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# THE INFLUENCE OF SEXUAL SELECTION ON BEHAVIORAL AND PHYSIOLOGICAL MECHANISMS UNDERLYING REPRODUCTIVE SUCCESS IN MALE CAPE GROUND SQUIRRELS (*XERUS INAURIS*)

by

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A dissertation submitted in partial fulfillment of the requirements for the degree of
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in the Department of Biological Sciences
in the College of Sciences
at the University of Central Florida
Orlando, Florida

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# ABSTRACT

Sexual selection is considered a powerful evolutionary force responsible for the enormous diversity found in reproductive morphology, physiology, and behavior. I addressed questions related to selection in the Cape ground squirrel (*Xerus inauris*), a species characterized as highly social and promiscuous. These attributes often are responsible for variance in male reproductive success and as such, sexual selection theory predicts increased opportunity for sexual selection. I confirm that the predominant mechanism underlying genital evolution and competition for paternity in *X. inauris* is sperm competition. I find evidence that investment in sperm competition is costly and may reflect immunocompetence. I quantify reproductive success as it relates to alternative male tactics and female resource distribution. I find that male *X. inauris* alternative reproductive tactics differ within and across populations most likely due to differences in female resource distribution. In areas where females are evenly distributed, dispersed males encounter more estrous females, and therefore have increased breeding opportunities. However, the decision to remain natal does not preclude reproduction. I determine that these tactics are most likely conditional with equal fitness payoffs. Males, regardless of tactic, invest more in post-copulatory competition (e.g. sperm competition, copulatory plugs) than males within a population with a clustered distribution of breeding females. In the latter area, males form dominance hierarchies that affect copulatory success and lead to greater skews in reproduction among males. Both sites have evidence of a highly skewed variance in reproduction and intense sexual selective pressure. My results suggest these populations have increased opportunities for selection but that different mechanisms of intrasexual competition may result in rapid evolutionary change within this species.

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# CHAPTER 1 – INTRODUCTION

## The Influence of Sexual Selection

Sexual selection, the process favoring traits that yield advantages in reproductive competition and ultimately reproductive, has long been recognized as a powerful force driving the evolution of behavior and morphology (Darwin, 1871; Darwin, 1859). Darwin defined sexual selection as simply a reproductive advantage that some males possess over other males for control of females (Darwin, 1871; Darwin, 1859). A century later, the mechanisms underlying sexual selection were revisited and all simplicity was lost. Robert Trivers introduced the idea that males and females have different evolutionary interests as a result of their unequal investments in reproduction (Trivers, 1972). These sex asymmetries in investment, often skewed towards greater female investment, begin with gamete formation for anisogamous species and continue throughout parental care. Because males and females have different genetic interests in reproduction, they maximize reproductive success in different ways (Bateman, 1948).

All interactions between males and females have the potential to lead to conflict in reproductive decisions and thus can explain patterns seen in morphology, physiology, and behavior (Arnqvist & Rowe, 2005; Eberhard, 2009). In 1970, Geoffrey Parker introduced the concept of sperm competition as a form of post-copulatory sexual selection. This competition occurs when ejaculates from multiple males compete for fertilization of a single ovum (Parker, 1970). Male post-copulatory success is maximized through various mechanisms such as increasing sperm numbers, sperm size or velocity relative to other competing sperm (Wedell &

Cook, 1999; Gomendio et al. 2006). The idea that competition and sexual selection can continue after copulation has extremely important implications for evolution due to increased opportunities for sexual selection in both males and females. As in interactions before mating, females can counter male manipulations and control insemination through cryptic female choice (e.g. selective sperm transport, varying oviposition, modifying internal conditions) (Thornhill & Alcock, 1983; Eberhard, 2009).

The degree to which sperm competition affects male mating strategies depends on fertilization success, which indicates how effective individuals are at preventing fertilizations by others (Waterman, 2007). Males attempt to reduce female promiscuity by limiting remating opportunities through the use of copulatory plugs (Koprowski, 1992), post-copulatory guarding (Sherman, 1989), or antiaphrodisiac accessory gland proteins (Chapman & Davis, 2004). Therefore, observed patterns of paternity reflect outcomes of both pre- and post-copulatory competition and patterns of parentage should play a central role in the study of diverse ecological and evolutionary topics such as sexual selection.

## Squirrels as “Model Systems”

Rodents are the most diverse mammalian order consisting of over 2000 species, 39% of known mammalian genera (Wolff, 2007). Species within this order are widely used in studies of genetics, physiology, psychology, and ecology and thus many aspects of rodent reproductive behavior are well characterized. The family Sciuridae consists of almost 300 species and 50 genera and covers the spectrum of variation in terms of life-history traits, social complexity, and mating systems (Murie & Michener, 1984; Blumstein & Armitage, 1998). The formation of complex social groups is thought to reflect a compromise between dispersal costs and foregoing reproduction by staying in the natal group. Because living in social groups requires a balance between competition and cooperation for resources, including reproductive opportunities, sociality often results in reproductive skew, the monopolization of breeding opportunities by a few individuals (Blumstein & Armitage, 1998). Reproductive skew has profound consequences on the mating system, where social monogamy is a result of complete reproductive skew and social polygamy reflects shared reproduction (Waterman, 2007). Sexual selection theory predicts that the opportunity for sexual selection is strongest when reproductive success varies widely among males.

## Goals of this Study

Here, I determine how sexual selection shapes different aspects of reproduction in the Cape ground squirrel (*Xerus inauris*), including various aspects of morphology, physiology, and mating behaviors. This species is characterized as highly social and promiscuous, characteristics that increase reproductive skew among individuals and theoretically should increase the intensity of sexual selection (Emlen & Oring, 1977; Wade & Arnold, 1980). In the first two chapters, I explore mechanisms underlying the evolution of male and female reproductive morphology. I specifically address predictions relating to sperm competition and male inferiority in immunocompetence due to greater selective pressures and negative effects of testosterone. In the last two chapters, I use molecular data to determine male reproductive success as it relates to different aspects of pre- and post-copulatory competition. Specifically, I address how reproductive opportunities are influenced by male dispersal tactics and female distribution and how these variations relate to the opportunity for sexual selection.

Questions relating to pre- and post-copulatory sexual selection are multidisciplinary and complex, encompassing the fields of anatomy, behavior, physiology, and genetics (Birkhead, 2010). Measuring the opportunity for sexual selection is crucial for addressing many questions in behavioral ecology including the evolution of morphological differences (e.g. sexual size dimorphism, male variation in ornamentation) and alternative mating tactics (Eberhard, 2009). Sexual selection often results in rapid trait divergence and accounts for much of the variation observed in natural populations. Understanding the influences of reproductive success can have important implications underlying the evolutionary trajectory of a species.

## References

Arnqvist, G. & Rowe, L. (2005). *Sexual Conflict*. Princeton: Princeton University Press. 330 pp.

Bateman, A. J. (1948). Intra-sexual selection in *Drosophila*. *Heredity* **2**, 349-368.

Birkhead, T. R. (2010). How stupid not to have thought of that: post-copulatory sexual selection. *J Zool Lond* **281**, 78-93.

Blumstein, D. T. & Armitage, K. B. (1998). Life history consequences of social complexity: a comparative study of ground-dwelling sciurids. *Behav Ecol* **9**, 8-19.

Chapman, T. & Davies, S. J. (2004). Functions and analysis of the seminal fluid proteins of male *Drosophila melanogaster* fruit flies. *Peptides* **25**, 1477-1490.

Darwin, C. (1859). *On the Origin of Species by Means of Natural Selection*. London: J. Murray.

Darwin, C. (1871). *The descent of man and selection in relation to sex*. London: Murray.

Eberhard, W. G. (2009). Postcopulatory sexual selection: Darwin's omission and its consequences. *Proc Nat Acad Sci* **106**, 10025-10032.

Emlen, S. T. & Oring, L. W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science* **197**, 215-223.

Gomendio, M., Martin-Coello, J., Crespo, C., Magana, C. & Roldan, E. R. S. (2006). Sperm competition enhances functional capacity of mammalian spermatozoa. *Proc Natl Acad Sci* **103**, 15113-15117.

Koprowski, J. L. (1992). Removal of copulatory plugs by female tree squirrels. *J Mammal* **73**, 572-576.

Murie, J. O. & Michener, G. R. (1984). The biology of ground-dwelling squirrels. *Annual cycles, behavioral ecology and sociality.* Lincoln: University of Nebraska press.

Parker, G. A. (1970). Sperm competition and its evolutionary consequences in the insects. *Biol Rev Phil Soc* **45**, 525-567.

Sherman, P. W. (1989). Mate guarding as paternity insurance in Idaho ground squirrels. *Nature* **338**, 418-320.

Thornhill, R. and Alcock, J. (1983). The evolution of insect mating systems. *Harvard University Press* Cambridge, MA.

Trivers, R. L. (1972). Parental investment and sexual selection. In: Sexual Selection and the Descent of Man (B. Cambell, ed). Aldine, Chicago, p. 136-179.

Wade, M. J. & Arnold, S. J. (1980). The intensity of sexual selection in relation to male sexual behaviour, female choice, and sperm precedence. *Anim Behav* **28**, 446-461.

Waterman, J.M. (2007). Male mating strategies in rodents. *Rodent societies: an ecological and evolutionary perspective* 27-41.

Wedell, N. & Cook, P. A. (1999). Butterflies tailor their ejaculate in response to sperm competition risk and intensity. *Proc R Soc B Biol Sci* **266**, 1033-1039.

Wolff, J. O. (2007). Social biology of rodents. *Int Zool* **2**, 193-204.

# CHAPTER 2 – STRUCTURE AND ALLOMETRY OF GENITALIA IN MALES AND FEMALES OF A SOCIAL AFRICAN GROUND SQUIRREL WITH HIGH POLYGYNANDRY[1]

## Introduction

Genitalia typically undergo rapid and divergent evolution across species if under sexual selection (Eberhard, 1985; Arnqvist, 1997; Arnqvist, 1998; House & Simmons, 2003; Hosken & Stockley, 2004). Often selected traits are larger and more ornamental and such trends, while also seen in external fertilizers (e.g. plants, Andersson & Iwasa, 1996), are more pronounced in species with internal fertilization (Arnqvist, 1998). In polygynandrous systems, genitalia have greater levels of diversity, when compared to monandrous systems, which is most likely attributed to variation in post-insemination paternity success (Arnqvist, 1998). Male genital morphology has been directly linked to fertilization success in both the water strider and the dung beetle (Arnqvist & Danielsson, 1999; House & Simmons, 2003) but the underlying mechanisms between genital morphology and fertilization success are difficult to disentangle. Furthermore, those mechanisms may not be mutually exclusive and can include female processes that affect male paternity success (cryptic female choice), the male-female antagonism over control of optimal fitness strategies (sexual conflict), and male gamete competition (sperm competition) (Arnqvist, 1998).

Levels of sperm competition (sperm from more than one male competing for the ova of one female) are expected to be greater in species with intense male-male competition and multiple

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partners in both sexes (Parker, 1970; Birkhead & Møller, 1998). The level of sperm competition can be determined based on the operational sex ratio (OSR), defined as the number of estrous females to sexually active males (Emlen & Oring, 1977). Increased levels of competition result in males investing more heavily in sperm production and ejaculates (Kenagy & Trombulak, 1986; Møller, 1989; Møller & Birkhead, 1989). This increased investment requires a greater amount of gonadal tissue for sperm production and storage such that levels of sperm competition often are indicated by the occurrence of large testes (Kenagy & Trombulak, 1986; Dixson & Anderson, 2004; Ramm, Parker & Stockley, 2005). Because testis size has evolved in response to factors beyond the first-order influence of body size, the relative testes size (RTS; as calculated in Kenagy & Trombulak, 1986) is expected to increase with increased levels of sperm competition (Kenagy & Trombulak, 1986; Parker et al., 1997; Gage & Freckleton, 2003).

Male and female reproductive tracts are thought to co-evolve and copulatory behaviour, physiology and morphology all have the potential to influence selection on genital traits (Eberhard, 1985; Birkhead, 1995; Presgraves, Baker & Wilkinson, 1999; Hosken & Stockley, 2004; Cordoba-Aguilar, 2005; Minder, Hosken & Ward, 2005; Beese, Beier & Baur, 2006; Brennan et al., 2007). For example, female waterfowl have developed longer and more complex vaginal morphology in relation to male phallus length and frequency of extra-pair copulations (Brennan et al., 2007). Likewise, male stalk-eyed flies (Diopsidae) have co-evolved longer sperm or dimorphic sperm, in relation to female reproductive morphology and sperm storage site (Presgraves et al., 1999). Sexually selected traits are commonly driven by directional selection resulting in both high levels of phenotypic variation (Pomiankowski & Møller, 1995; Hosken &

Stockley, 2004) and positive allometry (where a trait is proportionately larger with increasing organismal size) (Green, 1992; Petrie, 1992). A study on mole rats (Bathyergidae) found positively allometric penises and vaginas relative to body size suggesting the penis may act as a 'good genes' indicator with which the vagina co-evolved (Kinahan et al., 2007). Similar studies on harp seals (*Pagophilus groenlandicus*) (Miller and Burton, 2001), bats (*Nyctalus noctula*) (Lüpold, McElligott & Hosken, 2004), and muskrats (*Ondatra zibethicus*) (Tasikas et al., 2007) also found positive allometry of reproductive traits, e.g. baculum or penis length, potentially due to directional sexual selection, as well as higher levels of phenotypic variation in these traits. Such allometric relationships have been attributed to mating strategies that prohibit females assessing males prior to copulation and thus rely on cryptic, post-copulatory choice with a reproductive advantage being incurred as a result of proportionally longer genitalia (Miller, Stewart & Stenson, 1998; Miller & Burton, 2001; Lüpold, McElligott & Hosken, 2004; Kinahan et al., 2007; Tasikas et al., 2007). Predictions regarding intraspecific variation in genital size, form and allometry in male and female mammals are therefore difficult to make without some understanding of male and female social structure and mating strategies (Arnqvist, 1997; Miller & Burton, 2001; Kinahan et al., 2008).

Cape ground squirrels (*Xerus inauris*) exhibit a highly skewed OSR (11M:1F) due to year round breeding and asynchronous, spontaneous ovulation (Waterman, 1998; Bouchie et al., 2006). *Xerus inauris* have a level of social organization unlike that described for any other ground squirrel where males and females form separate social groups that persist year round (Waterman, 1995). Males are not territorial or aggressive, but rather they compete for females through competitive searching (Waterman, 1998). Females are not forced into copulation and mate with an average of four males per estrus (Waterman, 1994). While females may exert some level of mate choice by retreating underground with specific males, older more dominant males typically obtain the first copulation of a female's estrus (Waterman, 1998). The dominance hierarchy of males, however, is unrelated to external testes size or body size (Waterman, 1998); males have very obviously large external testes, about 20% of the head-body length (Waterman, 1998), and show no seasonality in external testicle size (Waterman, 1996).

The lack of direct male-male competition in *X. inauris* amid a high level of polygynandry make this species an excellent subject for examining the association between mating strategies and genital allometry. Because mating often occurs above ground where female *X. inauris* are not coerced into mating and are able to assess males prior to copulation (Waterman, 1998), this study differs from mammals previously studied which may have less female choice due to the environments in which mating occurs (Miller & Burton, 2001; Lüpold, McElligott & Hosken, 2004; Kinahan et al., 2007; Tasikas et al. 2007). The objectives of this paper are to (1) describe male and female reproductive anatomy that have evolved in a competitive and polygynandrous mating system, and (2) examine allometry of male and female genitalia. I predict that due to the level of polygynandry, males will experience high sperm competition, resulting in investment in the testes and epididymes. However, due to the mating environment, I predict that *X. inauris* will not exhibit positive allometry in reproductive tract length since no reproductive advantage would ensue.

## Methods

I sampled twenty six adult male and twenty one adult female *X. inauris* from private farm lands throughout South Africa and Namibia, where animals were being removed for control measures. I included reproductive adults but excluded pregnant females; reproductive condition was assessed based on size of testes for males and vaginal swelling and elongated nipples for females (Waterman, 1996). I trapped squirrels using Tomahawk® (Tomahawk Live Trap Co., Tomahawk, Wisconsin) live traps baited with peanut butter and chicken feed and euthanized them on site with a halothane or chloroform overdose. All handling was in accordance with the American Mammal Association guidelines (Gannon et al., 2007) and was approved by the University of Central Florida IACUC committee (#07-43W).

I recorded body mass (measured with a spring scale to ± 5.0 g), head-body length (from nasal bone to base of tail), tail length (base of tail to end of caudal vertebrate), and hind foot length (s.u.) for each animal as well as external testes length and width for all males. For internal genital measurements, I dissected the testes and penis of each male and recorded length and mass of both testes separately, mass of the surrounding epididymis (including the caput, corpus and cauda), length of the entire penis *in situ*, and mass of the dissected penis. I also recorded length of what I call the ‘intromittent’ portion of the penis of *X. inauris* as it has a well-defined flexure, or ‘doubling-back’, just beneath the foreskin. This flexure has been found in other sciurids, e.g. as illustrated in Prasad (1954), and I speculated that this may be the only part of the penis that enters the female, and hence measured it separately (hereafter ‘intromittent’ penis). I measured depth of the female vaginal tract by inserting a probe into the vagina of the dissected female and measuring the distance from the point of insertion to the cervix at the point of uterine horn separation. All lengths and widths were recorded using Mitutoyo electronic calipers to the nearest ± 0.1 cm for head/body and tail lengths and ± 0.1 mm for all other lengths and widths; mass was recorded on an AccuLab digital scale to ± 0.01 g.

### *Statistical Analysis*

Data were natural log-transformed to meet the assumptions of normality. Males and females were compared for differences in body size and coefficients of variation (CV) were calculated using the standard method: $\frac{s}{\bar{x}}$ (Sokal & Rohlf, 1995). For allometry analyses, I used an ordinary least squares (OLS) model, which examines relationships between log-log regressions of trait size on body length. When OLS slopes show a significant deviation ($\alpha < 0.1$) from zero, I determined deviations from isometry, where the slope is equal to one, using reduced major axis (RMA) regression (Lüpold, McElligott & Hosken, 2004; Kinahan et al., 2007). The latter method is more appropriate when variables are subject to measurement error and to overcome scale dependence (Sokal & Rohlf, 1995). Positively allometric traits result in $\beta > 1$, isometric traits $\beta = 1$, and negatively allometric traits $\beta < 1$. SAS 9.1 (SAS Institute Inc., Cary, North Carolina) was used for all statistical analyses.

## **Results**

The structures of male and female genitalia are illustrated in Figures 2.1a and 2.1b. Tables 2.1a and 2.1b provide the mean and SE for all traits measured for males and females respectively. Relative to body length, males have an extremely long penis ( $\bar{x}$ = 10.3 cm) that is approximately

42.4% of head/body length and a mean ‘intromittent’ length that is 12.1% of the head/body length. Males have a mean relative testes size of 2.2 (as calculated for rodents in Kenagy and Trombulak, 1986) with testes mass accounting for 1.5% of the total body mass. Females have a mean vaginal depth of 5.4 cm which is 22.4% of the head/body length.

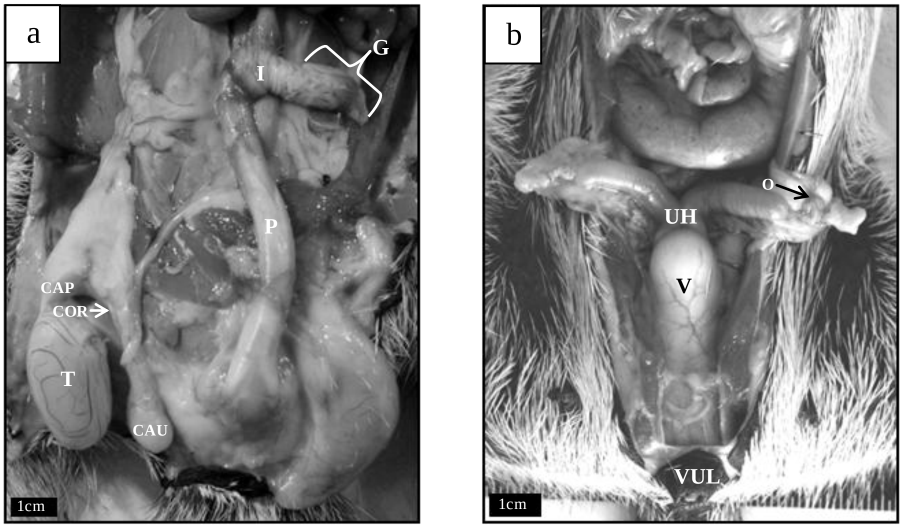

**Figure 2.1.** (a) *In situ* reproductive anatomy of an adult male *Xerus inauris*; T = testis, CAP = caput epididymis, COR = corpus epididymis, CAU = cauda epididymis, P = penis, I = intromittent, G = glans. (b) *In situ* reproductive anatomy of an adult female Xerus inauris; UH = uterine horns, O = ovary, V = vagina, VUL = vulva.

**Table 2.1.** Summary data of morphological measurements for male (a) and female (b) *Xerus inauris*. Masses are recorded in grams and lengths are in millimetres. % CV = percentage coefficient of variation.

| **(a) Male traits** | **n** | **Mean ± SE** | **Range** | **% CV** |
|---|---|---|---|---|
| Body mass | 26 | 678.0 ± 15.0 | 515 - 805 | 11.97 |
| Head/Body length | 26 | 258.3 ± 3.8 | 232.6 - 310.0 | 7.51 |
| L hind foot | 26 | 62.1 ± 0.4 | 58.3 - 66.2 | 3.60 |
| Penis length | 26 | 108.9 ± 1.9 | 93.3 - 130.6 | 9.05 |
| 'Intromittent' length | 12 | 29.2 ± 0.7 | 24.9 - 34.1 | 8.40 |
| Testes mass | 25 | 10.3 ± 0.5 | 4.9 - 15.3 | 23.68 |
| Epididymis mass | 25 | 5.6 ± 0.3 | 2.4 - 9.8 | 27.58 |

| **(b) Female traits** | **n*** | **Mean ± SE** | **Range** | **CV** |
|---|---|---|---|---|
| Body mass | 21 | 603.0 ± 10.0 | 545 - 750 | 8.02 |
| Head/Body length | 20 | 244.5 ± 3.3 | 213.1 - 277.0 | 5.95 |
| L hind foot | 21 | 60.2 ± 0.4 | 54.8 - 63.3 | 3.33 |
| Vaginal depth | 18 | 54.1 ± 1.9 | 40.4 - 67.4 | 14.77 |
| Vaginal mass | 12 | 6.1 ± 1.2 | 1.6 - 14.0 | 67.61 |

*excluding pregnant females

OLS regressions on the reproductive traits of males showed a significant relationship between body length and penis length, and body length and epididymal mass (Table 2.2). Females demonstrated no relationship between body length and reproductive tract depth or mass (Table 2.2). RMA regressions show that total penis length is isometric (i.e. increases in direct proportion) with respect to body size while epididymal mass and testes mass are positively allometric in relation to body size (Table 2.2). The male traits exhibiting positive allometry also had higher phenotypic CVs than isometric traits.

**Table 2.2**. Results of linear ordinary least squares (OLS) and reduced major axis (RMA) regressions for male and female *Xerus inauris* morphological traits (y-axis) regressed against body length (x-axis).

| | OLS | | | | | | | RMA | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | *r* | Slope | ± | SE | *t* value | *P* | Slope | ± | SE | *t* value | *P* | Allometry |
| Penis length | 26 | 0.57 | 0.68 | ± | 0.18 | 3.39 | <0.01 | 1.19 | ± | 0.20 | 0.95 | 0.35 | Isometric |
| 'Intromittent' length | 12 | 0.27 | 0.48 | ± | 0.54 | 0.90 | 0.39 | | | | | | |
| Testis mass | 25 | 0.36 | 1.25 | ± | 0.67 | 1.85 | 0.08 | 3.47 | ± | 0.67 | 3.67 | <0.01 | Positive |
| Epididymal mass | 25 | 0.67 | 2.65 | ± | 0.61 | 4.34 | <0.01 | 3.95 | ± | 0.61 | 4.84 | <0.01 | Positive |
| Vaginal depth | 18 | 0.19 | 0.07 | ± | 0.09 | 0.77 | 0.45 | | | | | | |
| Vaginal mass | 12 | 0.30 | 0.03 | ± | 0.03 | 0.98 | 0.35 | | | | | | |

## Discussion

The Cape ground squirrel has a relative testes size of 2.2 (*sensu* Kenagy and Trombulak, 1986) which, to the best of my knowledge, is one of the greatest proportional testes sizes recorded for any squirrel species (Kenagy & Trombulak, 1986). Levels of competition, as indicated by the OSR, can be high in other squirrel species, such as *Spermophilus beecheyi* (14:1) and *Sciurus carolinensis* (10.6:1), (Koprowski, 1993; Boellstorff et al., 1994; Waterman, 1998) but *X. inauris* has a larger relative testes size than either of these species (2.03 and 1.63 respectively) (Kenagy & Trombulak, 1986). The large relative testes size could be a product of the male grouping system and year-round breeding, which does not occur in *S. beecheyi* or *S. carolinensis*, such that sperm competition risk is ever present and intensity is often high. Testes size is directly related to sperm competition intensity and has been shown to increase in polygynandrous mating systems (Parker et al., 1997; Hosken & Ward, 2001; Pitnick et al., 2001; Ramm et al., 2005). Given that female Cape ground squirrels mate with multiple males (Waterman, 1998), a large relative testes size is expected, as males should invest more in testicular tissue to increase number of sperm per ejaculate. However, unlike other rodents where larger testes also are correlated with multiply sired litters (Ramm et al., 2005), Cape ground squirrels, with an average litter size of 1.6, are less likely to have multiply sired litters (Waterman, 1996). A small litter size does not, however, preclude a high level of sperm competition; in fact, it may encourage greater investment in competitive ejaculates due to the low paternity returns expected from each female.

Due to the rate of spermatogenesis in species with high levels of sperm competition, the role of the epididymis in the storage of mammalian ejaculates is more important than its role in maturation of sperm (Jones, 1999). Mammalian testes typically provide sperm for 0.5–2.0 ejaculates per day but storage capacity of the epididymis allows for continuous spermatogenesis and for controlled delivery of spermatozoa during each mating (Jones, 1999). Investing in proportionally larger epididymes may give a reproductive advantage to those males by enabling a greater capacity for the accumulation and storage of spermatozoa, resulting in the observed positively allometric relationship.

In *X. inauris*, the frequency of repeated copulations per male increases as females encounter and mate with subsequent males (Waterman, 1998). For males, the presence of another male or their knowledge of whether a female has already mated, indicates the 'risk' of sperm competition, which may induce males to produce more sperm. Increasing numbers of rival males results in diminishing returns for increased sperm production and indicates 'intensity' of sperm competition, where males may be predicted to invest fewer sperm per mating or forego mating entirely (Wedell, Gage & Parker, 2002). For *X. inauris*, the mating system and social structure (Waterman, 1998) generate both a high risk and high intensity of sperm competition (Parker & Ball, 2005). This high level of intensity and risk can lead to an increase in testis size as well as sperm expenditure (Parker & Ball, 2005), although the latter will require further study.

Optimal ejaculate expenditure is affected by social or dominance status that influences the order of mating and/or access to females (Parker, 1990). Male *X. inauris* have a dominance hierarchy based on age, not external testes size, and older males are typically able to find estrous females earlier and obtain the first copulation (Waterman, 1998). However, females copulate with younger males throughout the estrus (Waterman, 1998) suggesting that all males may have access to the female and should theoretically optimize ejaculate expenditure depending on perceived risk and intensity of sperm competition.

Female *X. inauris* have deep, thick-walled vaginas that demonstrate no allometric relationship to body length. In mating systems where pre-copulatory choice is not possible, females may gain greater control of conception by selecting for increased reproductive tract length (Birkhead, 1995), resulting in a positive allometric relationship with body size. Since male and female genitalia are thought to co-evolve (Arnqvist, 1997), males may respond by developing a proportionally longer penis (Kinahan et al. 2007) so that sperm can be placed closer to the oviducts thus increasing chances of fertilization (Birkhead, 1995). However, female *X. inauris* assess males prior to copulation (Waterman, 1998). In addition, polyandrous species not only have significantly longer oviducts, but they are also more convoluted than in monogamous species (Anderson, Dixson & Dixson, 2006). The additional complexity and length within the female reproductive tract further challenges the sperm of competing males such that females may be able to select sperm of males with the greatest reproductive potential (Anderson, Dixson & Dixson, 2006). Hence, no reproductive advantage is gained by either sex investing in proportionally longer genitalia. However, the penis in male *X. inauris* is long relative to body length. Greater levels of sperm competition increase genital length in rodents (Ramm, 2007) and the penis length and its potential relationship to sperm competition in the Cape ground squirrel merits further empirical study.

Despite the lack of positive allometry in females, I did see a high coefficient of variation in both vaginal depth and mass. Because *X. inauris* are aseasonal breeders, reproductive females within a population are in various stages of their estrous cycles at any one time. A considerable loss of collagen is associated with the changes in uterine size during an estrous cycle (Van Veen & Peereboom-Stegeman, 1987). I was unable to distinguish where females were in their cycle and suspect that these collagen changes are the cause of the high variability in my data. Seasonally breeding Cape dune mole-rats (*Bathyergus suillus*) have a positively allometric relationship only during the breeding season, suggesting that physiological changes may modify female reproductive anatomy (Kinahan et al., 2007).

This study shows strong support that in the highly competitive, highly polygynandrous *X. inauris*, the predominant mechanism underlying the genital evolution and competition for paternity is sperm competition. This is evident by the large testes size, long penis, and positively allometric epididymis. As sperm competition risk and intensity models predict, genitalia are affected by both the level of competition as well as the mating rate (Parker & Ball, 2005), both of which are correlated with mating systems. The unique male social structure of the Cape ground squirrel sets this species apart from other mammals previously studied and further supports the hypothesis that positively allometric genitalia should not be considered the rule with regards to mammals but rather a reflection of their mating strategies (Kinahan et al., 2008).

## References

Anderson, M. J., Dixson, A. S. & Dixson, A. F. (2006). Mammalian sperm and oviducts are sexually selected: evidence for co-evolution. *J Zool (Lond)* **270**, 682-686.

Andersson, M., & Iwasa, Y. (1996). Sexual selection. *Trends Ecol Evol* **11**, 53-58.

Arnqvist, G. (1997). The evolution of animal genitalia: distinguishing between hypotheses by single species studies. *Biol J Linn Soc* **60,** 365-379.

Arnqvist, G. (1998). Comparative evidence for the evolution of genitalia by sexual selection. *Nature* **393,** 784-786.

Arnqvist, G., & Danielsson, I. (1999). Copulatory behavior, genital morphology, and male fertilization success in water striders. *Evolution* **53,** 147-156.

Beese, K., Beier, K. & Baur, B. (2006). Coevolution of male and female reproductive traits in a simultaneously hermaphroditic land snail. *J Evol Biol* **19,** 410-418.

Birkhead, T. R. (1995). Sperm competition: Evolutionary causes and consequences. *Reprod Fertil Dev* **7,** 755-775.

Birkhead, T. R. & Møller, A. P. (eds) (1998). *Sperm competition and sexual selection*. London: Academic Press.

Boellstorff, D. E., Owings, D. H., Penedo, M. C. T. & Hersek, M. J. (1994). Reproductive behaviour and multiple paternity of California ground squirrels. *Anim Behav* **47,** 1057-1064.

Bouchie, L., Bennet, N. C., Jackson, T. & Waterman, J. M. (2006). Are Cape ground squirrels (*Xerus inauris*) induced or spontaneous ovulators? *J Mammal* **87,** 60-66.

Brennan, P. L. R., Prum, R. O., McCracken, K. G., Sorenson, M. D., Wilson, R. E. & Birkhead, T. R. (2007). Coevolution of male and female genital morphology in waterfowl. *PLoS ONE* **2,** e418.

Cordoba-Aguilar, A. (2005). Possible coevolution of male and female genital form and function in a calopterygid damselfly. *J Evol Biol* **18,** 132-137.

Dixson, A. F. & Anderson, M. J. (2004). Sexual behavior, reproductive physiology and sperm competition in male mammals. *Physiol Behav* **83,** 361-371.

Eberhard, W. G. (1985). *Sexual selection and animal genitalia*. Cambridge, MA: Harvard University Press.

Emlen, S. T. & Oring, L. W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science* **197,** 215-223.

Gage, M. J. G. & Freckleton, R. P. (2003). Relative testis size and sperm morphometry across mammals: no evidence for an association between sperm competition and sperm length. *Proc R Soc Lond B* **270,** 625-632.

Gannon, W. L., Sikes, R. S. & the Animal Care and Use Committee of the American Society of Mammalogists (2007). Guidelines of the American Society of Mammalogists for the use of wild animals in research. *J Mammal* **88,** 809-823.

Green, A. J. (1992). Positive allometry Is likely with mate choice, competitive display and other functions. *Anim Behav* **43,** 170-172.

Hosken, D. J. & Stockley, P. (2004). Sexual selection and genital evolution. *Trends Ecol Evol* **19,** 87-93.

Hosken, D. J. & Ward, P. I. (2001). Experimental evidence for testis size evolution via sperm competition. *Ecol Lett* **4,** 10-13.

House, C. M. & Simmons, L. W. (2003). Genital morphology and fertilization success in the dung beetle *Onthophagus taurus*: an example of sexually selected male genitalia. *Proc R Soc Lond B* **270,** 447-455.

Jones, R. C. (1999). To store or mature spermatozoa? The primary role of the epididymis. *Int J Androl* **22,** 57-67.

Kenagy, G. J. & Trombulak, S. C. (1986). Size and function of mammalian testes in relation to body size. *J Mammal* **67,** 1-22.

Kinahan, A. A., Bennett, N. C., O'Riain, M. J., Hart, L. & Bateman, P. W. (2007). Size matters: genital allometry in an African mole-rat (Family: Bathyergidae). *Evol Ecol* **21,** 201-213.

Kinahan A.A., Belton, L., Bennett N.C., & Bateman P.W. (2008). Do reproductive strategies determine genital allometry in mole-rats (Bathyergidae)? *J Zool (Lond)* **274,** 312-317.

Koprowski, J. L. (1993). Alternative reproductive tactics in male eastern gray squirrels: "making the best of a bad job". *Behav Ecol* **4,** 165-171.

Lüpold , S., McElligott, A. G. & Hosken, D. J. (2004). Bat genitalia: allometry, variation and good genes. *Biol J Linn Soc* **83,** 497-507.

Miller, E. H. & Burton, L. E. (2001). It's all relative: allometry and variation in the baculum (os penis) of the harp seal, *Pagophilus groenlandicus* (Carnivora: Phocidae). *Biol J Linn Soc* **72,** 345-355.

Miller, E. H., Stewart, A. R. J. & Stenson, G. B. (1998). Bacular and testicular growth, allometry, and variation in the harp seal (*Pagophilus groenlandicus*). *J Mammal* **79,** 502-513.

Minder, A. M., Hosken, D. J. & Ward, P. I. (2005). Co-evolution of male and female reproductive characters across the Scathophagidae (Diptera). *J Evol Biol* **18,** 60-69.

Møller, A. P. (1989). Ejaculate quality, testes size and sperm production in mammals. *Funct Ecol* **3,** 91-96.

Møller, A. P. & Birkhead, T. R. (1989). Copulation behaviour in mammals: evidence that sperm competition is widespread. *Biol J Linn Soc* **38,** 119-131.

Parker, G. A. (1970). Sperm competition and its evolutionary consequences in insects. *Biol Rev* **45,** 525-567.

Parker, G. A. (1990). Sperm competition games: sneaks and extra-pair copulations. *Proc R Soc Lond B* **242,** 127-133.

Parker, G. A., Ball, M. A., Stockley, P. & Gage, M. J. G. (1997). Sperm competition games: a prospective analysis of risk assessment. *Proc R Soc Lond B* **264,** 1793-1802.

Parker, G. A. & Ball, M. A. (2005). Sperm competition, mating rate and the evolution of testis and ejaculate sizes: a population model. *Biol Lett* **1,** 235-238.

Petrie, M. (1992). Are all secondary sexual display structures positively allometric and, if so, why? *Anim Behav* **43,** 173-175.

Pitnick, S., Miller, G. T., Reagan, J. & Holland, B. (2001). Males' evolutionary responses to experimental removal of sexual selection. *Proc R Soc Lond B* **268,** 1071-1080.

Pomiankowski, A. & Møller, A. P. (1995). A resolution of the lek paradox. *Proc R Soc Lond B* **260,** 21-29.

Prasad, M. R. N. (1954). The male genital tract of two genera of Indian squirrels. *J Mammal* **35,** 471-485.

Presgraves, D. C., Baker, R. H. & Wilkinson, G. S. (1999). Coevolution of sperm and female reproductive tract morphology in stalk-eyed flies. *Proc R Soc Lond B* **266**, 1041-1047.

Ramm, S. A. (2007). Sexual selection and genital evolution in mammals: a phylogenetic analysis of baculum length. *Am Nat* **169**, 360-369.

Ramm, S. A., Parker, G. A. & Stockley, P. (2005). Sperm competition and the evolution of male reproductive anatomy in rodents. *Proc R Soc Lond B* **272**, 949-955.

Sokal, R. R. & Rohlf, F. J. (1995). *Biometry*. 3rd edn. New York: W.H. Freeman and Co.

Tasikas, D. E., Fairn, E. R., Laurence, S., & Schulte-Hostedde, A. I. (2007). Baculum variation and allometry in the muskrat (*Ondatra zibethicus*): a case for sexual selection. *Evol Ecol* DOI 10.1007/s10682-007-9216-2

Van Veen, H. A. & Peereboom-Stegeman, J. H. (1987). The influence of the estrous cycle on the volume density and appearance of collagen containing vacuoles in fibroblasts of the rat uterus. *Virchows Arch B Cell Pathol Incl Mol Pathol* **53,** 23-31.

Waterman, J. M. (1994). The social organization of the Cape ground squirrel *Xerus inauris*. PhD thesis, University of Minnesota, St. Paul.

Waterman, J. M. (1995). The social organization of the Cape ground squirrel (*Xerus inauris*; Rodentia: Sciuridae). *Ethology* **101,** 130-147.

Waterman, J. M. (1996). Reproductive biology of a tropical, non-hibernating ground squirrel. *J Mammal* **77,** 134-146.

Waterman, J. M. (1998). Mating tactics of male Cape ground squirrels, *Xerus inauris*: consequences of year-round breeding. *Anim Behav* **56,** 459-466.

Wedell, N., Gage, M. J. G. & Parker, G. A. (2002). Sperm competition, male prudence, and sperm-limited females. *Trends Ecol Evol* **17,** 313-320.

# CHAPTER 3 – TRADE-OFFS IN IMMUNITY IN A SPECIES WITH INTENSE SPERM COMPETITION[2]

## Introduction

Sexual selection imposes different selective pressures on males and females resulting in a dichotomy in the way sexes maximize fitness (Zuk & McKean, 1996). Studies on mate choice, and tradeoffs between immunity, reproduction, and post-mating processes indicate that immunity and reproductive success are tightly linked in multiple diverse taxa (Lawniczak et al., 2006). Among many behavioral and physiological processes affected is immunocompetence, the ability to respond to foreign antigens (Owen & Wilson, 1999). In vertebrates, males typically show increased susceptibility to disease, due to greater intensity of sexual selection and possible negative effects of androgens (Folstad & Karter, 1992; Zuk, 1996; Møller et al., 1998; Schmid-Hempel, 2003). In monogamous species, where pressures to compete for females are less intense, sexes are less likely to differ in immunocompetence (Zuk & McKean, 1996). As competition for mates increases, however, males invest more energy into courtship displays, intrasexual competition, and sperm competition. Such competitive investment is energetically expensive and requires high levels of testosterone to express secondary sex characters (Folstad & Karter, 1992). Testosterone is hypothesized to have a dualistic effect, stimulating character development while simultaneously reducing immunocompetence. Folstad and Karter (1992) formalized the ‘immunocompetence handicap hypothesis’ (ICHH) based on the assumption that testosterone suppresses immune function during spermatogenesis because sperm are

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recognized as foreign bodies. Testosterone-dependent characters represent honest signals of quality because only highly immunocompetent males are capable of trading-off between reproduction and immunity (Folstad & Karter, 1992; Møller et al., 1998).

The ICHH predicts species with high investments in spermatogenesis are more likely to demonstrate a trade-off between reproductive and immunological investment (Folstad & Karter, 1992; Hosken & O'Shea, 2001). This relationship is not always found, possibly because immune response is also dependent on social and environmental circumstances. Characteristics such as increased sociality and promiscuity increase the likelihood of infection and transmission of pathogens altering immune response (Nunn et al., 2000). I addressed sex differences in immunity in the Cape ground squirrel (*Xerus inauris*), a species that has extremely high investment in sperm competition (Manjerovic et al., 2008), and is highly social and promiscuous (Waterman, 1995; Waterman, 1998). The absence of aggression and territoriality among males, in an extremely competitive system, results in huge investments in reproductive morphology in order to outcompete other males (Waterman, 1998; Manjerovic et al., 2008). I predict males able to invest more in sperm competition do so at a cost to their immune system. Males carry higher ectoparasite loads compared to females, which has been attributed to increased testosterone (Hillegass et al., 2008). Therefore, I predict males are under greater selective pressure and their investment in reproductive morphology will result in lower immunocompetence compared to females.

## Methods

I sampled *X. inauris* from May-June 2007 at S.A. Lombard Nature Reserve near Bloemhof, South Africa (27°35'S, 35°23'E). I trapped squirrels using Tomahawk® traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin) and euthanized a subset of adult animals on site with a halothane overdose. I took blood samples from the femoral artery of live, restrained animals or from the internal cavity of euthanized animals. I handled and euthanized animals in accordance with American Mammal Association guidelines (Gannon et al., 2007) with approval from the University of Central Florida IACUC (#07-43W).

To assess immunity, I measured spleen size in euthanized animals and percentage of red and white blood cells in all animals. Although such measures are proxies for immunity, they are frequently used to assess immunocompetence (Hosken & O'Shea, 2001; Corbin et al., 2008; Nunn et al., 2009). I recorded body mass (± 5.0 g) with a spring scale and spleen mass (± 0.01 g) on an AccuLab digital scale (Edgewood, NY). To control for differences in body mass, I compared spleen sizes using the residuals of spleen size regressed on body mass. I measured percentage of red blood cells (RBCs) by collecting blood in a heparinized capillary tube and spinning for two minutes in a portable microhematocrit (International Medical Associates, Inc.). For white blood cells (WBCs), I counted 100 cells on a single layer blood smear stained with eosin nigrosin and recorded the numbers of each type: basophils, eosinophils, lymphocytes, neutrophils, and monocytes (Zuk 1996). I normalized all blood cell percentages using an arcsine transformation. I analyzed data using JMP® v.8.0 (SAS Institute Inc., Cary, North Carolina), comparing males and females for all variables using a t-test and considering results significant if $\alpha \leq 0.05$ (Sokal & Rohlf, 1995).

To measure male reproductive investment, I measured mass (± 0.01 g) of each testis, and prostate and bulbourethral glands. To remove effects of body size, I calculated the residuals of a least squares regression of each organ on body mass. I then compared relationships between residuals (spleen versus each reproductive gland) using a least squares regression (Hosken & O'Shea, 2001).

## Results

I euthanized 26 males and 11 females and found no significant differences in body mass ($t_{13.02} = -0.26$, $P = 0.797$) or spleen size ($t_{35} = -0.26$, $P = 0.800$). I found a significant negative correlation between the residuals of spleen mass and testes mass but no relationship with spleen and bulbourethral or prostate glands (Figure 3.1). I found percentage of red blood cells to be significantly lower in males than females ($t_{62} = 2.97$, $P = 0.004$; Table 3.1). I also found significant differences in white blood cells, with higher percentages of basophils ($t_{40.2}=-5.65$, $P <0.0001$) and lymphocytes ($t_{28.2}=-2.82$, $P =0.009$) in males, and higher neutrophils ($t_{16.7}=2.58$, $P=0.020$) in females (Table 3.1).

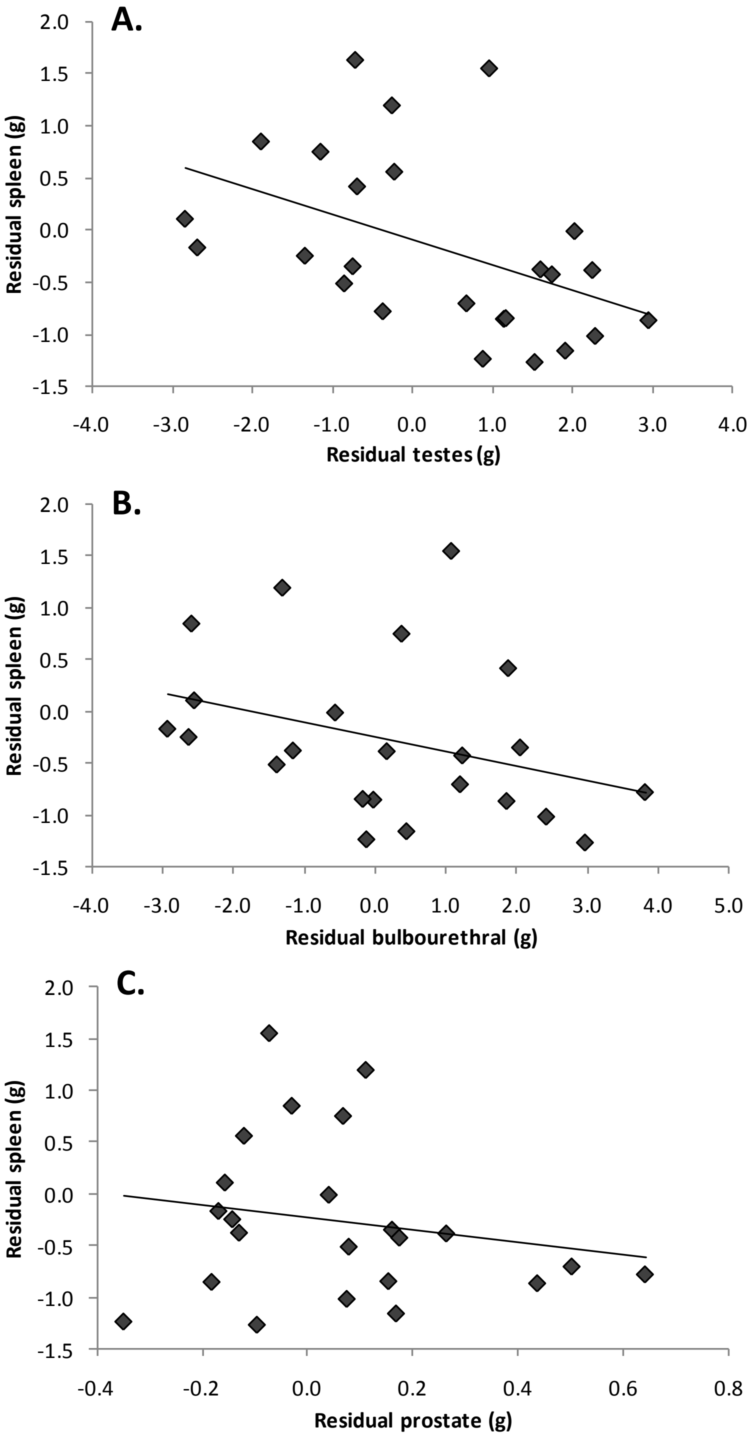


**Figure 3.1.** Relationship between residual masses of spleen and A. testes ($r^2 = 0.21$, F = 6.07, *P* = 0.022), B. bulbourethral glands ($r^2 = 0.12$, F = 2.78, *P* = 0.110), and C. prostate gland ($r^2 = 0.03$, F = 0.70, *P* = 0.412) calculated from least squares regression against body mass in *Xerus inauris*.

**Table 3.1.** Average blood cell percentages for male and female *Xerus inauris* (bold text indicates significance).

| | Males | | | Females | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SE | n | Mean | SE | n | t | df | *P* |
| **red blood cells** | **45.4** | **2.02** | **27** | **53.3** | **1.10** | **25** | **2.97** | **62.0** | **0.004** |
| **basophils** | **4.4** | **0.53** | **33** | **1.1** | **0.23** | **33** | **-5.64** | **40.2** | **<0.001** |
| eosinophils | 2.0 | 0.38 | 33 | 1.1 | 0.35 | 33 | -1.69 | 31.0 | 0.102 |
| **neutrophils** | **43.5** | **2.62** | **33** | **56.1** | **3.83** | **33** | **2.58** | **16.7** | **0.020** |
| **lymphocytes** | **32.2** | **2.29** | **33** | **23.1** | **2.38** | **33** | **-2.82** | **28.2** | **0.009** |
| monocytes | 17.9 | 1.34 | 33 | 18.6 | 2.16 | 33 | 0.26 | 16.7 | 0.803 |

## Discussion

Compared to other ground squirrels, male *X. inauris* have one of the highest testes size relative to the amount of competition (Manjerovic et al., 2008). On average, 11 males compete for a single, estrous female who mates with an average of 4 males (Waterman, 1998). As predicted by the ICHH, I found a significant negative relationship between testes size and spleen size suggesting male investment in reproduction carries immunological costs (Hosken & O'Shea, 2001). However, I did not find a relationship between accessory glands and spleen size. These glands are positively associated with sperm competition due to their role in copulatory plug formation (Ramm et al., 2005), and have been shown to be under sexual selection in invertebrates (Fairn et al., 2007). Because testosterone influences bulbourethral gland development (Gottreich et al., 2001), I predicted a trade-off would also exist between these glands and immunity.

Despite the negative relationship between spleen and testes size, I found no sex differences in spleen size even though males are under intense selection. When comparing other measures of immunity, I found significant differences in both RBCs and leukocyte types, which I attribute to variations in parasite type. Endoparasites and ectoparasites are known to affect hosts differently (Christe et al., 2002) but rarely are accounted for separately in studies addressing sexual dichotomy of immune response. Hillegass et al. (2008) found both male and female *X. inauris* have high parasite loads but males have significantly higher ectoparasite loads while females have significantly higher endoparasite loads. Similarities in spleen size may be attributed to both sexes being highly infected. Lower concentrations of RBCs in males are a likely response to higher numbers of fleas, as those parasites can induce anemia in rodents (Hawlena et al., 2008). Males have significantly more lymphocytes and basophils compared to females, possible because of higher ectoparasite loads. Basophils are part of an allergic response to ectoparasites (Falcone et al., 2001); lymphocytes are involved in recognition of antigens and increase in response to ectoparasites (Christe et al., 2002). Conversely, neutrophils, which were higher in females, have been shown to increase in response to endoparasites (Beardsell & Howell, 1984).

Previous studies of immunosuppression imply selection only imposes stress on males during the energetically expensive breeding season (Møller et al., 1998). *X. inauris*, however, are year-round, asynchronous breeders, requiring both sexes to continuously invest in reproduction (Waterman, 1996; Waterman, 1998). On average, 70% of estruses fail to produce offspring, which has been attributed to costs associated with parasites rather than scarce resources

(Waterman, 1996; Pettitt et al., 2008; Hillegass et al., 2010). Parasitic infection often increases when animals reproduce, due to transmission through contact, endocrine changes, or reallocation of resources (Deerenberg et al., 1997). Removal of parasites from female *X. inauris* significantly increases reproductive success suggesting females also are immunologically challenged (Hillegass et al., 2010). Given the costs associated with reproduction, females likely face a similar trade-off between investing in reproduction or immune response. Immunocompetence in both sexes in this species may be under intense selection.

In this system, both males and females have high energetic constraints and significant differences in parasite loads. While my findings support the ICHH and the male's response to testosterone, I present a larger issue rarely addressed in the literature. Parasite types trigger different immune responses. Therefore, questions addressing 'overall' immunity may fail to detect differences and may contribute to mixed support for the ICHH. As the field of ecological immunity progresses, additional immune responses (e.g. timing and specificity) are being considered (Winterhalter & Fedorka, 2009). I suggest future studies on this sexual dichotomy should incorporate multiple types of parasites and leukocyte type not just overall numbers of WBCs.

# References

Beardsell, P. L., & Howell, M. J. (1984). Killing of *Taenia hydatigena* oncospheres by sheep neutrophils. *Z Parasitenkd* **70**, 337-344.

Christe, P., Møller, A. P., González, G., & de Lope, F. (2002). Intraseasonal variation in immune defence, body mass and hematocrit in adult house martins *Delichon urbica. J Avian Biol* **33**, 321-325.

Corbin, E., Vicente, J., Martin-Hernando, M. P., Acevedo, P., Perez-Rodriguez, L., & Gortazar, C. (2008). Spleen mass as a measure of immune strength in mammals. *Mammal Rev* **38**, 108-115.

Deerenberg, C., Arpanius, V., Daan, S., & Bos, N. (1997). Reproductive effort decreases antibody responsiveness. *Proc R Soc Lond B* **264**, 1021-1029.

Fairn, E. R., Schulte-Hostedde, A. I., & Alarie, Y. (2007). Sexual selection on accessory glands, genitalia and protarsal pads in the whirligig beetle *Dineutus nigrior roberts* (Coleoptera: Gyrinidae). *Ethology* **113**, 257-266.

Falcone, F. H., Pritchard, D. I., & Gibbs, B. F. (2001). Do basophils play a role in immunity against parasites? *Trends Parasitol* **17**, 126-129.

Folstad, I., & Karter, A. J. (1992). Parasites, bright males, and the immunocompetence handicap. *Am Nat* **139**, 603-622.

Gannon, W. L., Sikes, R. S. & the Animal Care and Use Committee of the American Society of Mammalogists (2007). Guidelines of the American Society of Mammalogists for the use of wild animals in research. *J Mammal* **88,** 809-823.

Gottreich, A., Hammel, I., Yogev, L., Bartoov, B., & Terkel J. (2001). Structure and function of accessory sex glands in the male blind mole rat (*Spalax ehrenbergi*). *J Mammal* **82**, 201-208.

Hawlena, H., Krasnov, B. R., Abramsky, Z., Khokhlova, I. S., Goüy de Bellocq, J., & Pinshow, B. (2008). Effects of food abundance, age, and flea infestation on the body condition and immunological variables of a rodent host, and their consequences for flea survival. *Comp Biochem Physiol A* **150**, 66-74.

Hillegass, M. A., Waterman, J. M., & Roth, J. S. (2010). Parasite removal increases reproductive success in a social African ground squirrel. *Behav Ecol* **21**, 696-700.

Hillegass, M. A., Waterman, J.M., & Roth, J.D. (2008). The influence of sex and sociality on parasite loads in an African ground squirrel. *Behav Ecol* **19**, 1006-1011.

Hosken, D. J. & O'Shea, J. E. (2001). Sperm production and immune function in two Australian bats, *Chalinolobus morio* and *Nyctophilus geoffroyi*. *Ethol Ecol Evol* **13**, 173-180.

Lawniczak, M. K. N., Barnes, A. I., Linklater, J. R., Boone, J. M., Wigby, S., & Chapman, T. (2007). Mating and immunity in vertebrates. *Trends Ecol Evol* **22**, 48-55.

Manjerovic, M. B., Kinahan, A. A., Waterman, J. M., Bennett, N. C., & Bateman, P. W. (2008). Structure and allometry of genitalia in males and females of a social African ground squirrel with high polygynandry. *J Zool Lond* **275**, 375-380.

Møller, A. P., Sorci, G., & Erritz, J. (1998). Sexual dimorphism in immune defense. *Am. Nat* **152**, 605-619.

Nunn, C. L., Gittleman, J. L., & Antonovics, J. (2000). Promiscuity and the primate immune system. *Science* **290**, 1168-1170.

Nunn, C. L., Linderfors, P., Pursall, E. R., & Rolff, J. (2009). On sexual dimorphism in immune function. *Philos T Roy Soc B* **364**, 61-69.

Owens, I. P. F., & Wilson, K. (1999). Immunocompetence: a neglected life history trait or conspicuous red herring? *Trends Ecol Evol* **14**, 170-171.

Pettitt, B. A., Waterman, J. M., & Wheaton, C. J. (2008). Assessing the effects of resource availability and parity on reproduction in female Cape ground squirrels: resources do not matter. *J Zool Lond* **276**, 291-298.

Ramm, S. A., Parker, G. A., & Stockley, P. (2005). Sperm competition and the evolution of male reproductive anatomy in rodents. *Proc R Soc Lond B* **272**, 949-955.

Schmid-Hempel, P. (2003). Variation in immune defence as a question of evolutionary ecology. *Proc R Soc. Lond B* **270**, 357-366.

Sokal, R. R., & Rohlf, F. J. (1995). Biometry, 3rd edn. New York: W.H. Freeman and Co.

Waterman, J. M. 1995 The social organization of the Cape ground squirrel (*Xerus inauris*: Rodentia: Sciuridae). *Ethology* **101**, 130-147.

Waterman, J. M. (1996). Reproductive biology of a tropical, non-hibernating ground squirrel. *J. Mammal*. **77**, 134-146.

Waterman, J. M. (1998). Mating tactics of male Cape ground squirrels, *Xerus inauris*: consequences of year-round breeding. *Anim Behav* **56**, 459-466.

Winterhalter, W. E. & Fedorka, K. M. (2009). Sex-specific variation in the emphasis, inducibility and timing of the post-mating immune response in *Drosophila melanogaster. Proc R Soc Lond B* **276**, 1109-1117.

Zuk, M. (1996). Disease, endocrine-immune interactions, and sexual selection. *Ecology* **77**, 1037-1042.

Zuk, M. & McKean, K. A. (1996). Sex differences in parasite infections: patterns and processes. *Int J Parasitol* **26**, 1009-1024.

# CHAPTER 4 – 'SHOULD I STAY OR SHOULD I GO': INTRASPECIFIC VARIATION IN DISPERSAL LEADS TO DIFFERENCES IN PRE- AND POST-COPULATORY COMPETITION IN MALE CAPE GROUND SQUIRRELS

## Introduction

Variations in reproductive patterns, behaviors and morphology commonly are observed across taxa as mechanisms to maximize fertilization success (Gross, 1996; Shuster & Wade, 2003). The existence of alternative reproductive tactics (ARTs) is the result of reproductive competition driven by either intersexual competition and/or natural selection (Brockmann, 2001; Brockmann & Taborsky, 2008). These alternatives, often driven by social and ecological environments, can be classified based on average fitness pay-offs as (1) condition dependent with equal pay-offs where tactics are based on resources and population density, or (2) 'making the best of a bad job', where individuals adopt a less successful tactic based on factors such as dominance, age, or size (Gross, 1996; Wolff, 2008). Such reproductively inferior tactics often reflect an individual opportunistic response to the local social and ecological conditions and thus may change throughout a season or lifetime, depending on an individual's current status compared to the competitors (Kodric-Brown, 1986; Koprowski, 1993).

The occurrence of alternative tactics is related to the intensity of sexual selection and to the extent to which same-sex conspecifics can exploit their competitors in order to increase their probability of acquiring mates or fertilizations. In addition, high variance in fitness often is a result of intense sexual competition related to female accessibility, male interference competition, and sperm competition. Thus it should not be surprising that ARTs are commonly seen in males and vary spatially and temporally in response to female distribution (Shuster & Wade, 2003; Brockmann & Taborsky, 2008; Wolff, 2008). Males often invest disproportionately in pre- and post-copulatory competition depending on tactics and may derive unequal benefits despite equally high reproductive investments (Brockmann & Taborsky, 2008). Quantification of reproductive benefits to males in promiscuous species is impossible without the use of genetic data of individually known subjects.

Alternative reproductive tactics have been extensively studied in invertebrates (Gross, 1996; Brockmann, 2001) but also are observed in mammals, including both tree and ground squirrels (Koprowski, 1993; Scantlebury et al., 2008). Sciurids represent a diverse spectrum of breeding and social systems such that variations in reproductive tactics often can be characterized by mating systems and intensity of sperm competition. The majority of sciurids have discrete breeding seasons in which males have a fixed interval to compete for reproductive opportunities. These discrete intervals often result in males that are territorial and/or aggressive to maximize mating opportunities (Koprowski, 1993). In species that lack these behaviors or species that breed year-round, additional aspects of behavior (e.g. dispersal or search ability) may confer reproductive advantages (Young et al., 2007). Male Cape ground squirrels (*Xerus inauris*) reach reproductive maturity between 8-10 months, at which point they either disperse and join a roving all-male band, or do not disperse and remain in their natal burrow for up to four years (Waterman, 1995; Waterman, 1997; Scantlebury et al., 2008). Males are classified as either ‘dispersers’ or ‘natals’ but these tactics are not fixed; all natal males eventually disperse and there have been a few cases where dispersed males briefly return to natal burrows (Waterman, 1995; J. Waterman, personal observation). There are multiple factors influencing why individuals remain at their natal site, especially if this tactic results in delayed breeding and reduction in overall reproductive success (Koenig et al., 1992). Often the decision to remain at the natal site is attributed to indirect fitness benefits obtained by helping raise kin (Solomon, 1991) or, in the absence of helping, direct benefits of group-living (Kokko & Ekman, 2002). Because male *X. inauris* who disperse still obtain benefits of group-living (Waterman, 1997), the decision of 'should I stay or should I go' may be an integral part of the reproductive tactics of *X. inauris* depending on factors such as age, indirect fitness benefits, and likelihood of successful reproduction. Females do copulate with younger males throughout the estrus suggesting that all males, regardless of dispersal tactic, have access to females and potential for direct reproductive success (Waterman, 1998). While it has been suggested that there is a first male advantage based on older, dominant males obtaining first matings, fertilization success has never been quantified thus there is no measure of direct fitness between tactics (Waterman, 1998; Scantlebury et al. 2008).

In this study, I examined pre- and post-copulatory competition and the resulting fertilization success between alternative dispersal tactics used by male *X. inauris*. I hypothesize that differences in reproductive potential and investment should result in differences in the probability of reproduction. Specifically, I predict dispersed males would have increased reproductive potential due to larger home ranges, and a higher likelihood of encountering estrous females and mating with females first. Previous research found no differences in body condition or testosterone levels between dispersal tactics (Scantlebury et al, 2008). Therefore, I predicted males would invest equally in reproductive morphology for post-copulatory sperm competition.

Differing competitive abilities between tactics do not always result in unequal average fitness gains (Gross, 1996). If dispersal is a conditional strategy influenced by ecological and environmental factors, I would expect males to have equal average fitness (Gross, 1996). However, if male *X. inauris* are 'making the best of a bad job' in response to the frequency and competitive ability of rival males, I would expect unequal reproductive payoffs between natal and dispersed males (Gross, 1996). The decision to remain natal could therefore depend on both the direct and indirect fitness benefits that would be obtained compared to the risks associated with dispersing. If inclusive fitness benefits were gained by remaining natal, I predicted that natal males would be more likely to have their mother in the colony and that dispersal would be more likely to occur if an individual's mother disappears.

## Biology of the study species

*Xerus inauris* is a promiscuous, social species in which both males and females form independent social groups. Roving male bands exist independently of females and are often observed sleeping together and searching for estrous females (Waterman, 1997). Past research on *X. inauris* supports multiple behavioral and physical differences between individuals using these dispersal tactics (Scantlebury et al., 2008). Compared to natal males, dispersed males have higher resting metabolic rates, higher responses to gonadotropin challenges and lower cortisol levels. Dispersed males are older and spend more time moving and less time foraging

(Scantlebury et al., 2008). Female *X. inauris* are asynchronous breeders with spontaneous ovulation that breed year round (Waterman, 1996; Bouchie et al., 2006), thus males constantly have the potential to mate. On the single day of estrus there is intense competition, and females mate with an average of 4 males during a brief 3 hour estrus (Waterman, 1994; Waterman, 1998). The operational sex ratio, measured as the number of males attending an estrus, is highly skewed (11M:1F; range 3-18); despite this, males are not territorial or aggressive and compete for access to females via competitive searching (Waterman, 1995; Waterman, 1998) and sperm competition (Manjerovic, 2008). The promiscuous nature of this species is difficult to interpret because litter size is small (1-2 offspring), suggesting likelihood of multiple paternity is low, albeit not non-existent (Waterman, 1996).

## Methods

I trapped *X. inauris* from June 2002 until December 2006 at S. A. Lombard Nature Reserve, South Africa (27°35'S, 25°35'E) with peak trapping occurring between June and September. The site is divided into two areas where burrows are clustered hereto referred to as floodplain and house (Unck et al. 2009). I trapped squirrels using Tomahawk live traps (Model 202, Tomahawk Live Trap, Tomahawk, Wisconsin) baited with peanut butter and chicken feed, and placed traps on level ground near burrow entrances. Upon capture, I recorded body mass (± 0.5 g), sex, reproductive condition, and age based on mass, morphological characteristics, or known date of birth (Waterman, 1995; Waterman, 1996; Waterman, 1998). For individuals who were caught as juveniles, I was able to estimate age using a growth regression curve (Pettitt, 2006);

this allowed me to estimate average age of male dispersal. I used monthly trapping and observation occurrences to calculate date of last known occurrence. For permanent identification, I marked all animals using a single PIT tag (AVID, Norco, CA, USA) inserted subcutaneously above the right hind limb. For long distance field identification, I placed a unique dye mark (Rodol D, Lowenstein and Sons Inc., New York, NY, USA) and freeze-mark (Quick Freeze; Rood, 1980) combination on the back. For genetic analyses, I collected a 1-3 mm tail tissue sample stored in 95% ethanol. I released animals at the site of capture; all methods of handling were in accordance with the American Mammal Association guidelines (Gannon et al., 2007), and were approved by the University of Central Florida’s Institutional Animal Care and Use Committee (#07-43W).

I observed squirrels from trees, vehicles, blinds, and observation towers using 10x50 binoculars and 15-45x60 spotting scopes. From June 2003-July 2006, I collected estrous data opportunistically recording all interactions using all-occurrence sampling (Altmann, 1974) and behaviors based on Waterman (1995). I recorded 47 estrous events, of which 38 had sufficient data to include in this study. Because copulations occur above and below ground, I estimated copulation when a male closely pursued the estrous female down the same burrow and remained underground for over a minute (Waterman, 1998). I concluded observations when males no longer actively pursued the estrous female and began foraging. Because all males eventually disperse, I classified males based on year of dispersal such that males who may have been natal in earlier years were reclassified and counted as dispersed males in subsequent years.

## Reproductive potential

### *Body condition*

I created an index of body condition following methods outlined in Schulte-Hostedde et al. (2005). I calculated body condition using the residuals of the ordinary least squares regression of log spine length and log body mass compared between natal and dispersed males (Schulte-Hostedde et al., 2005). Individuals considered to be in better body condition would therefore have positive residual values.

### *Home range estimates*

For home range analysis, I fit a subset of males (n = 16) with Model SOM-2380 radiocollars (Wildlife Materials, Inc., Murphysboro, Illinois). I released collared animals at the site of capture and allowed 24-48 hours before radiotracking to allow for a period of acclimation. Between May and July 2006, I located animals daily or nightly for a minimum of 50 locations. Due to the subterranean nature of this burrow-dwelling sciurid, I was able to record an exact position where each squirrel resided for the night. For home range estimates, I also included locations based on observations and trapping during 2006. I categorized animals as natal or dispersed only if their natal burrow was known or if I was able to assign a burrow based on paternity analysis. Otherwise, I classified animals as having an unknown tactic and excluded them from analysis. I estimated home range using the animal-movement extension (Beyer, 2004) in ArcMap v.9.3.1 (ESRI, Redlands, California). I generated 95% fixed kernel estimate and calculated smoothing factors per individuals using Animal Space Use 1.3 (Horne & Garton, 2009). I calculated both the likelihood cross validation (CVh), which minimizes the Kullback-Leibler distance, and h-ref, based on the variance in locality data (Horne & Garton, 2006). I also calculated a 95% minimum convex polygons (MCP) and used these areas to estimate the number of adult breeding females within each male home range. Because the site had been trapped and observed extensively before and during the time of this study and because of the extremely philopatric nature of females (Waterman, 1995), I am confident of the locations of all adult females within the study area.

## Reproductive investment

### *Sperm competition*

I measured testes mass and accessory glands (bulbourethral and prostate) to the nearest 0.01 g, (correcting all measurements for body size) using a subset of individuals euthanized with a halothane overdose. In order to assess sperm characteristics, I collected epididymal semen samples on slides and stained slides with Spermac (Stain Enterprises Inc.). I fixed additional samples in 2.5% gluteraldehyde for scanning electron microscopy (Figure 4.1). Once pictures were obtained, I measured tail length of 10 sperm for each individual male using the NIH public domain program ImageJ (available at http://rsb.into.nih.gov/nih-image/); previous research suggests 10 spermatozoa per male gives adequate estimates of intramale variability (Schulte-Hostedde & Miller, 2004; Laskemoen et al., 2007).

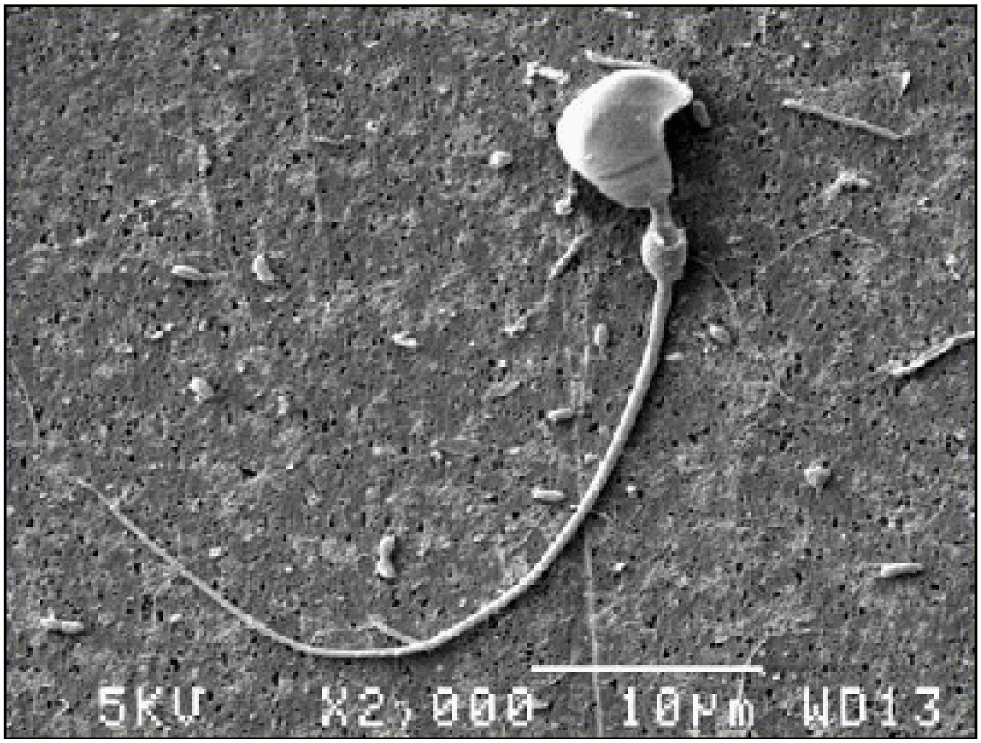


**Figure 4.1.** Scanning electron microscopic image of *Xerus inauris* sperm.

## Reproductive success

### *DNA Extraction and Genotyping*

I extracted genomic DNA from tail tissue using a DNeasy Kit (Qiagen, USA). I genotyped all individuals using eight species-specific di- and tetra-nucleotide repeat microsatellite loci. Primer sequences are available on GenBank and description of polymerase chain reactions can be found in Manjerovic et al. (2009) and in Appendix A. I used both fluorescently labeled, 5'-end forward primers, and forward primers with an m13 tag (5'-TGTAAAACCGACGGCCAGT-3'; Schuelke, 2000). I visualized amplified PCR products and internal size standards on a Beckman 8000 CEQ and used the corresponding software to size alleles. I tested for deviations from Hardy-Weinberg equilibrium and linkage disequilibrium using Genepop with $\alpha = 0.05$.

*Parentage analyses*

I obtained genetic estimates of reproductive success based on parentage assignments of juveniles and subadults, calculated using CERVUS v.3.0 (Marshall et al., 1998; Kalinowski et al., 2007). This likelihood-based approach assigns parentage based on confidence levels calculated using a simulation of population allele frequencies, proportion of population sampled and genotyped, and mistyping error. The advantage to incorporating genotyping errors and mutations is that parents are not excluded based on 1 mismatch with offspring allowing identification of the most likely parent from among multiple non-excluded parents (Kalinowski et al., 2007). However, I did not include dam-sire-offspring relationships with more than 1 mismatch. I calculated separate parentage analyses each year to take into account variation in sampling effort and success, as well as changes in natal group and candidate male composition among years.

I first calculated maternity using all adult breeding females for each colony (range: 1 – 8 individuals). Given the potential for multiple females to be pregnant simultaneously and difficulty in behavioral interpretation of true dam-offspring relationships, I calculated average female candidates per year and proportion sampled based on my knowledge of group composition (Table 4.1). For maximum reliability of paternity assignments, I excluded all juveniles with no assigned dam from paternity analysis; total exclusionary power for the dataset was over 98% (Jamieson et al. 2007). I used two different candidate male scenarios: 1) all males trapped or observed for the year in question and 2) males captured or observed in floodplain separate from those at the house each year. Estimated proportion of candidate males sampled was determined by subtracting the proportion of unknown adult males captured each year, which averaged around 30%. Number of potential candidates, averaged per year, varied based on the two simulations (range: 39 – 97 for first analysis; range: 19 – 55 when separated by area) with average proportion typed ranging from 0.94 – 0.98 (Table 4.1). All maternity and paternity analyses were conducted for each year separately with individuals typed at a minimum of 4 loci, although very few individuals were missing more than 2 loci; standard confidence levels were set at 95% strict and 80% relaxed. Once paternity was assigned, I separated candidate sires by tactic (dispersed versus natal) by year to account for changing tactics. I accepted parentage assignments when there was no more than 1 mismatch for assumed dam-sire-offspring relationships.

**Table 4.1.** Summary of parentage analyses by year: CERVUS input parameters and parentage assignment success

**A. Maternity Analysis Parameters**

| Year | No. of young | No. candidate females | Proportion typed | Proportion candidates sampled | No. assigned >80% | % assigned |
|---|---|---|---|---|---|---|
| 2002 | 33 | 4 | 0.96 | 0.85 | 23 | 0.70 |
| 2003 | 22 | 3 | 0.96 | 0.98 | 19 | 0.86 |
| 2004 | 61 | 5 | 0.96 | 0.98 | 57 | 0.93 |
| 2005 | 48 | 4 | 0.97 | 0.97 | 42 | 0.88 |
| 2006 | 63 | 4 | 0.95 | 0.93 | 56 | 0.89 |
| Total | 227 | | | | 197 | 0.87 |

**B. Paternity Analysis Parameters**

| Year | No. of young | No. candidate males | Proportion typed | Proportion candidates sampled | No. assigned >80% | % assigned |
|---|---|---|---|---|---|---|
| 2002 | 23 | 19 | 0.94 | 0.70 | 9 | 0.39 |
| 2003 | 19 | 28 | 0.94 | 0.65 | 10 | 0.53 |
| 2004 | 57 | 44 | 0.97 | 0.57 | 19 | 0.33 |
| 2005 | 42 | 33 | 0.96 | 0.87 | 33 | 0.79 |
| 2006 | 56 | 55 | 0.98 | 0.70 | 24 | 0.43 |
| Total | 197 | | | | 95 | 0.48 |

## Results

### Reproductive potential

For males with age estimates (n = 65), I found less than half were observed past one year of age; after two years, 11 of those males were still present, nine of them natal. I found adult body condition to be significantly different between tactics (t-test: t = 1.99, df = 16.29, *P* = 0.032) with dispersed males in better condition than natal males (Figure 4.2). I was able to assign tactics to 12 of the radio-collared males (5 dispersed, 7 natal). Dispersed males had significantly larger home ranges compared to natal males independent of method of home range estimation

(Figure 4.3; MCP: t=-2.76, df=4.35, *P*=0.023; 95% CVh: t=-2.74, df=7.32, *P*=0.014; 95% href: t=-3.26, df=4.61, *P*=0.013). Consequently, dispersed males overlapped with significantly more burrows and adult females compared to natal males (Table 4.2).

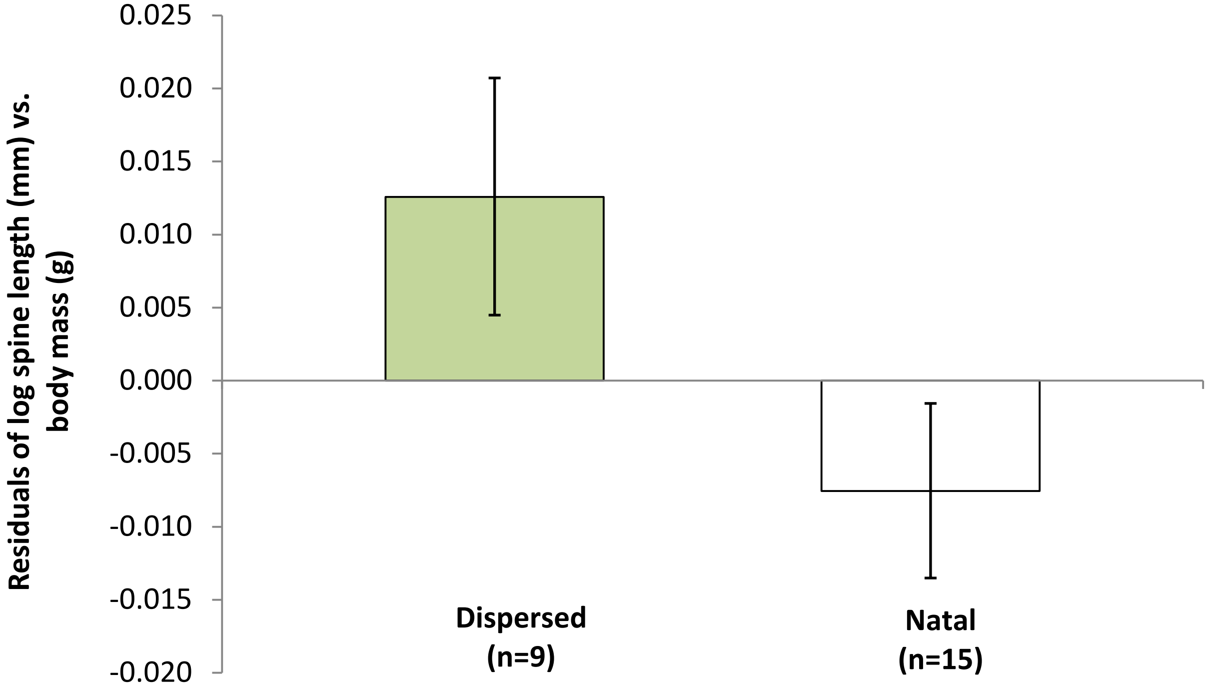


**Figure 4.2.** Comparison of body condition index between dispersed and natal male *Xerus inauris* calculated as the residuals of log spine length (mm) versus mass (g). Positive values indicate better body condition.

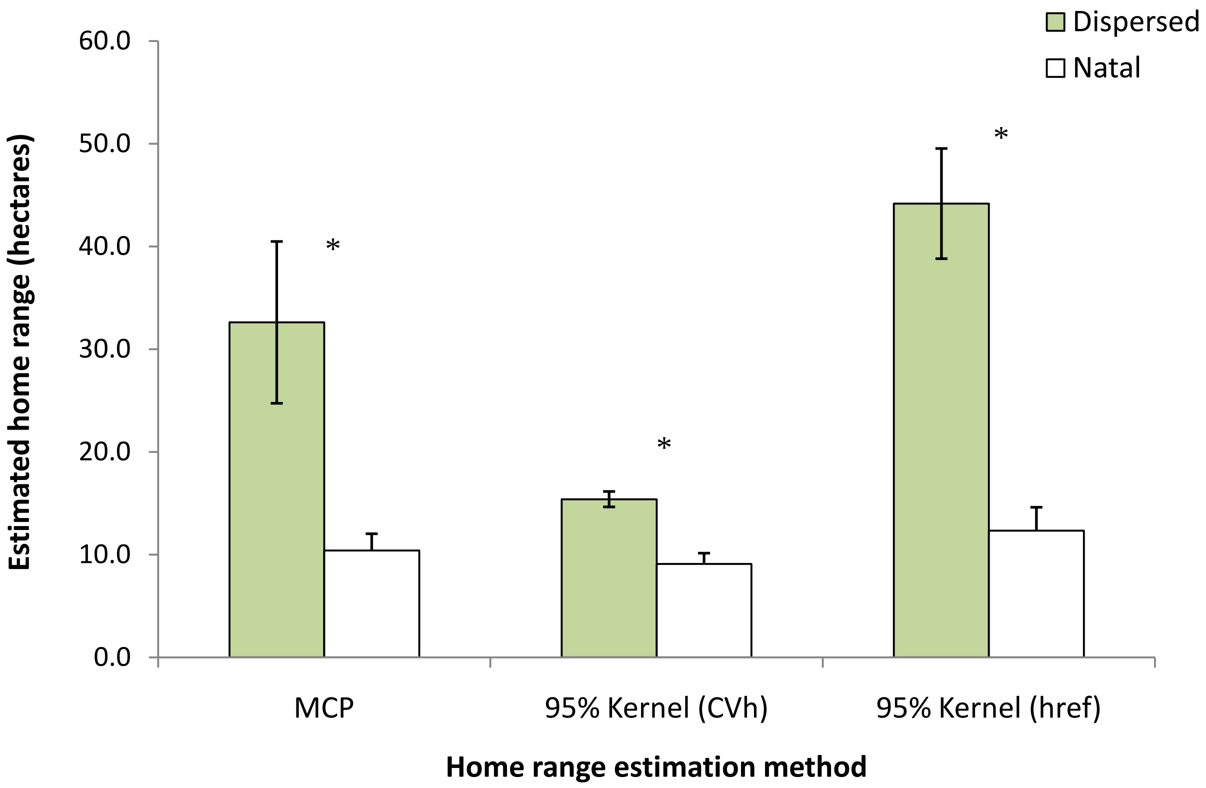


**Figure 4.3.** Home range estimates for dispersed (n = 5) and natal (n = 7) *Xerus inauris* males. Asterisks indicate significant differences between tactics (α = 0.05).

**Table 4.2.** Comparison between dispersed and natal *Xerus inauris* male home range overlap of number of burrow clusters and number of adult females living in each burrow cluster using minimum convex polygon home range estimates.

| | | | # of burrows clusters | | | | # of adult females | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **N** | **Mean # of locations*** | **Mean ± SD** | **t** | **df** | **P** | **Mean ± SD** | **t** | **df** | **P** |
| **Dispersed** | 5 | 98.0 | 30.4 ± 12.97 | -3.03 | 4.12 | 0.019 | 31.0 ± 2.55 | -5.27 | 8.49 | <0.001 |
| **Natal** | 7 | 117.5 | 12.3 ± 1.75 | | | | 23.0 ± 2.53 | | | |

*used to calculate minimum convex polygon

### Reproductive investment

Comparison of reproductive morphology suggested there were no differences in male investment between tactics (Table 4.3). I collected sperm from 24 individuals and was only able to assign tactics to 13 males (7 dispersed, 6 natal) after excluding individuals for which I lacked prior knowledge of natal burrows. I found dispersed males had significantly longer sperm tails compared to natal males (Table 4.3).

**Table 4.3.** Comparison of reproductive characteristics and sperm morphology between dispersed and natal *Xerus inauris* males.

| | **Testes (g)** | | | | **Bulbourethral (g)** | | | | **Prostate (g)** | | | | **Sperm tail length (µM)** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Tactic** | **Mean ± SD** | **N** | **t** | ***P*** | **Mean ± SD** | **N** | **t** | ***P*** | **Mean ± SD** | **N** | **t** | ***P*** | **Mean ± SD** | **N** | **t** | ***P*** |
| **Dispersed** | 12.99 ± 1.15 | 7 | -1.23 | 0.12 | 8.15 ± 1.45 | 6 | 0.12 | 0.55 | 1.48 ± 0.19 | 7 | 1.21 | 0.87 | 42.63 ± 0.14 | 7 | -1.73 | 0.06 |
| **Natal** | 12.22 ± 1.03 | 6 | | | 8.51 ± 2.12 | 6 | | | 1.65 ± 0.31 | 5 | | | 42.31 ± 0.12 | 6 | | |

### Reproductive success

I genotyped 383 individuals (223 males, 160 females) with 87.5% typed at all 8 loci and only 0.013% missing more than 2 loci. The majority of males were typed at all 8 loci with 0.018% missing one locus. Out of 227 juveniles and subadults, I assigned maternity to 145 individuals at 95% confidence and an additional 47 at 80% (Table 4.1). Based on trapping and observation records, I included 5 additional dam-offspring pairs for a total of 197 juveniles with known dams used in paternity analysis. I assigned mothers to 21 natal males and 22 dispersed males that were older than six months, to account for all potential dispersal even though males disperse between eight to ten months (Waterman, 1995). Because indirect fitness benefits may be contributing to males remaining within their natal burrow, I compared timing of dispersal in relation to presence or absence of mother for both natal and dispersed males. I removed 5 males (1 natal, 4 dispersed) who disappeared at the same time as their mother. I found that the remaining individuals were equally likely to disperse regardless if their mother was present or not ($\chi^2 = 0.85$, df = 1, $P = 0.358$; Figure 4.4).

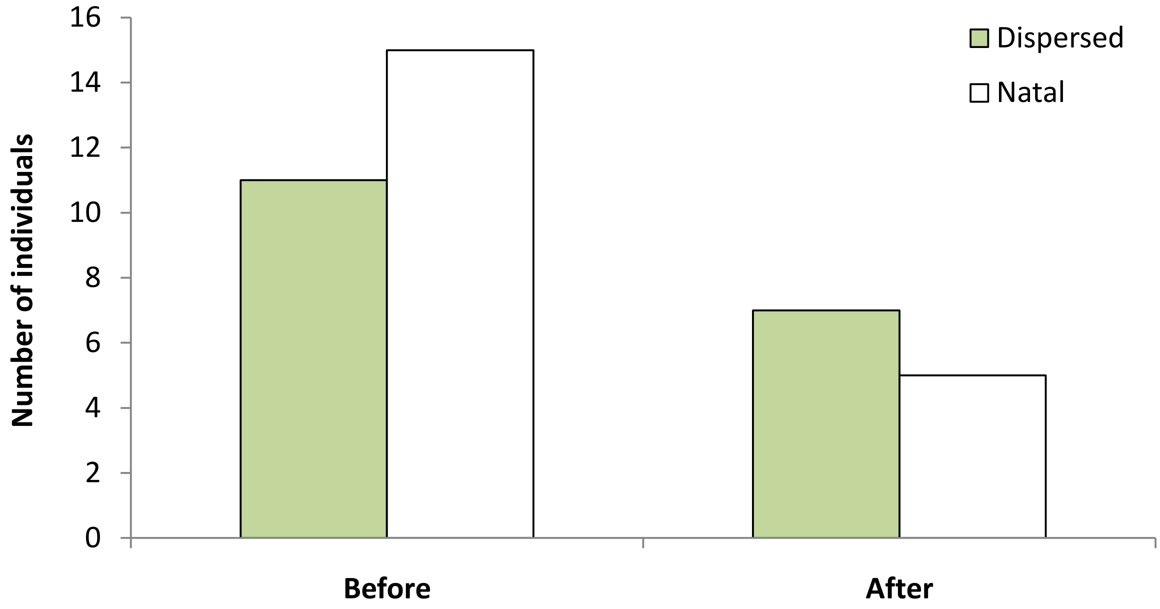


**Figure 4.4.** Number of *Xerus inauris* males who dispersed or disappeared while their mother was still present (before) and those that dispersed or disappeared after their mother disappeared (after) ($\chi^2 = 0.85$, df = 1, $P = 0.36$).

I found paternity assignments were more compatible with field observations when using males grouped according to area within the site (scenario 2). Out of 223 candidate males, I assigned sires to 48.7% of juveniles; only 33.2% of all candidate males successfully sired offspring. I found no difference between tactics in the average number of offspring sired (Mann-Whitney: Z = 0.00, $P = 1.000$; Figure 4.5). The 70 juveniles assigned to dispersed males represented 55 unique individuals with 13 males siring more than one offspring. Of the 25 assignments to natal males, 5 individuals sired more than a single juvenile. Although dispersed males had a higher percentage of assigned offspring, this was not significantly different than expected given the higher percentage of dispersed males that attend each estrus (χ2 = 0.26, df = 1, $P = 0.61$). Out of 38 observed estruses, natal males made up 28.6% of the males in attendance and excluding unknown males, 57.8% were dispersed males. The ratio of dispersed to natal males during an estrus was 6:3. Unknown males made up 13.6% of males in attendance although this value is most likely an overestimate due to difficulties in distinguishing among unknown individuals.

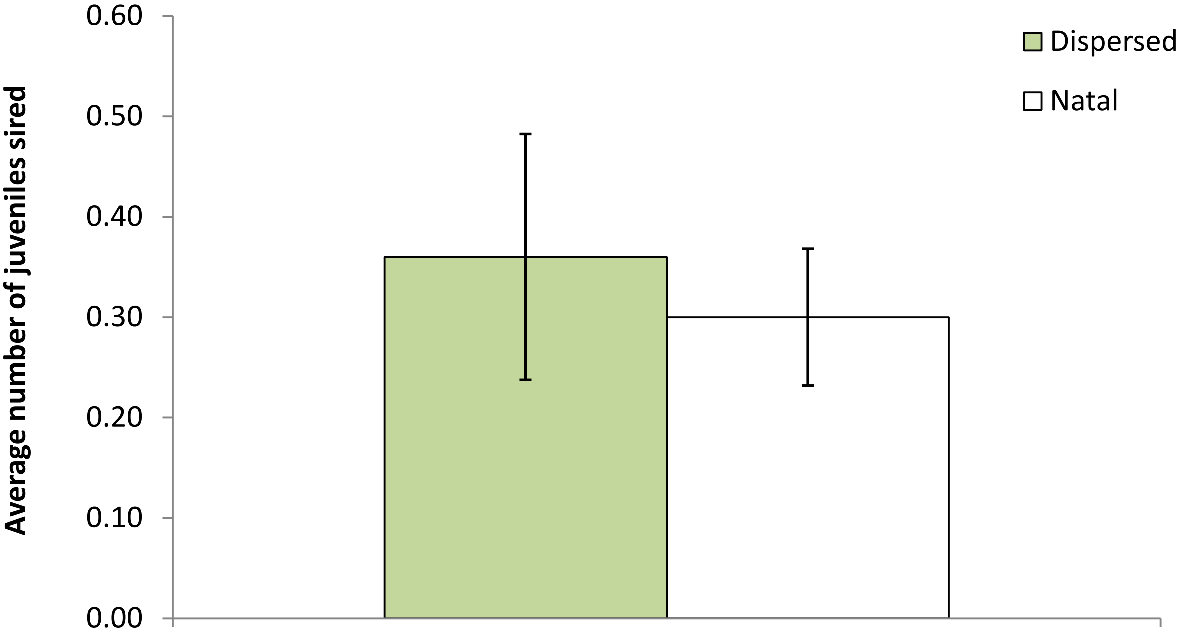


**Figure 4.5.** Average number of offspring per individual for all dispersed and natal *Xerus inauris* males based on genetic paternity assignments.

I recorded 158 copulations from 32 estruses and estimated intermale variability in copulatory success between tactics based on coefficient of variation, CV = SD/mean, and intensity of sexual selection index, $I_s = SD^2/mean^2$ (Wade & Arnold, 1980; Shuster, 2009). Copulations were unevenly distributed among all males attending an estrus (31.5%; 0.47 ± 0.80 copulations/male; CV = 170.9; $I_s = 2.92$) with over 65% of males never obtaining any copulations. Although both tactics copulated, dispersed males obtained significantly more copulations per male (t=-3.8, df = 62, $P < 0.001$; Table 4.4). When factoring in percentages of tactics in attendance, I found no significant differences between copulation frequency and tactic (χ2 = 0.10, df = 1, *P* = 0.75). I found no differences between tactics in first copulation (χ2 = 1.15, df = 1, *P* = 0.28), location of copulation (Above: χ2 = 0.01, df = 1, *P* = 0.79; Below: χ2 < 0.01, df = 1, *P* = 0.97), or location of first copulation (Above: χ2 = 0.01, df = 1, *P* = 0.923; Below: χ2 = 1.77, df = 1, *P* = 0.18). These comparisons did not change if I included unknowns in the analyses as dispersed males or if I removed from analyses.

**Table 4.4.** Measure of intertactic variation in copulatory success for adult male *Xerus inauris*

| | Tactic | |
|---|---|---|
| Measure of copulatory success | Dispersed | Natal |
| Number of copulations | 111 (70.3%) | 47 (29.7%) |
| # Above copulations | 36 (72.0%) | 14 (28.0%) |
| # Below copulations | 75 (69.4%) | 33 (30.6%) |
| Mean number of copulations/male/estrus ± SD | 0.35 ± 0.22 | 0.15 ± 0.18 |
| Coefficient of variation | 64.9 | 122.2 |
| Index of sexual selection intensity* | 0.42 | 1.49 |

*SD$^2$/mean$^2$

## Discussion

Alternative reproductive tactics of *X. inauris* affect male reproductive potential, investment, and ultimately influence fertilization success. Over half of male *X. inauris* in this population remain natal past sexual maturity (Scantlebury et al. 2008) but this does not imply the majority of males at the site are natal as individuals are always moving in from surrounding areas. All natal males dispersed by four years of age (Scantlebury et al. 2008). While I observed natal males are observed for longer periods of time, I cannot conclude if males that disperse leave the observation area or die. Dispersal is widely accepted as a costly life history tactic due to vulnerability to predation, and due to stress and malnutrition that dispersers may experience while moving through unfamiliar terrain (Gaines & McClenaghan Jr., 1980). The arid environment may further exacerbate these costs; S. A. Lombard Nature Reserve receives an average rainfall of 502 mm (range: 241-965 mm; Pettitt et al. 2008). Prior to joining an all-male roving band, dispersed *X. inauris* are initially solitary (Waterman, 1997). The primary benefit to forming groups is thought to be enhanced predator detection and avoidance (Waterman, 1997), suggesting this period of isolation may be most costly in terms of predation (Ridley et al., 2008).

Lone male *X. inauris* spend significantly more time alert and less time foraging (Scantlebury et al. 2008) which has been shown in birds to result in a significant loss of body mass (Ridley et al., 2008). While dispersed male *X. inauris* appear to be in better body condition than natal males, this value reflects their condition after joining an all-male band. The period prior to joining a male band but after leaving the natal group may be extremely costly such that initial dispersal is condition dependent. The significantly larger home range of dispersed males also implicates body condition as an important variable when considering additional energy requirements required for maintaining larger home ranges (Mace & Harvey, 1983). The differences in home range between tactics results in dispersed males overlapping with more adult females compared to natal males. Because females are spontaneous ovulators, males competitively search out estrous females (Waterman, 1998; Bouchie et al., 2006). It has been suggested that one benefit to dispersing and joining a roving male band is an increased likelihood of finding the females as they approach estrus (Waterman, 1997). While this information exchange hypothesis was not supported when comparing large and small male groups, it is a possible benefit to joining a group seeking out estrous females (dispersed) rather than remaining in a female group (natal).

Although dispersed males are likely to encounter additional estrous females compared to natal males, I found no differences between tactics in likelihood of obtaining the first copulation, and whether the first copulation or any copulation occurred above or below ground. There was higher variability in copulatory success among natal males suggesting fewer individual natal males successfully copulated. Alternative reproductive tactics commonly evolve when variance in reproductive success in high (Brockmann & Taborsky, 2008). I demonstrated extremely high variance in copulatory success and fertilization success with only 31.5% of males attending estruses copulating with females and 33.2% of all males in the population successfully siring offspring. Although more offspring were sired by dispersed males, I found no difference in average number of offspring sired per male between the tactics when incorporating males that never successfully sired offspring. Previous research suggests the majority of individuals remain natal for some period of time (Scantlebury et al. 2008), but 83% of males dispersed or disappeared by 2 years throughout the duration of this study. Because males can live over 5 years, dispersed males likely make up the majority of the population. My low assignment of paternity suggests there are many unknown males mating with estrous females. While I cannot rule out the possibility that these males are natal from surrounding burrows, it is unlikely given the typical home range of natal males and my knowledge of the burrow clusters surrounding estrous events.

Given the large relative testes size and positive allometry of testes and epididymis in this species, sperm competition is likely a strong selective pressure (Manjerovic et al. 2008). The lack of morphological differences in these reproductive characteristics between tactics suggests both natal and dispersed males have similar competitive pressures. This was further supported by the similar likelihood of tactics to obtain the first copulation. However, the propensity for female *X. inauris* to mate with multiple males, sometimes repeatedly with the same male (Waterman, 1998), suggests variation may occur in individual ejaculate allocation depending on the perceived risk of sperm competition (Pizzari et al., 2003). Differences in sperm production and velocity have also been shown to rapidly shift based on an individual's current reproductive or social status (Rudolfsen et al., 2006). Males in disfavored roles often invest more in ejaculates to counter disadvantages in mating, e.g. satellite males or second male to mate (Pizzari et al., 2003; Cornwallis & Birkhead, 2006; Rudolfsen et al., 2006). I found older, dispersed males had significantly longer sperm, a trait that has been found to be positively correlated to sperm competition risk with longer tails increasing sperm velocity (Gage, 1994). Although this relationship is debatable and most likely species specific (Gage & Freckleton, 2003), differences in sperm tail length suggest males may invest differently in ejaculate characteristics for sperm competition. I cannot rule out age or body condition as a confounding factor and suggest further study of this component of sperm competition in *X. inauris*.

Natal philopatry typically is attributed to indirect fitness benefits and group living benefits (Solomon, 1991; Kokko & Ekman, 2002). I found no differences between tactics in timing of dispersal relative to the presence or absence of their mother suggesting that indirect fitness is unlikely to contribute to the probability of remaining natal. However, it is possible that presence or absence of a female sibling could be contributing to indirect fitness. Because male *X. inauris* continue to live in groups after dispersing, both dispersal tactics also are likely to receive benefits of group living. However, these group living benefits may be unequal between dispersal tactics. Past research has demonstrated that female *X. inauris* are more likely to be reproductively suppressed while in the presence of a natal male (Pettitt, 2006) suggesting there is a conflict between sexes related to timing of male dispersal. Natal males have also been shown to have higher cortisol levels compared to dispersed males which may be a response to increased aggression from resident females (Scantlebury et al., 2008). Therefore, although alternative dispersal tactics of male *X. inauris* result in similar direct fitness benefits, multiple additional factors are likely to influence timing of male dispersal including localized female density, conditional ability to disperse, and social group composition.

## Conclusions

Although male *X. inauris* who are able to disperse encounter more estrous females, the decision to remain natal does not preclude reproduction. Natal males attend estruses and successfully copulate, but smaller home ranges reduce encounters with estrous females resulting in fewer breeding opportunities. All males, regardless of dispersal tactic, invest heavily in reproductive morphology, although males may be investing differently in ejaculate allocation. This investment, when compared to other rodents, likely reflects high risk and intensity of sperm competition. My inferences about sperm competition are supported by the male-biased operational sex ratio, the promiscuous nature of both sexes (Waterman, 1994; Waterman, 1998; Manjerovic et al., 2008), and likelihood of multiple paternity. Although there has been evidence that natal males help raise kin (Waterman, personal observation), there appears to be no dependence on dispersal tactic relative to the presence or absence of a mother. Although indirect fitness benefits may accrue to natal males, it is more likely that males are obtaining direct survival benefits by remaining in their natal group (Kokko & Ekman, 2002). The decision of 'should I stay or should I go' has direct impacts on the reproductive success of *X. inauris* and suggests that further research should consider potential differences in survival and lifetime reproductive success.

# References

Andersson, M. & Simmons, L. W. (2006). Sexual selection and mate choice. *Trends Ecol Evol* **21**, 296-302.

Arnqvist, G. & Rowe, L. (2005). *Sexual Conflict*. Princeton: Princeton University Press. 330 pp.

Bateman, A. J. (1948). Intra-sexual selection in *Drosophila*. *Heredity* **2**, 349-368.

Beyer, H. L. (2004). Hawth's Analysis Tools for ArcGIS. http:www.spatialecology.com/htools

Bouchie, L., Bennet, N. C., Jackson, T. & Waterman, J. M. (2006). Are Cape ground squirrels (*Xerus inauris*) induced or spontaneous ovulators? *J Mammal* **87**, 60-66.

Brockmann, H. J. (2001) The evolution of alternative strategies and tactics. *Adv Study Behav* **30**, 1-52.

Brockmann, H. J. & Taborsky, M. (2008). Alternative reproductive tactics and the evolution of alternative allocation phenotypes. In: *Alternative Reproductive Tactics: An Integrative Approach*: 25-51. R. F. Oliveira, M. Taborsky & H. J. Brockmann (eds.). Cambridge University Press, New York.

Cornwallis, C. K. & Birkhead, T. R. (2006). Social status and availability of females determine patterns of sperm allocation in the fowl. *Evolution* **60**, 1486-1493.

Darwin, C. (1859). *On the Origin of Species by Means of Natural Selection*. London: J. Murray.

Darwin, C. (1871). *The descent of man and selection in relation to sex.* London: Murray.

Gage, M. J. G. (1994). Associations between body size, mating pattern, testis size and sperm lengths across butterflies. *Proc R Soc Lond B* **258**, 247-254.

Gage, M. J. G. & Freckleton, R. P. (2003). Relative testis size and sperm morphometry across mammals: no evidence for an association between sperm competition and sperm length. *Proc R Soc Lond B* **270**, 625-632.

Gaines, M. S. & McClenaghan, L. R.,Jr. (1980). Dispersal in small mammals. *Annu Rev Ecol Syst* **11**, 163-196.

Gannon, W. L., Sikes, R. S. & the Animal Care and Use Committee of the American Society of Mammalogists (2007). Guidelines of the American Society of Mammalogists for the use of wild animals in research. *J Mammal* **88**, 809-823.

Gross, M. R. (1996). Alternative reproductive strategies and tactics: Diversity within sexes. *Trends Ecol Evol* **11**, 92-98.

Horne, J. S. & Garton, E. O. (2006). Likelihood cross-validation versus least squares cross-validation for choosing the smoothing parameter in kernel home-range analysis. *J Wildl Mgmt* **70**, 641-648.

Horne, J. S. & Garton, E. O. (2009). Animal Space Use v.1.3: *www.cnr.uidaho.edu/population_ecology/animal_space_use*.

Jamieson, A. & Taylor, S. C. S. (1997). Comparisons of three probability formulae for parentage exclusion. *Anim Gen* **28**, 397-400.

Jones, A. G. & Avise, J. C. (1997). Microsatellite analysis of maternity and the mating system in the Gulf pipefish *Syngnathus scovelli*, a species with male pregnancy and sex-role reversal. *Mol Ecol* **6**, 203-213.

Jones, A. G., Small, C. M., Paczolt, K. A. & Ratterman, N. L. (2010). A practical guide to methods of parentage analysis. *Mol Ecol Res* **10**, 6-30.

Kalinowski, S. T., Taper, M. L. & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol* **16**, 1099-1106.

Kenagy, G. J. & Trombulak, S. C. (1986). Size and function of mammalian testes in relation to body size. *J Mammal* **67**, 1-22.

Kodric-Brown, A. (1986). Satellites and sneakers: opportunistic male breeding tactics in pupfish (*Cyprinodon pecosensis*). *Behav Ecol Sociobiol* **19**, 425-432.

Koenig, W. D., Pitelka, F. A., Carmen, W. J., Mumme, R. L., & Stanback, M. T. (1992). The evolution of delayed dispersal in cooperative breeders. *Q Rev Biol* **67**, 11a1-150.

Kokko, H. & Ekman, J. (2002). Delayed dispersal as a route to breeding: territorial inheritance, safe havens, and ecological constraints. *Am Nat* **160**, 468-484.

Koprowski, J. L. (1993). Alternative reproductive tactics in male eastern gray squirrels - making the best of a bad job. *Behav Ecol* **4**, 165-171.

Laskemoen, T., O. Kleven, F. Fossoy & Lifjeld, J. T. (2007). Intraspecific variation in sperm length in two passerine species, the Bluethroat *Luscinia svecica* and the Willow Warbler *Phylloscopus trochilus*. *Ornis Fenn* **84**, 131-139.

Mace, G. M. & Harvey, P. H. (1983). Energetic constraints on home-range size. *Am Nat* **121**, 120-132.

Manjerovic, M. B., Kinahan, A. A., Waterman, J. M., Bennett, N. C. & Bateman, P. W. (2008). Structure and allometry of genitalia in males and females of a social African ground squirrel with high polygynandry. *J Zool (Lond)* **275**, 375-380.

Manjerovic, M. B., Waterman, J. M., Hoffman, E. A., & Parkinson, C. L. (2009). Characterization of nine microsatellite loci in the Cape ground squirrel, *Xerus inauris*, and their cross-utility in other species. In: Abercrombie, et al. Permanent genetic resources added to Molecular Ecology Resources database 1 January 2009 – 30 April 2009. *Mol Ecol Res* **9**, 1375-1379.

Marshall, T. C., Slate, J. , Kruuk, L. E. B. & Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Mol Ecol,* **7**, 639-655.

Parker, G. A. (1970). Sperm competition and its evolutionary consequences in the insects. *Biol Rev Camb Philos Soc* **45**, 525-567.

Pettitt, B. A. (2006). Reproductive delay in the female Cape ground squirrel, *Xerus inauris*. In: *Biology.* University of Central Florida, Orlando.

Pettitt, B. A., Waterman, J. M. & Wheaton, C. J. (2008). Assessing the effects of resource availability and parity on reproduction in female Cape ground squirrels: resources do not matter. *J Zool (Lond)* **276**, 291-298.

Pizzari, T., Cornwallis, C. K., Lovlie, H., Jakobsson, S. & Birkhead, T. R. (2003). Sophisticated sperm allocation in male fowl. *Nature* **426**, 70-74.

Ridley, A. R., Raihani, N. J. & Nelson-Flower, M. J. (2008). The cost of being alone: the fate of floaters in a population of cooperatively breeding pied babblers *Turdoides bicolor*. *J Avian Biol* **39**, 389-392.

Rood, J. P. & Nellis, D. W. (1980). Freeze marking mongooses. *J Wild Mgmt* **44**, 500-502.

Rudolfsen, G., Figenschou, L., Folstad, I., Tveiten, H. & Figenschou, M. (2006). Rapid adjustments of sperm characteristics in relation to social status. *Proc. R. Soc. B-Biol. Sci.* **273**, 325-332.

Scantlebury, M., Waterman, J. M. & Bennett, N. C. (2008). Alternative reproductive tactics in male Cape ground squirrels *Xerus inauris*. *Physiol Behav* **94**, 359-367.

Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nat Biotechnol* **18**, 233-234.

Schulte-Hostedde, A. I. & Millar, J. S. (2004). Intraspecific variation of testis size and sperm length in the yellow-pine chipmunk (*Tamias amoenus*): implications for sperm competition and reproductive success. *Behav Ecol Sociobiol* **55**, 272-277.

Schulte-Hostedde, A. I., Zinner, B., Millar, J. S. & Hickling, G. J. (2005). Restitution of mass-size residuals: validating body condition indices. *Ecology* **86**, 155-163.

Shuster, S. M. & Wade, M. J. (2003). *Mating systems and strategies*. Princeton Univ Pr.

Shuster, S. M. (2009). Sexual selection and mating systems. *Proc Natl Acad Sci* **106**, 10009-10016.

Solomon, N. G. (1991). Current indirect fitness benefits associated with philopatry in juvenile prairie voles. *Behav Ecol Sociobiol* **29**, 277-282.

Thornhill, R. & Alcock, J. (1983). *The evolution of insect mating systems*. Harvard University Press Cambridge, MA.

Trivers, R. L. (1972). Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man*: 136-179. B. Cambell (ed.). Aldine, Chicago.

Unck, C. E., Waterman, J. M., Verburgt, L. & Bateman, P. W. (2009). Quantity versus quality: how does level of predation threat affect Cape ground squirrel vigilance? *Anim Behav* **78**, 625-632.

Wade, M. J. & Arnold, S. J. (1980). The intensity of sexual selection in relation to male sexual behaviour, female choice, and sperm precedence. *Anim Behav* **28**, 446-461.

Waterman, J. M., (1994). The social organization of the Cape ground squirrel *Xerus inauris*. University of Minnesota, St. Paul.

Waterman, J. M. (1995). The social organization of the Cape ground squirrel (*Xerus inauris* Rodentia, Sciuridae). *Ethology* **101**, 130-147.

Waterman, J. M. (1996) Reproductive biology of a tropical, non-hibernating ground squirrel. *J Mammal* **77,** 134-146.

Waterman, J. M. (1997). Why do male Cape ground squirrels live in groups? *Anim Behav* **53**, 809-817.

Waterman, J. M. (1998). Mating tactics of male Cape ground squirrels, Xerus inauris: consequences of year-round breeding. *Anim Behav* **56**, 459-466.

Wolff, J. O. (2008). Alternative reproductive tactics in nonprimate male mammals. In: *Alternative Reproductive Tactics: An Integrative Approach*: 356-372. R. F. Oliveira, M. Taborsky & H. J. Brockmann (eds.). Cambridge University Press, New York.

Young, A. J., Spong, G., & Clutton-Brock, T. (2007). Subordinate male meerkats prospect for extra-group paternity: alternative reproductive tactics in a cooperative mammal. *Proc. R. Soc. B-Biol. Sci.* **274**, 1603-1609.

# CHAPTER 5 – INTRASPECIFIC VARIATION IN MALE MATING TACTICS IN RESPONSE TO FEMALE DISTRIBUTION

## Introduction

Mating systems reflect the outcome of sex-specific tactics used to maximize reproductive opportunities. In species where males do not contribute to parental care, male reproductive success generally is limited by the number of acquired mates. In order to maximize reproductive success, males must balance current and future reproductive opportunities depending on whether females are economically defendable in space and time (Emlen & Oring, 1977; Clutton-Brock, 1989). Female density, dispersion, and ultimately social organization, largely are dependent on the distribution and abundance of resources and therefore can vary across a species range (Crook, Ellis & Goss-Custard; 1976; Clutton-Brock & Harvey, 1978; Clutton-Brock, 1989). Male mating tactics often are facultative and commonly vary depending on differences in the social and ecological environment including female distribution and intrasexual competition (Trivers, 1972; Emlen & Oring, 1977; Waterman, 2007; Wolff & Sherman, 2007). For example, in the side-blotched iguanan lizard (*Uta palmeri*), female distribution changed in response to increases in food abundance. Male lizards increased courtship rates in response to changes in female distribution, effectively altering mating opportunities (Hews, 1993).

As variance in reproductive success increases among males, the opportunity for sexual selection intensifies (Emlen & Oring, 1977; Shuster, 2009). However, reproductive skew can be difficult to predict if (1) intrasexual competition is not explicit, (2) female behavior aids or counters male mating success, or (3) fertilization success does not reflect mating success due to postcopulatory processes (Kokko & Rankin, 2006). In promiscuous species, fertilization success reflects outcomes of gamete interactions, sperm competition, or cryptic female choice resulting in opportunities for post-copulatory selection (Andersson & Simmons, 2006). The importance of post-copulatory competition is becoming more apparent (Birkhead & Pizzari, 2002; Eberhard, 2009; Birkhead, 2010) and explains many aspects of the variation observed in male and female reproductive traits (Cordoba-Aguilar, 2005; Minder et al., 2005; Ramm et al, 2005), sperm physiology and morphology (Wedell & Cook, 1999; Dixson & Anderson, 2004; Gomendio et al., 2006), seminal fluid allocation (Wigby et al., 2009), and mating behaviors (delBarco-Trillo & Ferkin, 2006). Because patterns of mating and parentage are not always equal, understanding factors affecting both mating success and paternity can play a central role in addressing ecological and evolutionary patterns of sexual selection (Emlen & Oring, 1977; Shuster, 2009).

It is well-established that the number of potential mates influences reproductive strategies and affects reproductive success (Emlen & Oring, 1977; Clutton-Brock, 1989; Kokko & Rankin, 2006). Given the likelihood of sociality increasing competition for breeding opportunities, it is not surprising that all-male groups are uncommon among mammals (Trivers, 1972; Clutton-Brock, 1989; Waterman, 1997). The Cape ground squirrel, *Xerus inauris*, differs from most social species in that males form all-male groups independent of females that persist throughout the year (Waterman, 1995; Waterman, 1997). These male groups move throughout the landscape searching for sexually receptive females and thus sire offspring in multiple social groups

(Waterman, 1998; Chapter 4). The pattern of male grouping in *X. inauris* seems to present somewhat of an evolutionary quagmire in that males lack aggressive competition to defend females or maintain territories (Waterman, 1995; Waterman, 1998). Rather, male *X. inauris* search for estrous females in the presence of other competitors (Waterman, 1995; Waterman, 1997). In a population in Namibia, mate order was determined by a linear dominance hierarchy among males maintained by non-aggressive displacements (Waterman, 1995; Waterman, 1998). Although dispersed males ranked highest and are likely to have greater reproductive success (Waterman, 1997), males have been known to remain within their natal social group well past sexual maturity before leaving to join an all-male group (Scantlebury et al., 2008). Previous research has demonstrated that rates of dispersal vary between *X. inauris* populations (Scantlebury et al., 2008) suggesting that individual behaviors in differing environments may alter the relative fitness of mating tactics.

Interactions between mating systems and population dynamics are complex, but linked by feeding behavior, population dispersion, breeding populations and morphology (Clutton-Brock & Harvey, 1978; Kokko & Monaghan, 2001). I present data from two populations of *X. inauris* that differ in resource distribution in order to address how resources affect female distribution and consequently how female distribution influences pre- and post-copulatory reproductive competition. I predict that as females become more aggregated, males would be more likely to monopolize mates based on a dominance hierarchy, increasing pre-copulatory reproductive competition. If females are evenly distributed within a population, males would be more likely to competitively search for females and compete via sperm competition. If there are differences in competitive intensity, I predict greater sexual selection pressures would result in greater variance in reproductive success.

## Biology of the Study Species

Breeding in *X. inauris* is not limited by hibernation and occurs year round (Waterman, 1996). There is no predictability to female receptivity as females are asynchronous breeders with spontaneous ovulation (Waterman, 1996; Bouchie et al., 2006). In the days preceding an estrus, males increase attention to the estrous female suggesting that competitive searching is important for determining when a female is ready to mate (Waterman, 1997). In Namibia, the establishment of a male dominance hierarchy based on age also is important to male mating success as dominant males locate more estrous females, are usually the first to mate, and obtain more underground copulations where they are less likely to be disturbed (Waterman, 1997; Waterman, 1998). During a brief 3 hour estrus, the operational sex ratio (OSR; Emlen & Oring, 1977) ranges from 3 to 18, with an average of 11 males, of which females mate with up to 10 individuals (average 4.1; Waterman, 1994). While it has been suggested that there is a first male advantage given the likelihood of dominant males having first access to females, alternative male tactics also may be successful. There is evidence that all adult males compete via sperm competition (Manjerovic et al., 2008), regardless of age, dominance, or dispersal status (Waterman, 1998; Chapter 4). Given the propensity for females to remate (Waterman, 1998) and the probability for multiple paternity, additional postcopulatory tactics may be important to both male and female reproductive success.

## Methods

### *Population sampling*

I conducted fieldwork between January 2004 and December 2006 at two sites with known variation in rainfall and consequently resource availability (Pettit et al., 2008). S.A. Lombard Nature Reserve, South Africa (27°35’S, 25°35’E), a 3660 ha reserve characterized as Cymbopogon-Themeda veld surrounded by *Eragrostis* spp. (van zyl, 1965), receives an average of over 500 mm rainfall per year (Pettitt et al., 2008). I considered this the high resource site due to a contiguous distribution of grasses surrounding suitable squirrel habitat. My low resource site, located in the Kalahari bushveld region 185 km southeast of Windhoek, Namibia (23°25’S, 18°00’E), was a private 3500 ha farm receiving 220 mm yearly rainfall average (Waterman, 1995). This site is predominantly *Acacia* bush with patchy distributions of grasses (e.g. *Schmidtia kalahariensis*; Waterman, 1995). Individuals from these two sites are known to be of the same phylogenetic clade (Herron et al., 2004).

I trapped squirrels using live traps (Tomahawk Live Trap®, Tomahawk, Wisconsin) using methods outlined in detail in Waterman (1995). On site, I recorded body mass (± 0.5 g), sex, reproductive condition, and age; I inserted a single PIT tag (AVID, Norco, CA, USA) subcutaneously above the right hind limb in all animals. For long distance field identification, I placed a unique dye mark (Rodol D, Lowenstein & Sons Inc., New York, NY, USA; Melchior & Iwen, 1965) and freeze-mark (Quick Freeze; Rood & Nellis, 1980) combination on the back. For genetic analyses, I collected 1-3 mm of tail tissue in 95% ethanol. I released animals at the site of capture; handling was in accordance with the American Mammal Association guidelines

(Gannon et al., 2007) and was approved by the University of Central Florida's Institutional Animal Care and Use Committee (#07-43W).

### ***Movement and density patterns***

Home range analysis was only conducted for South African male squirrels; Namibian home ranges are published in Waterman (1995). I fitted 16 males with Model SOM-2380 radiocollars (Wildlife Materials, Inc., Murphysboro, Illinois) and released collared animals at the site of capture. I allowed 24-48 hours before radiotracking to allow for a period of acclimation. Between May and July 2006, I located animals for a minimum of 50 locations; including locations based on observations and trapping increased average points per individual to 96. I used ArcMap v.9.3.1 (ESRI, Redlands, California) and the animal-movement extension (Beyer, 2004) to generate 95% minimum convex polygons for male home ranges and to calculate the total area of known burrow clusters. Typically, a single social group resides in a burrow cluster, which is defined as an aggregation of multiple burrow openings separated from adjacent clusters by areas without burrows (Herzig-Straschil, 1978; Waterman, 1995). I plotted all known burrow cluster locations within these areas and used the multi-distance spatial cluster analysis to calculate dispersion of burrow clusters based on Ripley's K (Ripley, 1976). This tool generates an expected pattern of complete spatial randomness across multiple distances. Calculated K-values that fall outside and above this expected confidence interval represent significant clustering while values below represent significant dispersion. In order to compare female density between sites, I estimated adult breeding females per hectare within similar sized core areas. I also measured average distance between all burrow clusters and average nearest neighbor distance within these core areas.

### ***Mating behaviors***

I observed squirrels from trees, vehicles, blinds, and observation towers using 10x50 binoculars and 15-45x60 spotting scopes. I collected detailed behavioral data on the day of estrus opportunistically throughout the duration of the study; I combined Namibian estrous data from the current study with data collected at the same site from 1989 – 1991 (Waterman, 1998). I used focal sampling of the estrous female and recorded all interactions with other individuals including identity, location, and behavior; all behaviors recorded were based on Waterman (1995). Because copulations occur above and below ground, I estimated copulation to have occurred when a male closely pursued the estrous female down the same burrow and remained underground for over a minute (Waterman, 1995; Waterman, 1998). I defined mate guarding as one male actively preventing other males from approaching the estrous female. I used all male-male approach-displacement interactions (e.g. approach-move away, chase) to calculate dominance relationships among males at South Africa; Namibia dominance analysis previously published in Waterman (1995). I used Matman v.1 (Noldus Information Technology; de Vries et al., 1993) to calculate Landau’s index of linearity, a value ranging from 0 (no hierarchy) to 1 (complete linear hierarchy) (Lehner, 1998). I concluded estrous observations when males no longer actively pursued the estrous female and began foraging (Waterman, 1998).

To compare sexual selection intensity, I calculated the operational sex ratio as the ratio of attending males at each estrus (Emlen & Oring, 1977). Because this value treats males that mate and males that do not mate equally, it may not accurately reflect the strength of selection (Shuster & Wade, 2003; Shuster, 2009). Therefore, I also calculated the opportunity for sexual selection in both copulatory and fertilization rates as the variance in success divided by the squared mean of success ($I_s = SD^2/mean^2$; Wade & Arnold, 1980; Shuster & Wade, 2003).

***Sperm competition***

In order to quantify sperm competition investment, I euthanized a subset of adult animals on site with a halothane overdose. I measured testes, epididymal, and accessory gland (bulbourethral and prostate) masses to the nearest 0.01 g. I corrected all measurements for body size before comparing between sites. Larger testes often are attributed to selection for increased sperm quantity (Hosken, 1998; Parker & Ball, 2005) and have been shown to be positively correlated with sperm competition intensity (Ramm et al., 2005). Accessory gland products have been shown to maintain or enhance sperm quality (Chapman, 2001) or secrete coagulated proteins in the form of copulatory plugs (Hart & Greenstein, 1968). Similar to testes mass, size of accessory glands also appears to be positively correlated with sperm competition intensity (Ramm et al. 2005).

I tested that all data were normal and homoscedastic; data that did not meet those assumptions were either log-transformed or tested using nonparametric statistics. All data were tested for significance in JMP v.8 (SAS Institute Inc., Cary, NC, USA) and considered significant at α = 0.05.

### *DNA extraction and statistical analysis*

I extracted total genomic DNA from tail tissue using a DNeasy Kit (QIAGEN®, Valencia, Ca, USA) and genotyped all individuals using eight microsatellite loci. Primer sequences are available on GenBank and description of polymerase chain reactions can be found in Manjerovic et al. (2009) and in Appendix A. I visualized amplified PCR products and internal size standards on a Beckman CEQ8000 (Beckman-Coulter, Fullerton, CA) and used the corresponding software to size alleles. I initially checked for high null allele frequencies (> 0.09), allelic dropout, stuttering, and scoring errors using MICROCHECKER v.2.23 (van Oosterhout et al., 2004). I tested for deviations from Hardy-Weinberg equilibrium and linkage disequilibrium using GENEPOP (Raymond & Rousset, 1995).

To quantify male reproductive success, I assigned parentage of all juveniles and subadults using a likelihood-based approach in CERVUS V.3.0 (Marshall et al., 1998; Kalinowski et al., 2007). This program calculates an expected distribution of log-likelihood scores based on population specific simulations and compares the difference between the most-likely and next most-likely parent to randomly chosen individuals (Marshall et al., 1998). I calculated separate parentage analyses each year using a specific simulation to account for differences in sampling effort and genotyping success, as well as changes in candidate male composition between years. I ran 100,000 cycles, with > 90% of loci typed each year, 0.7 proportion sampled (based on capture rate of unknown, adult males), and an error rate of 0.1. I used standard 80% (relaxed) and 95% (strict) confidence levels to assign dam-father-offspring relationships.

## Results

### *Movement and density patterns*

I found burrow clusters occurred at a lower density in Namibia (0.26/ha) compared to South Africa (8.42/ha) (Figure 5.1). Burrow clusters in Namibia were significantly aggregated compared to a random distribution of burrow clusters in South Africa (Figure 5.2). The average distance (m) between all burrow clusters was significantly shorter in South Africa (n = 9, 154.12 ± 9.2; Namibia: n = 7, 248.04 ± 27.2 ; t = -3.62, df = 14, *P* = 0.001). Average nearest neighbor distance (m) was not significantly larger in Namibia (n = 7, 105.5 ± 27.1; South Africa: n = 10, 79.9 ± 10.6; t = -0.99, df = 15, *P* = 0.169). The average home range of dispersed radio-collared males in South Africa was 32.6 ± 7.9 ha (n = 5; range: 14.0 – 61.0), overlapping an average of 31.0 ± 2.6 adult females. Number of adult breeding females per social group within core areas did not differ between sites (South Africa: n = 10, range: 2 – 8, mean: 3.7 ± 0.4; Namibia: n = 7, range: 2 – 10, mean: 4.6 ± 0.5; t-test: t = -1.48, df = 15, *P* = 0.08).

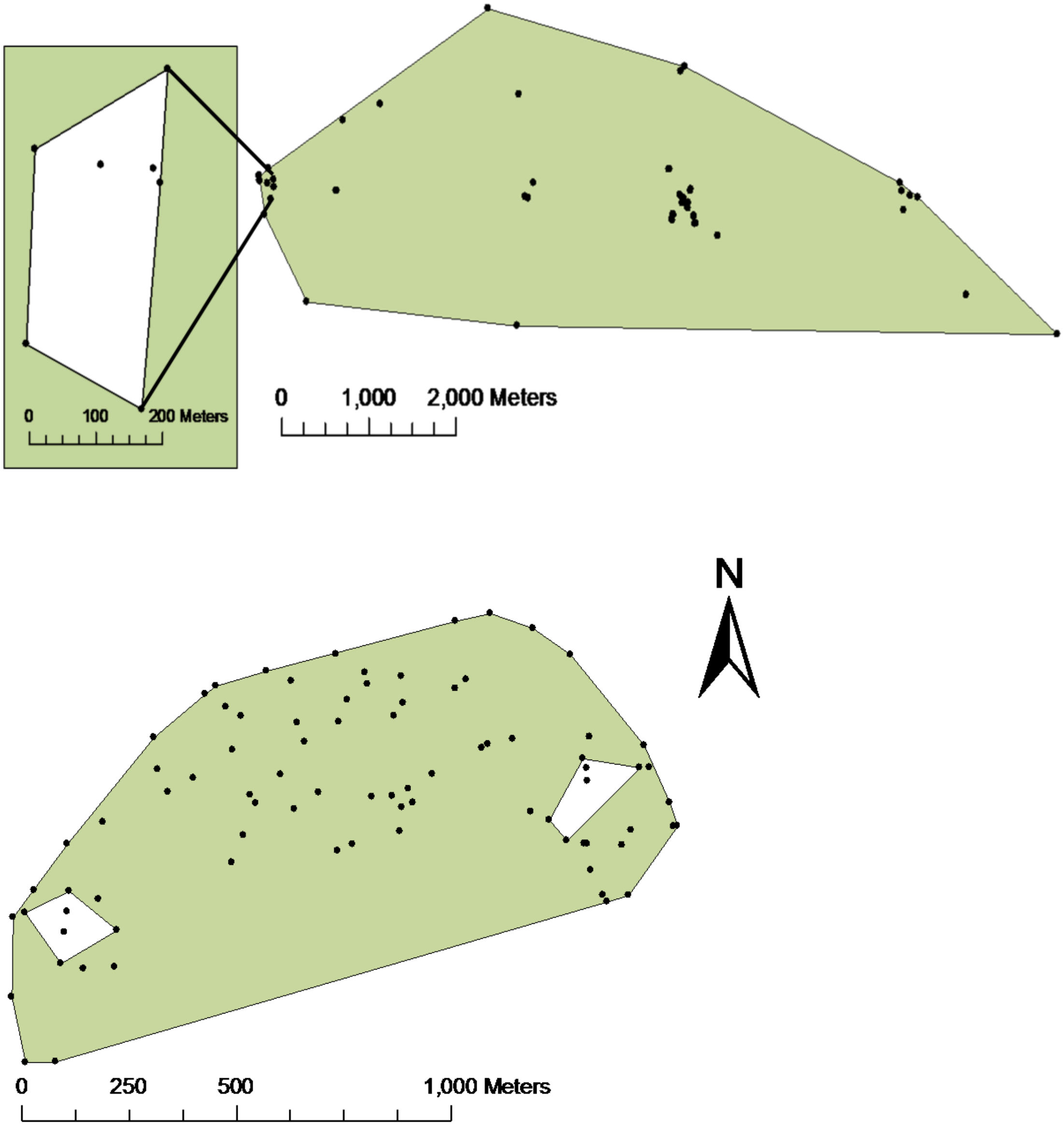
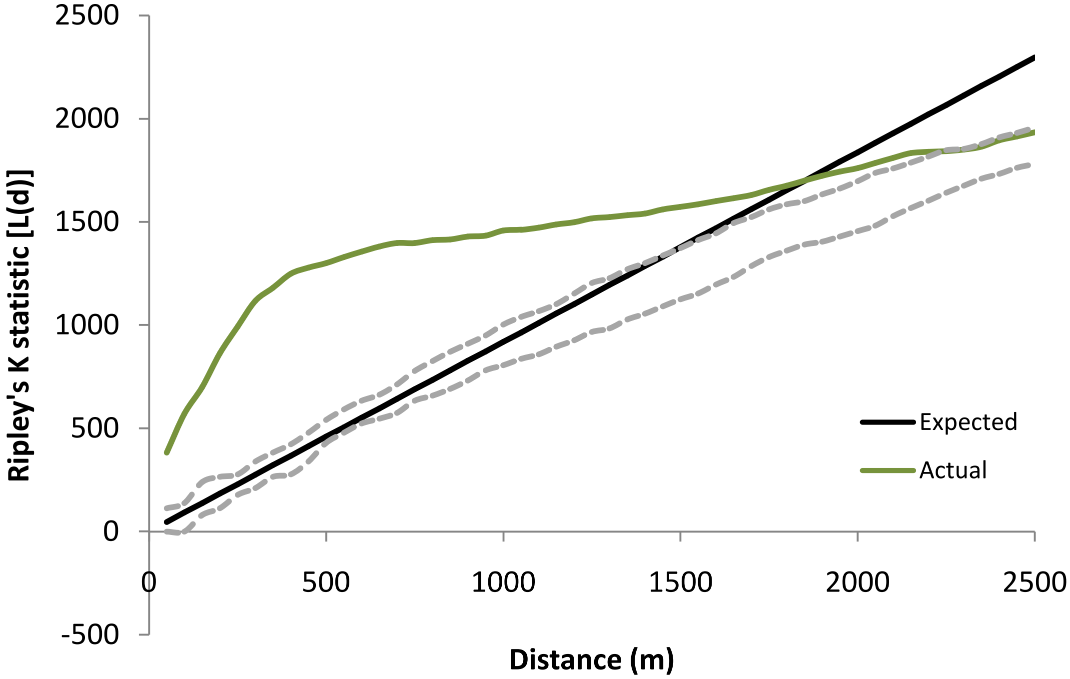


**Figure 5.1.** Distribution of *Xerus inauris* burrow clusters within study sites at Namibia (above) and South Africa (below). White polygons represent core areas of approximately 6 hectares that were used to compare female density. All polygons represent 95% minimum convex polygons; differences in scale should be noted.



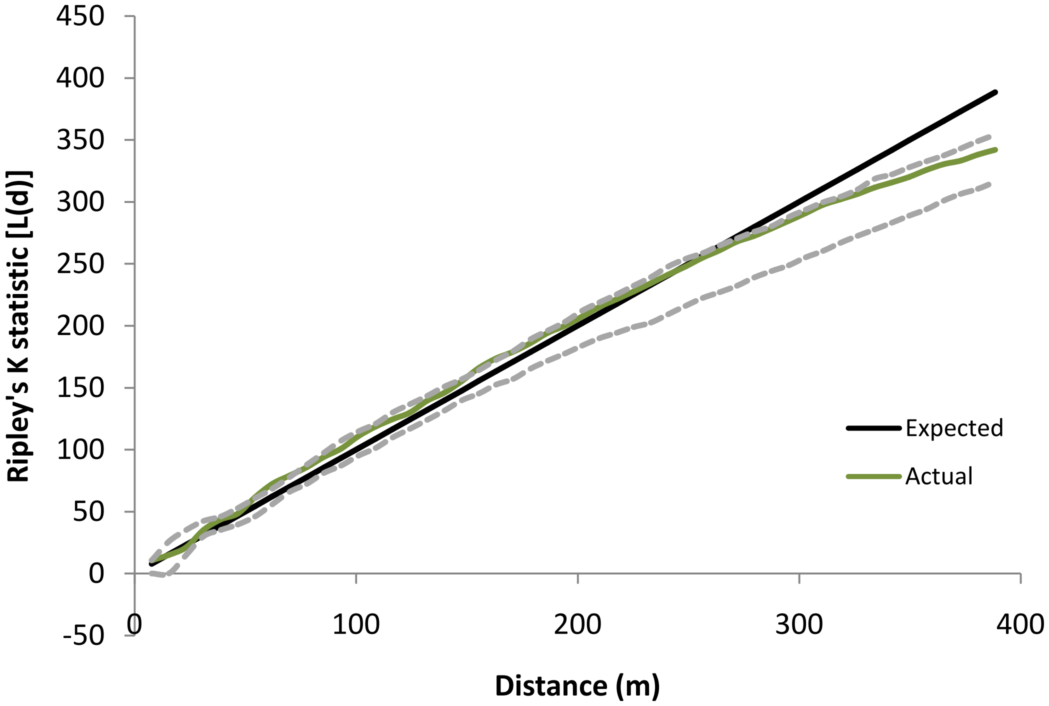


**Figure 5.2.** Distance (m) versus modified Ripley's K (L-Distance function) of *Xerus inauris* burrow clusters in Namibia (top) and South African (below). Expected line indicates complete spatial randomness; dashed lines represent upper and lower confidence estimates.

### ***Mating system***

Females were receptive throughout the entire year at both sites. I compared 34 estruses from Namibia and 38 from South Africa and found no differences in estrous duration (South Africa: 163.5 ± 8.8 min; Namibia: 185.7 ± 15.8 min) or in the intensity of male-male competition as measured by the operational sex ratio (Table 5.1). Sexual selection intensity was high in both sites (South Africa $I_s$ = 2.9; Namibia $I_s$ = 2.7). Despite similar intensities, I found female *X. inauris* in Namibia have a tendency to mate with more individual males and copulate significantly more times compared to females in South Africa (Table 5.1). Consequently, average copulatory success was greater in Namibia with males attending estruses averaging 0.75 copulations per individual compared to 0.47 copulations per individual in South Africa. I observed significantly more unknown males attending each estrus in South Africa.

### ***Pre- and post-copulatory competition***

I found no evidence of a dominance hierarchy among South African males. I calculated a Landau's index of linearity of less than 0.2 across all three study years (range: 0.04 – 0.18). Approach-displacement behaviors included in the analysis were rare; mean number of interactions per individual was 2.2 ± 0.4 (n = 238) over the three year period.

I found no differences in body mass or body condition of males between the two sites (Table 5.2). Even after controlling for body size, South African males had significantly larger testes size, larger combined epididymal mass and larger bulbourethral glands than males in Namibia (Table 5.2). Throughout the duration of the study, I recovered nine copulatory plugs from South Africa females during trapping; no copulatory plugs were ever recovered from Namibia females. Mate guarding was more likely to occur in South African compared to Namibia ($\chi^2 = 5.87$, $P = 0.015$). Males guarded females in 26% of South African estruses (10 out of 38) compared to 6% of the estruses in Namibia (2 out of 34). The majority of mate guarding occurred after the guarding males had copulated (82%) but in eight occasions, females did remate with another male.

**Table 5.1.** Comparison of breeding behaviors of *Xerus inauris* between South Africa and Namibia. Operational sex ratio is measured as number of males present and attentive to estrous female.

| | South Africa | | | Namibia | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | Mean ± SE | range | N | Mean ± SE | range | t | df | *P* |
| Operational sex ratio | 38 | 10.84 ± 0.6 | 5 - 19 | 34 | 11.32 ± 0.6 | 3 - 18 | -0.58 | 70 | 0.567 |
| Number of mates | 33 | 3.24 ± 0.3 | 1 - 6 | 34 | 4.12 ± 0.4 | 1 - 10 | -1.53 | 65 | 0.067 |
| Number of copulations* | 33 | 5.00 ± 0.6 | 1 - 13 | 34 | 8.09 ± 1.3 | 1 - 32 | -1.81 | 65 | 0.038 |
| Number of unknowns | 38 | 1.47 ± 0.3 | 0 - 8 | 34 | 0.97 ± 0.2 | 0 - 4 | 1.68 | 70 | 0.048 |
| Estrus duration (min) $^{+}$ | 38 | 163.53 ± 8.8 | 70 - 290 | 33 | 185.73 ± 15.8 | 40 - 375 | -1.27 | 69 | 0.104 |

*log-transformed

$^{+}$one outlier removed

**Table 5.2**. Comparison of male morphology of *Xerus inauris* between South Africa and Namibia. Averages and ranges indicate actual values before correcting for body size; statistics run on values corrected for body size.

| | South Africa | | | | Namibia | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Morphological character | N | Mean ± SE | range | CV | N | Mean ± SE | range | CV | t | df | *P* |
| Body mass (g) | 31 | 671.8 ± 9.2 | 575 - 800 | 7.62 | 25 | 657.2 ± 15.2 | 515 - 805 | 11.54 | 0.86 | 54 | 0.396 |
| Testes mass (g) | 29 | 12.50 ± 0.3 | 8.32 - 16.64 | 12.46 | 25 | 9.02 ± 0.5 | 4.49 - 13.96 | 24.79 | 6.06 | 52 | <0.0001 |
| Epididymal mass (g) | 29 | 6.71 ± 0.2 | 4.72 - 9.83 | 17.64 | 24 | 4.66 ± 0.3 | 2.43 - 6.42 | 26.36 | 6.19 | 51 | <0.0001 |
| Bulbourethral gland mass (g) | 22 | 7.66 ± 0.4 | 3.82 - 11.49 | 27.21 | 14 | 5.78 ± 1.1 | 1.18 - 13.61 | 69.6 | 1.92 | 34 | 0.032 |

### *Reproductive success*

I genotyped a total of 387 individuals from South Africa and 322 individuals from Namibia. I did find evidence of Hardy-Weinberg disequilibrium in three loci from South Africa and two loci from Namibia (Table 5.3), along with significant linkage disequilibrium after correcting for multiple comparisons (Rice, 1989). In a previous analysis of a random sample of individuals from South Africa, I found Hardy-Weinberg assumptions were met in all loci (Manjerovic et al., 2009). Therefore, deviations seen in this current data set are most likely due to the inclusion of family groups (Li & Leal, 2009). The largest discrepancy between expected and observed heterozygosity was in Xin4 in Namibia, most likely due to the presence of null alleles. I calculated parentage with and without this locus present and found assignments did not change significantly; therefore I included all loci in final assignments. Furthermore, CERVUS includes corrections for scoring errors, mutations, and null alleles (Jones & Ardren 2003, Kalinowski et al., 2007). Including maternity estimates resulted in combined second-parent exclusion probabilities of $Pr_e = 0.982761$ in South Africa and $Pr_e = 0.996384$ in Namibia (Jamieson & Taylor, 1997). I assigned paternity to 76 of 155 juveniles in South Africa (49%) and 66 of 102 juveniles in Namibia (65%) (Table 5.4). There were five instances (1 South Africa, 4 Namibia) where the most likely candidate was a natal male from that colony.

**Table 5.3.** Microsatellite genetic variation of *Xerus inauris* between two main study sites

| | | | South Africa | | | Namibia | | |
|---|---|---|---|---|---|---|---|---|
| | N | Ar | A | $H_e$ | $H_o$ | A | $H_e$ | $H_o$ |
| Xin1 | 13 | 10.3 | 7 | 0.70 | 0.67 | 11 | 0.74 | 0.74 |
| Xin3 | 14 | 10.0 | 9 | 0.69 | 0.66 | 12 | 0.70 | 0.65 |
| Xin4 | 11 | 8.9 | 4 | 0.61 | 0.53 | 11 | **0.70** | **0.47** |
| Xin5 | 18 | 15.5 | 4 | **0.64** | **0.60** | 18 | 0.86 | 0.84 |
| Xin8 | 10 | 8.4 | 7 | 0.64 | 0.65 | 10 | 0.69 | 0.71 |
| Xin9 | 10 | 8.5 | 9 | **0.61** | **0.59** | 7 | 0.73 | 0.72 |
| Xin10 | 10 | 9.3 | 8 | **0.75** | **0.67** | 10 | **0.73** | **0.70** |
| Xin12 | 5 | 4.6 | 4 | 0.59 | 0.57 | 5 | 0.48 | 0.51 |
| Mean | | 9.4 | 6.5 | 0.65 | 0.62 | 10.5 | 0.70 | 0.67 |
| # of males | | | 223 | | | 188 | | |
| # of females | | | 164 | | | 144 | | |
| # of juveniles | | | 203 | | | 161 | | |
| Total # of individuals | | | 387 | | | 332 | | |

Number of alleles per locus (N), allelic richness ($A_r$), number of alleles per population (A), expected heterozygosity ($H_e$) and observed heterozygosity ($H_o$) by locus for each population; mean, average $H_e$ and $H_o$ and number of alleles over eight loci. Significant deviations between observed and expected levels of heterozygosoty in each population and locus by locus after sequential Bonferroni correction (Rice, 1989) are shown in bold.

**Table 5.4.** Characteristics of juvenile paternity assignments of *Xerus inauris* in two study sites across all three years.

| | South Africa | | | | | Namibia | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Year | Candidate males | # of juveniles | # of assigned juveniles | # of sires | % of male sires | Candidate males | # of juveniles | # of assigned juveniles | # of sires | % of male sires |
| 2004 | 78 | 57 | 19 (33%) | 17 | 0.22 | 23 | 57 | 36 (63%) | 11 | 0.48 |
| 2005 | 60 | 42 | 33 (79%) | 21 | 0.35 | 32 | 13 | 12 (92%) | 7 | 0.22 |
| 2006 | 97 | 56 | 24 (43%) | 21 | 0.22 | 38 | 32 | 18 (56%) | 10 | 0.26 |

Reproduction was extremely skewed among individuals (Figure 5.3) but was not significantly different between sites (Wilcoxon Ranked Sums: Z = 1.41; $P = 0.159$). Both sites had approximately 65% of the males sire no offspring resulting in extremely high intensities of sexual selection (Namibia $I_s = 4.81$, South Africa $I_s = 3.53$). Of males that successfully sired offspring, more individuals sired a single offspring in South Africa (68.8%) compared to 39.1% in Namibia. This skew resulted in more individuals who sired multiple offspring and a higher variance in male mating success for Namibia (4.20) compared to South Africa (0.85). Based on estimated date of juvenile emergence, I recorded 14 babies from the 38 estruses at South Africa (36.8%) and assigned paternity to five of those offspring. In none of those five occurrences was the assigned male the first to copulate with the estrous female; only once was the assigned male the last mate.

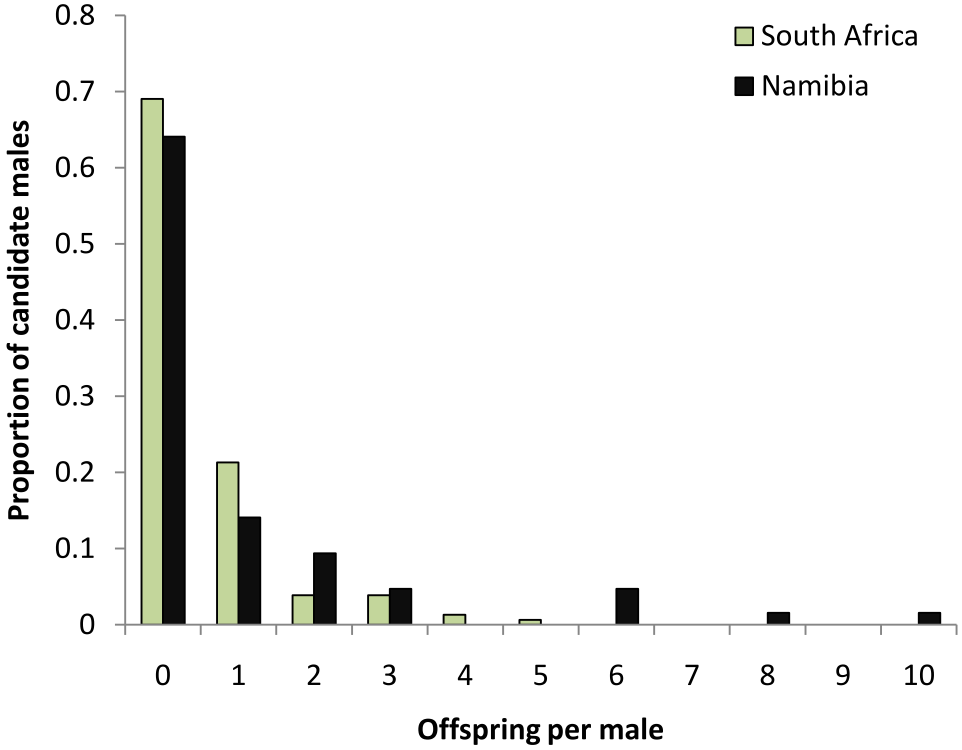


**Figure 5.3.** Reproductive skew among *Xerus inauris* based on number of juveniles sired.

## Discussion

Male mating behaviors have long been recognized as a response to the spatial and temporal distribution of receptive females (Emlen & Oring, 1977; Shuster & Wade, 2003). Female *X. inauris* do not differ in their temporal availability between sites but do differ in their spatial distribution (Figure 5.1). Burrow clusters in Namibia are significantly further away from one another and not as evenly distributed as in South Africa. This patchiness is likely a response to the amount of suitable habitat patches with higher habitat heterogeneity in Namibia compared to South Africa possibly reflecting higher unpredictability of rainfall (Pettitt et al., 2008). Differences in male home ranges between sites reflect these differences in female distribution. Males that disperse in South Africa had an average home range of 32.6 ha, which is over three times larger than the previously published average home range for dispersed squirrels from Namibia (12.5 ± 2.5 ha; Waterman, 1995). However, males in Namibia most likely have to disperse further to find additional female social groups. I have documented multiple males dispersing up to 5 km in Namibia. Increasing dispersal distance to find suitable mates suggests that dispersal may be more costly in Namibia compared to South Africa. Dispersal appears to be a conditional strategy (Chapter 4), which suggests timing of male dispersal from the natal burrow site may vary between sites based on an individual's status (Gross, 1996).

Variance in timing of dispersal between sites is likely to influence male reproductive success. All male *X. inauris* competitively search for estrous females, but only males in Namibia form a dominance hierarchy, with older, more dominant males more likely to copulate first with females and younger, subordinate males copulating after or not at all (Waterman, 1995;

Waterman, 1998). These mating behaviors suggest a first male advantage exists in Namibia related to age (Waterman, 1998). First male biases in reproduction are common in ground squirrels [*Urocitellus beldingi* (Sherman, 1989); *Ictidomys tridecemlineatus* (Schwagmeyer & Foltz, 1990); *U. parryii* (Lacey et al., 1997); *U. columbianus* (Raveh et al., 2010)]. In these species, paternity is biased towards the first males but mate precedence does not always decrease with mate order (Lacey, et al., 1997; Raveh et al., 2010). The degree of bias is not necessarily correlated with litter size suggesting variation exists in post-copulatory competition. These species all have average litter sizes of over three (see Waterman, 1996), increasing the probability for multiple paternity. Reproduction in these species is also constrained by a discrete breeding season, where multiple females may be receptive at a single time. Therefore, males may gain greater reproductive advantages by leaving to search for additional females after the initial mating bout.

Unlike North American squirrels, female *X. inauris* are asynchronous in receptivity allowing males opportunities to mate throughout the entire year. This lack of constraint on additional breeding opportunities for males allows multiple opportunities for selection, hence the high sexual selection intensities I observed in both populations. All male *X. inauris* are highly sperm competitive (Manjerovic et al., 2008) as the small litter size does not necessarily preclude the possibility of multiple paternity. The differences seen in reproductive morphology between the sites, with South Africa have significantly larger testes and accessory glands, support increased investment in sperm competition at the South African site (Ramm et al., 2005). The lack of dominance relationships in South Africa may reflect the larger home ranges and fewer consistent interactions with other males. Males in South Africa may be off-setting a potential first male mating advantage with increasing ejaculate investments and by discharging copulatory plugs. However, I cannot rule out the possibility of female mate choice both in selective sperm use (Birkhead, 1998), or in removal of copulatory plugs (Koprowski, 1992). Guarding females after insemination, as seen in *U. brunneus*, does delay mate searching but may increase paternity assurance by reducing subsequent matings and sperm competition (Sherman, 1989). Given the low probability for multiple females to come into estrus on the same day (Waterman, 1996), mate guarding does not impose a cost to male *X. inauris* in terms of a fitness trade-off. Guarding was significantly more likely to occur in South Africa after mating and several occurrences of post-copulatory calls have been observed in South Africa but never Namibia (J.M. Waterman, M.B. Manjerovic, personal observation). Post-copulatory calls may be produced by dominant individuals in order to deter other males from attempting to mate or produced by males trying to attract additional females (Grady & Hoogland, 1998; McElligott & Hayden, 2001). Because males are unlikely to be attracting other females and the lack of a dominance hierarchy in South Africa, additional research would be necessary to determine why these calls are produced. These post-copulatory behaviors suggest the likelihood of a last male advantage in South Africa (Sherman, 1989; Waterman, 2007), the opposite of what is thought to occur in Namibia (Waterman, 1998). I did find evidence that males that sire offspring in South Africa were not necessarily the first males to copulate with the estrous female, nor were they always the last, suggesting there may be no relationship between fertilization success and mate order.

Sperm competition intensifies sexual selection beyond nonrandom mating when those males who have an opportunity to mate are also disproportionately successful (Shuster, 2009). I found variance in copulatory success among individuals was lower than variance in reproductive success in both sites. This was not surprising due to a high number of copulations despite low likelihood of paternity for each male attending an estrus. Despite differences in female burrow distribution and number of burrow clusters per hectare, I found both sites had high sexual selection intensities whether calculated as the number of sexually active males attending each estrus or the opportunity for sexual selection ($I_s$). However, differences in competitive strategies likely contribute to the variance I calculated in fertilization success. In Namibia, dominant males obtain a greater proportion of copulations (Waterman, 1998) and I saw higher variance in mating success with fewer individuals siring the majority of the offspring. Males in South Africa appear to be investing more in sperm competition in regards to sperm quantity resulting in a lower within sire variance. Despite these differences in male mating tactics, both sites had estimates of sexual selection intensity similar to what is seen in lekking species (DuVal & Kempenaers, 2008) suggesting that mating is extremely skewed towards specific individuals within a population of adult males increasing the intensity of sexual selection.

## Conclusions

Many studies address variations in male mating tactics caused by changes in female distribution and how these changes affect the environmental potential for polygamy and the intensity of male-male competition. In a species that mates promiscuously, where a single male is unlikely to monopolize the female mating, I demonstrated that differences in the spatial distribution of females did not affect mating intensity. However, the social structure and promiscuous nature of *X. inauris* results in high variance in reproductive success among males and likely contributes to different mechanisms of pre- and post-copulatory competition, specifically site differences in dominance and sperm competition. Such high opportunities for selection suggest the potential for rapid evolutionary change within this species related to discrepancies in the breeding system and how breeding behavior impacts population structure.

# References

Andersson, M., & Simmons, L. W. (2006). Sexual selection and mate choice. *Trends Ecol Evol* **21**, 296-302.

Beyer, H. L. (2004). Hawth's Analysis Tools for ArcGIS. http://www.spatialecology.com/htools (ed.).

Birkhead, T. R. (1998). Cryptic female choice: criteria for establishing female sperm choice. *Evolution* **52**, 1212-1218.

Birkhead, T. R. (2010). How stupid not to have thought of that: post-copulatory sexual selection. *Journal of Zoology* **281**, 78-93.

Birkhead, T. R. & Pizzari, T. (2002). Postcopulatory sexual selection. *Nat Rev Genet* **3**, 262-273.

Bouchie, L., Bennet, N. C., Jackson, T. & Waterman, J. M. (2006). Are Cape ground squirrels (*Xerus inauris*) induced or spontaneous ovulators? *J Mammal* **87**, 60-66.

Chapman, T. (2001). Seminal fluid-mediated fitness traits in Drosophila. *Heredity* **87**, 511-521.

Clutton-Brock, T. H. (1989). Mammalian mating systems. *Proc R Soc B-Biol Sci* **236**, 339-372.

Clutton-Brock, T. H. & Harvey, P. H. (1978). Mammals, resources and reproductive strategies. *Nature* **273**, 191-195.

Cordoba-Aguilar, A. (2005). Possible coevolution of male and female genital form and function in a calopterygid damselfly. *J Evol Biol* **18**, 132-137.

Crook, J. H., Ellis, J. E. & Goss-Custard, J. D. (1976). Mammalian social systems: structure and function. *Anim Behav* **24**, 261-274.

de Vries, H., Netto, W. J. & Hanegraaf, P. L. H. (1993). Matman: a program for the analysis of sociometric matrices and behavioural transition matrices. *Behaviour* **125**, 157-175.

delBarco-Trillo, J. & Ferkin, M. H. (2006). Male meadow voles respond differently to risk and intensity of sperm competition. *Behav Ecol* **17**, 581-585.

Dixson, A. F. & Anderson, M. J. (2004). Sexual behavior, reproductive physiology and sperm competition in male mammals. *Physiol Behav* **83**, 361-371.

DuVal, E. H. & Kempenaers, B. (2008). Sexual selection in a lekking bird: the relative opportunity for selection by female choice and male competition. *Proc R Soc B-Biol Sci* **275**, 1995-2003.

Eberhard, W. G. (2009). Postcopulatory sexual selection: Darwin's omission and its consequences. *Proc R Soc Lond B* **106**, 10025-10032.

Emlen, S. T. & Oring, L. W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science* **197**, 215-223.

Evanno, G., Regnaut, S. & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* **14**, 2611-2620.

Koprowski, J. L. (1992). Removal of copulatory plugs by female tree squirrels. *J Mammal* **73**, 572-576.

Hews, D. (1993). Food resources affect female distribution and male mating opportunities in the iguanian lizard (*Uta palmeri*). *Anim Behav* **46**, 279-291.

Gannon, W. L. & Sikes, R. S. (2007). Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *J Mammal* **88**, 809-823.

Gomendio, M., Martin-Coello, J., Crespo, C., Magana, C. & Roldan, E. R. S. (2006). Sperm competition enhances functional capacity of mammalian spermatozoa. *Proc Natl Acad Sci* **103**, 15113-15117.

Grady, R. M. & Hoogland, J. L. (1986). Why do male black-tailed prairie dogs (*Cynomys ludovicianus*) give a mating call? *Anim Behav* **34**, 108-112.

Gross, M. R. (1996). Alternative reproductive strategies and tactics: Diversity within sexes. *Trends Ecol Evol* **11**, 92-98.

Hart, R. G. & Greenstein, J. S. (1968) A newly discovered role for Cowper's gland secretion in rodent semen coagulation. *Reproduction* **17**, 87-94.

Herzig-Straschil, B. (1978). On the biology of *Xerus inauris* (Zimmerman, 1780) (Rodentia, Sciuridae). *Zeitschrift Fur Saugetierkunde* **43**, 262-278.

Herron, M. D., Castoe, T. A. & Parkinson, C. L. (2004). Sciurid phylogeny and the paraphyly of Holarctic ground squirrels (Spermophilus). *Mol Phylogenet Evol* **31**, 1015-1030.

Hosken, D. J. (1998). Testes mass in megachiropteran bats varies in accordance with sperm competition theory. *Behav Ecol Sociobiol* **44**, 169-177.

Jamieson, A. & Taylor, S. C. S. (1997). Comparisons of three probability formulae for parentage exclusion. *Anim Genet* **28**, 397-400.

Jones, A. G. & Ardren, W. R. (2003). Methods of parentage analysis in natural populations. *Mol Ecol* **12**, 2511-2523.

Kalinowski, S. T., Taper, M. L. & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol* **16**, 1099-1106.

Kokko, H. & Monaghan, P. (2001). Predicting the direction of sexual selection. *Ecol Lett* **4**, 159-165.

Kokko, H. & Rankin, D. J. (2006). Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Phil Trans Roy Soc B* **361**, 319-334.

Lacey, E. A., Wieczorek, J. R. & Tucker, P. K. (1997). Male mating behaviour and patterns of sperm precedence in Arctic ground squirrels. *Anim Behav* **53**, 767-779.

Lehner, P. N. (1998). *Handbook of ethological methods*. Cambridge Univ Pr.

Li, B. & Leal, S. M. (2009). Deviations from hardy-weinberg equilibrium in parent and unaffected sibling genotype data. *Hum Hered* **67**, 104-115.

Manjerovic, M. B., Kinahan, A. A., Waterman, J. M., Bennett, N. C. & Bateman, P. W. (2008). Structure and allometry of genitalia in males and females of a social African ground squirrel with high polygynandry. *J Zool (Lond)* **275**, 375-380.

Manjerovic, M. B., Waterman, J. M., Hoffman, E. A., & Parkinson, C. L. (2009). Characterization of nine microsatellite loci in the Cape ground squirrel, *Xerus inauris*, and their cross-utility in other species. In: Abercrombie, et al. Permanent genetic resources added to Molecular Ecology Resources database 1 January 2009 – 30 April 2009. *Mol Ecol Res* **9**, 1375-1379.

Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Mol Ecol* **7**, 639-665.

McElligott, A. G. & Hayden, T. J. (2001). Postcopulatory vocalizations of fallow bucks: who is listening? *Behav Ecol* **12**, 41-46.

Melchior, H. R. & Iwen, F. A. (1965). Trapping, restraining, and marking arctic ground squirrels for behavioral observations. *J Wildl Mgmt* **29**, 671-678.

Minder, A. M., Hosken, D. J. & Ward, P. I. (2005). Co-evolution of male and female reproductive characters across the Scathophagidae (Diptera). *J Evol Biol* **18**, 60-69.

Parker, G. A. & Ball, M. A. (2005) Sperm competition, mating rate and the evolution of testis and ejaculate sizes: a population model. *Bio Lett* **1**, 235-238.

Pettitt, B. A., Waterman, J. M. & Wheaton, C. J. (2008). Assessing the effects of resource availability and parity on reproduction in female Cape ground squirrels: resources do not matter. *J Zool (Lond)* **276**, 291-298.

Ramm, S. A., Parker, G. A. & Stockley, P. (2005). Sperm competition and the evolution of male reproductive anatomy in rodents. *Proc R Soc B-Biol Sci* **272**, 949-955.

Raveh, S., Heg, D., Dobson, F. S., Coltman, D. W., Gorrell, J. C., Balmer, A. & Neuhaus, P. (2010). Mating order and reproductive success in male Columbian ground squirrels (*Urocitellus columbianus*). *Behav Ecol* **21**, 537-547.

Raymond, M. & Rousset, F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Hered* **86**, 248-249.

Ripley, B. D. (1976). The second-order analysis of stationary point processes. *J Appl Prob* **13**, 255-266.

Rood, J. P. & Nellis, D. W. (1980). Freeze marking mongooses. *J Wildl Mgmt* **44**, 500-502.

Scantlebury, M., Waterman, J. M. & Bennett, N. C. (2008). Alternative reproductive tactics in male Cape ground squirrels *Xerus inauris*. *Physiol Behav* **94**, 359-367.

Schwagmeyer, P. L. & Foltz, D. W. (1990). Factors affecting the outcome of sperm competition in thirteen-lined ground squirrels. *Anim Behav* **39**, 156-162.

Schwartz, O. A. & Armitage, K. B. (1980). Genetic variation in social mammals: the marmot model. *Science* **207**, 665-667.

Sherman, P. W. (1989). Mate guarding as paternity insurance in Idaho ground squirrels. *Nature* **338**, 418-320.

Shuster, S. M. (2009). Sexual selection and mating systems. *Proc Natl Acad Sci* **106**, 10009-10016.

Shuster, S. M. & Wade, M. J. (2003). *Mating systems and strategies*. Princeton Univ Pr.

Storz, J. F. (1999). Genetic consequences of mammalian social structure. *J Mammal* **80**, 553-569.

Sugg, D. W., Chesser, R. K., Dobson, F. S. & Hoogland, J. L. (1996) Population genetics meets behavioral ecology. *Trends Ecol Evol* **11**, 338-342.

Trivers, R. L., (1972). Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man*: 136-179. B. Cambell (ed.). Aldine, Chicago.

van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. & Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* **4**, 535-538.

van zyl, J. H. M. (1965). The vegetation of the S.A. Lombard Nature Reserve. *Zoologica Africana* **1**, 55-71.

Wade, M. J. & Arnold, S. J. (1980). The intensity of sexual selection in relation to male sexual behaviour, female choice, and sperm precedence. *Anim Behav* **28**, 446-461.

Waterman, J. M. (1994). The social organization of the Cape ground squirrel *Xerus inauris*. University of Minnesota, St. Paul.

Waterman, J. M. (1995). The social organization of the Cape ground squirrel (*Xerus inauris* Rodentia, Sciuridae). *Ethology* **101**, 130-147.

Waterman, J. M. (1996). Reproductive biology of a tropical, non-hibernating ground squirrel. *J Mammal* **77**, 134-146.

Waterman, J. M. (1997). Why do male Cape ground squirrels live in groups? *Anim Behav* **53**, 809-817.

Waterman, J. M. (1998). Mating tactics of male Cape ground squirrels, *Xerus inauris*: consequences of year-round breeding. *Anim Behav* **56**, 459-466.

Waterman, J.M. (2007). Male mating strategies in rodents. *Rodent societies: an ecological and evolutionary perspective* 27-41.

Wedell, N. & Cook, P. A. (1999). Butterflies tailor their ejaculate in response to sperm competition risk and intensity. *Proc R Soc B-Biol Sci* **266**, 1033-1039.

Wigby, S., Sirot, L. K., Linklater, J. R., Buehner, N, Calboli, F. C. F., Bretman, A., Wolfner, M. F. & Chapman, T. (2009). Seminal fluid protein allocation and male reproductive success. *Curr Biol* **19**, 751-757.

Wolff, J. & Sherman, P. W. (2007). *Rodent societies: an ecological & evolutionary perspective*. University Of Chicago Press.

# CHAPTER 6 – CONCLUSIONS

My research corroborates much of what is known in highly competitive, promiscuous systems while addressing additional aspects of sexual selection. The predominant mechanism underlying genital evolution and competition for paternity in *X. inauris* is sperm competition. This should not come as a surprise given the fact that *X. inauris* have the largest known relative testes size of any sciurid. While large testes size often is attributed to increasing sperm numbers, there are multiple other ways in which species maximize sperm competition (e.g. increasing sperm motility, viability, or velocity). Many of these sperm attributes can be quickly altered by an individual male in response to level of competition and mating or remating rate. Past research on *X. inauris* found that as females increased number of mates, males were more likely to copulate repeatedly (Waterman, 1998). Theoretically, this translates to increased sperm competition intensity affecting remating rate and may cause males to allocate ejaculates differently depending on the competitive environment in which mating occurs. I did find differences in sperm length but not testes size between males utilizing different dispersal tactics suggesting sperm competition strategies may be related to sperm physiology or possibly body condition rather than simply increasing sperm numbers.

Costs associated with increasing investment in sperm competition likely occur in *X. inauris*, where increased investment in spermatogenesis was significantly related to decreased spleen size. This supports the 'immunocompetence handicap hypothesis', which suggests investment in sperm competition is an honest signal of quality because immunocompetent males are more capable of trading-off investment between reproduction and immunity. However, unlike other vertebrates in which males show increased susceptibility to disease compared to females, i.e. reduced immunocompetence, I did not find sexual dimorphism in overall immunocompetence as measured by spleen size. Other species, in which males show reduced immunocompetence, often attribute this sexual dimorphism to the greater intensity of sexual selection and negative effects of androgens. This relationship is especially true in promiscuous species where males have greater investments in energetically expensive aspects of pre- and post-competition (e.g. courtship displays, combat, and mate-guarding). What differs in *X. inauris* compared to other promiscuous species is that breeding year-round likely imposes large immunological costs on both males and females. Previous research, in which parasites were removed from females, demonstrated a significant increase in reproductive success suggesting that females are under strong selective pressures as well as males (Hillegass et al., 2010).

Although males and females did not significantly differ in overall immunity, I did find differences when looking at more specific immunological attributes, specifically white and red blood cells. It is possible that sex-biases in parasites types trigger different immune responses, especially given past research where females have higher endoparasite loads and males have higher ectoparasite loads. Sexual dimorphisms in blood cells may be a response to different selective pressures, e.g. males to intrasexual competition and females to costs associated with reproduction, causing overall immunity to be similar while specific responses vary. Given the promiscuous nature of this species, it is also extremely probable that sexually transmitted infections are rampant and would affect both males and females, albeit in potentially different ways. I suggest future studies in the field of sexual dimorphism and ecological immunity should incorporate these differences as failure to do so may underestimate sexual dimorphism in immune response.

In promiscuous systems, there are multiple pre-copulatory reproductive opportunities in which males can outcompete same-sex conspecifics in order to increase their probability of acquiring mates. In male *X. inauris*, dispersed males are likely to encounter more estrous females and therefore have increased breeding opportunities, although I found no differences between tactics in number of estruses attended. Surprisingly, the decision to remain natal does not preclude reproduction as natal males attend estruses and successfully copulate with females. The success of natal males was unexpected and leads me to change my question from 'why stay' to 'why leave'. In order to address this, future research should explore the social group composition and relatedness among individuals. Natal males encounter increased aggression from female social group members but this degree of aggression may change based on the relatedness between individuals. Past research has determined that the presence of a natal male suppresses female reproduction (Pettitt, 2006). If this is an inbreeding avoidance mechanism, degree of relatedness would be positively correlated with aggression.

Alternative mating tactics often vary in response to female distribution altering the intensity of male-male competition. I demonstrated that differences in the spatial distribution of female *X. inauris* did not affect mating intensity but did affect mating behaviors. In a population where females were spatially aggregated, males may disperse further from their natal social group presumably due to greater distances between suitable breeding areas. Mating success, in this population, is dependent on a linear dominance hierarchy based on dispersal and age. If dispersal is condition dependent, as it appears to be in South African populations, then it could be used by females as an honest indicator of male quality, hence female preference for breeding with older, dispersed males. I found no evidence of a dominance hierarchy in a population where females were distributed over a more uniform area. However, in this population, males invested more in sperm competition and were more likely to mate-guard, deposit post-copulatory plugs, and vocalize after mating. Females do not appear to choose males based on external testes size but it is possible female control insemination based on additional cryptic mechanisms. Given the almost certainty for females to mate multiply, it is likely that cryptic female choice does occur in this species.

The promiscuous nature of *X. inauris* results in high variance in reproductive success among males and likely contributes to the evolution of alternative mating tactics. In addition, females are strongly clustered into family groups that can lead to opportunities for kin selection as well as influence inbreeding rates, and influence the rate at which genetic diversity is lost from natural population. Differences in social group composition between sites may also result in differences in timing of male dispersal and consequently rates of male dispersal especially if degree of relatedness causes increased aggression towards natal males. Therefore, variations in the breeding system and population structure of this species results in increased opportunities for selection suggesting the potential for rapid evolutionary change within this species.

## References

Hillegass, M. A., Waterman, J. M., & Roth, J. S. (2010). Parasite removal increases reproductive success in a social African ground squirrel. *Behav Ecol* **21**, 696-700.

Pettitt, B. A. (2006). Reproductive delay in the female Cape ground squirrel, *Xerus inauris*. In: *Biology.* University of Central Florida, Orlando.

Waterman, J. M. (1998). Mating tactics of male Cape ground squirrels, *Xerus inauris*: consequences of year-round breeding. *Anim Behav* **56**, 459-466.

# APPENDIX – CHARACTERIZATION OF NINE MICROSATELLITE LOCI IN THE CAPE GROUND SQUIRREL, *XERUS INAURIS*, AND THEIR CROSS-UTILITY IN OTHER SPECIES

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[1] **Published as:** Manjerovic, MB, JM Waterman, EA Hoffman, and CL Parkinson. Characterization of nine microsatellite loci in the Cape ground squirrel, *Xerus inauris*, and their cross-utility in other species. In: Abercrombie, et al. 2009. Permanent genetic resources added to Molecular Ecology Resources database 1 January 2009 – 30 April 2009. *Mol Ecol Res* **9**, 1375-1379.

Cape ground squirrels (*Xerus inauris*) are arid-adapted rodents found throughout southern Africa. Unlike North American ground-dwelling sciurids that reproduce seasonally, *X. inauris* individuals do not hibernate and breed year-round (Waterman, 1996). Additionally, *Xerus inauris* has a form of social organization unique among ground squirrels. Females live in highly philopatric, matrilineal kin groups and males live in independent all-male bands, with both groups persisting year round (Waterman, 1996; Waterman, 1998). Males are not territorial or aggressive, but rather compete for females through competitive searching and high levels of sperm competition (Manjerovic et al., 2008; Waterman, 1998). The level of social organization and cryptic competition has made this species ideal for investigating questions regarding sociality and male mating tactics. The microsatellite markers I develop here will provide important tools to investigate parentage, population structure, and kinship in *X. inauris*.

I extracted genomic DNA from tail tips using DNeasy Kits (Qiagen). Following the microsatellite enrichment protocol of Hoffman et al. (2003), I generated random DNA fragments with known flanking sequences through degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) incorporating the K6-MW primer developed by Macas *et al.* (1996). I combined these fragments with a 3’-biotinylated repeat motif (GATA or CA) bound to streptavidin-coated particles (Promega), which allows for enrichment via magnetic separation. I cloned these enriched products using TOPO TA cloning kits (Invitrogen) and ran PCRs following the procedure from Cabe and Marshall (2001). I ran two PCRs per sample, including either T3 and T7 primers or an additional GATA or CA primer. Visualization of the PCR products side-by-side on a 2.0% agarose gel allowed me to see positive clones as a smear next to the distinct band produced in the T3-T7 reaction. I screened 216 clones of which 83 (38.4%) indicated the presence of the target repeat. Sequences were aligned and edited with Sequencher version 4.1 (Gene Codes Corp.) to identify potential microsatellite loci. I developed primers flanking repeat motifs longer than eight repeat units in length using PRIMER3.0 (Rozen and Skaletsky 2000).

For genotyping, I direct labelled four loci (Xin04, Xin06, Xin08, and Xin10) on the 5'-end of the forward primer with a fluorescent dye. The five remaining loci were labelled with an M13(-21) tail (5'-TGTAAAACCGACGGCCAGT-3') attached to the forward primer for fluorescent labelling (M13) (Schuelke 2000). I ran all PCRs on a MJ Research PTC 200 thermal cycler using approximately 10 ng DNA template, 1x PCR buffer with 20 µL final volumes. I performed M13 PCRs containing 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 U Sigma *Taq* polymerase, 0.2 µM forward primer, and 0.8 µM of both reverse primer and M13 tag. Xin04 and Xin06 included 2.5 mM $MgCl_2$, 0.12 mM dNTPs, 0.75 U *Taq* polymerase, and 0.16 mM forward and reverse primer. Xin08 and Xin10 reactions used 1x PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.3 U *Taq* polymerase, and 0.2 µM forward and reverse primer. Thermocycling profiles for M13 loci are as follows: initial denaturation at 94 °C for 5 min; 30 cycles of 94 °C for 30 s, optimal annealing temperature ($T_a$, Table A.1) for 45 s, 72 °C for 45 s, followed by 8 additional cycles as above with reduced annealing temperature of 53 °C, and a final extension at 72 °C for 10 min. Direct labelled PCR conditions begin with initial denaturation at 94 °C for 1 min and one cycle of 94 °C for 30 s, 58 °C for 20 s, and 72 °C for 5 s followed by 32 cycles of 94 °C for 15 s, $T_a$ for 20 s, and 72 °C for 5 s with a final extension at 72 °C for 5 min. I confirmed amplification on a 2% agarose gel and assessed polymorphism using the CEQ 8000 genetic analysis system and software (Beckman-Coulter).

To assess genetic variability, I used 46 animals from a single population in Bloemhof, South Africa. I found the number of alleles per locus ranging from 2 to 7 and averaging 4.7 per locus, with expected heterozygosities ranging from 0.29 to 0.74 (Table A.1). Exact tests for Hardy-Weinberg and linkage disequilibria were conducted in GENEPOP V.3.3 (Raymond and Rousset, 1995). I found no deviation from Hardy-Weinberg proportions at the 5% level and no significant gametic disequilibrium for pairwise comparisons after applying the sequential Bonferroni correction (Rice, 1989).

Using the same PCR and cycling conditions, I tested these primer sets for cross-species amplification in the following sciurids: Mountain ground squirrel (*X. princeps*; n=1), thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*; n=2), and eastern gray squirrel (*Sciurus carolinensis*; n=1). Visualization on a 2% agarose gel demonstrated successful amplification for all loci in *X. princeps* but not for the two other species. This low degree of cross-species amplification is most likely explained by the phylogenetic relationship of the Xerini tribe to that of other sciurids (Herron et al., 2004). To the best of my knowledge, these novel microsatellites are the first created for the tribe Xerini. The utility of these markers will allowed me to examine questions relating to paternity and social structure in the highly promiscuous Cape ground squirrel, while their cross utility in closely related species will be useful for comparative studies to understand selective pressures on those systems.

# References

Cabe, P. R. & Marshall, K. E. (2001). Microsatellite loci from the house wren (*Troglodytes aedon*). *Mol Ecol Notes* **1**, 155-156.

Herron, M. D., Castoe, T. A. & Parkinson, C. L. (2004). Sciurid phylogeny and the paraphyly of Holarctic ground squirrels (Spermophilus). *Mol Phylogenet Evol* **31**, 1015-1030.

Hoffman, E. A., Ardren, W. R., & Blouin, M. S. (2003). Nine polymorphic microsatellite loci for the northern leopard frog (*Rana pipiens*). *Mol Ecol Notes* **3**, 115-116.

Macas, J., Gualberti, G., Nouzová, M., Samec, P., Lucretti, S., & Dolezel, J. (1996). Construction of chromosome-specific DNA libraries covering the whole genome of field bean (*Vicia faba* L.). *Chromo Res* **4**, 531-539.

Manjerovic, M. B., Kinahan, A. A., Waterman, J. M., Bennett, N. C. & Bateman, P. W. (2008). Structure and allometry of genitalia in males and females of a social African ground squirrel with high polygynandry. *J Zool (Lond)* **275**, 375-380.

Raymond, M. & Rousset, F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Hered* **86**, 248-249.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223-225.

Rozen, S., & Skaletsky, H. (2000). Primer 3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds. Krawetz S, Misener S), pp. 365-386. Humana Press, Totowa, New Jersey, (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

Schuelke, M. (2000) An economic method for the fluorescent labeling of PCR fragments. *Nature Biotech* **18**, 233-234.

Waterman, J. M. (1996). Reproductive biology of a tropical, non-hibernating ground squirrel. *J Mammal* **77**, 134-146.

Waterman, J. M. (1998). Mating tactics of male Cape ground squirrels, *Xerus inauris*: consequences of year-round breeding. *Anim Behav* **56**, 459-466.

**Appendix A.1.** Characterization for nine polymorphic microsatellite loci used in this study. Locus name, GenBank Accession number, repeat motif, primer sequence, and annealing temperature ($T_a$) are shown. Population data (allele size range; number of diploid genotypes obtained, *n*; number of alleles, *A*; and observed and expected heterozygosities) for *Xerus inauris* individuals are given. Allele sizes for M13 tagged primers (*) do not include 18 bp M13(-21) primer.

| Locus | GenBank Accession no. | Repeat motif | Primer sequence (5' - 3') | $T_a$ (°C) | Allele size range (bp) | *n* | *A* | $H_O$ | $H_E$ |
|---|---|---|---|---|---|---|---|---|---|
| Xin01* | FJ823123 | $(GATA)_8$ | F - AGA ATC CAA CAG ACA GAA AAC AA<br>R - CAA CGC AGC TGG CAT AGT AA | 63 | 339-355 | 44 | 5 | 0.86 | 0.71 |
| Xin03* | FJ823124 | $(GATA)_{15}$ | F - CGT GGG TTC AAT TCC TGG TA<br>R - AGG GCG ATA GCT CAG TGG TA | 62 | 211-231 | 46 | 7 | 0.70 | 0.73 |
| Xin04 | FJ823125 | $(CTAT)_{10}$ | F - GGA CAA AGT TAA GCT GGG TCA G<br>R - CGA CAT GTG GTG CGA CTT TA | 58 | 226-234 | 46 | 3 | 0.65 | 0.60 |
| Xin05* | FJ823126 | $(CA)_{12}$ | F - TCT TGA GCT GCC AAG TTT CTC<br>R - AGG TTC AAA GTT CTT GCC TGA | 59 | 234-238 | 46 | 4 | 0.52 | 0.64 |
| Xin06 | FJ823127 | $(CA)_5(CA)_7$ imperfect | F - CCC TAC ACA CAG AGG AAA GGA<br>R - CAA AGG GAA TGT GAG CGA AG | 60 | 107-109 | 46 | 2 | 0.35 | 0.29 |
| Xin08 | FJ823128 | $(GT)_{16}$ | F - AAG TGG TGC TAT AAT TGC TTT<br>R - GCA GTT ACA GAG CCT GGA GTT | 57 | 173-189 | 46 | 6 | 0.59 | 0.67 |
| Xin09* | FJ823129 | $(GT)_8$ | F - CCT CAT CAC AAC CAA GAC AG<br>R - GGT GAA TAC ATG ACC CAC AC | 56 | 186-200 | 46 | 6 | 0.52 | 0.55 |
| Xin10 | FJ823130 | $(GT)_{12}$ | F - CAG ATT GAG AGT GAG AGG TG<br>R - CTA TTC TGG GCA AAC GTG | 59 | 221-233 | 46 | 6 | 0.74 | 0.74 |
| Xin12* | FJ823131 | $(GT)_9$ | F - CCT TGA ACC TCC AGA AGT GT<br>R - GTT CAA TCC CTG GTA CAC GA | 57 | 201-207 | 46 | 3 | 0.41 | 0.50 |