54 offspring over the two years. We combined the data from these two years after determining that there were no significant differences between the years using a GLMM analysis in R v 4.2.2 (GLMM: $\beta = -1.74 \pm 0.78$ $z = -1.64$, $p = 0.08$). The mean family group size was 9.6 ± 1.37 (range 4 to 20 individuals), consisting of 3.06 ± 0.38 (range 1 to 7) adult females, 2.1 ± 0.24 (range 0 to 3) natal males, 0.47 ± 0.22 (range 0 to 4) sub-

adult males, 0.47 ± 0.21 (range 0 to 3) sub-adult females, and 4.4 ± 0.72 (range 1 to 10) juveniles. Our study consisted of six family groups that had no natal males and nine groups that had natal males. Of these nine groups, all nine groups had a 1-year-old natal male; three groups also contained older natal males (one with a 3-year-old male and two with 4-year-old males), and one group had 2-year-old natal males. As only young natal males give care (1-2 years of age; Chapter 4), we focused our analysis on only these males. We could not examine the presence or absence of non-mother helpers, as there was only one group in which a non-mother helper was absent.

Offspring Care

Not all mothers and non-mothers provided care, either maternal or alloparental. Maternal care was provided by 18 of 22 mothers, representing 82% of the observed mothers, with a mean rate of 0.70 ± 0.22 acts/hour. Alloparental care was provided by 22 females, including 16 mothers (73% of all mothers) at a mean rate of 0.25 ± 0.07 acts/hour, and 6 non-mothers (27% of all non-mothers) at a mean rate of 0.45 ± 0.24 acts/hour. Young natal males gave care to offspring at 0.60 ± 0.27 acts/hour. The presence of natal males did not affect the amount of maternal care provided to offspring ($\beta = -0.48 \pm 0.79$ SE, $z = -1.06$, $p = 0.29$; Figure 5.1), nor did it affect the amount of alloparental care mothers provided to other offspring ($\beta = -0.15 \pm 0.17$ SE, $z = -0.86$, $p = 0.40$; Figure 5.2). When considering mothers only, natal male presence also did not affect female alloparental care ($\beta = -0.15 \pm 0.18$ SE, $z = -0.86$, $p = 0.39$). Group identity accounted for moderate variation (variance = 0.051, $SD = 0.227$), while residual variance was higher (variance = 0.092, $SD = 0.304$; $ICC \approx 0.36$).

In contrast, the presence of natal males decreased the amount of alloparental care provided by non-mothers ($\beta = -1.61 \pm 0.12$ SE, $z = -13.02$, $p < 0.001$; Figure 5.3).

The amount of alloparental care given by natal males or non-mothers did not affect the amount of maternal care given (GLMM: natal male $\beta = -0.25 \pm 0.35$ SE, $z = -0.72$, $p = 0.47$; non-mother females $\beta = 0.78 \pm 0.72$ SE, $z = 1.08$, $p = 0.28$, Figure 5.4).

A mother's vigilance did not differ from that of non-mothers during foraging near offspring (mean 0.06 ± 0.01 ; $z = 0.03$, $p = 0.62$, $SE = 0.03$; Figure 5.5). The presence of natal males did not affect vigilance in mothers ($\beta = -0.02 \pm 1.08$ SE, $z = -0.02$, $p = 0.98$; Figure 5.6) or non-mothers ($\beta = -0.14 \pm 0.11$ SE, $z = -1.31$, $p = 0.19$; Figure 5.6).

Foraging and Body Condition

A mother's foraging time did not differ from that of non-mothers (mother: $45\% \pm 3\%$, non-mother: $49\% \pm 7\%$; $\beta = -0.06 \pm 0.07$ SE, $z = -0.85$, $p = 0.40$). The presence of natal males did not affect foraging time for mothers ($\beta = 0.11 \pm 0.08$ SE, $z = 1.47$, $p = 0.14$) or non-mothers ($\beta = 0.21 \pm 0.15$ SE, $z = 1.41$, $p = 0.16$; Figure 5.7). Care provided by natal males also did not influence foraging time in mothers ($\beta = -0.05 \pm 0.03$ SE, $z = -1.65$, $p = 0.10$) or non-mothers ($\beta = 0.19 \pm 0.33$ SE, $z = 0.57$, $p = 0.57$).

The presence of natal males did not affect maternal body condition ($\beta = -0.02 \pm 0.02$ SE, $z = -1.12$, $p = 0.26$; Figure 5.8), but significantly increased body condition in non-mothers ($\beta = 0.08 \pm 0.02$ SE, $z = 3.91$, $p < 0.001$; Figure 5.8).

Reproduction and survival

The presence of natal males during winter also did not significantly affect successful pregnancy for mothers ($\beta = 0.05 \pm 0.30$ SE, $z = 0.17$, $p = 0.86$, Figure 5.9) nor did it affect non-mother successful pregnancy ($\beta = -0.03 \pm 0.08$ SE, $z = -0.41$, $p = 0.69$, $SE = 1.0$, Figure 5.9) with negligible variation among groups in both cases.

The presence of natal males did not affect the survival of either mothers or non-mothers. Among mothers, natal male presence was not a significant predictor of survival (Cox Model: $\text{coef} = 0.35 \pm 2.69 \text{ SE}$, $z = 0.13$, $p = 0.90$). Similarly, in the model for non-mothers, natal male presence did not influence survival (Cox Model: $\text{coef} = -2.58 \pm 3.57 \text{ SE}$, $z = -0.72$, $p = 0.47$). However, greater maternal care was associated with reduced maternal survival; the amount of care mothers provided significantly decreased their survival probability (Cox model: $\text{coef} = -0.56 \pm 0.28 \text{ SE}$, $z = -2.03$, $p = 0.043$), indicating that higher levels of care increased a mother's hazard of mortality.

Discussion

This study aimed to examine whether alloparental care by non-mother females and natal males provides a load-lightening effect for mothers in Cape ground squirrels, and how such assistance influences maternal effort, self-maintenance, reproductive success, and survival. Contrary to expectations, natal male presence did not influence maternal behavior or maternal fitness in Cape ground squirrels (*Xerus inauris*), but it did affect non-mother adult females. Non-mother alloparents reduced their care when natal males were present, and their body condition increased, suggesting an energetic benefit. The presence of natal males did not influence litter loss of either mothers or non-mothers during pregnancy. Importantly, however, maternal care was costly, as higher investment was associated with reduced mother survival.

In many cooperative species, breeders adjust their investment in offspring care in response to helper availability, often reducing their own effort when helpers are abundant, whereas non-breeders tend to maintain consistent levels of care (Gilchrist and Russell 2007). For example, dominant female suricates (*Suricata suricatta*) increase care when helpers are scarce and reduce care when more helpers are present (Clutton-Brock et al. 2004), while in communal-

breeding banded mongooses (*Mungos mungo*), non-breeding females provide less care overall than mothers and males and do not modify their contributions with helper number (Gilchrist 2007, Gilchrist and Russell 2007). In Cape ground squirrels, non-mother contributions to alloparental care similarly appear to be shaped by helper availability, specifically the presence of natal male helpers; when natal males assist, non-mothers may reduce care, either because collective care is sufficient or because conserving energy benefits future reproduction. Comparable patterns occur in other cooperative breeders, such as Seychelles warblers (*Acrocephalus sechellensis*), where alloparental effort varies with helper numbers (Komdeur 1994). However, only non-mothers in Cape ground squirrels showed this flexibility. Mothers did not reduce care, likely because their direct reproductive success depends on the survival of their own offspring, making decreased maternal investment risky even when helpers are present (Boland et al. 1997, Russell and Lummaa 2009).

Helper presence can offset female investment by freeing time for other essential behaviors (Heldstab et al. 2017). Cape ground squirrels, as granivorous herbivores (Herzig-Straschil 1978), spend most of their day foraging (Waterman 1995). Although reproduction should increase maternal energy demands (McNab 2006, Heldstab et al. 2017), foraging time did not differ between mothers and non-mothers. The foraging time likely reflects that mothers had already weaned offspring, reducing energetic costs, and that feeding already occupies over 55% of a female's activity budget (Waterman 1995). Still, the high alloparental contributions of young natal males may allow non-mothers to maintain feeding levels while allocating less energy to alloparental care.

The positive association between natal male presence and non-mother body condition may reflect preparation for future reproduction. Similar effects have been observed in other

systems: in social weavers, helpers increase clutch frequency (Paquet et al. 2013), while in suricates, better maternal condition is linked to heavier offspring and greater reproductive success (Russell et al. 2003). In Cape ground squirrels, as well, better pre-pregnancy condition positively affects reproductive success (Chapter 3).

Cape ground squirrels are plural breeders, with multiple related females reproducing within the same group (Waterman 1995, 1996, Chapter 3). While non-mother females will provide care to non-descendent offspring (Chapter 4), we found that natal male care provided an additional layer of support to their family group, potentially aiding non-mother reproduction. Unlike in suricates, however, natal male presence did not influence litter survival or reproductive output in Cape ground squirrels. This difference likely reflects life history. Female ground squirrels isolate during parturition and remain solitary until weaning (Waterman 1996), preventing helpers from affecting early offspring survival. By contrast, species such as suricates, wolves (*Canis lupus*), and many cooperative birds involve helpers across multiple reproductive stages (Rood 1974, Legge 2000, Heldstab et al. 2017, Groenewoud and Clutton-Brock 2021). In suricates, care is communal; while pups are initially fed by their mothers, they are soon provisioned by other group members, a behavior essential for survival (Clutton-Brock et al. 2001*b, a*, 2004). In wolves, helpers contribute by guarding, babysitting, and provisioning pups at the den (Ausband et al. 2016).

In Cape ground squirrels, natal male presence shaped the behavior and condition of non-mothers but had little influence on maternal investment or reproductive success. Mothers maintained consistent levels of care regardless of the presence of helpers, reflecting the high costs of reducing investment in dependent offspring, whereas non-mothers strategically reduced their alloparental contributions when males provided care and gained improved condition in their

presence. These patterns highlight the flexible nature of alloparental care in cooperative breeders, where the benefits of helping depend on group composition and breeding system, reflecting a form of load-lightening in which additional helpers allow some individuals to reduce their alloparental investment without compromising overall offspring care. In this case, the benefits were most evident for non-mothers, who could conserve energy, most likely for their own future reproduction. Importantly, maternal care was associated with reduced mother survival, emphasizing the costs of caregiving. These results illustrate how the costs of care and the need to balance current versus future reproduction influence the strategies of mothers and non-mothers in a cooperative plural breeding system. They demonstrate that while maternal care in Cape ground squirrels remained resilient to social variation, non-maternal strategies were flexibly shaped by helper availability, underscoring how life history trade-offs and cooperative breeding dynamics jointly structure the costs and benefits of care.

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Tables

Table 5.1: Behavioral description of Cape ground squirrel (*Xerus inauris*) behaviors used in alloparental care.

Behavior		Description
Allogroom		One individual grooms another, including licking, sniffing, and nibbling
Affiliative behavior	Approach	Individual walks to within 10cm of a second individual
	Greet	Individual places their nose within 1 cm of another individual's mouth
Escorting behavior		
	Follow/followed	When individuals moved together with one individual within 1m behind the leading individual

Figures

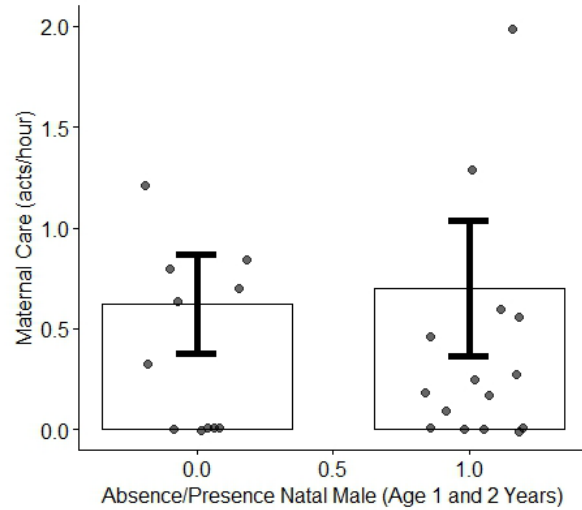


Figure 5.1: The absence (n = 2 groups)/presence (n = 8 groups) of natal male Cape ground squirrels on the amount of maternal care (acts/hour) in South Africa.

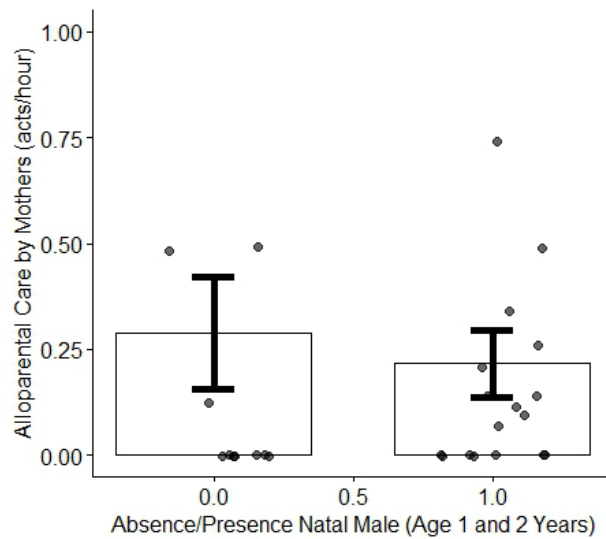


Figure 5.2: The absence (n = 2 groups)/presence (n = 8 groups) of natal male Cape ground squirrels on the amount of alloparental care (acts/hour) a mother gave to other offspring in their group.

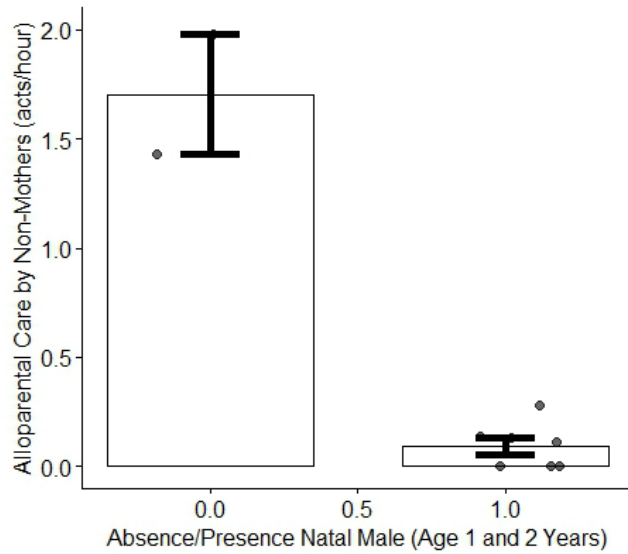


Figure 5.3: The absence ($n = 5$ groups)/ presence ($n = 2$ groups) of natal male Cape ground squirrels on the amount of alloparental care by non-mothers in South Africa.

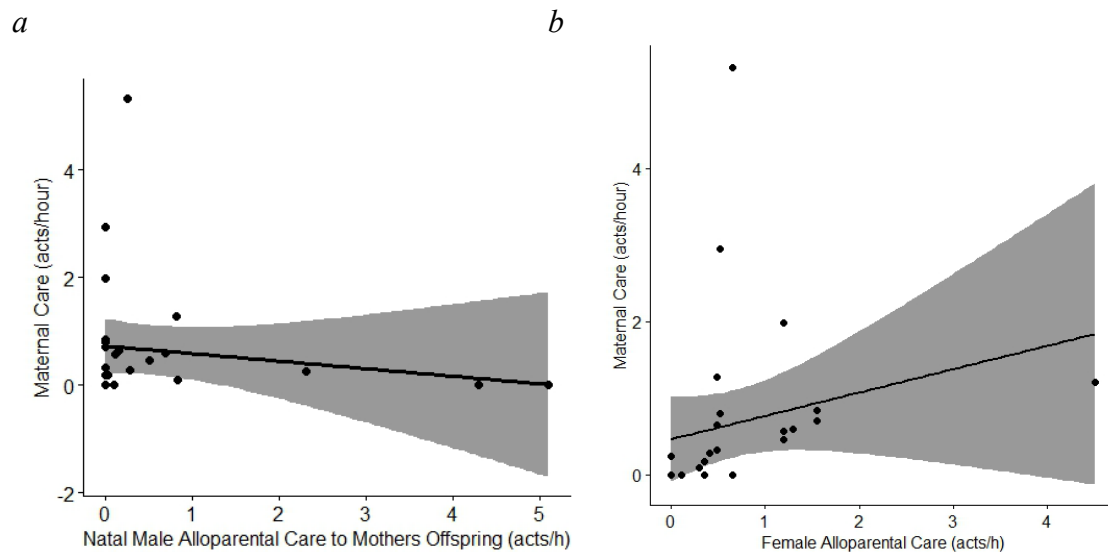


Figure 5.4: The amount of alloparental care (acts/hour) given by *a*) natal males ($n = 14$ natal males and *b*) female alloparents (non-mothers, $n = 8$) on the amount of maternal care (acts/hour) given in South Africa.

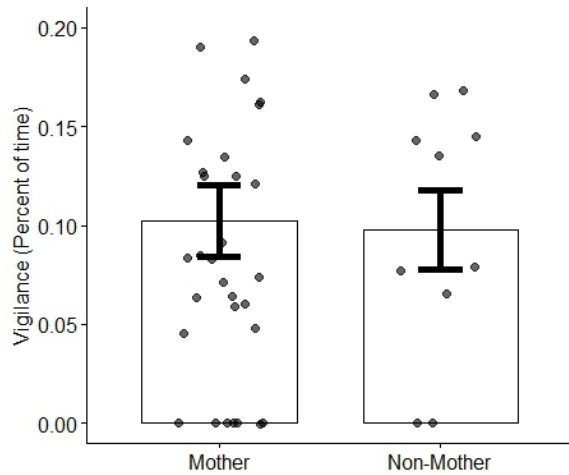


Figure 5.5: Vigilance (percent of time) of mothers (n = 32) and non-mothers (n = 11) in the presence of juveniles.

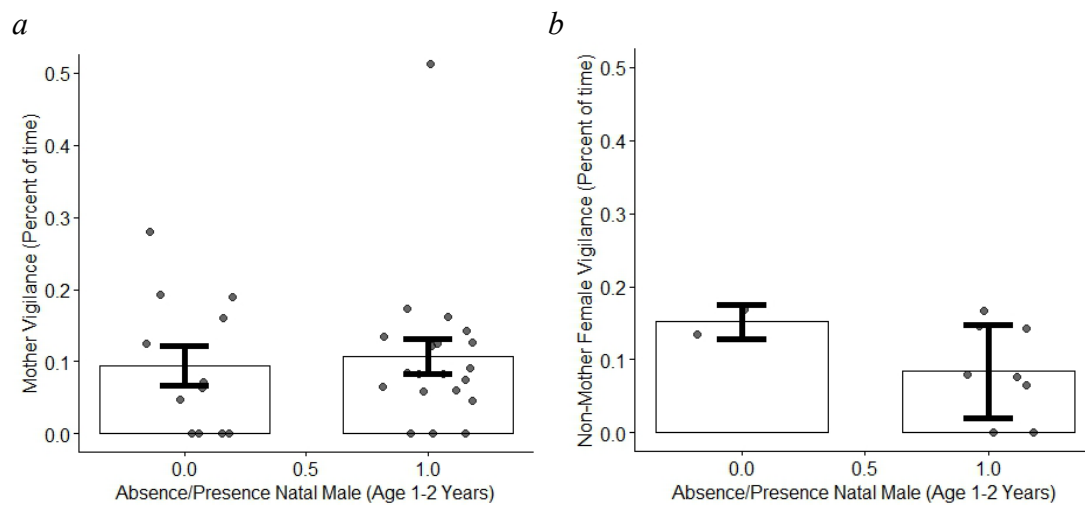


Figure 5.6: Mother vigilance (percent of time, $n = 32$) *a*) and non-mother vigilance ($n = 11$) *b*) in the absence/presence of natal male Cape ground squirrels.

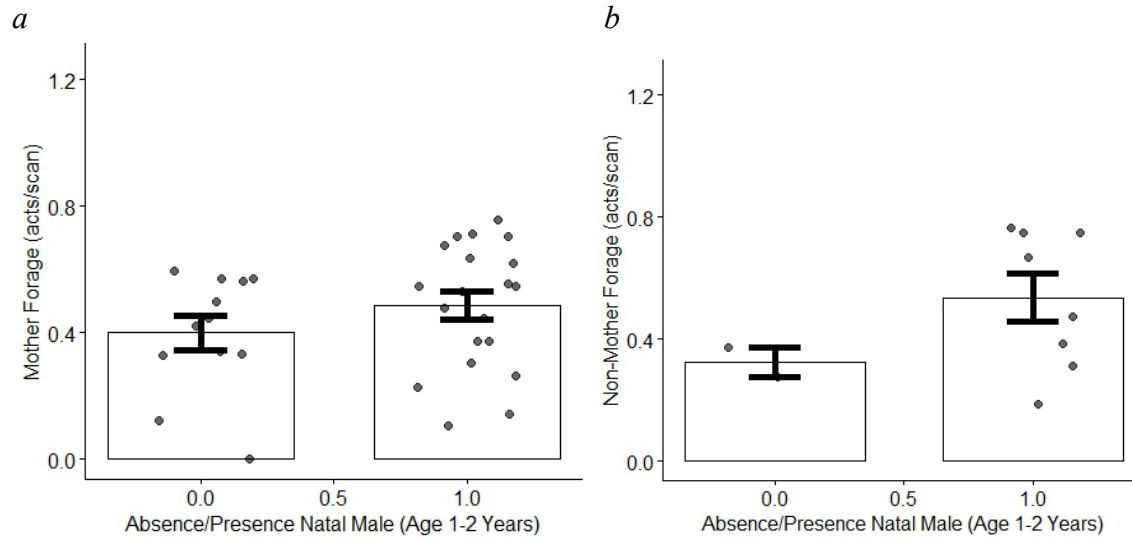


Figure 5.7: The absence and presence of natal male Cape ground squirrels on *a*) mother ($n = 32$) and *b*) non-mother ($n = 11$) female foraging time.

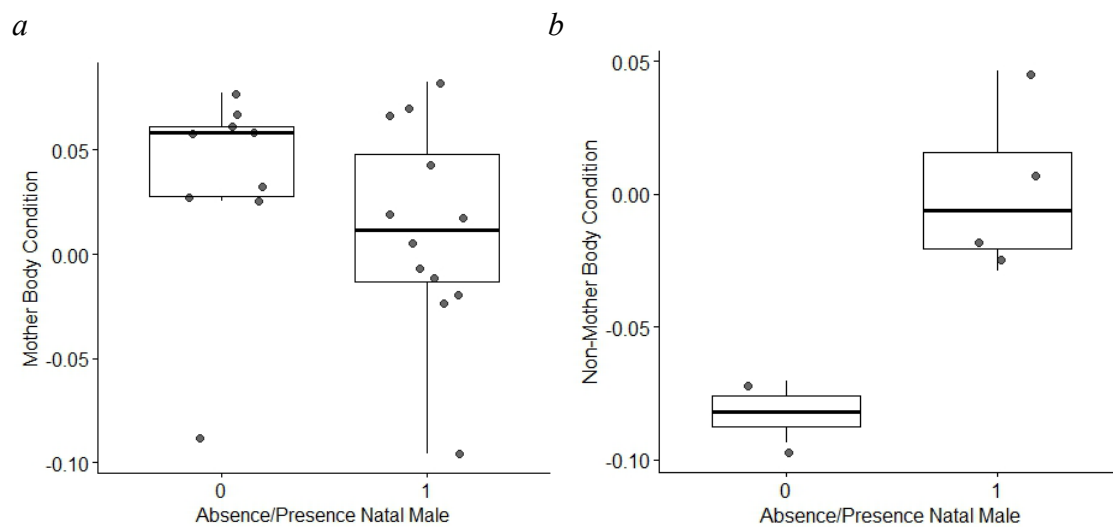


Figure 5.8: The absence and presence of natal male Cape ground squirrel on *a*) mother ($n = 32$) and *b*) non-mother ($n = 11$) body condition.

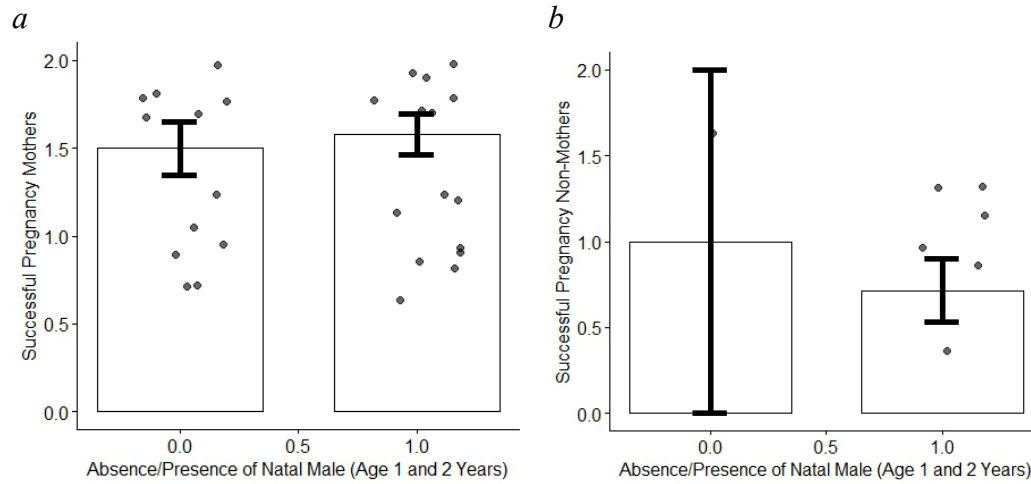


Figure 5.9: The absence/presence of natal males Cape ground on successful pregnancies for *a*) mothers (n = 32) and *b*) non-mothers (n = 11).

Chapter 6 General Discussion

The four chapters of this thesis addressed sexual selection and reproductive skew (Chapter 2), effects of extrinsic (rainfall and temperature) and intrinsic factors (body condition and age) on reproduction (Chapter 3), and alloparental care on offspring and female fitness (Chapters 4 and 5). Together, these studies provide an integrated view of the reproductive strategies of Cape ground squirrels, illustrating how sexual selection, ecological variability, and cooperative behaviors shape fitness in a promiscuous, cooperatively breeding mammal.

Cooperative breeding has long been recognized as a strategy influenced by ecological constraints, life-history trade-offs, and social dynamics (Stearns 1989, Blumstein and Armitage 1997, Clutton-Brock 2009, Koenig and Dickinson 2016). Across vertebrates, the costs and benefits of caregiving vary with sex, age, and ecological context, producing a striking diversity of cooperative systems (Cockburn 1998, Clutton-Brock and Lukas 2012). The findings from Cape ground squirrels contribute to this broader framework by highlighting how promiscuous mating, dispersal patterns, and ecological variability shape the evolution of cooperative care.

Reproductive skew (Chapter 2), observed in both sexes, reflects the operation of strong sexual selection in a species without dominance hierarchies. Male variance in reproductive success is mediated by scramble competition, mate guarding, and sperm competition, while female variance arises through reproductive failure, senescence, and resource limitation. These findings align with comparative work demonstrating that high skew can occur under both competitive and cooperative systems, often shaped by life-history and mating strategies (Stearns 1989, Emlen et al. 1998, Clutton-Brock 2017). Importantly, they underscore that reproductive variance is not solely a product of dominance but can emerge from ecological and social processes that differentially constrain the sexes.

Environmental effects further affect reproductive success (Chapter 3). The non-linear effects of rainfall and temperature on survival and reproduction mirror patterns reported in other cooperative species, where both drought and flooding reduce fitness (Thorley et al. 2025*a, b*). Social thermoregulation buffered individuals against cold stress, consistent with evidence that group living provides resilience in harsh climates (Hayes 2000, Scantlebury et al. 2012). Yet, high maximum temperatures reduced litter size and juvenile survival, emphasizing that cooperative sociality does not eliminate vulnerability to climate extremes. These findings add to a growing body of work suggesting that while cooperative breeding may buffer variability in resource availability, these species may remain sensitive to climatic challenges (Jetz and Rubenstein 2011, Guindre-Parker and Rubenstein 2018).

The results on alloparental care reinforce the flexibility and costs of cooperation (Chapters 4 and 5). Young natal males provided substantial care, often more than adult female helpers, despite being the dispersing sex. This contrasts with the general pattern that philopatric individuals provide the bulk of care (Cockburn 1998, Marshall et al. 2016) and highlights the diversity of strategies through which cooperative systems can evolve. Mothers maintained consistent care regardless of helper presence, reflecting the high costs of reducing investment in dependent offspring, whereas non-mothers adjusted their investment depending on group composition. Such plasticity echoes findings from birds and mammals where helper effort is contingent on the presence of others and the ecological context (Komdeur 1994, Clutton-Brock et al. 2004, Paquet et al. 2013).

Taken together, these results position Cape ground squirrels within a continuum of cooperative strategies, bridging communal breeding and more “classical” cooperative systems. They illustrate how caregiving roles can shift across age, sex, and social context, and how

ecological variability and life-history trade-offs shape the balance of cooperation and competition. More broadly, they support comparative work arguing that cooperative breeding cannot be understood solely in terms of kinship or reproductive suppression but must be viewed as a dynamic strategy integrating ecology, demography, and individual life-history trajectories (Cant and Field 2001, Koenig and Dickinson 2016, Mocha et al. 2023).

As global climates become increasingly variable, the resilience of cooperative breeders will depend on their capacity to flexibly allocate care and buffer environmental fluctuations. The Cape ground squirrel provides a valuable model for understanding this interplay: a promiscuous, long-lived mammal in an unpredictable environment where care is shared among both sexes, but unevenly distributed in its benefits and costs. By situating these findings within broader cooperative breeding theory, this thesis underscores the importance of integrating sexual selection, ecological constraints, and life-history trade-offs to understand the evolution and maintenance of cooperation in vertebrates.

Future research should expand on this research by examining not only how the number of helpers influences juvenile survival and growth, but also how helper quality, measured through age, experience, relatedness, or condition, affects offspring fitness. Such work could determine whether some helpers contribute disproportionately to juvenile fitness and whether these effects persist into adulthood. Equally important is assessing the long-term consequences of alloparental care on both recipients and providers, including whether juveniles that receive more care exhibit higher survival, earlier reproduction, or increased lifetime reproductive success, and whether helpers themselves incur delayed costs or benefits in terms of future fitness. To continue to evaluate how rising temperatures and shifting rainfall patterns amplify reproductive skew, alter dispersal strategies, or shift the costs and benefits of cooperative care. Incorporating age

structure and senescence into these analyses will help clarify how reproductive variance and caregiving strategies change across the lifespan. Such studies will provide a more complete picture of how cooperation interacts with life-history trade-offs and ecological variability to shape the persistence and evolution of cooperative breeding systems. Ultimately, the results presented in this thesis open far more questions than they answer, underscoring that there are many possible future directions to pursue in this system.

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