

Behavioural and endocrine correlates to the
mating system of the aardwolf *Proteles*
cristata

by

David Gary Marneweck

Submitted in partial fulfillment of the requirements for the degree

Master of Science

In the Faculty of Natural & Agricultural Sciences

University of Pretoria

Pretoria

March 2013

Behavioural and endocrine correlates to the mating system of the aardwolf *Proteles cristata*

by

David Gary Marneweck

Supervisor:

Dr. Fredrik Dalerum

Mammal Research Institute & Centre for Wildlife Management
Department of Zoology and Entomology
Private Bag X20
University of Pretoria
Pretoria
0028
South Africa

Co-supervisor:

Prof. Elissa Z. Cameron

School of Zoology
University of Tasmania
Hobart
Australia

Abstract

Mating systems are classifications of the outcome of individual strategies aimed at maximising reproductive success. These systems have two components; one describes how individuals socially relate and bond to mating partners and the other describes the genetic outcome of actual mating activities. Reproductive activity is under strong endocrine and behavioural regulation where inter-sexual discrepancies in the regulation of reproductive success have resulted in the majority of mammal species exhibiting polygynous mating systems, with only 5% of mammals being socially monogamous. However, in monogamous species there can be a discrepancy between social pair bonds and sexual mating activities. Aardwolves are extreme diet specialists

on a temporally fluctuating resource, *Trinervitermes* spp. A number of studies have described them as socially monogamous but at the same time observed a high frequency of extra pair copulations (EPCs). A recent study also linked sex variation in den use to polygamous mating. However, it is unclear to what extent these EPC's contribute to individual fitness, and how these contrasting mating strategies influence space use and reproductive physiology. The aim of this project was to determine if there was seasonality in reproductive activity and to test if endocrine physiology, home range size and space use were similar between males and females as predicted for a socially monogamous species. We assessed reproductive endocrinology in zoo-housed aardwolves and quantified if physiological and behavioural data in wild aardwolves relate more to predictions based on social monogamy or polygamy. We found physiological support for previous behavioural observations of reproductive seasonality in both zoo-housed and wild aardwolves. We suggest that the seasonal breeding strategy in aardwolves is as a direct consequence of their strong dietary preference on a temporally fluctuating resource, where it is important for aardwolves to time periods of high physiological investment in reproduction with high seasonal abundance of these termites. Our data on zoo-housed aardwolves also showed that the social environment appeared to modify physiological responses to variation in environmental conditions. We suggest that due to strict seasonal breeding in wild aardwolves females are time constrained in receptivity which, combined with their largely solitary behaviour, implies that males have to be dynamic in the onset of their reproductive activity to closely match that of locally receptive females. Therefore, it may be adaptive for aardwolves to retain social receptivity even if resource distributions cause these animals to forage alone. We also found that physiological and behavioural traits correspond better to predictions based on social monogamy than polygamous mating in a population of wild aardwolves. However, earlier studies in the same population found that behavioural traits were more related to predictions based on polygamous mating. Therefore, our data and other

studies show how a difference between traits can relate to either social monogamy or polygamous mating in wild aardwolves in the same study area. We suggest that social mating system components regulate the observed endocrine and behavioural parameters more than actual mating patterns, which implies that social components pose a stronger selective pressure on physiology and behaviour than sexual mating patterns. Overall, we conclude that due to the discrepancy in traits that correspond to predictions based on different mating systems, aardwolves do not fit discreetly into any current mating system classification. We suggest that the evolutionary causes for the potentially conflicting mating strategies as well as the fitness benefits of these strategies need to be further investigated.

Declaration

I, David Gary Marneweck declare that the thesis, which I hereby submit for the degree of Master of Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE: _____

DATE: _____

Acknowledgements

Firstly, I would like to thank De Beers Consolidated Mines (Ltd.) for allowing us to conduct this study on Benfontein Nature Reserve. Additionally, I would like to acknowledge the various funding we received and thank those people and organisations. This study was financially supported by a National Research Foundation (NRF) focal area grant (Prof. Elissa Z. Cameron), an NRF incentive fund (Dr. Fredrik Dalerum), an NRF SARCHi chair in mammal behavioural ecology and physiology (Prof. Nigel C. Bennett) and a research fellowship from the University of Pretoria for Dr. Dalerum. I also received numerous help in the field on Benfontein and I owe my sincere gratitude to a number of important people. I want to thank the Manager on Benfontein, Mr. Finlay Markam, for his friendship, interest and field support during the project. I also would like to thank Mr. Koos Jooste and Mr. Andrew Soul who often helped dig and tow me out of the mud, regardless of the time of day or night. I would also like to thank Ms. Monique Engelkes and Ms. Charlotte Muilwijk for teaching me the fine skills of radio-tracking at the onset of the project; I learnt a great deal in our short time together. I would also like to thank Mr. Wouter Brekelmans and Mr. Rick Beshuizen for their hard work, which without, numerous aspects of the data collection phase would not have gone as smoothly. Finally, I would like to thank Miss. Charmaine Theron who often helped as a friendly spot-light operator in long grass sections of the reserve.

Numerous people needed to be thanked for their academic support during this project. In this respect, I wish to thank Prof. Elissa Cameron, for her supervision and financial support during the project. Big thanks must be given to Mrs. Stefanie Munscher who patiently taught me how to prepare faecal samples for hormone assay analysis. In this respect, I also wish to thank Dr. André Ganswindt for his supervision during the physiology analysis and for his support whenever a new aspect of endocrinology baffled me! Thanks must also be given to Dr.

Nadja Wielebnowski who provided the Brookfield data set that allowed us to base the structure of our understanding into the aardwolf mating system. Undoubtedly, the biggest acknowledgement I can give must go to my main supervisor, Dr. Fredrik Dalerum. Fred's kindness when things got very hard at the beginning of the project, his patience, belief in me, dedication, hard work and teaching have allowed me to grow tremendously in the past few years. The steps he facilitated allowed me to achieve the results obtained in this thesis and the standard of work he set will serve me well in the years to come as a scientist. Thank you for looking at numerous drafts and providing the foundation for me to expand my thinking and my skills base.

I would like to thank my mom, Dr. Lorraine Marneweck, for supporting me not only through this project but through the trials that we went through in order to get to this point. Thank you for editing my thesis in such vigorous detail; your comments and suggestions made a big difference. Thank you for your patience, love, care, support and interest in my life; your love has always made things better and easier.

I also would like to thank my wife, Courtney Marneweck, who helped for a long 6 months during the extreme cold of Benfontein nights! Thank you for deciding to take on the position of field assistant on Benfontein for if you had not, we would not have met. Thank you for reading and editing every draft, listening patiently as I rattled off more ideas about aardwolf mating systems and thank you for all your support and understanding during this project.

Finally, I wish to dedicate this thesis to my brother, Steven Marneweck. When times got hard in the field and with the write up, you always calmed and helped me more than words can express. I miss and love you. Never forget.

Table of Contents

Abstract	i
Declaration	iv
Acknowledgements	v
List of tables and figures	x
CHAPTER 1	
General Introduction	1
1.1. Social systems	1
1.2. Social systems in mammalian carnivores	7
1.3. Effects of resource use on carnivore social systems	9
1.4. The aardwolf	10
1.5. Aim and predictions	13
1.6. References	14
CHAPTER 2	
Reproductive endocrinology of zoo-housed aardwolves	23
2.1 Abstract	23
2.2 Introduction	25
2.3 Materials and methods	26
2.3.1. <i>Study animals and animal housing</i>	26
2.3.2. <i>Sample collection and extraction protocol</i>	27
2.3.3. <i>Enzyme immunoassays</i>	28
2.3.4. <i>Biological validations of immunoassays</i>	29
2.3.5. <i>Data analyses</i>	30
2.4 Results	32
2.4.1. <i>Biological validations of immunoassays</i>	32
2.4.2. <i>Variation in fA concentrations</i>	33
2.4.3. <i>Variation in fE and fP concentrations</i>	33

2.4.4. Relationships between fGC concentrations and reproductive hormone metabolite levels	33
2.5 Discussion	34
2.5.1. Temporal variation in reproductive hormone metabolites	35
2.5.2. Relationships between fGC concentrations and reproductive hormone metabolites	37
2.5.3. Conclusions	37
2.6 Acknowledgements	38
2.7 References	38
 CHAPTER 3	
Reproductive seasonality and stress-related adrenocortical hormones in wild aardwolves	50
3.1 Abstract	50
3.2 Introduction	51
3.3 Materials and methods	55
3.3.1. Study Area	55
3.3.2. Aardwolf capture and immobilisation	56
3.3.3. Definition of reproductive seasons	56
3.3.4. Faecal sample collection, extraction protocol and hormone assays	57
3.3.5. Biological validation of immunoassays	60
3.3.6. Statistical analyses	61
3.4 Results	62
3.4.1. Biological validation of EIAs for reproductive hormones	62
3.4.2. Seasonal variation in reproductive hormone metabolite concentrations	62
3.4.3. Seasonal variation in faecal glucocorticoid metabolite concentrations	63
3.4.4. Anecdotal copulation observations	64
3.5 Discussion	64
3.5.1. Reproductive seasonality in wild aardwolves	64
3.5.2. Endocrine correlations to the mating system in wild aardwolves	65
3.5.3. Conclusions	66
3.6 References	67

CHAPTER 4

Behavioural correlates to the aardwolf mating system	80
4.1 Abstract	80
4.2 Introduction	81
4.3 Materials and methods	83
4.3.1. <i>Study area and study animals</i>	83
4.3.2. <i>Spatial and behavioural data collection</i>	84
4.3.3. <i>Revised definition of seasons</i>	85
4.3.4. <i>Estimation of home range size, utilisation and evenness</i>	85
4.3.5. <i>Temporal and spatial patterns in scent marking and time spent foraging</i>	86
4.3.6. <i>Statistical analyses</i>	87
4.4 Results	88
4.4.1. <i>Effects of sex on home range size and utilisation</i>	88
4.4.2. <i>Effects of sex and season on rate of scent marking and foraging</i>	88
4.4.3. <i>Effects of sex and seasons on the spatial distribution of scent marking and foraging</i>	89
4.5 Discussion	89
4.5.1. <i>Conclusions</i>	92
4.6 References	92

CHAPTER 5

Conclusions	105
5.1 References	108
Appendix	112
Common and scientific names of species cited in text	112

List of tables and figures

Chapter 2:

Table 2.1. Life history data of five zoo-housed aardwolves, including variation in housing organisation with conspecifics during the data collection period, as well as sample sizes during each housing arrangement.....44

Table 2.2. Summary of mixed-linear model parameters of fA for two males and fE for two females. Beta values indicates the difference between the nominate month January and consecutive months. Data are from when both the males and the females were housed alone45

Figure 2.1. Longitudinal profiles of fA concentrations in a male (a) and female (b) aardwolf over a 10 month period encompassing observed mating during February 200346

Figure 2.2. Longitudinal profiles of fE (a-c) and fP (d-f) concentrations for three aardwolf females during the study period (Safina: a,d; Mwena: b, e; Mlia: c,f)47

Figure 2.3. Monthly variation (mean \pm 1sd) in fA concentrations for two male aardwolves when housed alone (a), one male aardwolf housed together with a female (b) and monthly variation in fE concentrations for two female aardwolves when housed alone (c) and when housed together with a male (Safina with Mutosi) and a female (Mwena with Mlia) (d).....48

Figure 2.4. Relationships between fA and fGC concentrations in two male aardwolves when housed alone (a) and one male housed together with a female (b), relationships between fE and fGC concentrations in two female aardwolves when housed alone (c) and when housed with a male (Safina with Mutosi) and a female (Mwena with Mlia; d), and the relationship between fP and fGC concentrations in one aardwolf female (e). Regression lines represent parameters from linear (a-d) and non-linear (e) mixed models.....49

Chapter 3:

Table 3.1. Observed mating events, number of litters sired and scat samples collected for seven VHF collared aardwolves on Benfontein Nature Reserve. Scat samples are broken down into the described aardwolf reproductive seasons, where the 2009 parental care season was from Nov 2009-Jan 2010, 2010 parental care season from Nov 2010-Jan 2011 and 2011 parental care season being Nov 2011 77

Figure 3.1. Geographic location of Benfontein Nature Reserve, enclosed in the square box, situated approximately 10km south-east of Kimberley, Northern Cape Province, South Africa. Male aardwolf annual home ranges are represented by solid lines, while female annual home ranges are represented by dashed lines. Home ranges delineated by 100% Minimum Convex Polygons (MCPs)..... 78

Figure 3.2. Seasonal and sex-specific fA (a), fE (b) and fGC (d) hormone metabolite concentrations for male and female wild aardwolves. Seasonal inter-individual means and variances for female fP (c) are also shown. Data was averaged across years for each individual and represented as means across all individuals within sex \pm SE of inter-individual differences in these mean concentrations. Data are expressed as $\mu\text{g/g}$ or ng/g dry organic material. NRP = non-reproductive season, Gest = gestation season, Parental = parental care season. ● = outlier in fA and fGC for females 79

Chapter 4:

Table 4.1. Number of spatial tracks and sessions where behavioural data was recorded for each of the seven aardwolves on Benfontein Nature Reserve 100

Figure 4.1. Seasonal home range sizes (a) and spatial utilisation indices (b) for male (n=4) and female (n=3) aardwolves on Benfontein. Home range sizes were delineated using the 95% MCP method and the evenness of spatial utilisation was calculated with the Shannon spatial diversity index. Data are represented as a mean of individual means \pm SE 102

Figure 4.2. Seasonal scent marking rates (scent marks / 10 minutes: a), foraging rates (foraging bouts / 10 minutes: b) and time spent foraging (proportion of observed time: c) for males (n=4) and female (n=3) aardwolves. Data are represented as individual means \pm SE 103

Figure 4.3. Spatial utilisation intensity (metres of movement tracks per square metre of area) and distance to nearest home range border of locations used for scent marking (a, b) and for foraging (c, d). Utilisation intensity was determined from the intersection of spatial coordinates of scent marking and foraging with the home range utilisation estimate. Home range borders were delineated from 95% MCP's. Data are represented as individual means \pm SE 104

CHAPTER 1

General Introduction

1.1. Social systems

Social systems form the basis of how we describe the organisation of individuals within animal societies. There is a high degree of inter- and intra-specific variation within social systems (Lott 1991). There is also much redundancy and overlap in terminology and hence little congruency in the uniform classifications of different social systems. Much of the vertebrate social system frameworks and analyses have stemmed from studies on apes and monkeys (Whitehead 2008). For example, Kappeler and van Schaik (2002) used social organisation, mating system and social structure as three distinct components when defining primate social systems. Within this framework the social organisation of a species describes the spatio-temporal distribution of individuals within a society; the mating system describes the behavioural interactions associated with mating and their genetic consequences; and the social structure describes the patterns of social interactions and relationships between the members of a society. For the remainder of the thesis we will adopt the theoretical framework from Kappeler and van Schaik (2002) as an appropriate basis for the classification of social systems amongst other mammalian species, carnivores in particular. All three components are closely interrelated, thus it is important to note the differences between each component (Clutton-Brock & Harvey 1978).

1.1.1. Social organisation

Most characterisations of mammalian social organisations have adapted a discrete classification containing three types: solitary, pair living and group living species (Kappeler &

van Schaik 2002; Dalerum 2007). However, these discrete categorical constructions have been overlaid upon a continuous trait of organisations ranging from animals that spend almost all their time solitarily to ones that constantly live in complex social groups (Dalerum 2007). In this context it is also important to note that some species can shift between categories in order to maximise reproductive success; these species are referred to as facultatively social as their organisations are flexible. An example is the male mountain gorilla (*Gorilla gorilla beringei*) that has a highly varied social organisation from one-male to multi-male to all male groups (Robbins 1995).

Solitary social organisation is where spatio-temporal movements of individuals are not synchronised (Charles-Dominique 1978) except during the mating season (Sandell 1989). In these species, female space use is typically determined by the distribution of resources whereas male space use is determined by females' spacing patterns (Bradbury & Vehrencamp 1977; Emlen & Oring 1977; Erlinge & Sandell 1986; Sandell 1989). For example, adult male and female bushbuck (*Tragelaphus scriptus*) walk, forage and rest on their own, with no spatial cohesion between males and females (Waser 1974). However, bushbucks have greatly overlapping home ranges and males search for females during the mating season (Waser 1974). Sometimes males and females of the same species have different social organisations. For example, adult male white rhinoceros (*Ceratotherium simum*) are solitary, whereas females can live in groups with their current offspring and unrelated sub-adults (Owen-Smith 1973; Shrader & Owen-Smith 2002).

A pair living social organisation occurs when there is an association of one adult male and one adult female, where their ranges show strong spatial synchrony even if consistent spatial association is loose between the pair (Kappeler & van Schaik 2002). For example, dwarf antelope and some duikers live in permanent pair bonds with close spatio-temporal associations of the pair where they walk, forage and rest together. Some species living in pairs are not

always closely spatially associated yet they share a common home range. For example, Hilgartner et al. (2012) found that red-tailed sportive lemurs (*Lepilemur ruficaudatus*) were found to live in dispersed pairs, characterised by low cohesion and low encounter rates within a common home range.

A group living social organisation occurs when multiple males and/or females have a high degree of consistent spatial synchrony when foraging, resting, defending resources or mating (Kappeler & van Schaik 2002). For example, African elephants (*Loxodonta africana*) form large groups ranging from 2-24 individuals of a mother and young with her grown daughters accompanied by their offspring (Douglas-Hamilton 1972; Moss & Poole 1983; Moss 1988). All members of the herd synchronise their movement patterns and are seldom more than 50 metres away from their nearest neighbour. In addition, the vast majority of primates live in groups although the amount of fission-fusion and multilevel societies complicates primate social organisation (Kappeler & van Schaik 2002).

1.1.2. Mating system

A mating system refers to the way in which individuals relate to mating partners as well as to the genetic effects of mating activities (Emlen & Oring 1977). Mating systems can be viewed as the outcomes of individual behaviour aimed at maximising reproductive success (Clutton-Brock 1989; Davies 1991). Clutton-Brock (1989) highlighted three important generalisations about animal mating systems. First, mating systems are the outcome of the reproductive strategies of individuals rather than evolved characteristics of species (Bradbury & Vehrencamp 1977; Clutton-Brock & Harvey 1978; Rubenstein & Wrangham 1986; Dunbar 1988). Therefore, variation in mating behaviour is expected within and between populations as a result of the adaptive adjustment of individual female and male behaviour to differences in the social and ecological environment and to variation in individual capabilities (Rubenstein

1980; Dunbar 1981). Second, all social relationships lead to conflicts of interest between individuals which results in diverse forms of competition between mating partners and between parents and offspring (Trivers 1974; Davies 1985). Third, variation in mating systems represent varied forms of mate guarding adapted to the spatio-temporal distribution of receptive females which depend on variation in resource distribution, predation pressure, costs of social living and the activities of other males (Bradbury & Vehrencamp 1977; Emlen & Oring 1977; Clutton-Brock & Harvey 1978; Kruuk & Macdonald 1985; Rubenstein & Wrangham 1986; Wrangham 1987).

Mating systems are typically divided into four main classes (Clutton-Brock 1989; Davies 1991): (1) A single male mates with the same female in several mating attempts (monogamy), (2) a single male mates with the same group of females in successive mating attempts (polygyny), (3) a single female mates with multiple males in successive mating attempts (polyandry) and (4) both males and females mate with numerous partners in successive mating attempts (promiscuity). The males of more than 90% of mammalian species are habitually polygynous (Kleiman 1977; Rutberg 1983) whereas 90% of bird species are habitually monogamous (Lack 1968). There are subsets within each of these four main classes as can be seen for mammals (Figure 1.1 in Clutton-Brock 1989) and for birds (Figure 1.2 in Davies 1991), where males and females employ different reproductive strategies to maximise individual reproductive success. The two most widely used classifications, by Clutton-Brock (1989) and Davies (1991), base their frameworks on female spacing patterns and levels of paternal care. However, both classifications omit a special case of polygyny, where groups of males and groups of females mate with different groups of females and males, respectively, in successive reproductive attempts (polygynandry). In addition, some species have a high degree of mating system variation. For example, the Red Sea damselfish (*Dascyllus aruanus*) employs

three different mating systems; monogamy, polygyny and polygynandry depending on the size of the coral heads where they live (Fricke 1977).

Genetic mating system studies in birds have revealed a high degree of extra-pair paternities (EPPs: Griffith et al. 2002). Contrary to prior expectations (e.g., Lack 1968) birds are rarely sexually monogamous with extra-pair offspring found in 86% of species (Griffith et al. 2002). Therefore, the genetic consequences of actual mating activities cannot simply be predicted from observations of social bonds. Why should a species maintain a socially monogamous pair bond while mating promiscuously? Social mating system components are understood by social and/or spatial arrangement of individuals and the necessity for paternal care, while genetic mating systems refer to situations where molecular analyses can resolve the parental outcomes of mating activities (Reichard 2003). The differences between social and genetic mating system components arise from benefits of utilising a different strategy for each component. For females, genetic promiscuity may allow for increased sperm competition permitting highest quality offspring (e.g., Birkhead 1998), whereas for males it may allow increased opportunities for paternities with limited extra costs of caring for young. However, a study on dark-eyed juncos (*Junco hemalis*) showed that both sexes gain an advantage in promiscuous mating by producing offspring with higher lifetime fitness (Gerlach et al. 2012).

1.1.3. Social structure

Social structures are formed by the nature, frequency and intensity of relationships between individuals both within and between social units (Lott 1991; Kappeler & van Schaik 2002; Whitehead 2008). Although social organisations and mating systems have been extensively described, quantified and classified, the social structures of animal societies have not been subjected to rigorous definitions. This is probably caused by difficulties in observing and quantifying the nature of relationships between individuals, particularly in solitary species.

A first distinction between groups with different social structures can be made between social groups versus social aggregations. In social groups there are explicit relationships among individuals whereas such individual relationships are typically lacking in social aggregations. For example, in warthogs (*Phacochoerus aethiopicus*), groups are usually comprised of females and their young of the year where adult males are only permitted during oestrous (Cumming 1975). These are matriarchal groups with close social bonds between group members. In contrast, social aggregations of white rhinoceros around a water source (e.g., Owen-Smith 1973) provide an example of a group of animals that show no direct social relationships among them, while still tolerating each other's presence with almost no agonistic interactions.

Within societies with defined social associations, dominance relationships often develop where some individuals monopolise access to resources and thus increase fitness. These resources can be mates, food and/or water. Despite dominance hierarchies being overlaid from human observed inter-relationships, interactions tend to be pair-wise where hierarchies are not always biologically relevant. Further, numerous examples exist where one individual is more dominant than all others. For example, in the hippopotamus (*Hippopotamus amphibius*), a single male is typically dominant over all other adult males as well as over other females and their offspring (Karstad & Hudson 1986). This is an example of a despotic society. In egalitarian societies, on the other hand, dominance relationships do not exist or are very weak so that all individuals are of equal social status with equal reproductive opportunities (e.g., banded mongoose [*Mungos mungo*], De Luca & Ginsberg 2001). Most species, however, tend to fall along a continuum from completely despotic to completely egalitarian societies. Therefore, it seems justified to think of classifying social structure along a flexible scale ranging from truly egalitarian societies to societies with a highly defined dominance structure.

1.2. Social systems in mammalian carnivores

Carnivora is a mammalian order which consists of 271 species that are divided among 13 families. Much attention has been paid to certain families of the Carnivora (e.g., *Canidae* and *Felidae*) where many studies have revealed interesting variations in behaviour, morphology and ecology leading to a high variation in sociality (Bekoff et al. 1984). The social system exhibited by a single species can vary due to the ecological constraints within local areas (Emlen & Oring 1977). For example, grey wolves (*Canis lupus*) live solitarily, in pairs or in large packs and have home ranges that differ 50-100 fold between populations (Mech 1970; Zimen 1981; Harrington & Pacquet 1982).

Inter-specific variation in carnivore social organisations is high (Macdonald 1983; Bekoff et al. 1984; Gittleman 1989; Sandell 1989). For example, while some carnivore species are exclusively solitary (e.g., most felid, ursid, and mustelid species), others are pair living (e.g., most fox-like canid species). In addition, some carnivore species form large cohesive social groups of cooperatively behaving individuals such as the African lion (*Panthera leo*), African wild dog (*Lycaon pictus*), spotted hyena (*Crocuta crocuta*), Eurasian badger (*Meles meles*), coyote (*Canis latrans*), dwarf mongoose (*Helogale parvula*) and suricate (*Suricata suricata*). Carnivore social organisations are not best described as a dichotomy consisting of solitary or group living species as most species seem to fall within a continuum of social organisations, varying from solitary to group living social organisations (Dalerum 2007).

Generally, solitary carnivores have either a polygynous (e.g., most viverrids, Bekoff et al. 1984) or promiscuous mating system (e.g., most felid species, Eaton 1973, 1974, 1976, 1977; Gittleman 1985). All canids tend to have a monogamous mating system (Kleiman & Eisenberg 1973) although Wright et al. (2010) found 9.8% of successful EPPs from extra-pair copulations (EPCs) in bat-eared foxes (*Otocyon megalotis*), showing that genetic promiscuity exists in some behaviourally monogamous canids. Komers and Brotherton (1997) showed that

neither the degree of biparental care nor the ability of males to monopolise females were indicators of monogamy. Instead, female space use was found as the best predictor of monogamy. Polyandry is rare in the Carnivora, like in other mammal groups, although it occurs in the striped hyena (*Hyaena hyaena*, Wagner et al. 2008). There are exceptions to these broad mating systems for the Carnivora, for example, polygynandry in the African lion. A single species may also employ multiple mating strategies (e.g., swift fox [*Vulpes velox*] Kitchen et al. 2006) highlighting the complexity of defining carnivore mating systems.

Social structure in Carnivora has been poorly documented for solitary species as they tend to be small, nocturnal and shy (Bekoff et al. 1984). Therefore, the majority of empirical data on carnivore social structure has stemmed from group living carnivores (but see Gehrt & Fritzell 1998 for an exception), where observing and interpreting social interactions is easier and more frequent. For example, in the spotted hyena interactions are in the form of a competitive system in which access to kills, mating opportunities and male dispersal age depend on individuals dominating other members of the clan, where intra- and inter-sexual competition exists (Kruuk 1972; Tilson & Hamilton 1984; Frank 1986). Interestingly, female spotted hyenas are more aggressive, larger and more dominant than all other clan males as a result of similar levels of testosterone in both sexes (Dloniak et al. 2004; Dloniak et al. 2006a; Dloniak et al. 2006b). However, among females there is a clear dominance structure with a single matriarch claiming the largest share of kills with her cubs gaining privileges over lower classed cubs. Most group living carnivores are despotic but there are examples of egalitarian structure in some social species (e.g., banded mongoose; De Luca & Ginsberg 2001). Solitary carnivores can be expected to be weakly despotic due to low spatio-temporal cohesion between individuals. However, in brown bears (*Ursus arctos*) there is a strict dominance hierarchy that dictates where and when each individual has access around salmon-spawning runs (Stonorov & Stokes 1972; Pullianinen et al. 1984; Mattson et al. 1991; Craighead et al. 1995).

1.3. Effects of resource use on carnivore social systems

Resources tend to fluctuate both spatially and temporally in their abundance, availability and distribution. Carnivores have adapted their behaviour to that of the resources they utilise (Wrangham & Rubenstein 1986). For instance, ursids exhibit a wide variation of social organisations that can be attributed to the distribution, density, and temporal availability of food. Polar bears (*Ursus maritimus*) have very large (50 000-250 000km²), loosely defined and broadly overlapping home ranges as a response to living in an environment where habitat (the pack ice) and food availability are transitory (Ramsay & Stirling 1986; Garner et al. 1990; Ferguson et al. 1997). Similarly, brown bears eat a variety of widely dispersed foods and thus have large non-exclusive home ranges (females: 281-2434km²; males: 874-8171km², LeFranc et al. 1987; Mace & Waller 1997; Dahle & Swenson 2003). However, giant pandas (*Ailuropoda melanoleuca*) are highly specialised with 99% of their diet comprised of bamboo and thus have small (males and females: 5-15km²) and highly overlapping ranges (Hu et al. 1985). The sloth bear (*Melursus ursinus*) is also highly specialised but to a myrmecophagous diet with small (females: 2km², males: 4km² in Sri Lanka and 25-100km² in Panna National Park, India) and extensively overlapping seasonal home ranges (Joshi et al. 1999). In the hyaenids, striped hyenas are solitary, have medium sized ranges (females: 44-68km²; males: 72-81km²) with high inter-sexual overlap and scavenge almost all food resources from other carnivores (Kruuk 1976; Wagner et al. 2008). On the other hand, spotted hyenas have large home ranges (up to 1500km² in the Kalahari National Park, Mills 1990) and often live in large groups in order to successfully hunt large and diverse prey species (Kruuk 1972).

The spatio-temporal distribution of resources, the defendability of these resources, the availability of mates and thereby the potential for polygamy are important factors determining mating systems (Emlen & Oring 1977; Clutton-Brock & Harvey 1978; Clutton-Brock 1989). For example, brown bears have a two month mating period, are solitary and occur in low

densities. Therefore it is impossible for males to defend females (Clutton-Brock 1989) and brown bears subsequently exhibit polygyny or promiscuity (Craighead et al. 1995). Further, carnivores that specialise on seasonal resources tend to breed cooperatively with paternal care being necessary. For example, bat-eared foxes are insectivore specialists and experience periods of resource depletion. Successively, there is a period of high resource availability where they forage intensively to replenish reserves. Offspring are small and unable to travel with the mother to acquire these necessary resources. Therefore, the necessity for paternal care while the mother is foraging is fundamental for offspring survival in this socially monogamous carnivore (Lamprecht 1979; Malcolm 1986; Wright 2004; Wright et al. 2010).

1.4. The aardwolf

1.4.1. Morphology

The aardwolf (*Proteles cristata*) is a small hyaenid with a slender build, sloping back, long legs with vertical brown torso stripes and horizontal stripes on the forelimbs. There is little sexual dimorphism in aardwolves with both sexes weighing approximately 8-12kg and being 40-50cm in height. Aardwolves have a long mane (16-20cm) and bushy tail that when the hairs are erected is used to signal aggression (Koehler & Richardson 1990). Like other hyaenids, aardwolves have an anal gland that they use to scent mark grass stalks via pasting a yellow-orange secretion in various parts of their territories (Kruuk & Sands 1972; Nel & Bothma 1983; Richardson 1985; Koehler & Richardson 1990; Sliwa 1996; Sliwa & Richardson 1998).

1.4.2. Distribution

The aardwolf has a discontinuous distribution, favouring the semi-arid grasslands in eastern and southern Africa (Koehler & Richardson 1990). Within these areas, aardwolves live in old

aardvark (*Orycteropus afer*), porcupine (*Hystrix africaeaustralis*) and springhare (*Pedetes capensis*) burrows and utilise different dens at various intensities throughout the year (Kotze et al. 2012). Aardwolf distribution is highly regulated by the availability and abundance of termites. They therefore prefer open, dry habitats with short grass, especially areas that have been overgrazed by ungulates (Nel & Bothma 1983).

1.4.3. Diet and resource use

The aardwolf is an extreme resource specialist that feeds almost exclusively on a single genus of termites, *Trinervitermes* spp. (Richardson 1987a; Koehler & Richardson 1990). For example, in east Africa, Kruuk and Sands (1972) found that 97.5% of the 79 collected faecal samples were comprised of a single species of termites, *Trinervitermes bettonianus*. In fact, *T. bettonianus* was the sole species present in 46% of samples. Kruuk and Sands (1972) also found that the aardwolf diet becomes only slightly more varied in the wet season. Termite resource availability fluctuates seasonally, where in the cold winter months termite abundance is decreased but peaks in the wet summer months.

1.4.4. Social organisation

Aardwolves were first thought to have a solitary social organisation. However, detailed studies have revealed that aardwolves have a dispersed pair living social organisation, where an adult male and female share a perennial territory although the pair is spatially cohesive only during mating periods (Nel & Bothma 1983; Koehler & Richardson 1990; Richardson 1987b; Sliwa 1996). Further, both home range and behaviour alters according to season (Kruuk & Sands 1972; Bothma & Nel 1980; Richardson 1985; Sliwa 1996). For example, aardwolves increase the rate of scent marking along the edge of their range during the mating season to maintain

communication with conspecifics in and around their home range (Kruuk & Sands 1972; Skinner & van Aarde 1986; Sliwa 1996; Sliwa & Richardson 1998).

1.4.5. Mating system

Aardwolves are seasonal breeders and mate during the dry winter months of decreased resource availability (Richardson 1987a; Koehler & Richardson 1990; Sliwa 1996). Sliwa (1996) found the onset of the breeding season differed between three consecutive years due to variation in resource availability although the duration of the breeding season was one month in all three years. All three breeding seasons overlapped in July, indicating a degree of oestrous synchrony in females. During this period, males increased scent marking rates and expanded their home ranges to find receptive females (Sliwa 1996). Richardson (1985) and Koehler and Richardson (1990) described the aardwolf as behaviourally monogamous due to their pair living social organisation. In addition, paternal care is necessary for offspring survival where males spent up to six hours each night on guard at the den while females foraged to replenish their reserves (Richardson 1987a; Richardson & Coetzee 1988; Sliwa 1996). However, aardwolves are sexually promiscuous (Richardson 1987a; Richardson 1987b; Sliwa 1996), and Sliwa (1996) found that between 62-72% of all copulations observed between aardwolves were EPCs. However, without genetic markers it cannot be confirmed that EPCs translate into EPPs. More recently, Kotze et al. (2012) suggested that the temporal utilisation of dens in aardwolves corresponded more closely to polygamous mating patterns than social monogamy.

1.4.6. Social structure

Sliwa and Richardson (1998) experimentally translocated scent marks from known males and females into the territories of other known aardwolves. Over-marking occurred significantly more in inter-sexual than intra-sexual scent marks in the mating season. Once a resident

aardwolf discovered the “inturder’s” scent mark it went to the border of the territory and increased its scent marking rate. These results showed that there is a highly territorial system within aardwolves, with scent marks acting as behavioural cues in communicating territorial ownership and social dominance (e.g., Johnson 1973; Brown 1979; Müller-Schwarze & Silverstein 1983; Brown & Macdonald 1985). Generally, pair-wise interactions between adults entail erecting their manes when approached and avoidance by both individuals, making assumptions about social structure difficult in this species. When interactions do become agonistic, aardwolves chase each other until one retreats. When fighting occurs they sit on their behinds, face to face, and bite at each other’s necks (Richardson 1985; Richardson 1987a; Koehler & Richardson 1990; Sliwa 1996).

1.5. Aim and predictions

The aim of this project was to test if behavioural and physiological variables related to the aardwolf mating system corresponded to predictions based on aardwolves’ extreme diet specialisation and to test if they correlated with expectations from a behavioural monogamous mating system. Therefore, based on the aardwolf’s extreme specialisation on a resource that undergoes drastic seasonal fluctuations but that is uniformly distributed, we can predict the following with respect to variables of mating system components:

1. Strong seasonality in reproductive activity and related behavioural and physiological parameters.
2. Symmetry between males and females in terms of their endocrine physiology, home range size and space use due to similarities expected for a pair living, behaviourally monogamous species.

1.6. References

- Bekoff, M., Daniels, T. J. & Gittleman, J. L. 1984. Life history patterns and the comparative social ecology of carnivores. *Annual Review of Ecology and Systematics* 15: 191-232
- Birkhead, T. R. 1998. Sperm competition in birds: mechanisms and function. In: Birkhead, T. R. & Möller, A. P. (Eds.), *Sperm Competition and Sexual Selection*. Academic Press, London, UK, pp. 579-622
- Bothma, J. P. & Nel, J. A. J. 1980. Winter food and foraging behaviour of the aardwolf *Proteles cristatus* in the Namib-Naukluft Park. *Madoqua* 12: 141-145
- Bradbury, J. W. & Vehrencamp, S. L. 1977. Social organization and foraging in emballonurid bats III: Mating systems. *Behavioral Ecology and Sociobiology* 2: 1-17
- Brown, R. E. 1979. Mammalian social odors: a critical review. *Advances in the Study of Behavior* 10: 103-162
- Brown, R. E. & Macdonald, D. W. 1985. *Social Odours in Mammals*. Oxford Clarendon Press, Oxford, UK
- Charles-Dominique, P. 1978. Solitary and gregarious prosimians: Evolution of social structures in primates. In: Chivers, D. J. & Joysey, K. A. (Eds.), *Recent Advances in Primatology, Vol. 3. Evolution*. Academic Press, New York, USA, pp. 139-149
- Clutton-Brock, T. H. 1989. Mammalian mating systems. *Proceedings of the Royal Society of London, B: Biological Sciences* 236: 339-372
- Clutton-Brock, T. H. & Harvey, P. H. 1978. Mammals, resources and reproductive strategies. *Nature* 17: 191-195
- Craighead, J. J., Summer, J. S. & Mitchell, J. A. 1995. *The grizzly bears of Yellowstone: their ecology in the Yellowstone Ecosystem, 1959-1992*. Island Press, Washington DC, USA

- Cumming, D. H. 1975. *A field study of the ecology and behaviour of Warthog*. Trustees of the National Museums and Monuments of Rhodesia, Harare, Zimbabwe
- Dahle, B. & Swenson, J. E. 2003. Seasonal range size in relation to reproductive strategies in brown bears *Ursus arctos*. *Journal of Animal Ecology* 71: 660-667
- Dalerum, F. 2007. Phylogenetic reconstruction of carnivore social organizations. *Journal of Zoology* 273: 90-97
- Davies, N. B. 1985. Cooperation and conflict among dunnocks, *Prunella modularis*, in a variable mating system. *Animal Behaviour* 33: 628-648
- Davies, N. B. 1991. Mating systems. In: Krebs, J. R. & Davies, N. B. (Eds.), *Behavioural Ecology: An evolutionary approach, 3rd Edition*. Blackwell Scientific Publications, Oxford, UK, pp. 263-294
- De Luca, D. W. & Ginsberg, J. R. 2001. Dominance, reproduction and survival in banded mongooses: towards an egalitarian social system? *Animal Behaviour* 61: 17-30
- Dloniak, S. M., French, J. A., Place, N. J., Weldele, M. L., Glickman, S. E. & Holekamp, K. E. 2004. Non-invasive monitoring of fecal androgens in spotted hyenas (*Crocuta crocuta*). *General and Comparative Endocrinology* 135: 51-61
- Dloniak, S. M., French, J. A. & Holekamp, K. E. 2006a. Rank-related maternal effects of androgens on behaviour in wild spotted hyenas. *Nature* 440: 1190-1193
- Dloniak, S. M., French, J. A. & Holekamp, K. E. 2006b. Faecal androgen concentrations in adult male spotted hyenas, *Crocuta crocuta*, reflect interactions with socially dominant females. *Animal Behaviour* 71: 27-37
- Douglas-Hamilton, I. 1972. On the ecology and behaviour of the African elephant: Elephants of Lake Manyara. D.Phil. thesis. University of Oxford, Oxford, UK
- Dunbar, R. I. M. 1981. Intraspecific variations in mating strategy. In: Klopfer, P. & Bateson, P. (Eds.), *Perspectives in Ethology, Vol. 5*. Plenum Press, New York, USA, pp. 385-431

- Dunbar, R. I. M. 1988. *Primate social systems*. Croom Helm, Beckenham, UK
- Eaton, R. L. 1973. *The World's Cats: Ecology and Conservation*. Feline Research Group, Seattle, USA
- Eaton, R. L. 1974. *The World's Cats: Biology, Behavior and Management of Reproduction*. Feline Research Group, Seattle, USA
- Eaton, R. L. 1976. *The World's Cats: Contributions to Biology, Ecology, Behavior and Evolution*. Carnivore Research Institute, Seattle, USA
- Eaton, R. L. 1977. *The World's Cats: Contributions to Breeding Biology, Behavior and Husbandry*. Carnivore Research Institute, Seattle, USA
- Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection and the evolution of mating systems. *Science* 197: 215-223
- Erlinge, S. & Sandell, M. 1986. Seasonal changes in the social organization of male stoats, *Mustela erminea*: an effect of shifts between two decisive resources. *Oikos* 47: 57-62
- Ferguson, S. H., Taylor, M. K. & Messier, F. 1997. Space-use of polar bears in and around Auyuittuq National Park, North-west Territories, during the ice-free period. *Canadian Journal of Zoology* 75: 1585-1594
- Frank, L. G. 1986. Social organization of the spotted hyaena (*Crocuta crocuta*) II: Dominance and reproduction. *Animal Behaviour* 34: 1510-1527
- Fricke, H. W. 1977. Community structure, social organization, and ecological requirements of coral reef fish (Pomacentridae). *Helgoländer Wissenschaftliche Meeresuntersuchungen* 30: 412-426
- Garner, G. W., Knick, S. T. & Douglas, D. C. 1990. Seasonal movements of adult female polar bears in the Bearing and Chuckchi seas. *International Conference on Bear Research and Management* 8: 219-226

- Gehrt, S. D. & Fritzell, E. K. 1998. Resource distribution, female home range dispersion and male spatial interactions: group structure in a solitary carnivore. *Animal Behaviour* 55: 1211-1227
- Gerlach, N. M., McGlothlin, J. W., Parker, P. G. & Ketterson, E. D. 2012. Promiscuous mating produces offspring with higher lifetime fitness. *Proceedings of the Royal Society of London, B: Biological Sciences* 279: 860-866
- Gittleman, J. L. 1985. Functions of communal care in mammals. In: Greenwood, P. J. & Slatkin, M. (Eds.), *Evolution: Essays in Honour of John Maynard Smith*. Cambridge University Press, Cambridge, UK, pp. 187-205
- Gittleman, J. L. 1989. Carnivore group living: comparative trends. In: Gittleman, J. L. (Ed.), *Carnivore Behavior, Ecology and Evolution, Vol. 1*. Cornell University Press, New York, USA, pp. 183-207
- Griffith, S. C., Owens, I. P. F. & Thuman, K. A. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology* 11: 2195-2212
- Harrington, F. H. & Pacquet, P. C. 1982. *Wolves of the World: Perspectives of behaviour, Ecology and Conservation*. Noyes Publications, New Jersey, USA
- Hilgartner, R., Fichtel, C., Kappeler, P. M. & Zinner, D. 2012. Determinants of pair-living in red-tailed sportive lemurs (*Lepilemur ruficaudatus*). *Ethology* 118: 466-479
- Hu, J., Schaller, G. B., Pan, W. & Zhu, J. 1985. *The giant panda of Wolong*. Sichuan Science and Technology Publishing House, Chengdu, China
- Johnson, R. P. 1973. Scent marking in mammals. *Animal Behaviour* 21: 521-535
- Joshi, A. R., Smith, J. L. D. & Garshelis, D. L. 1999. Sociobiology of the myrmecophagous sloth bear in Nepal. *Canadian Journal of Zoology* 77: 1690-1704
- Kappeler, P. M. & van Schaik, C. P. 2002. Evolution of primate social systems. *International Journal of Primatology* 23: 707-740

- Karstad, E. L. & Hudson, R. J. 1986. Social organization and communication of riverine hippopotami in southwestern Kenya. *Mammalia* 50: 153-164
- Kitchen, A. M., Gese, E. M., Waits, L. P., Karki, S. M. & Schauster, E. R. 2006. Multiple breeding strategies in the swift fox, *Vulpes velox*. *Animal Behaviour* 71:1029-1038
- Kleiman, D. G. 1977. Monogamy in mammals. *Quarterly Review of Biology* 52: 39-69
- Kleiman, D. G. & Eisenberg, J. 1973 Comparison of canid and felid social systems from an evolutionary perspective. *Animal Behaviour* 21: 637-659
- Koehler, C. E. & Richardson, P. R. K. 1990. *Proteles cristatus*. *Mammalian Species* 363: 1-6
- Komers, P. E. & Brotherton, P. N. M. 1997. Female space use is the best predictor of monogamy in mammals. *Proceedings of the Royal Society of London, B: Biological Sciences* 264: 1261-1270
- Kotze, R., Bennett, N., Cameron, E., De Vries, L., Marneweck, D., Pirk, C. & Dalerum, F. 2012. Temporal patterns of den use suggest polygamous mating patterns in the socially monogamous aardwolf. *Animal Behaviour* 84: 1573-1578
- Kruuk, H. 1972. *The spotted hyena: a study of predation and social behaviour*. University of Chicago Press, Chicago, USA
- Kruuk, H. 1976. Feeding and social behaviour of the striped hyaena (*Hyaena vulgaris Desmarest*). *East African Wildlife Journal* 14: 91-111
- Kruuk, H. & Sands, W. A. 1972. The aardwolf (*Proteles cristatus*, Sparrman 1783) as a predator of termites. *East African Wildlife Journal* 10: 211-227
- Kruuk, H. & Macdonald, D. 1985. Group territories of carnivores: empires and enclaves. In: Sibly, R. M. & Smith, R. H. (Eds.), *Behavioural Ecology*. Blackwell Publishing Press, Oxford, UK, pp. 521-536
- Lack, D. 1968. *Ecological Adaptations for Breeding in Birds*. Methuen Publishing Ltd, London, UK

- Lamprecht, J. 1979. Field observations on the behavior and social system of the bat-eared fox *Otocyon megalotis*. *Zeitschrift für Tierpsychologie* 49:260-284
- Lefranc, M. N., Moss, M. B., Patnode, K. A. & Sugg, W. C. 1987. *Grizzly Bear Compendium*. Interagency Grizzly Bear Committee, National Wildlife Federation, Washington DC, USA
- Leyhausen, P. 1965. The communal organization of solitary mammals. *Symposia of the Zoological Society of London* 14: 249-263
- Lott, D. F. 1991. *Intraspecific variation in the social systems of wild vertebrates*. Cambridge University Press, Cambridge, UK
- Macdonald, D. W. 1983. The ecology of carnivore social behaviour. *Nature* 301: 379- 84
- Mace, R. D. & Waller, J. S. 1997. Spatial and temporal interaction of male and female grizzly bears in northwestern Montana. *Journal of Wildlife Management* 61: 39-52
- Malcolm, J. R. 1986. Socio-ecology of bat-eared foxes *Otocyon megalotis*. *Journal of Zoology* 208: 457-467
- Mattson, D. J., Blanchard, B. M. & Knight, R. R. 1991. Food habits of Yellowstone grizzly bears, 1977-1987. *Canadian Journal of Zoology* 69: 2430-2435
- Mech, L. D. 1970. *The Wolf: The Ecology and Behavior of an Endangered Species*. Doubleday Publishing, New York, USA
- Mills, M. G. L. 1990. *Kalahari Hyenas: the Behavioural Ecology of Two Species*. Unwin Hyman Publishers Ltd, London, UK
- Moss, C. J. 1988. *Elephant Memories*. University of Chicago Press, Chicago, USA
- Moss, C. J. & Poole, J. H. 1983. Relationships and social structure of African elephants. In: Hinde, R. A. (Ed.), *Primate Social Relationships: an Integrated approach*. Black-well Scientific Publications, London, UK, pp. 315-325

- Müller-Schwarze, D. & Silverstein, R. M. 1983. *Chemical Signals in Vertebrates 3*. Plenum Publishing, New York, USA
- Nel, J. A. J. & Bothma, L. Du P. 1983. Scent marking and midden use by aardwolves (*Proteles cristatus*) in the Namib Desert. *African Journal of Ecology* 21: 25-39
- Owen-Smith, R. N. 1973. The behavioural ecology of the white rhinoceros. Ph.D. thesis, University of Wisconsin, Wisconsin, USA
- Puliainen, E., Luukkonen, Y. & Hietajärvi, T. 1984. Formation of a dominance hierarchy among wild brown bears (*U. arctos*). *Zeitschrift für Säugetierkunde* 49: 58-59
- Ramsay, M. A. & Stirling, I. 1986. On the mating system of polar bears. *Canadian Journal of Zoology* 64: 2142-2151
- Reichard, U. H. 2003. Monogamy: past and present. In: Reichard, U. H. & Boesch, C. (Eds.), *Monogamy: mating strategies and partnerships in birds, humans and other mammals*. Cambridge University Press, Cambridge, UK, pp. 3-25
- Richardson, P. R. K. 1985. The social behaviour and ecology of the aardwolf, *Proteles cristatus*, (Sparman, 1783) in relation to its food resources. Ph.D. thesis, University of Oxford, Oxford, UK
- Richardson, P. R. K. 1987a. Aardwolf mating system: overt cuckoldry in an apparently monogamous mammal. *South African Journal of Science* 83: 405-410
- Richardson, P. R. K. 1987b. Food-consumption and seasonal-variation in the diet of the aardwolf *Proteles cristatus* in Southern Africa. *Zeitschrift für Säugetierkunde* 52: 307-325
- Richardson, P. R. K. & Coetzee, M. 1988. Mate desertion in response to female promiscuity in the socially monogamous aardwolf *Proteles cristatus*. *South African Journal of Zoology* 23: 306-308

- Robbins, M. M. 1995. A demographic analysis of male life history and social structure of mountain gorillas. *Behaviour* 132: 21-47
- Rubenstein, D. I. 1980. On the evolution of alternative mating strategies. In: Staddon, J. E. R. (Ed.), *Limits to Action*. Academic Press, London, UK, pp. 65-100
- Rubenstein, D. I. & Wrangham, R. W. 1986. Socioecology: origins and trends. In: Rubenstein, D. I. & Wrangham, R. W. (Eds.), *Ecological aspects of social evolution*. Princeton University Press, New Jersey, USA, pp. 3-20
- Rutberg, A. T. 1983 The evolution of monogamy in primates. *Journal of Theoretical Biology* 104: 93-112
- Sandell, M. 1989. The mating tactics and spacing patterns of solitary carnivores. In: Gittleman, J. L. (Ed.) *Carnivore Behavior, Ecology and Evolution*. Cornell University Press, New York, USA, pp. 164-182
- Schaller, G. B. 1972. *The Serengeti Lion: a Study of Predator-Prey Relations*. Chicago University Press, Chicago, USA
- Shrader, A. M. & Owen-Smith, N. 2002. The role of companionship in the dispersal of white rhinoceros (*Ceratotherium simum*). *Behavioral Ecology and Sociobiology* 52: 255-261
- Skinner, J. D. & van Aarde, R. J. 1986. The use of space by the aardwolf *Proteles cristatus*. *Journal of Zoology* 209: 299-301
- Sliwa, A. 1996. A functional analysis of scent marking and mating behaviour in the aardwolf, *Proteles cristatus* (Sparrman, 1783). Ph.D. thesis, University of Pretoria, Pretoria, RSA
- Sliwa, A. & Richardson, P. R. K. 1998. Responses of aardwolves, *Proteles cristatus*, Sparrman 1783, to translocated scent marks. *Animal Behaviour* 56: 137-146
- Stonorov, D. & Stokes, A. 1972. Social behaviour of the Alaska brown bear. In: Herrero, S. (Ed.) *Bears - Their Biology and Management: Proceedings of the Second International*

- Conference on Bear Research and Management*. International Union for the Conservation of Nature and Natural Resources, Alberta, Canada, pp. 232-242
- Tilson, R. L. & Hamilton, W. J. 1984. Social dominance and feeding patterns of spotted hyaenas. *Animal Behaviour* 32: 715-24
- Trivers, R. L. 1974. Parent-offspring conflict. *American Zoologist* 14: 249-264
- Wagner, A. P., Frank, L. G. & Creel, S. 2008. Spatial grouping in behaviourally solitary striped hyaenas, *Hyaena hyaena*. *Animal Behaviour* 75: 1131-1142
- Waser, P. M. 1974. Spatial associations and social interactions in a “solitary” ungulate: the bushbuck (*Tragelaphus scriptus*). *Zeitschrift für Tierpsychologie* 37: 24-36
- Whitehead, H. 2008. *Analyzing animal societies: Quantitative methods for vertebrate social analysis*. Chicago University Press, Chicago, USA
- Wrangham, R. W. 1987. Evolution of social structure. In: Smuts, B. B., Cheney, D. L., Seyfarth, R. M., Wrangham, R. W. & Struhsaker, T. T. (Eds.), *Primate societies*. University of Chicago Press, Chicago, USA, pp. 282-298
- Wrangham, R. W. & Rubenstein, D. I. 1986. Ecological aspects of social evolution. In: Rubenstein, D. I. & Wrangham, R. W. (Eds.), *Social evolution in birds and mammals*. Princeton University Press, New Jersey, USA, pp. 452-470
- Wright, H. W. Y. 2004. Monogamy in the bat-eared fox, *Otocyon megalotis*. Ph.D. thesis, University of Warwick, Coventry, UK
- Wright, H. W. Y, Gray, M. M., Wayne, R. K. & Woodroffe, R. B. 2010. Mating tactics and paternity in a socially monogamous canid, the bat-eared fox (*Otocyon megalotis*). *Journal of Mammalogy* 91: 446-2010
- Zimen, E. 1981. *The Wolf: A Species in Danger*. Delacorte Press, New York, USA

CHAPTER 2

Reproductive endocrinology of zoo-housed aardwolves

Accepted and published in: Acta Theriologica, Online early, DOI:

10.1007/s13364-012-0101-1

David G. Marneweck, Andre Ganswindt, Stephanie Rhodes, Astrid Bellem, Jocelyn Bryant, Nadja Wielebnowski, Fredrik Dalerum

D. G. Marneweck, A. Ganswindt, F. Dalerum

Mammal Research Institute, Department of Zoology and Entomology, Private Bag X20, University of Pretoria, Pretoria 0028, South Africa

A. Ganswindt

Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

S. Rhodes

Animal Programs, Chicago Zoological Society, 3300 Golf Road, Brookfield, IL 60513, USA

A. Bellem, J., Bryant, N. Wielebnowski

Department of Conservation Science, Chicago Zoological Society, 3300 Golf Road, Brookfield, IL 60513, USA

F. Dalerum, Correspondence

Centre for Wildlife Management, Hatfield Experimental Farm, Private bag X20, University of Pretoria, Pretoria 0028, South Africa

E-mail: fredrik.dalerum@zoology.up.ac.za; Tel.: +27 12 420 2627; Fax: +27 12 420 6096

2.1 Abstract

Knowledge regarding the relationship between endocrine parameters and reproductive activity can offer important insights into how social and environmental factors influence the reproductive success of mammals. Although components of both the physical and social environment affect endocrine regulation of reproduction, less is understood about the potential

role of interactions between different endocrine axes on reproductive activity. We evaluated temporal patterns of reproductive and adrenocortical steroids in two male and three female aardwolves (*Proteles cristata*) housed in captivity at Brookfield Zoo, Chicago, IL, USA. We found seasonal variation in faecal androgens, estrogens, and progestagens, which provide support for previous observations of the aardwolf as a seasonal breeder. However, the timing of peak endocrine activity did not correspond to observations from wild populations. Our interpretation is that this discrepancy is caused by photoperiodic regulation of reproductive activity. We found a positive relationship between faecal androgens and faecal glucocorticoid metabolites in males and a positive relationship between faecal estrogens and faecal glucocorticoid metabolites in females when housed with conspecifics but not when housed alone. We also found a positive but asymptotic relationship between faecal progestagens and faecal glucocorticoid metabolites. We argue that these observations indicate a potential effect of reproductive endocrine activity on the hypothalamic–pituitary–adrenal axis, which could result in interesting physiological trade-offs in male reproductive tactics and female pre-partum maternal investment because of the negative effects of long-term glucocorticoid elevation on reproductive performance. Finally, our results suggest that social and environmental factors interact in regulating many aspects of endocrine fluctuations in this mostly solitary species.

Key Words: *Aardwolf, Hypothalamic–pituitary–adrenal axis, Seasonality, Stress, Reproductive timing*

2.2 Introduction

Knowledge of how social and environmental factors influence animal reproductive physiology is important for our ability to understand the regulation of reproductive success. Reproductive activity in mammals is often either seasonal or non-seasonal, with subsequent different longitudinal patterns of reproductive hormones. In non-seasonal breeders, females exhibit ovulatory cycles and males are typically reproductively active throughout the year (Brown 2006). Contrarily, in seasonally breeding species reproductive hormones are elevated but highly variable during a defined period of time in both males and females (Monfort et al. 1989; Wingfield 1990; Monfort et al. 1997; Kraaijeveld-Smit et al. 2002; Kretzschmar et al. 2004; Hesterman et al. 2005; Dloniak et al. 2006b; Fanson et al. 2010a, b). The ultimate causes for seasonal breeding strategies are linked to temporal variation in resource abundance, whereas the most important proximate regulator of reproductive activity is photoperiod (Scott 1986; Goldman 1999), typically with the relative rate of change in daylight patterns being more important than the photoperiod per se (McAllan and Dickman 1985). However, social factors may also influence such environmental regulation of circannual hormonal rhythms (Scott 1986).

The physiological stress response is primarily regulated by glucocorticoids mediated through the hypothalamic–pituitary–adrenal (HPA) axis. Although there are well-documented negative effects of persistent elevated glucocorticoid levels on reproduction (Sapolsky 2002), comparatively little attention has been given to the possible impact of reproductive endocrine activity on HPA activity (but see, e.g., Rasmussen et al. 2008; Ganswindt et al. 2010). Although it is unlikely that there is a negative feedback of gonadal activity on the HPA axis, reproductive activity could act as a stressor since males often compete for mating opportunities through aggressive interactions and gestation and lactation are physiologically demanding periods for

females (Goymann et al. 2001; Weingrill et al. 2004; Monclus et al. 2009). Because of the observed suppressive effects of elevated glucocorticoid levels on reproduction, a potential impact of reproductive activity on the HPA axis could lead to interesting physiological trade-offs.

The aardwolf (*Proteles cristata*) is a small hyaenid species (8-12 kg) that lives in semi-arid regions of southern and eastern Africa. Aardwolves are solitary foragers but have been described as socially monogamous and show little sexual dimorphism. Data from wild populations suggest that they are strictly seasonal breeders (Richardson 1987). Aardwolves give birth to litters of one to four offspring during the rainy season, which coincides with time of peak food abundance in the wild. However, physiological data related to reproductive activity are lacking. Therefore, we used non-invasive monitoring of faecal hormone metabolites in zoo-housed aardwolves to provide basic information on longitudinal variation of reproductive hormones in males and females. We also quantified the effects of the social environment on reproductive endocrine activity as well as the relationships between reproductive and adrenocortical hormones.

2.3 Materials and methods

2.3.1. Study animals and animal housing

The study included five zoo bred aardwolves (two males, three females) housed at Brookfield Zoo, Chicago, IL, USA (Table 2.1). One of the males (Mutosi) and one of the females (Mwena) were the biological parents of the other three animals. The social groupings varied over time. At first, all animals were housed together. However, they were gradually separated due to increasing aggressive behaviour. The male Mutosi was given an epididectomy in 1998 in an effort to prevent potential inbreeding while allowing for group housing. The male Rafiki was

separated from the other animals at the age of 6 months (October 1997) and housed alone with no direct contact with other aardwolves until his death on 16 November 2010. The remaining group of Mutosi and the females Mwena, Safina and Mlia was separated on 20 February 2001 into a pair of Mwena and Mlia and another pair of Mutosi and Safina. On 10 June 2002, Mwena and Mlia were separated and on 29 May 2003, Safina was separated from Mutosi. These four animals were housed adjacent to one another (mean cage size: 4.3×3.9m; $n=13$) and therefore could hear and smell one another regularly and occasionally see each other. Rafiki was housed separately in another area in the building (mean cage size: 3.8×2.8m, $n=2$), but could likely still smell and hear the other aardwolves. However, he had no visual access to them. All aardwolves were fed 226–300 g daily of a mix which consisted of 25% ground Hill's Feline Maintenance chow and 25% ground Marion Leaf eater chow mixed with 50% water. Aardwolves were also fed 10–20 insects/day. To enable individual identification of faecal samples, each animal had their food marked with commercial food colour when housed together with conspecifics (Fuller et al. 2009). Table 2.1 shows life history data and arrangements of housing situation at Brookfield Zoo from birth until the end of the study period.

2.3.2. Sample collection and extraction protocol

A total of 1,709 faecal samples were collected from July 2001 until April 2004. Details regarding sample sizes for each individual during the different housing situations are given in Table 1. Samples were collected by the zookeepers every 2–3 days and kept frozen until extraction at -20°C . Aliquots of 0.5 g well-mixed, defrosted faecal material were extracted with 5.0 ml of 80% ethanol in distilled water by horizontal shaking for 14–18 h at room temperature. After centrifuging for 15 min at $1,500\times g$, 1 ml of supernatant was added to 1 ml

of assay buffer (0.1M phosphate buffered saline containing 1% BSA, pH 7.0), and the mixture stored frozen at $-20\text{ }^{\circ}\text{C}$ until assaying.

2.3.3. Enzyme immunoassays

Diluted faecal extracts were measured for immunoreactive androgen (fA), estrogen (fE) and progestagen (fP) metabolites using previously described enzyme immunoassay systems from Coralie Munro's laboratory at the University of California, Davis, CA, USA (Graham et al. 2001; deCatanzaro et al. 2003; Atsalis et al. 2004; Dloniak et al. 2004; Fanson et al. 2010a,b). We quantified fA using the testosterone polyclonal antibody R156/7, and cross-reactions of relevant steroids are given by deCatanzaro et al. (2003). Sensitivity of the assay (at 90% binding) was 0.04 ng/well. Intra-assay coefficients of variation (CV), determined by repeated measurements of low and high value quality controls ranged between 7.5% and 10.2%. The inter-assay CV was 19.6% and 23.8%, respectively. Recovery of exogenous hormone was $85.1\pm 5.8\%$ (mean \pm standard deviation [SD], $n=7$).

We used the estradiol-17 β antibody R4972 to quantify fE. Cross-reactions of relevant steroids are given by deCatanzaro et al. (2003). Assay sensitivity at 90% binding was 0.39 ng/well. Intra-assay CV was 9.4% and 12.2% for low and high controls, respectively. Inter-assay CV was 16.7% and 18.9%, respectively. Recovery of exogenous hormone was $80.6\pm 2.8\%$ ($n=7$).

We used the progesterone monoclonal antibody CL425 to quantify fP. Relevant cross-reactivities are given by Graham et al. (2001). Sensitivity of the assay at 90% binding was 0.05 ng/well. Intra-assay CV for low and high value quality controls was 8.3% and 10.6%, respectively, and inter-assay CV was 18.7% and 22.6%. Recovery of exogenous hormone was $72.2\pm 4.3\%$ ($n=7$).

We quantified faecal glucocorticoid metabolites (fGC) using a commercially available enzyme-immunoassay (Corticosterone; Assay Designs, Ann Arbor, MI), which successfully have been used on several similar species (e.g., river otter [*Lontra Canadensis*], Rothschild et al. 2008, Canada lynx [*Lynx Canadensis*], Fanson et al. 2012). The antibody cross-reacts with corticosterone (100%), deoxycorticosterone (28.6%), progesterone (1.7%), and less than 1% for other tested steroids. Assay sensitivity was 0.03 ng/well. Intra-assay CV was 12.3% and 13.7% for low and high controls, respectively, and inter-assay CV were 18.2% and 16.8%. Recovery of exogenous hormone was 82.0 ± 4.7 % ($n=7$).

Samples were assayed in duplicate, and serial dilutions of extracted faecal samples gave displacement curves which were parallel to the respective standard curves in all assays. Data are expressed as μg per g wet faecal weight.

2.3.4. *Biological validations of immunoassays*

To evaluate the biological relevance of the fA assay, we contrasted baseline levels of fA in one male (Mutosi) and one female (Safina) to levels found during periods of mating activity. Since mating activity is typically regulated by testosterone in mammalian males, but not in females, contrasting correlations between mating activity and fA's in males and females can function as a biological validation for fA. The baseline fA value was calculated using an iterative process in which values that exceeded the mean plus 1.5 SD were excluded. The average was then recalculated and the elimination process was repeated until no values exceeded the mean plus 1.5 SD (Brown et al. 1994b).

To evaluate the biological relevance of the fE and fP assays, we similarly contrasted baseline concentrations, calculated as described above, to those found during and after periods of mating activity.

To evaluate the biological relevance of the fGC assay, we used samples from a previously described study on wild aardwolves, in which exogenous adrenocorticotrophic hormone (ACTH) were injected to one male and one female to determine stress-related physiological responses using another enzyme immunoassay (Ganswindt et al. 2012). We used contrasts in fGC levels between samples collected prior to injection and within 20 h after ACTH administration to verify the reliability of the fGC assay used.

2.3.5. *Data analyses*

We used mixed linear models to test for variation among months in reproductive hormone metabolites, as well as to test for the relationship between reproductive hormone metabolite and fGC concentrations. We created two separate pairs of models to test for monthly variation in reproductive hormone metabolite levels. First, we created two models that only included data collected during time periods when animals were housed alone, one for variation in fA concentrations in males and one for variation in fE concentrations in females. In these models we included data from both males (fA) and two of the females (Mwena and Mlia: fE) since we did not have data from the third female when she was housed alone. We used fA and fE concentrations as response variables, respectively, month as a fixed effect predictor and controlled for non-independence within individuals as well as temporal pseudoreplication by adding sample day blocked over each individual as random effects. Secondly, we created two models to test for possible interactions between housing arrangements and monthly variation in fA and fE concentrations. In these two models, we only included data from one male (Mutosi) and from one female (Mwena), because these two were the only individuals for which we had data from both when they had been housed alone and together with a conspecific. Similarly to the models described above, we used fA concentrations as response, and added

month, housing and a two way interaction as fixed effects. Since the models only included one animal, we only added sample day as random variable.

To test for possible effects of reproductive hormone metabolites on fGC concentrations, and whether or not these effects were influenced by the presence of conspecifics, we first created three models using fGC concentrations as the response variable and reproductive hormone metabolite concentrations (fA and fE, respectively) as fixed effect. In these models, we included both males when housed alone (for the model on fA), two of the females (Mweni and Mlia) when housed alone (fE) and in the last model data from one female housed together with a male (since we had no data for this female when she was housed alone). Secondly, we tested if housing influenced the effect of hormone metabolites on fGC by creating two models only including one male (Mutosi) and one female (Mwena), respectively. In these models, we included faecal reproductive hormone metabolite concentrations, housing (for the model on fA this consisted of alone or housing together with a female, Safina; for the model on fE this consisted of housed alone or together with another female, Mlia) and the two-way interaction as fixed effects. We added similar random effect structures to all these models as described above.

We used a nonlinear mixed-effects model to test for an asymptotic relationship between fGC and fP concentrations for data from one female (Safina). We tested an asymptotic rather than a linear relationship since visual inspection of the data suggested this to be a more appropriate representation of the relationship, and a likelihood ratio test confirmed that an asymptotic model provided a better fit to the data than a linear one ($\chi^2=247.69$, $df=1$, $p<0.001$). The model used three parameters as fixed effects: the asymptotic value of fGC, the intercept of the function and the slope until the asymptote. Similarly to the linear models including only one individual, we added sample day as random effect.

All models were fitted to raw endocrine data, but a variance power function was used to account for heteroscedasticity (Pinheiro and Bates 2000). Statistical significance was set to 0.05 and all tests were two tailed. Statistical analyses were performed with the software R, version 2.15.1 for Linux (<http://www.r-project.com>) using functions in the user contributed package nlme (Pinheiro et al. 2012).

2.4 Results

2.4.1. *Biological validations of immunoassays*

In the male Mutosi, fA concentrations were elevated over five times above the baseline during February 2003 where frequent copulations with a female were observed (Fig. 2.1a, baseline=0.19 $\mu\text{g/g}$ wet weight vs. average 1.12 $\mu\text{g/g}$ wet weight during February 2003). For the female Safina, fA concentrations were not as elevated above baseline concentrations during this mating period (Fig. 2.1b, baseline=0.13 $\mu\text{g/g}$ wet weight vs. average 0.39 $\mu\text{g/g}$ wet weight during February 2003), but fE concentrations were (Fig. 2.2a, baseline=0.13 $\mu\text{g/g}$ wet weight vs. average 0.78 $\mu\text{g/g}$ wet weight during February 2003). For the same female, fP concentrations were above baseline post elevated estrogen levels and observed copulations (Fig. 2.2d, baseline=0.30 $\mu\text{g/g}$ wet weight vs. average 19.0 $\mu\text{g/g}$ wet weight during March/April 2003). For fGC concentrations, post injection samples had 2.5 times higher concentrations compared to pre sample average in the male (pre sample average=0.29 $\mu\text{g/g}$ wet weight vs. post sample=0.72 $\mu\text{g/g}$ wet weight) and 2.1 times higher concentrations in the female (pre sample average=0.27 vs. post sample average=0.57 $\mu\text{g/g}$ wet weight).

2.4.2. Variation in fA concentrations

When housed alone, fA concentrations for both males were significantly higher during February, March, June, July, and December compared to the nominate month January (Table 2.2; Fig. 2.3a). However, for the male Mutosi, there was a significant interaction effect of month and housing on fA concentrations (Mutosi; $F=2.07$, $df=10,375$, $p=0.026$; Fig. 2.3b), with elevated fA concentrations during October 2002 and during February 2003 when frequent copulations occurred (Fig. 2.1a). During this same period, there were no distinct fluctuations in fA concentrations in the female (Fig. 2.1b).

2.4.3. Variation in fE and fP concentrations

When housed alone, females had significantly higher fE concentrations in February compared to the nominate month January and lower levels in April, May, August, September, and November (Table 2.2; Fig. 2.3c). However, as with one of the males, there was a significant interaction effect of month and housing on fE concentrations in the one female that was housed both alone and together with a conspecific (another female: $F=10.49$, $df=11391$, $p<0.001$). There were more pronounced monthly fluctuations when housed with a male and drastically lower monthly fluctuations when housed with a female (Fig. 2.3d). In general, both fE and fP concentrations varied among the three females (Fig. 2.2), but the most drastic fluctuations were observed in the female that was housed together with a male, with elevated fE concentrations during a period with frequent copulations followed by a sharp increase in fP concentrations (Fig. 2.2d).

2.4.4. Relationships between fGC concentrations and reproductive hormone metabolite levels

When housed alone, fA concentrations had a positive effect on fGC concentrations for the two males ($\beta=4.54$, $df=445$, $t=5.41$, $p<0.001$; Fig. 2.4a). In addition, the one male that was housed

both alone and together with a female had higher fGC concentrations when he was together with the female compared to when he was alone ($\beta=8.84$, $df=419$, $t=6.26$, $p<0.001$). However, there was an almost significant interaction effect between fA concentration and housing arrangement for this male ($F=3.34$, $df=1,419$, $p=0.068$), suggesting that fA had a stronger effect on fGC when he was housed together with a female than when he was alone ($\beta=6.43$, $df=419$, $t=1.82$, $p=0.068$; Fig. 2.4b). Similarly, there was a positive effect of fE concentrations on fGC concentrations for females when housed alone ($\beta=5.61$, $df=674$, $t=2.76$, $p=0.006$; Fig. 2.4c) and together with a male ($\beta=62.38$, $df=288$, $t=8.00$, $p<0.001$; Fig. 2.4d). For the one female that was housed both alone and with a female, there was a significant interaction effect of fE concentrations and housing on fGC concentrations with a more pronounced effect of fE concentrations on fGC concentrations when animals were housed with a female compared to when housed alone ($\beta=115.71$, $df=411$, $t=12.04$, $p<0.001$; Fig. 2.4c,d). All parameters in the non-linear model of the effects of fP concentrations on fGC concentrations were significant (Asymptote=32.19, $t=13.34$, $df=287$, $p<0.001$; Intercept $t=2.89$, $t=4.35$, $df=287$, $p<0.001$; $\beta=2.23$, $t=17.58$, $df=287$, $p<0.001$), supporting an asymptotic relationship between fP and fGC concentrations in this female (Fig. 2.4e).

2.5 Discussion

This is the first study to present reproductive endocrine data for aardwolves, and the first study to present longitudinal data on glucocorticoid hormone metabolites for this species. Our results demonstrate that monitoring faecal hormone metabolites can be a useful non-invasive tool for assessing both gonadal and adrenocortical activity in this species. A previous study on wild aardwolves similarly validated a different EIA for glucocorticoid metabolites (Ganswindt et al.

2012), which further lends support for the usefulness of non-invasive monitoring of endocrine parameters in aardwolves.

2.5.1. Temporal variation in reproductive hormone metabolites

Zoo-housed aardwolves exhibited monthly fluctuations in both male and female reproductive hormone metabolites. Although there were some variations in these monthly fluctuations, both within and between sexes, both males and females had elevated fA and fE concentrations during February when housed alone. However, these fluctuations were more pronounced in the male and the female that were housed together. Our interpretation of these observations is that there is an inherent seasonality in aardwolf reproductive physiology, but that gonadal activity has remained receptive to the presence of opposite sex conspecifics. For males in particular, an endocrine response to female estrogen levels has also been found in many other carnivore species (Wingfield et al. 1990; Hesterman et al. 2005; Brown 2006; Dloniak et al. 2006b), and has been suggested to promote a close timing of mating activity to ovulation as a way of maximising reproductive success (Kraaijeveld-Smit et al. 2002).

The timing of increased reproductive hormone metabolite concentrations differed from observations of wild aardwolf populations in African savannas, where mating activity has been recorded during May to July (Richardson 1987). In wild populations, drastic seasonal fluctuations in food supply linked to rainfall patterns are likely the ultimate cause for reproductive seasonality in the aardwolf. However, rainfall and food supply are unlikely proximate regulators of seasonal fluctuations in reproductive physiology. Since our observations of mating activity in February were done in the northern hemisphere, they correspond to the same season as field observations from the southern hemisphere. We therefore suggest that our observations of contrasting periods of mating activity between the

northern and southern hemisphere points to a photoperiodic regulation of reproductive endocrine activity in this species.

There was some discrepancy in the timing of peak fA concentrations in the two males, with the male who was consistently housed in isolation showing some distinct elevations in fA concentrations in June and July. Numerous studies have demonstrated that captivity can adversely affect an animal's behaviour and physiology (reviewed by Lindburg and Fitch-Snyder 1994; Carlstead 1996; Estep and Dewsbury 1996), and lack of exposure to an appropriate social environment may disrupt the ability to correctly respond physiologically to environmental changes (Meier 1965; Bekoff 1972; Marchlewska-Koj 1997; Tilbrook et al. 2000; Blanchard et al. 2001; Lovic et al. 2006). We suspect that long term isolation caused one of the males to lose the ability to respond appropriately to environmental cues and hence time his peak in fA concentrations to periods of peak oestrus activity in females. This would further support that both environmental and social cues may be necessary for developing coordinated temporal fluctuations in gonadal activity between males and females in this species.

Pregnancy and pseudo-pregnancy in mammalian females are reflected by elevated progesterone levels caused by an increased production of progesterone first from the corpus lutea and later from the placenta (Schwarzenberger 1996; Monfort et al. 1997; Dloniak et al. 2006a; Schwarzenberger 2007; Van Meter et al. 2009; Fanson et al. 2010b). For felids, starting 1 to 2 days after ovulation, progestagen secretion from corpora lutea increases and concentrations remain elevated for 64–67 days in pregnant cats and approximately half that in not pregnant cats (Paape et al. 1975; Wildt et al. 1981; Tsutsui and Stabenfeldt 1993). We propose that the 300 fold increase in fP concentrations observed in one of the females may be indicative of a pseudo-pregnancy since the male Mutosi was epidectomized and should have therefore been incapable of producing viable, fertile sperm. However, since we did not observe

distinct elevations in fP during the period of repeated mating activity, our data do not suggest that the aardwolf is an induced ovulator.

2.5.2. Relationships between fGC concentrations and reproductive hormone metabolites

Due to the well documented negative effects of HPA activity on reproductive function (Dobson and Smith 2000), we regard it to be highly unlikely that our observations of positive associations between fGC concentrations and reproductive hormone metabolites were caused a positive effect of fGC on gonadal activity. Therefore, we suggest that our results points to possible effects of reproductive hormones on the activity of the HPA axis, and that reproductive activity could function as a physiological stressor. In females, fGC concentrations showed a stronger relationship with reproductive hormone metabolites when animals where housed with a conspecific than when kept alone. The observed asymptotic relationship between fP and fGC concentrations suggests that ovulatory state may also function as a physiological stressor. Since long-term elevations of glucocorticoids can compromise reproductive function, including terminating pregnancies (Sapolsky 2002), a positive association between reproductive endocrine parameters and glucocorticoids could lead to interesting physiological trade-offs. However, observations from wild populations suggest that aardwolves mostly live solitarily and mainly interact with conspecifics during the breeding season (Richardson 1987). Therefore, these results may partly be confounded by the forced social situation that showed variation in faecal glucocorticoid and reproductive hormone levels when housed with a conspecific.

2.5.3. Conclusions

To conclude, we suggest that our study provided physiological support for the aardwolf as a seasonal breeder, and that this seasonality may be regulated by photoperiod. We also found a

positive relationship between reproductive and adrenocortical endocrine activity, and we argue that this association is caused by a physiological stress response to reproductive activity. Such a HPA response to gonadal activity could result in interesting physiological trade-offs in male reproductive tactics and female pre-partum maternal investment. Finally, our results suggest that social and environmental factors interact in regulating reproductive activity in this mostly solitary species.

2.6 Acknowledgements

We thank the ‘Fragile Kingdom’ manager and keeper staff at Brookfield Zoo for their dedicated scat sample collection and for providing details on housing and husbandry of the aardwolves. We also thank zoo intern Jaime Pape and several volunteers who helped with weighing of faecal samples, some aspects of sample processing, and data entry in the Brookfield Zoo Endocrine Laboratory. D. Marneweck, F. Dalerum and A. Ganswindt were supported by the National Research Foundation of South Africa, and F. Dalerum and A. Ganswindt were further supported by research fellowships from University of Pretoria.

2.7 References

- Atsalis S, Margulis S, Bellem A, Wielebnowski N (2004) Sexual behavior and hormonal estrus cycles in captive aged lowland gorillas (*Gorilla gorilla*). *Am J Primatol* 62:123–132
- Bekoff M (1972) The development of social interaction, play, and metacommunication in mammals: an ethological perspective. *Quart Rev Biol* 47:412–434
- Blanchard RJ, McKittrick CR, Blanchard DC (2001) Animal models of social stress: effects on behaviour and brain neurochemical systems. *Physiol Behav* 73:261–271

- Brown JL, Bellem AC, Fouraker M, Wildt DE, Roth TL (1994b) Comparative analysis of gonadal and adrenal activity in the black and white rhinoceros in North America by noninvasive endocrine monitoring. *Zoo Biol* 20:463–486
- Brown JL (2006) Comparative endocrinology of domestic and nondomestic felids. *Theriogenology* 66:25–36
- Carlstead K (1996) Effects of captivity on the behaviour of wild mammals. In Kleiman DG, Allen ME, Thompson KV, Lumpkin S (eds) *Wild mammals in captivity*, University of Chicago Press, Chicago, pp, 317–333
- deCatanzaro D, Muir C, Beaton E, Jetha M, Nadella K (2003) Enzymeimmunoassay of oestradiol, testosterone and progesterone in urine samples from female mice before and after insemination. *Reproduction* 126:407–414
- Dloniak SM, French JA, Place NJ, Weldele ML, Glickman SE, Holekamp KE (2004) Non-invasive monitoring of fecal androgens in spotted hyenas (*Crocuta crocuta*). *Gen Comp End* 135:51–61
- Dloniak SM, French JA, Holekamp KE (2006a) Rank-related maternal effects of androgens on behaviour in wild spotted hyenas. *Nature* 440:1190–1193
- Dloniak SM, French JA, Holekamp KE (2006b) Faecal androgen concentrations in adult male spotted hyenas, *Crocuta crocuta*, reflect interactions with socially dominant females. *Anim Behav* 71:27–37
- Dobson H, Smith RF (2000) What is stress, and how does it affect reproduction? *Anim Repr Sci* 60-61:743–752
- Estep DQ, Dewsbury DA (1996) Mammalian reproductive behavior. In Kleiman DG, Allen ME, Thompson KV, Lumpkin S (eds) *Wild mammals in captivity*. University of Chicago Press, Chicago, pp 379–389

- Fanson KV, Wielebnowski NC, Shenk TM, Lucas JR (2012) Comparative patterns of adrenal activity in captive and wild Canada lynx (*Lynx Canadensis*). *J Comp Phys B* 182:157–165
- Fanson KV, Wielebnowski NC, Shenk TA, Jakubas WJ, Squires JR, Lucas JR, 2010a. Patterns of testicular activity in captive and wild Canada lynx (*Lynx canadensis*). *Gen Comp End* 169:210–216
- Fanson KV, Wielebnowski NC, Shenk TA, Jakubas WJ, Squires JR, Lucas JR (2010)b. Patterns of ovarian and luteal activity in captive and wild Canada lynx (*Lynx canadensis*). *Gen Comp End* 169:217–224
- Fuller G, Margulis SG, Santymire R (2009) The effectiveness of indigestible markers for identifying individual animal feces and their prevalence of use in North American zoos, *Zoo Biol* 30:379–398
- Ganswindt A, Muenscher S, Henley M, Henley S, Heistermann M, Palme R, Thompson P, Bertschinger H (2010) Endocrine correlates of musth and the impact of ecological and social factors in free-ranging African elephants (*Loxodonta africana*). *Horm Behav* 57:506–510
- Ganswindt A, Muijlwijk C, Engelkes M, Muenscher S, Bertschinger H, Paris M, Palme R, Cameron EZ, Bennett NC, Dalerum F (2012) Validation of non-invasive monitoring of adrenocortical endocrine activity in ground feeding aardwolves (*Proteles cristata*); exemplifying the influence of consumption of inorganic material for fecal steroid analysis. *Phys Biochem Zool* 85:194–199
- Goldman BD (1999) The circadian timing system and reproduction in mammals. *Steroids* 64:679–685
- Goymann W, East WL, Wachter B, Höner OP, Möstl E, Van't Hof TJ, Hofer H (2001) Social, state dependent and environmental modulation of faecal glucocorticoid levels in free ranging female spotted hyenas. *Proc Roy Soc B* 268:2453–2459

- Graham L, Schwarzenberger F, Möstl E, Galama W, Savage A (2001) A versatile enzyme immunoassay for the determination of progestogens in feces and serum. *Zoo Biol* 20:227–236
- Hesterman H, Wasser SK, Cochrem JF (2005) Longitudinal monitoring of fecal testosterone in male Malayan sun bears (*U. malayanus*). *Zoo Biol* 24:403–417
- Kraaijeveld-Smit FJL, Ward SJ, Temple-Smith PD, Paetkau D (2002) Factors influencing paternity success in *Antechinus agilis*: last-male sperm precedence, timing of mating and genetic compatibility. *J Evol Biol* 15:100–107
- Kretzschmar P, Ganslosser U, Dehnhard M (2004) Relationship between androgens, environmental factors, and reproductive behavior in male white rhinoceros (*Ceratotherium simum simum*). *Horm Behav* 45:1–9
- Lindburg DG, Fitch-Snyder H (1994) Use of behaviour to evaluate reproductive problems in captive mammals. *Zoo Biol* 13:433–455
- Lovic V, Gonzalez A, Madden M, Sinopoli K, Fleming AS (2006) Maternal and littermate deprivation disrupts maternal behaviour and social-learning of food preference in adulthood: Tactile stimulation, nest odor, and social rearing prevent these effects. *Dev Psych* 48:209–219
- Marchlewska-Koj A (1997) Sociogenic stress and rodent reproduction. *Neurosc Biobehav Rev* 21:699–703
- McAllan BM, Dickman, CR (1985) Trole of photoperiod in the timing of reproduction in the Dasyurid marsupial *Antechinus stuartii*. *Med Biol Eff Light* 453:182–204
- Meier GW (1965) Other data on the effects of social isolation during rearing upon adult reproductive behaviour in the rhesus monkey (*Macaca mulatta*). *Anim Behav* 13:228–232

- Monclus R, Palomares F, Tablado Z, Martinez-Fontúrbel A, Palme R (2009) Testing the threat-sensitive predator avoidance hypothesis: physiological responses and predator pressure in wild rabbits. *Oecologia* 158:615–623
- Monfort SL, Dahl KD, Czekala NM, Stevens L, Bush M, Wildt DE (1989) Monitoring ovarian function and pregnancy in the giant panda (*Ailuropoda melanoleuca*) by evaluating urinary bioactive FSH and steroid metabolites. *J Repr Fert* 85:203–212
- Monfort SL, Wasser SK, Mashburn KL, Burke M, Brewer BA, Creel SR (1997) Steroid metabolism and validation of noninvasive endocrine monitoring in the African wild dog (*Lycaon Pictus*). *Zoo Biol* 16:533–548
- Paape SR, Shille VM, Seto H, Stabenfeldt GH (1975) Luteal activity in the pseudopregnant cat. *Biol Reprod* 13:470–474
- Pinheiro JC, Bates DM (2000) Mixed effect models in S and S-plus. Springer Verlag, New York
- Pinheiro JC, Bates DM, DebRoy S, Sarkar D (2012). nlme: linear and nonlinear mixed effects models. R package version 3.1-104
- Richardson PRK (1987) Aardwolf mating system: overt cuckoldry in an apparently monogamous mammal. *S Afr J Sc* 83:405–410
- Rasmussen HB, Ganswindt A, Douglas-Hamilton I, Vollrath F (2008) Endocrine and behavioural changes in male African elephants: linking hormone changes to sexual state and reproductive tactic. *Horm Behav* 54:539–548
- Rothschild DM, Serfass TL, Seddon WL, Hegde L, Fritz RS (2008) Using fecal glucocorticoids to assess stress levels in captive river otters. *J Wildl Man* 72:138–142
- Sapolsky RM (2002) Endocrinology of the stress response. In: Becker JB, Breedlove SM, Crews D, McCarthy M (eds) Behavioral endocrinology, 2nd edn. MIT Press, Cambridge, pp 409–450

- Schwarzenberger F, Möstl E, Palme R, Bamberg E (1996) Fecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. *Anim Repr Sc* 42:515–526
- Schwarzenberger F (2007) The many uses of non-invasive faecal steroid monitoring in zoo and wildlife species. *Int Zoo Yearb* 41:52–74
- Scott MP (1986) The timing and synchrony of seasonal breeding in the marsupial, *Antechinus stuartii*: Interaction of environmental and social cues. *J Mamm* 67:551–560
- Tilbrook AJ, Turner AI, Clarke IJ (2000) Effects of stress on reproduction in non-rodent mammals: the role of glucocorticoids and sex differences. *Rev Repr* 5:105–113
- Tsutsui T, Stabenfeldt GH (1993) Biology of ovarian cycles, pregnancy and pseudopregnancy in the domestic cat. *J Repr Fert* 47:29–35
- Van Meter PE, French JA, Dloniak SM, Watts HE, Kolowski JM, Holekamp KE (2009) Fecal glucocorticoids reflect socio-ecological and anthropogenic stressors in the lives of wild spotted hyenas. *Horm Behav* 55:329–337
- Weingrill T, Gray DA, Barrett L, Henzi SP (2004) Fecal corticosteroid levels in free-ranging female chacma baboons: relationships to dominance, reproductive state and environmental factors. *Horm Behav* 45:259–269
- Wildt DE, Chan SYW, Seager SWJ, Chakraborty PK (1981) Ovarian activity, circulating hormones, and sexual behavior in the cat: I. Relationships during the coitus induced luteal phase and the estrous period without mating. *Biol Repr* 25:15–28
- Wingfield JC, Hegner RE, Duffy Jr, AM, Ball GF (1990) The “challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am Nat* 136:829–846

Table 2.1 Life history data of five zoo-housed aardwolves, including variation in housing organisation with conspecifics during the data collection period, as well as sample sizes during each housing arrangement

Animal ID	Sex	Born	Housing organisation, dates and sample sizes					
			Alone		With Female		With Male	
			Period	<i>n</i>	Period	<i>n</i>	Period	<i>n</i>
Mutosi ^a	Male	16/05/1992	29/5/2003– 9/4/2004	129	18/7/2001– 28/5/2003 ^c	294		
Rafiki ^b	Male	30/04/1997	18/7/2001– 9/4/2004	319				
Mwena ^a	Female	14/05/1993	11/6/2002– 9/4/2004	259	18/7/2001– 10/6/2002 ^d	156		
Safina ^b	Female	30/04/1997					18/7/2001– 28/5/2003 ^f	290
Mlia ^b	Female	06/01/1998	11/6/2002– 9/4/2004	262	18/7/2001– 10/6/2002 ^e			

^a Unrelated pair

^b Offspring of Mutosi and Mwena

^c Housed together with Safina

^d Housed together with Mlia

^e Housed together with Mwena, no samples collected

^f Housed together with Mutosi

Table 2.2 Summary of mixed-linear model parameters of fA for two males and fE for two females

Sex	Month	β	SE β	df	t	p value
Males	Feb	0.132	0.063	435	2.08	0.038
	Mar	0.167	0.056	435	3.03	0.003
	April	0.008	0.040	435	0.20	0.842
	May	0.050	0.053	435	0.95	0.343
	June	0.154	0.071	435	2.16	0.031
	July	0.387	0.123	435	3.14	0.002
	Aug	0.097	0.056	435	1.71	0.089
	Sep	0.033	0.044	435	0.76	0.449
	Oct	-0.044	0.034	435	-1.30	0.195
	Nov	0.102	0.057	435	1.77	0.077
	Dec	0.122	0.062	435	1.97	0.050
	Females	Feb	0.049	0.019	664	2.59
Mar		0.013	0.014	664	0.97	0.333
April		-0.035	0.011	664	-3.14	0.002
May		-0.045	0.012	664	-3.90	<0.001
June		-0.012	0.017	664	-0.72	0.473
July		-0.015	0.011	664	-1.37	0.171
Aug		-0.025	0.010	664	-2.45	0.014
Sep		-0.025	0.010	664	-2.37	0.018
Oct		-0.016	0.011	664	-1.44	0.151
Nov		-0.023	0.011	664	-2.11	0.035
Dec		-0.015	0.011	664	-1.35	0.176

Beta values indicates the difference between the nominate month January and consecutive months. Data are from when both the males and the females were housed alone.

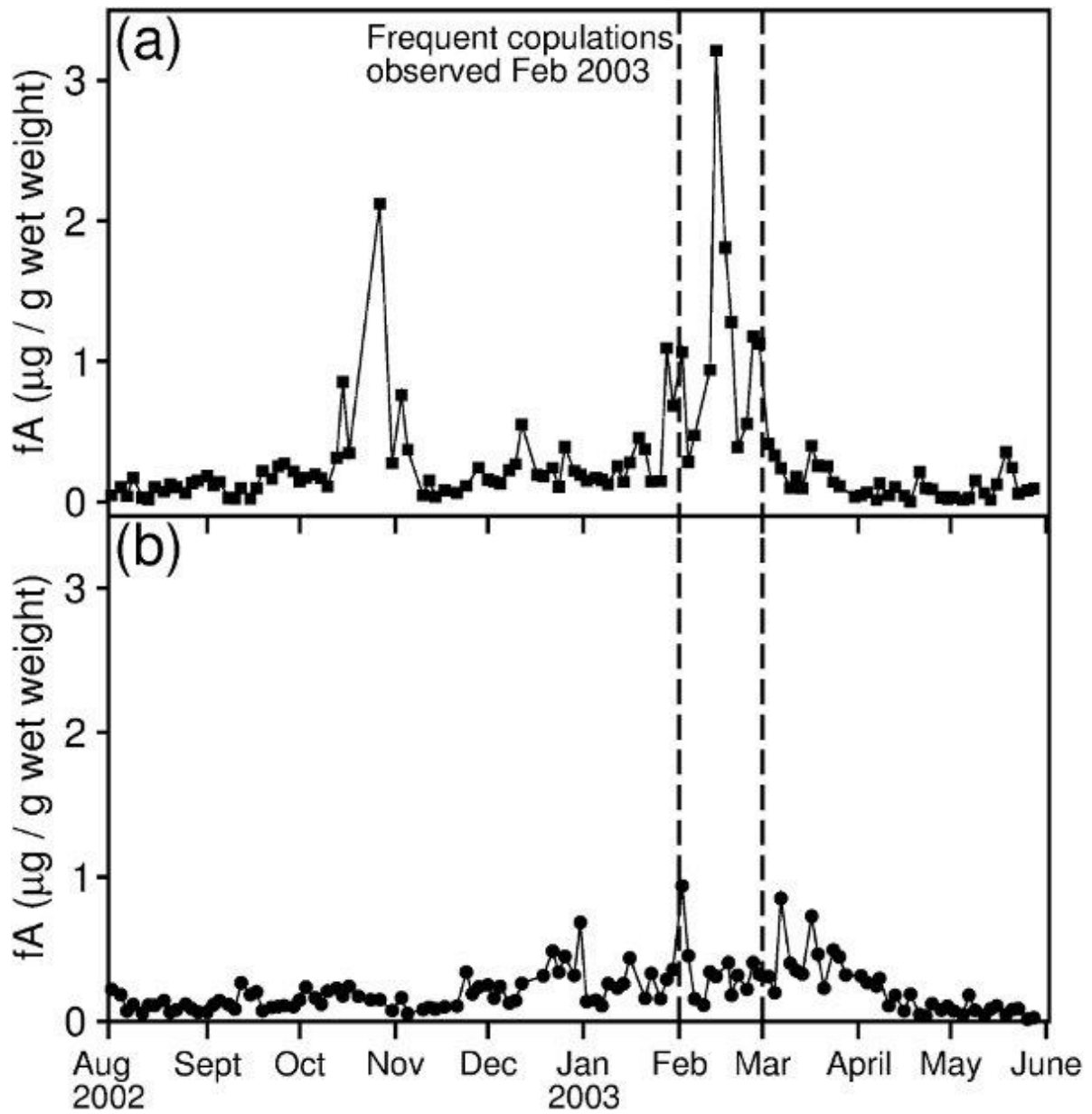


Fig. 2.1 Longitudinal profiles of fA concentrations in a male (a) and female (b) aardwolf over a 10-month period encompassing observed mating during February 2003

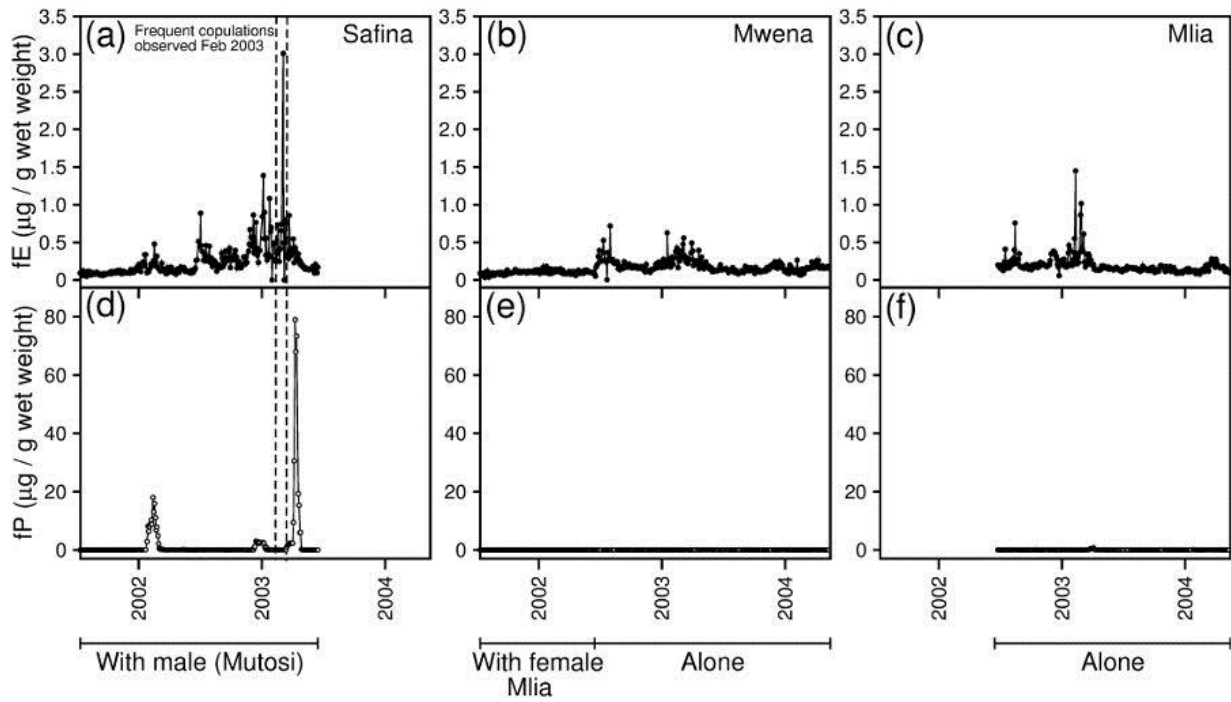


Fig. 2.2 Longitudinal profiles of fE (a-c) and fP (d-f) concentrations for three aardwolf females during the study period (Safina: a, d; Mwena: b, e; Mlia: c, f)

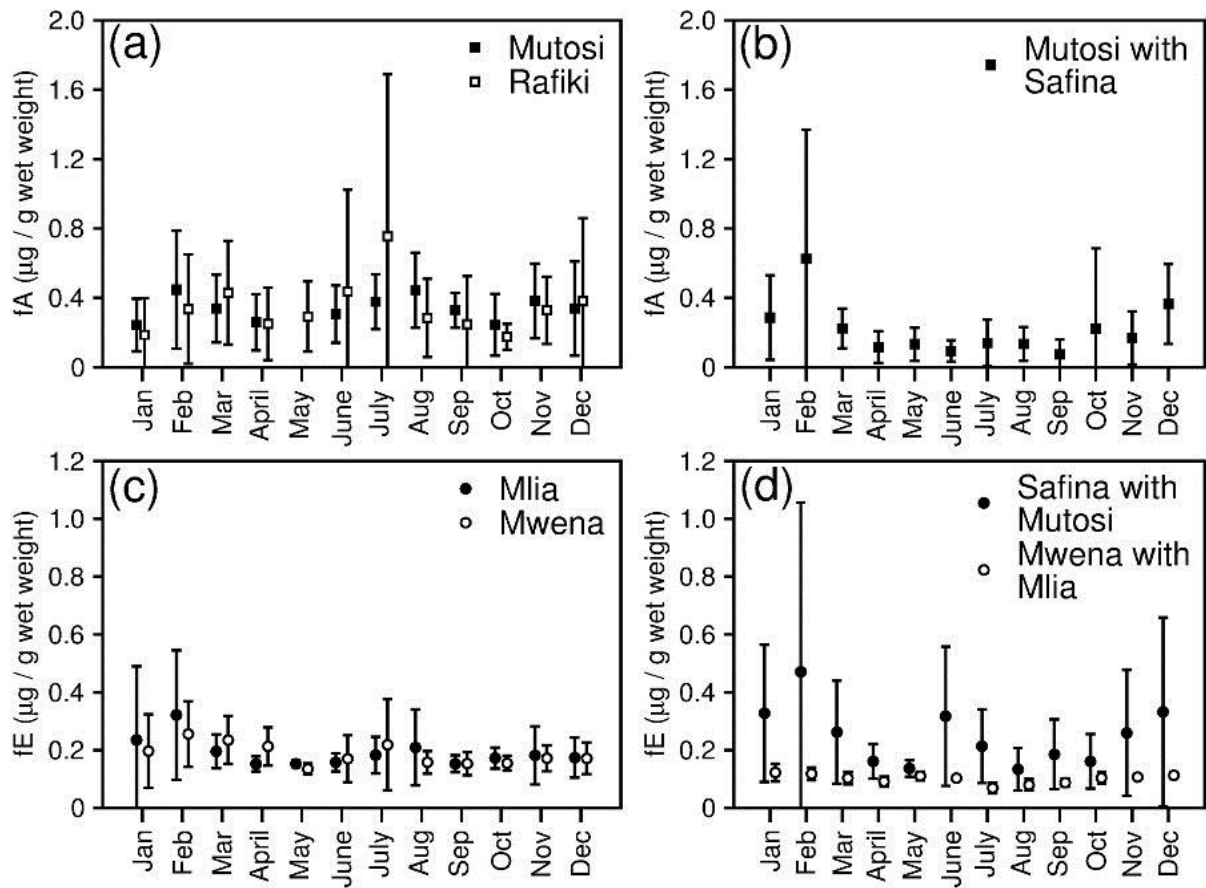


Fig. 2.3 Monthly variation (mean \pm 1SD) in fA concentrations for two male aardwolves when housed alone (a), one male aardwolf housed together with a female (b) and monthly variation in fE concentrations for two female aardwolves when housed alone (c) and when housed together with a male (Safina with Mutosi) and a female (Mwena with Mlia) (d)

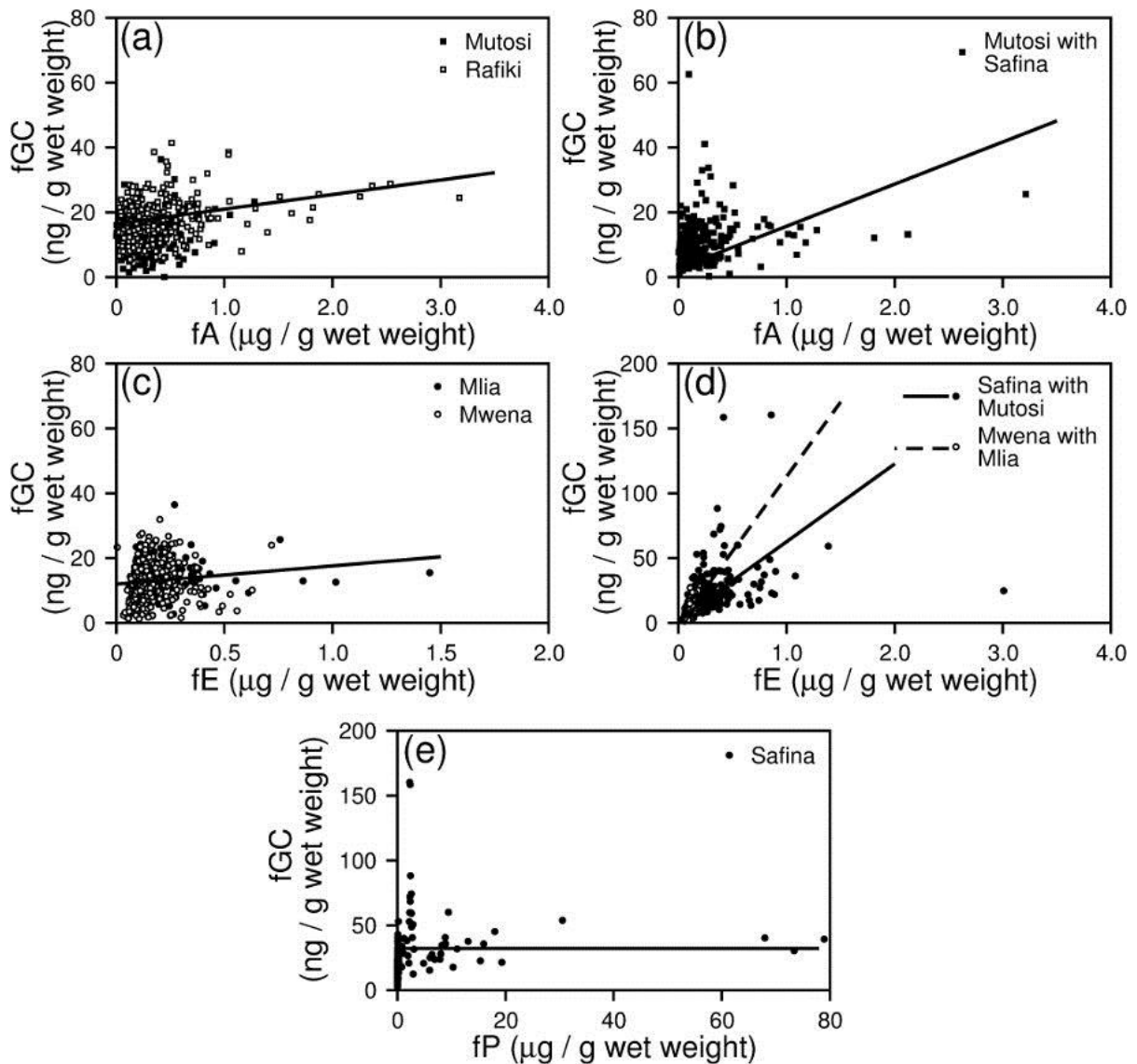


Fig. 2.4 Relationships between fA and fGC concentrations in two male aardwolves when housed alone (a) and one male housed together with a female (b), relationships between fE and fGC concentrations in two female aardwolves when housed alone (c) and when housed with a male (Safina with Mutosi) and a female (Mwena with Mlia; d), and the relationship between fP and fGC concentrations in one aardwolf female (e). Regression lines represent parameters from linear (a-d) and non-linear (e) mixed models

CHAPTER 3

Reproductive seasonality and stress-related adrenocortical hormones in wild aardwolves

3.1 Abstract

Inter-sexual discrepancies in the regulation of reproductive success have amounted to the majority of mammal species having a polygynous mating system, with only 5% of mammals being socially monogamous. Reproductive activity is under strong endocrine regulation which is closely connected to behaviours associated with mating patterns. In the seasonally breeding aardwolf, observations point to a discrepancy between behavioural observations of promiscuity and a described obligate social monogamy. In this study we describe sex-specific seasonal patterns in reproductive and adrenocortical hormones in wild aardwolves. In addition, we test if endocrine variations between and within sexes correspond to the suggested monogamous mating system or to behavioural observations related to polygamous mating. Our results provide physiological support for reproductive seasonality in wild aardwolves with elevated androgen concentrations in males during the mating season and elevated oestrogen and progesterone concentrations during the gestation season in females. We found no effect of season on the mean concentrations of faecal glucocorticoid metabolites (FGM) or on inter-individual variation of reproductive and FGM hormone levels in either males or females. Our results indicate inter-sexual symmetry in seasonal fluctuations of reproductive and stress-related adrenocortical hormones. We suggest that these similarities between males and females better fit predictions from social monogamy. Our physiological data therefore differ from

behavioural support for promiscuity in aardwolves, which could be related to greater plasticity in behavioural than physiology traits.

Key words: *Aardwolf, Behavioural plasticity, Faecal androgens, Faecal glucocorticoid metabolites, Monogamy, Reproductive seasonality*

3.2 Introduction

Mating systems are the outcome of individual strategies aimed at maximising reproductive success (Emlen & Oring 1977; Clutton-Brock 1989). Although there are intra-population differences in such strategies (e.g., Heymann 2000; Steenbeek et al. 2000; Struhsaker 2000), mating systems are often divided into four main classes (Clutton-Brock 1989; Davies 1991): (1) an individual mates with the same individual in several mating attempts (monogamy), (2) a single male mates with the same group of females in successive mating attempts (polygyny), (3) a single female mates with multiple males in successive mating attempts (polyandry) and (4) both males and females mate with numerous partners in successive mating attempts (promiscuity). Previous work has highlighted that mating activity cannot be directly assessed by social relationships (e.g., Griffith et al. 2002). Therefore, it is now widely recognised that mating systems should be regarded as having two components; one social, which describes how individuals socially relate to mating partners and one genetic, which describes the genetic effects of actual mating activities (Emlen & Oring 1977; Clutton-Brock 1989; Kappeler & van Schaik 2002).

Within mammals, there is a discrepancy between males and females in the regulation of reproductive success. This asymmetry is caused by the large physiological investment in gestation and lactation for females, which is contrasted by males who are largely regulated by

the number of successful mating attempts (Trivers 1972). This asymmetry has amounted to the majority of mammal species having polygynous mating systems, with only 5% of mammals being socially monogamous (Clutton-Brock 1989). It is not entirely clear why a male would confine himself to breeding with a single female (Clutton-Brock 1989). Therefore, much work has been dedicated to investigate the evolution of monogamy in mammals, where two main hypotheses have arisen (Wittenberger & Tilson 1980; Komers & Brotherton 1997). The first hypothesis stressed the importance of biparental care for reproductive success (i.e., obligate monogamy: Kleiman 1977; Wynne-Edwards 1987; Clutton-Brock 1989; Gubernick et al. 1993). The second hypothesis is focused on males' inability to monopolise more than one partner if females are widely dispersed (facultative monogamy: Emlen & Oring 1977; Rutberg 1983; van Schaik & van Hooff 1983; Barlow 1988; Clutton-Brock 1989; FitzGibbon 1997). Although males and females of both obligate and facultative monogamous mammals have been found to engage in extra-pair copulations (EPCs) (Richardson 1987; Agren et al. 1989; Reichard 1995; Cohan & Allaine 2009), there are lower degrees of extra-pair paternities (EPPs) for mammals within obligate compared to facultative monogamous mating systems (Clutton-Brock & Isvaran 2006; Cohan & Allaine 2009). These observations correspond to a predicted lower mating competition for obligate monogamous species.

Reproductive activity is under strong endocrine regulation where males attempt to synchronise their reproductive activity to that of receptive females (Kraaijeveld-Smit et al. 2002). Female receptivity is largely governed by an increase in oestrogen concentrations and male mating activity is governed by an increase in androgens. In females, oestrogen stimulates changes in reproductive organs and promotes the development of secondary characteristics. In males androgens stimulate and control the development and maintenance of sexual characteristics. Reproductive activity in mammals is either seasonal or aseasonal. For aseasonal breeders, females exhibit ovulatory cycles and males are typically constantly reproductively

active with reproductive hormones elevated throughout the year (Brown 2006). Contrastingly, for seasonally breeding species the reproductive hormones are elevated during a defined period of time in both males and females (Monfort et al. 1989; Monfort et al. 1997; Kraaijeveld-Smit et al. 2002; Kretzschmar et al. 2004; Hesterman et al. 2005; Dloniak et al. 2006; Fanson et al. 2010a, b). The ultimate cause for seasonal breeding strategies is linked to temporal variation in resource abundance, whereas the most important proximate regulator of reproductive activity is the relative rate of change in the photoperiod (McAllan & Dickman 1985; Scott 1986; Goldman 1999).

There is a close connection between behaviours related to mating activities and the endocrine regulation of reproduction. For example, experimental administration of exogenous androgens during both the mating and non-mating seasons in monogamous male birds caused males to seek EPCs, suppress paternal behaviour in favour of territorial aggression and mate guarding, thus adopting a polygynous mating system. (Silverin 1980; Wingfield 1984a; Hegner & Wingfield 1987; Wingfield et al. 1987; Wingfield et al. 1990). Behavioural traits related to mating activities are proximately regulated by the individual's hormonal environment. However, different mating strategies may also be reflected in contrasting reproductive and glucocorticoid hormone fluctuations, where high levels of glucocorticoids are widely accepted as indicators of increased stress (Möstl & Palme 2002; Schwarzenberger 2007; Hodges et al. 2010). For example, in the polygynous spotted hyena (*Crocuta crocuta*) there is a high degree of inter-individual variation in reproductive and adrenocortical stress hormones for both males and females associated with competition between differently ranked individuals (Lindeque & Skinner 1982; Glickman et al. 1992; Goymann et al. 2001; Goymann et al. 2003; Dloniak et al. 2004). These endocrine patterns related to polygynous mating stand in contrast to predictions from monogamous mating where we would expect low inter-individual variation in reproductive hormones due to a lack of mating competition (e.g., Creel et al. 1997). In

obligate monogamous species we presumably can expect elevated glucocorticoid levels in males during the parental care period due to the necessity for biparental care, as shown in red-fronted lemurs (*Eulemur fulvus rufus*, Ostner et al. 2008).

The aardwolf (*Proteles cristata*) is an extreme resource specialist belonging to the family Hyenidae (Kruuk & Sands 1972; Richardson 1987b; Matsebula et al. 2009; De Vries et al. 2011). Wild aardwolves have been described as strict seasonal breeders (Richardson 1987; Koehler & Richardson 1990; Sliwa 1996), observations that have been supported by endocrine data on captive animals (Marneweck et al. 2012). Aardwolves have also been described as obligate socially monogamous (Richardson 1985; Koehler & Richardson 1990), as a result of the necessity for a high level of paternal care caused by the increased need for females to forage away from offspring during lactation (Richardson & Coetzee 1988). However, aardwolves engage in a high frequency of EPCs (60-70% of all observed matings: Richardson 1987b; Sliwa 1996), and Kotze et al. (2012) suggested that the temporal utilisation of dens corresponded more closely with predictions from polygamous mating rather than social monogamy. Therefore, there seems to be a discrepancy between behavioural observations and the described mating system for the species.

The aim of this chapter is to describe sex-specific seasonal patterns in reproductive and stress-related adrenocortical hormone levels in wild aardwolves. We also test if endocrine variations between and within sexes correspond to the suggested monogamous mating system or to the behavioural observations related to polygamous mating. We predicted elevated androgen concentrations in males and elevated oestrogen concentrations in females during a defined mating season. Moreover, based on their described social monogamy we predicted; (1) that inter-individual variation in reproductive and stress-related adrenocortical hormone (FGM) levels will not differ between males and females and (2) that there will be increased FGM concentrations for both males and females during the parental care season due to the

necessity for biparental care. In contrast, under polygamous mating we would expect higher inter-individual variation of androgen concentrations in males compared to individual variation in female oestrogen concentrations during the mating season, caused by increased male mating competition. Furthermore, we would also predict higher FGM concentrations in males during the mating season compared to the parental care season, caused by the joint effect of increasing mating competition and lower paternal care associated with polygamous mating.

3.3 Materials and methods

3.3.1. Study Area

The study was conducted on Benfontein Nature Reserve, approximately 10km southeast of Kimberley, Northern Cape Province, South Africa (28.80°S, 24.77°E) (Fig. 3.1). Benfontein covers 11 400ha of semiarid terrain and lies within a transitional zone between dry Karoo, grassland and Kalahari thornveld (Schultze & McGee 1978). The reserve has a distinct cold and dry winter period (March-August) and a hot and wet summer period (September-February). The mean annual rainfall for the Kimberley area is 431 ± 127 mm (Weather Bureau in Pretoria) and the temperature in winter usually drops below freezing at night. Benfontein has a pan located in the northwest of the reserve where clay-rich soils are underlain by a calcium carbonate substrate (Sliwa 1996).

The sparse vegetation of the pan basin changes in a gradual incline to perennial grasses, short Karoo bushes and an area of Kalahari thornveld with *Acacia erioloba* in the southeast section (Richardson 1987b). While Benfontein has no large predators, it does have black-backed jackal (*Canis mesomelas*), caracal (*Caracal caracal*), martial eagle (*Polemaetus bellicosus*) and eagle owls (giant: *Bubo lacteus*; spotted: *Bubo africanus*). Benfontein has abundant termitaria throughout the reserve. The reserve has hosted the majority of previous

studies on aardwolf (Richardson 1985; Richardson 1987b; Richardson & Coetzee 1988; Koehler & Richardson 1990; Sliwa 1996; Sliwa & Richardson 1998; De Vries et al. 2011; Ganswindt et al. 2012; Kotze et al. 2012).

3.3.2. Aardwolf capture and immobilisation

Between November 2008 and May 2010 seven wild aardwolves (four males and three females; Table 3.1) were darted, immobilised and fitted with very high frequency radio collars (VHF; Sirtrack Ltd, Havelock North, New Zealand: weight $68.25\text{g} \pm 8\text{g}$, mean $\pm 1\text{sd}$). We located aardwolves by driving around the reserve and scanning with a hand-held spotlight. Once a non-collared individual was located it was followed by a 4x4 vehicle from a distance of $>100\text{m}$ until the animal became habituated to our presence. Aardwolves were remote injected with 36.0mg ketamine hydrochloride and 0.6mg medetomidine hydrochloride using a CO_2 -powered remote injection system. Generally, the darted animal took 5-10 minutes to become anaesthetised. Aardwolves were kept anaesthetised for 45-60 minutes. Sex, age and other anatomical data were recorded. Each of the seven individuals was fitted with a VHF radio collar and pit tag implanted between the shoulder blades. Collars were also fitted with a mortality setting in order to determine if the individual had slipped the collar or had died. The medetomidine was subsequently reversed with 3.0mg of atipamezole hydrochloride. All individuals were fully mobile within 10 minutes after reversal. We generally remained with animals for 30 minutes after full recovery. Animal handling procedures were approved by the Animal Use and Care Committee at University of Pretoria (EC031-07).

3.3.3. Definition of reproductive seasons

Both behavioural observations (Richardson 1987; Koehler & Richardson 1990; Sliwa 1996) and physiological data (Marneweck et al. 2012) have indicated that aardwolves are strictly

seasonal breeders. Female aardwolves come into pro-oestrus during the last week of June and mating has been observed during the last week of June and first two weeks of July (Richardson 1987; Koehler & Richardson 1990; Sliwa 1996). The gestation period is approximately 90 days with cubs generally born in early October (Richardson 1987; Koehler & Richardson 1990; van Jaarsveld et al. 1995; Sliwa 1996). Cubs are born in dens and usually stay underground until one month old, when they start to emerge while the male or female is present for protection (Koehler & Richardson 1990). Cubs start to forage on termites from two months and are weaned at approximately four months. During this weaning period cubs need intensive paternal protection as females are required to forage away from dens during lactation (Richardson & Coetzee 1988). The end of weaning to the beginning of mating generally occurs from February to May (Koehler & Richardson 1990). Based on these previous observations we have defined four seasons related to aardwolf reproductive activities: (1) the mating season (June & July), (2) the gestation season (August-October), (3) the weaning/parental care season (November-January) and (4) the non-mating season (February-May).

3.3.4. Faecal sample collection, extraction protocol and hormone assays

Aardwolves are primarily nocturnal and utilise dens during the day as thermal refugia during periods of inactivity (Williams et al. 1997) and for the rearing and protection of young (Anderson & Richardson 2005). Collared aardwolves were habituated to the presence of vehicles and were followed in a 4x4 from their resting den sites using a hand-held spotlight. Generally, aardwolves defecated within 10-15 minutes after leaving their den. They also defecated at random times throughout their active period (Ganswindt et al. 2012). The intensity of sample collection varied both over time and between study animals (Table 3.1). At each observed defecation event, we collected 10–15g of faeces within 10 minutes of defecation after the study animal had moved away from the defecation site. The faecal sample was collected

using rubber gloves and a thoroughly-mixed aliquot was stored in a glass vial. The glass vial containing the sample was placed on ice immediately and frozen at -20°C within 1 hour after collection and maintained at that temperature until analysis.

We lyophilised and pulverised the faecal samples and sifted them using a mesh strainer to remove fibrous material (Fieß et al. 1999). Subsequently, approximately 0.2g of faecal powder was extracted with 3ml of 80% ethanol in water (Ganswindt et al. 2012). After vortexing for 15 minutes and following centrifugation at 3300rpm for 10 minutes, the supernatants were transferred into microcentrifuge tubes and stored at -20°C until the hormone analysis. In addition, we determined the organic content of each sample according to the procedure described by Anestis et al. (2010). The remaining faecal pellets were air-dried at room temperature under a fume cupboard for at least 48 hours, where we weighed (AT261 Delta Range; Mettler-Toledo) the dried faecal material again (initial weight). Subsequently, the dried faecal material was ashed in a muffle furnace (Protea Incinerator; Protea Laboratory Equipment) at 460°C for 1 hour. After the remains were cooled in a desiccator for approximately 2 hours we weighed them (final weight) to calculate the dry weight of organic material combusted (Ganswindt et al. 2012).

Diluted faecal extracts were measured for immunoreactive androgen (fA), oestrogen (fE), progesterone (fP) and glucocorticoid (fGC) metabolites. We quantified fA using an epiandrosterone assay as first described by Palme and Möstl (1994). The epiandrosterone enzyme immunoassay (EIA) used an antibody raised in rabbit against a 5α -androstane- 3α -ol-17-on-HS:BSA and 5α -androstane-3,17-dione-thioether conjugated with N-biotinyl-1,8-diamino-3,6-dioxaoctane (DADOO-biotin) as label. The cross-reactivities of the antibody are described in Palme and Möstl (1994). Serial dilutions of faecal extracts gave displacement curves which were parallel to the respective standard curve of the assay. Sensitivity of the assay at 90% binding was 8pg/well. Intra- and inter-assay coefficients of variation (CV) were

determined by repeated measurements of low and high value quality controls ranging between 8.6% and 10.1% for intra-assay variation and between 13.5% and 14.6% for inter-assay variation. In brief, 50µl aliquots of epiandrosterone standard (range 9.8–2500pg), quality controls and diluted faecal extracts were pipetted in duplicate into microtiter plate wells. Fifty µl of label and antiserum were added and the plates incubated overnight at 4 °C. Following incubation the plates were washed four times and 150µl (20ng) of streptavidin–peroxidase added to each well. Following incubation in the dark for 45 minutes, plates were washed again before 150µl peroxidase substrate solution was added and plates further incubated for 30-60 minutes. The reaction was terminated by adding 50 µl of 4N H₂SO₄ and the absorbance measured at 450 nm.

We quantified fE using an oestrone assay as first described by Palme and Möstl (1994). The oestrone EIA used an antibody raised in rabbit against a 17β-oestradiol-17-HS:BSA and 17β-oestradiol-17-gluc coupled with N-biotinyl-1,8-diamino-3,6-dioxaoctane (DADOO-biotin) as label. The cross-reactivities of the antibody are described in Palme and Möstl (1994). Serial dilutions of faecal extracts gave displacement curves which were parallel to the respective standard curve of the assay. Sensitivity of the assay at 90% binding was 0.5 pg/well. Intra-assay CV was 3.1% and 5.6% for low and high controls, respectively. Inter-assay variation was 11.7% and 13.7% for low and high controls, respectively.

We quantified fP using a 5α-pregnan-3β-ol-20-one assay as first described by Hodges et al. (1997). The 5α-pregnan-3β-ol-20-one EIA used an antibody raised in rabbit against 5α-pregnane-3β-ol-20-one-3HS-BSA and 5α-pregnane-3β-ol-20-one-3HS conjugated with biotin as a label (Hodges et al. 1997). Cross-reactivities of the antibody are described in Hodges et al. (1997). Serial dilutions of faecal extracts gave displacement curves which were parallel to the respective standard curve of the assay. Sensitivity of the assay at 90% binding was 3 pg/well

and intra-assay CV was 3.6% and 4.8% for low and high controls, respectively; while inter-assay CV was 8.5% and 15.7% for low and high controls, respectively.

We quantified fGC using a cortisol assay as first described by Palme and Möstl (1997). This assay was previously validated for determining changes in fGC concentrations in the aardwolf (Ganswindt et al. 2012). The cortisol EIA used an antibody raised in rabbit against cortisol-3-CMO:BSA and cortisol-3-CMO a coupled with N-biotinyl-1,8-diamino-3,6-dioxaoctane (DADOO-biotin) as label. The cross-reactivities of the cortisol antibodies are described by Palme and Möstl (1997). Serial dilutions of faecal extracts gave displacement curves which were parallel to the respective standard curve of the assay. Accuracy of the assay was $108.1 \pm 10.2\%$ (mean \pm standard deviation) as from Ganswindt et al. (2012). Sensitivity of the assay at 90% binding was 1.5 pg/well. Intra-assay CV was 9.5% and 11.0% for low and high controls, respectively. Inter-assay variation was 6.7% and 14.6% for low and high controls, respectively.

Data are presented as $\mu\text{g/g}$ dry extracted organic material for fA and fP concentrations while data are presented as ng/g dry extracted organic material for fE and fGC concentrations.

3.3.5. Biological validation of immunoassays

Since male mating activity is typically regulated by androgens in mammals, we evaluated the biological relevance of the fA assay by contrasting baseline concentrations of fA in males to concentrations found during the mating season. The baseline fA value was calculated using an iterative process in which values that exceeded the mean plus 1.5 standard deviations (SD) were excluded. The average was then recalculated and the elimination process was repeated until no values exceeded the mean plus 1.5 SD (Brown et al. 1994).

Mating activity in females is predominantly regulated by oestrogens in mammals. Therefore, we contrasted baseline oestrogen concentrations in females, calculated as described

above, to those found in the mating season to evaluate the biological relevance of the fE assay. To evaluate the biological relevance of the fP assays we contrasted baseline concentrations in females, calculated as described above, to those found during the gestation season for females with pregnancies confirmed by subsequent observations of offspring.

3.3.6. *Statistical analyses*

We used linear mixed-effects (LME) models to evaluate the effect of season on mean concentrations of reproductive and adrenocortical hormone metabolites within each sex. We created six LME models, two for males using fA and fGC as response variables and four for females using fA, fE, fP and fGC as responses. All models were fitted with season as a fixed effect predictor. Within all six models, we controlled for non-independence within individuals as well as temporal pseudoreplication by fitting sample day nested within each individual as a random effect structure. Fixed effects were evaluated by conditional F-tests (Pinheiro & Bates 2000). To evaluate differences between specific seasons we conducted post-hoc tests using Tukey contrasts. We used variance ratio tests to evaluate pair wise seasonal differences in inter-individual variation on reproductive and adrenocortical hormones within each sex. These tests were conducted on fA and fGC concentrations for males and on fA, fE, fP and fGC concentrations for females. Each test was conducted on individual means, calculated for each year, and we adjusted p-values for multiple comparisons.

We omitted one outlier from the fA LME model for females and one outlier from the fGC LME model for females. Both of these outliers were tested against a standardized normal distribution using standardized residuals as z-values (fA lme outlier: $z = 7.41$, $p < 0.01$; fGC lme outlier: $z = 5.76$, $p < 0.01$). These outliers were then omitted and the fA LME and fGC LME models re-run for females. We regard that these outliers may have been caused by analytical error or mislabeling in the field rather than by endocrine fluctuations.

All models were fitted to raw endocrine data and statistical significance was set to 0.05 with all tests two tailed. Statistical analyses were performed with the software R version 2.15.1 for windows (<http://www.r-project.org>) using functions in the user contributed packages nlme (Pinheiro et al. 2012) and multcomp (Hothorn et al. 2012). Data represented in text and figures are mean \pm SE, unless otherwise stated.

3.4 Results

3.4.1. *Biological validation of EIAs for reproductive hormones*

In males, fA concentrations during the mating season were elevated over six times above the baseline (mating season 120.20 $\mu\text{g/g}$ vs. baseline 18.60 $\mu\text{g/g}$). In females, fE concentrations in the mating season were not as elevated, but nearly twice baseline concentrations (mating season 97.20 ng/g vs. baseline 59.00 ng/g). fP concentrations in pregnant females during the gestation season were elevated eight times above the baseline (gestation season 216.90 $\mu\text{g/g}$ vs. baseline 26.70 $\mu\text{g/g}$).

3.4.2. *Seasonal variation in reproductive hormone metabolite concentrations*

Season had a significant effect on fA concentration in males ($F_{2,51} = 4.92$, $p = 0.01$) but not in females ($F_{3,58} = 1.74$, $p = 0.17$; Fig. 3.2a). In males, the mating season produced significantly higher fA concentrations than both the non-reproductive ($p < 0.01$) and the parental care seasons ($p < 0.01$). However, there were no significant seasonal differences in the inter-individual variation of fA concentrations, either for males (mating vs. non-reproductive: $F_{3,4}=3.87$, $p_{\text{adj}} > 0.99$; mating vs. parental care: $F_{3,6}= 5.203$, $p_{\text{adj}} > 0.99$; non-reproductive vs. parental care: $F_{4,6}=1.34$, $p_{\text{adj}} > 0.99$) or for females (mating vs. gestation: $F_{1,2}=1.45$, $p_{\text{adj}} > 0.99$; mating vs. parental care: $F_{2,4}=0.01$, $p_{\text{adj}} = 0.12$; mating vs. non-reproductive: $F_{2,3}=0.04$, $p_{\text{adj}} =$

0.19; non-reproductive vs. parental care: $F_{3,4}=0.29$, $p_{adj} = 0.92$; parental care vs. gestation: $F_{1,4}=0.01$, $p_{adj} = 0.12$; gestation vs. non-reproductive: $F_{1,4}=0.05$, $p_{adj} = 0.20$).

Season had a significant effect on mean fE concentrations in females ($F_{3,59} = 4.42$, $p = 0.01$), where fE concentrations during the gestation season were higher than both the non-reproductive ($p < 0.01$) and the parental care seasons ($p < 0.01$: Fig. 3.2b). However, fE concentrations during the mating season were not significantly higher than the gestation ($p = 0.07$), non-reproductive ($p = 0.80$) or parental care ($p = 0.78$) seasons. There were no significant differences between seasons in terms of female inter-individual variation of fE concentrations (mating vs. gestation: $F_{1,2}=4.85$, $p_{adj} > 0.99$; mating vs. non-reproductive: $F_{2,3}=0.74$, $p_{adj} > 0.99$; mating vs. parental care: $F_{2,4}=3.33$, $p_{adj} > 0.99$; gestation vs. non-reproductive: $F_{1,3}=3.59$, $p_{adj} > 0.99$; gestation vs. parental care: $F_{1,4}=16.15$, $p_{adj} > 0.99$; parental care vs. non-reproductive: $F_{3,4}=4.50$, $p_{adj} > 0.99$).

Season had a significant effect on mean fP concentrations in females ($F_{3,59} = 9.49$, $p < 0.01$), with the gestation season concentrations being higher than all other seasons (vs. mating: $p < 0.01$; vs. non-reproductive: $p < 0.01$; vs. parental care: $p < 0.01$; Fig. 3.2c). There were no significant differences between seasons in terms of female inter-individual variation of fP concentrations (gestation vs. mating: $F_{1,2}=2.39$, $p_{adj} > 0.99$; gestation vs. non-reproductive: $F_{1,3}=76.80$, $p_{adj} > 0.99$; gestation vs. parental care: $F_{1,4}=12.92$, $p_{adj} > 0.99$; mating vs. non-reproductive: $F_{2,3}=32.18$, $p_{adj} > 0.99$; mating vs. parental care: $F_{2,4}=5.41$, $p_{adj} > 0.99$; parental care vs. non-reproductive: $F_{4,3}=5.95$, $p_{adj} > 0.99$).

3.4.3. Seasonal variation in faecal glucocorticoid metabolite concentrations

Season did not have a significant effect on fGC concentrations either in males ($F_{2,51} = 0.003$, $p > 0.99$) or in females ($F_{3,59} = 0.65$, $p = 0.59$; Fig. 3.2d). There were no significant differences between seasons in inter-individual variation of fGC concentrations in males (mating vs.

parental care: $F_{6,3}=0.59$, $p_{\text{adj}} > 0.99$; mating vs. non-reproductive seasons: $F_{3,4}=3.87$, $p_{\text{adj}} > 0.99$; parental care vs. non-reproductive: $F_{6,4}=6.60$, $p_{\text{adj}} > 0.99$) and in females (mating vs. gestation: $F_{1,2}=0.32$, $p_{\text{adj}} > 0.99$; mating vs. parental care: $F_{4,2}=0.47$, $p_{\text{adj}} > 0.99$; mating vs. non-reproductive: $F_{2,3}=1.58$, $p_{\text{adj}} > 0.99$; parental care vs. gestation: $F_{1,4}=0.68$, $p_{\text{adj}} > 0.99$; parental care vs. non-reproductive: $F_{4,3}=3.33$, $p_{\text{adj}} > 0.99$; non-reproductive vs. gestation: $F_{1,3}=0.20$, $p_{\text{adj}} > 0.99$).

3.4.4. Anecdotal copulation behaviour observed

We observed four copulations in four of the seven study individuals (Table 3.1). Of the four copulations observed, only two of these were quantifiable. Of these two copulations, only one was an EPC (Table 3.1).

3.5 Discussion

3.5.1. Reproductive seasonality in wild aardwolves

This is the first study to present reproductive hormone data for wild aardwolves. Our results confirm previous observations of the aardwolf as a strictly seasonal breeder (Richardson 1987a; Koehler & Richardson 1990; Sliwa 1996; Marneweck et al. 2012). Faecal androgen concentrations were under a seasonal influence in males where the elevation during the mating season was significantly higher compared to the other seasons. Normally, significantly higher oestrogen concentrations in females are correlated to a specific mating season (Monfort et al. 1989; Wingfield et al. 1994; Monfort 1997; Kraaijeveld-Smit et al. 2002; Kretzschmar et al. 2004; Hesterman et al. 2005; Dloniak et al. 2006; Fanson et al. 2010a, b). Although oestrogen and progesterone were under a seasonal influence in females, it is unusual that these hormone concentrations were not elevated during periods of mating activity. However, we do not know

if females are mono- or polyoestrous. Therefore, it could be difficult to detect a rise during the mating season if cyclic patterns were reflected by a peak in a single faecal sample (e.g., Brown 2006). We found indications of pro-oestrous in wild aardwolves where concentrations of oestrogen and progesterone during the mating season were increasing (aardwolves: Koehler & Richardson 1990; Canada lynx [*Lynx canadensis*]: Fanson et al. 2010b). We also found a subsequent peak in the concentrations of oestrogen and progesterone during the gestation season which is indicative of a pregnant or non-pregnant luteal phase (Schwarzenberger et al. 1996; Schwarzenberger 2007). In addition, two females were observed to have offspring in both parental care seasons confirming the pregnant luteal phase. Strictly seasonal breeding strategies in aardwolves are most likely to be proximately regulated by available food resources and by the rate of change in the photoperiod (Bronson & Heideman 1994).

3.5.2. *Endocrine correlations to the mating system in wild aardwolves*

Our physiological data from wild aardwolves appear to better fit predictions based on social monogamy. The results suggest inter-sexual symmetry in the seasonal inter-individual variation of reproductive hormones. This is also the first study to present stress-related adrenocortical hormone data for wild aardwolves. Our results showed no seasonal effect on mean or inter-individual variation of FGM concentrations in either males or females. Intra-sexual competition is strongly correlated with aggressive interactions which typically result in social rank relationships (Creel 2005). In species exhibiting such relationships there is typically a discrepancy between dominants and subordinates regarding their glucocorticoid concentrations, although the direction of these differences is related to the stability of the rank relationships (Creel et al. 1997; Creel 2005). Our results therefore suggest a lack of distinct rank relationships among wild aardwolves. The lack of variation in FGM values supports the data on androgens in that there appears to be no strong mating competition. We suggest that

the monogamous pair bonds may account for the constant levels and variances of glucocorticoids across the four seasons in both males and females. However, we highlight that these results were based on a low sample size.

Although recent data suggested behavioural support for promiscuity in wild aardwolves (Kotze et al. 2012), our study provides physiological data that more closely relate to monogamy. One potential reason for these contradictory results could be that behavioural traits have a larger plasticity compared to physiological traits (e.g., Hazlett 1995). Behaviour has long been considered the most plastic phenotypic trait because it is likely that behaviour shows the quickest response to temporal changes in extrinsic factors (e.g., Hazlett 1995; Sih et al. 2004; Briffa et al. 2008). However, this type of phenotypic plasticity may be costly (DeWitt et al. 1998) where proximate constraints such as physiology could limit behavioural plasticity (Hazlett 1995; Dall et al. 2004; Briffa et al. 2008). Therefore, there is a close connection between behavioural and physiological traits. We suggest that the discrepancy between hormonal versus behavioural support for mating promiscuity in aardwolves requires further investigation.

3.5.3. Conclusions

To conclude, our study provides physiological support for reproductive seasonality in wild aardwolves. In addition, our results suggest inter-sexual symmetry between males and females in the seasonal effect on reproductive and stress-related adrenocortical hormones. We suggest that these quantitative changes in reproductive and adrenocortical steroid hormone fluctuations in males and females fit predictions from social monogamy, but we acknowledge that our results were based on a small sample size. We suggest that the discrepancy between behavioural support for promiscuity and our physiological support for monogamy may be caused by a greater plasticity in behavioural versus physiological traits. This could allow

aardwolves to behaviourally deviate from social monogamy while retaining endocrine fluctuations related to this mating system.

3.6 References

- Agren, G., Zhou, Q. & Zhong, W. 1989. Ecology and social behavior of Mongolian gerbils, *Meriones unguiculatus*, at Xilinhot, Inner Mongolia, China. *Animal Behaviour* 37:11-27
- Anderson, M. D. & Richardson, P. R. K. 2005. The physical and thermal characteristics of aardwolf dens. *South African Journal of Wildlife Research* 35: 147-153
- Anestis, A., Pörtner, H. O., Karagiannis, D., Angelidis, P., Staikou, A. & Michaelidis, B. 2010. Response of *Mytilus galloprovincialis* (L.) to increasing seawater temperature and to martellosis: metabolic and physiological parameters. *Comparative Biochemistry and Physiology, Part A* 156: 57–66
- Barlow, G. W. 1988. Monogamy in relation to resources. In: Slobodchikoff, C. N. S. (Ed.), *The Ecology of Social Behavior*. Academic Press, London, UK, pp. 55-79
- Briffa, M., Rundle, S. D. & Fryer, A. 2008. Comparing the strength of behavioural plasticity and consistency across situations: Animal personalities in the hermit crab *Pagurus bernhardus*. *Proceedings of the Royal Society, B: Biological Sciences* 275: 1305-1311
- Bronson, F. H. & Heideman, P. D. 1994. Seasonal regulation of reproduction in mammals. In: Knobil, E., Neill, J. D., Greenwald, G. S., Markert, C. L. & Pfaff, D. W (Eds.), *The Physiology of Reproduction: Volume 1*. Raven Press, New York, USA, pp. 541-584
- Brown, J. L. 2006. Comparative endocrinology of domestic and nondomestic felids. *Theriogenology* 66: 25-36

- Brown, J. L., Bellem, A. C., Fouraker, M., Wildt, D. E. & Roth, T. L. 1994. Comparative analysis of gonadal and adrenal activity in the black and white rhinoceros in North America by noninvasive endocrine monitoring. *Zoo Biology* 20: 463-486
- Clutton-Brock, T. H. 1989. Mammalian mating systems. *Proceedings of the Royal Society of London, B: Biological Sciences* 236: 339-372
- Clutton-Brock, T. & Isvaran, K. 2006. Paternity loss in contrasting mammalian societies. *Biology Letters* 2: 513-516
- Cohas, A. & Allaine, D. 2009. Social structure influences extra-pair paternity in socially monogamous mammals. *Biology Letters* 5: 313-316
- Creel, S. 2005. Dominance, aggression, and glucocorticoid levels in social carnivores. *Journal of Mammalogy* 86: 255-264
- Creel, S., Creel, N. M., Mills, M. G. L. & Monfort, S. L. 1997. Rank and reproduction in cooperatively breeding African wild dogs: behavioural and endocrine correlates. *Behavioral Ecology* 8: 298-306
- Dall, S. R. X., Houston, A. I. & McNamara, J. M. 2004. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters* 7: 734-739
- Davies, N. B. 1991. Mating systems. In: Krebs, J. R. & Davies, N. B. (Eds.), *Behavioural Ecology: An evolutionary approach, 3rd Edition*. Blackwell Scientific Publications, Oxford, UK, pp. 263-294
- De Vries, J. L., Pirk, C. W. W., Bateman, P. W., Cameron, E. Z. & Dalerum, F. 2011. Extension of the diet of an extreme foraging specialist, the aardwolf (*Proteles cristata*). *African Zoology* 16: 194-196
- DeWitt, T. J., Sih, A. & Wilson, D. S. 1998. Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution* 13: 77-81

- Dloniak, S. M., French, J. A., Place, N. J., Weldele, M. L., Glickman, S. E., Holekamp, K. E. 2004. Non-invasive monitoring of fecal androgens in spotted hyenas (*Crocuta crocuta*). *General and Comparative Endocrinology* 135: 51-61
- Dloniak, S. M., French, J. A. & Holekamp, K. E. 2006. Faecal androgen concentrations in adult male spotted hyaenas, *Crocuta crocuta*, reflect interactions with socially dominant females. *Animal Behaviour* 71:27-37
- Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection and the evolution of mating systems. *Science* 197: 215-223
- Fanson, K. V., Wielebnowski, N. C., Shenk, T. A., Jakubas, W. J., Squires, J. R. & Lucas, J. R. 2010a. Patterns of testicular activity in captive and wild Canada lynx (*Lynx canadensis*). *General and Comparative Endocrinology* 169: 210-216
- Fanson, K. V., Wielebnowski, N. C., Shenk, T. A., Jakubas, W. J., Squires, J. R. & Lucas, J. R. 2010b. Patterns of ovarian and luteal activity in captive and wild Canada lynx (*Lynx canadensis*). *General and Comparative Endocrinology* 169: 217-224
- Fieß, M., Heistermann, M. & Hodges, J. K. 1999. Patterns of urinary and fecal steroid excretion during the ovarian cycle and pregnancy in the African elephant (*Loxodonta africana*). *General and Comparative Endocrinology* 115:76-89
- FitzGibbon, C. 1997. The adaptive significance of monogamy in the golden-rumped elephant-shrew. *Journal of Zoology* 242: 167-177
- Ganswindt, A., Rasmussen, H. B., Heistermann, M. & Hodges, J. K. 2005. The sexually active states of free ranging male African elephants (*Loxodonta africana*): defining musth and non-musth using endocrinology, physical signals, and behaviour. *Hormones and Behavior* 47: 83-91
- Ganswindt, A., Muilwijk, C., Engelkes, M., Muenscher, S., Bertschinger, H., Paris, M., Palme, R., Cameron, E. Z., Bennett, N. C. & Dalerum, F. 2012. Validation of non-invasive

- monitoring of adrenocortical endocrine activity in ground feeding aardwolves (*Proteles cristata*); exemplifying the influence of consumption of inorganic material for fecal steroid analysis. *Physiological and Biochemical Zoology* 85:194-199
- Glickman, S. E., Frank, L. G., Pavgi, S. & Licht, P. 1992. Hormonal correlates of “masculinization” in female spotted hyaenas (*Crocuta crocuta*). 1. Infancy to sexual maturity. *Journal of Reproduction and Fertility* 95: 451-462
- Goldman, B. D. 1999. The circadian timing system and reproduction in mammals. *Steroids* 64: 679-685
- Goymann, W., East, W. L., Wachter, B., Höner, O. P., Möstl, E., Van’t Hof, T. J. & Hofer, H. 2001. Social, state dependent and environmental modulation of faecal glucocorticoid levels in free ranging female spotted hyenas. *Proceedings of the Royal Society of London, B: Biological Sciences* 268: 2453-2459
- Goymann, W., East, M. L. & Hofer, H. 2003. Defense of females, but not social status, predicts plasma androgen levels in male spotted hyenas. *Physiological and Biochemical Zoology* 76: 586-593
- Griffith, S. C., Owens, I. P. F. & Thuman, K. A. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology* 11: 2195-2212
- Gubernick, D., Wright, S. & Brown, R. 1993. The significance of father’s presence for offspring survival in the monogamous California mouse, *Peromyscus californicus*. *Animal Behaviour* 46: 539-546
- Hazlett, B. A. 1995. Behavioural plasticity in crustacea: why not more? *Journal of Experimental Marine Biology and Ecology* 193: 57-66
- Hegner, R. E. & Wingfield, J. C. 1987. Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male house sparrows. *Auk* 104: 462-469

- Hesterman, H., Wasser, S. K. & Cochrem, J. F. 2005. Longitudinal monitoring of fecal testosterone in male Malayan sun bears (*U. malayanus*). *Zoo Biology* 24: 403-417
- Heymann, E. W. 2000. The number of males in callitrichine groups and its implications for callitrichine social evolution. In: Kappeler, P. M. (Ed.), *Primate Males*. Cambridge University Press, Cambridge, UK, pp. 64-71
- Hodges, J. K., Heistermann, M., Beard, A., van Aarde, R. J., 1997. Concentrations of progesterone and the 5 alpha-reduced progestins, 5 alpha-pregnane-3,20-dione and 3 alpha-hydroxy-5 alpha-pregnan-20-one, in luteal tissue and circulating blood and their relationship to luteal function in the African elephant, *Loxodonta africana*. *Biology of Reproduction* 56: 640-646
- Hodges, K., Brown, J. & Heisterman, M. 2010. Endocrine monitoring of reproduction and stress. In: Kleiman, D. G., Thompson, K. V. & Kirk Baer, C. (Eds.), *Wild mammals in captivity: Principles and techniques for zoo management*. The University of Chicago Press, Chicago, USA, pp. 447-468
- Hothorn, T., Bretz, F. & Westfall, P. 2012. *Simultaneous Inference in General Parametric Models*. R package version 1.2-13
- Kappeler, P. M. & van Schaik, C. P. 2002. Evolution of primate social systems. *International Journal of Primatology* 23: 707-740
- Kleiman, D. G. 1977. Monogamy in mammals. *Quarterly Review of Biology* 52: 39-69
- Koehler, C. E. & Richardson, P. R. K. 1990. *Proteles cristatus*. *Mammalian Species* 363: 1-6
- Komers, P. & Brotherton, P. 1997. Female space use is the best predictor of monogamy in mammals. *Proceedings of the Royal Society of London, B: Biological Sciences* 264: 1261-1270

- Kotze, R., Bennett, N., Cameron, E., De Vries, L., Marneweck, D., Pirk, C. & Dalerum, F. 2012. Temporal patterns of den use suggest polygamous mating patterns in the socially monogamous aardwolf. *Animal Behaviour* 84: 1573-1578
- Kraaijeveld-Smit, F. J. L., Ward, S. J., Temple-Smith, P. D. & Paetkau, D. 2002. Factors influencing paternity success in *Antechinus agilis*: last-male sperm precedence, timing of mating and genetic compatibility. *Journal of Evolutionary Biology* 15: 100-107
- Kretzschmar, P., Ganslöber, U. & Dehnhard, M. 2004. Relationship between androgens, environmental factors, and reproductive behavior in male white rhinoceros (*Ceratotherium simum simum*). *Hormones and Behavior* 45: 1-9
- Kruuk, H. & Sands, W. A. 1972. The aardwolf (*Proteles cristatus*, Sparrman 1783) as a predator of termites. *East African Wildlife Journal* 10: 211-227
- Lindeque, M. & Skinner, J. D. 1982. Fetal androgens and sexual mimicry in spotted hyaenas (*Crocuta crocuta*). *Journal of Reproduction and Fertility* 65: 405-410
- Marneweck, D. G., Ganswindt, A., Rhodes, S., Bellem, A., Bryant, J., Wielebnowski, N. & Dalerum, F. 2012. Reproductive endocrinology of zoo-housed aardwolves. *Acta Theriologica* DOI 10.1007/s13364 012-0101-1
- Matsebula, S. N., Monadjem, A., Roques, K. G. & Garcelon, D. K. 2009. The diet of the aardwolf, *Proteles cristatus* at Malolotja Nature Reserve, western Swaziland. *African Journal of Ecology* 47: 448-451
- McAllan, B. M. & Dickman, C. R. 1985. The role of photoperiod in the timing of reproduction in the Dasyurid Marsupial *Antechinus stuartii*. *Oecologia* 68: 259-264
- Monfort, S. L., Dahl, K. D., Czekala, N. M., Stevens, L., Bush, M. & Wildt, D. E. 1989. Monitoring ovarian function and pregnancy in the giant panda (*Ailuropoda melanoleuca*) by evaluating urinary bioactive FSH and steroid metabolites. *Journal of Reproduction and Fertility* 85: 203-212

- Monfort, S. L., Wasser, S. K., Mashburn, K. L., Burke, M., Brewer, B. A. & Creel, S. R. 1997. Steroid metabolism and validation of noninvasive endocrine monitoring in the African wild dog (*Lycaon Pictus*). *Zoo Biology* 16: 533-548
- Möstl, E. & Palme, R. 2002. Hormones as indicators of stress. *Domestic Animal Endocrinology* 23: 67-74
- Ostner, J., Kappeler, P. & Heistermann, M. 2008. Androgen and glucocorticoid levels reflect seasonally occurring social challenges in male redfronted lemurs (*Eulemur fulvus rufus*). *Behavioural Ecology and Sociobiology* 62: 627-638
- Palme, R. & Möstl, E. 1994. Biotin-streptavidin enzyme immunoassay for the determination of oestrogens and androgens in boar faeces. *Proceedings of the 5th Symposium on the Analysis of Steroids*. Szombathely, Hungary
- Palme, R. and Möstl, E. 1997. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. *Zeitschrift für Säugetierkunde* 62: 192-197
- Pinheiro, J. C. & Bates, D. M. 2000. *Mixed effect models in S and S-plus*. Springer-Verlag, New York, USA
- Pinheiro, J. C., Bates, D. M., DebRoy, S. & Sarkar, D. 2012. *Nlme: linear and nonlinear mixed effects models*. R package version 3.1-104
- Reichard, U. 1995. Extra-pair copulations in a monogamous gibbon *Hylobates lar*. *Ethology* 100: 99-112
- Richardson, P. R. K. 1985. The social behaviour and ecology of the aardwolf, *Proteles cristatus*, (Sparman, 1783) in relation to its food resources. Ph.D. thesis, University of Oxford, UK
- Richardson, P. R. K. 1987. Aardwolf mating system: overt cuckoldry in an apparently monogamous mammal. *South African Journal of Science* 83: 405-410

- Richardson, P. R. K. 1987a. Aardwolf mating system: overt cuckoldry in an apparently monogamous mammal. *South African Journal of Science* 83: 405-410
- Richardson, P. R. K. 1987b. Food-consumption and seasonal-variation in the diet of the aardwolf *Proteles cristatus* in Southern Africa. *Zeitschrift für Säugetierkunde* 52: 307-325
- Richardson, P. R. K. & Coetzee, M. 1988. Mate desertion in response to female promiscuity in the socially monogamous aardwolf *Proteles cristatus*. *South African Journal of Zoology* 23: 306-308
- Rutberg, A. T. 1983. The evolution of monogamy in primates. *Journal of Theoretical Biology* 104: 93-112
- Schulze, R. E. & McGee, O. S. 1978. Climatic indices and classifications in relation to the biogeography of southern Africa. In: Werger, M. J. A. & Junk, W. (Eds.), *Biogeography and Ecology of South Africa*. The Hague, Netherlands
- Schwarzenberger, F. 2007. The many uses of non-invasive faecal steroid monitoring in zoo and wildlife species. *International Zoo Yearbook* 41: 52-74
- Schwarzenberger, F., Möstl, E., Palme, R. & Bamberg, E. 1996. Fecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. *Animal Reproduction Science* 42: 515–526
- Scott, M. P. 1986. The timing and synchrony of seasonal breeding in the marsupial, *Antechinus stuartii*: Interaction of environmental and social cues. *Journal of Mammology* 67: 551-560
- Sih, A., Bell, A. & Johnson, J. C. 2004 Behavioural syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution* 19: 372-377
- Silverin, B. 1980. Effects of long-acting testosterone treatment on free-living pied flycatchers, *Ficedula hypoleuca*, during the breeding period. *Animal Behaviour* 28: 906-912

- Sliwa, A. 1996. A functional analysis of scent marking and mating behaviour in the aardwolf, *Proteles cristatus* (Sparrman, 1783). Ph.D. thesis, University of Pretoria, RSA
- Sliwa, A. & Richardson, P. R. K. 1998. Responses of aardwolves, *Proteles cristatus*, Sparrman 1783, to translocated scent marks. *Animal Behaviour* 56: 137-146
- Steenbeek, R., Sterck, E. H. M., de Vries, H. & van Hooff, J. A. R. A. M. 2000. Costs and benefits of the one-male, age-graded and all-male phases in wild Thomas's langur groups. In Kappeler, P. M. (Ed.), *Primate Males*. University of Cambridge Press, Cambridge, UK, pp. 130-145
- Struhsaker, T. T. 2000. Variation in adult sex ratios of red colobus monkey social groups: Implications for interspecific comparisons. In Kappeler, P. M. (ed.), *Primate Males*, Cambridge University Press, Cambridge, UK, pp. 108–119
- Trivers, R. L. 1972. Parental investment and sexual selection. In: Campbell, B. (Ed.), *Sexual selection and the descent of man*. Aldine, Chicago, USA, pp. 136-179
- van Jaarsveld, A. S., Richardson, P. R. K. & Anderson, M. D. 1995. Postnatal-growth and sustained lactational effort in the Aardwolf: life-history implications. *Functional Ecology* 9: 492-497
- van Schaik, C. P. & van Hooff, J. A. R. A. M. 1983. On the ultimate causes of primate social systems. *Behaviour* 85: 91-117
- Williams, J. B., Anderson, M. D. & Richardson, P. R. K. 1997. Seasonal differences in field metabolism, water requirements, and foraging behaviour of free-living aardwolves. *Ecology* 78: 2588-2602
- Wingfield, J. C. 1984a. Androgens and mating systems: testosterone-induced polygyny in normally monogamous birds. *Auk* 101: 665-671
- Wingfield, J. C., Ball, G. F., Dufty Jr, A. M., Hegner, R. E. & Ramenofsky, M. 1987. Testosterone and aggression in birds. *American Scientist* 75: 602-608

- Wingfield, J. C., Hegner, R. E., Dufty Jr, A. M. & Ball, G. F. 1990. “The challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *American Naturalist* 136: 829-846
- Wingfield, J. C., Whaling, C. S. & Marler, P. 1994. Communication in vertebrate aggression and reproduction: The role of hormones. In: Knobil, E., Neill, J. D., Greenwald, G. S., Markert, C. L. & Pfaff, D. W. (Eds.), *The Physiology of Reproduction: Volume 1*. Raven Press, New York, USA, pp. 303-342
- Wittenberger, J. F. & Tilson, R. L. 1980. The evolution of monogamy: hypotheses and evidence. *Annual Review of Ecology and Systematics* 11: 197-232
- Wynne-Edwards, K. 1987. Evidence for obligate monogamy in the Djungarian hamster, *Phodopus campbelli*: pup survival under different parenting conditions. *Behavioral Ecology and Sociobiology* 20:427-437

Table 3.1. Observed mating events, number of litters sired and faecal samples collected for seven VHF collared aardwolves on Benfontein Nature Reserve. Faecal samples are broken down into the described aardwolf reproductive seasons, where the 2009 parental care season was from Nov 2009-Jan 2010, 2010 parental care season from Nov 2010-Jan 2011 and 2011 parental care season being Nov 2011.

ID	Sex	Period followed (month/year)	No. faecal samples					Observed mating partner	No. litters sired
			Non- reproductive	Mating season	Gestation	Parental care	Total		
M2	Male	03/2009-06/2011	16	2	0	17	35	NA	NA
M8	Male	05/2009-04/2011	2	0	0	22	24	F6~, F9*	1 litter ¹
M12	Male	04/2010-11/2011	4	1	0	1	6	Unknown*#	NA
M14	Male	05/2010-11/2011	6	3	0	6	15	NA	1 litter ²
F6	Female	03/2009-12/2010	4	1	3	21	29	M8~	2 litters ^{1,2}
F9	Female	03/2009-09/2011	18	5	1	24	48	M8*	2 litters ^{1,2}
F15	Female	07/2010-07/2011	3	0	0	7	10	NA	NA
							Total	167	

~2009 Mating season; ¹2009 Parental care season

*2010 Mating season; ²2010 Parental care season

#2011 Mating season

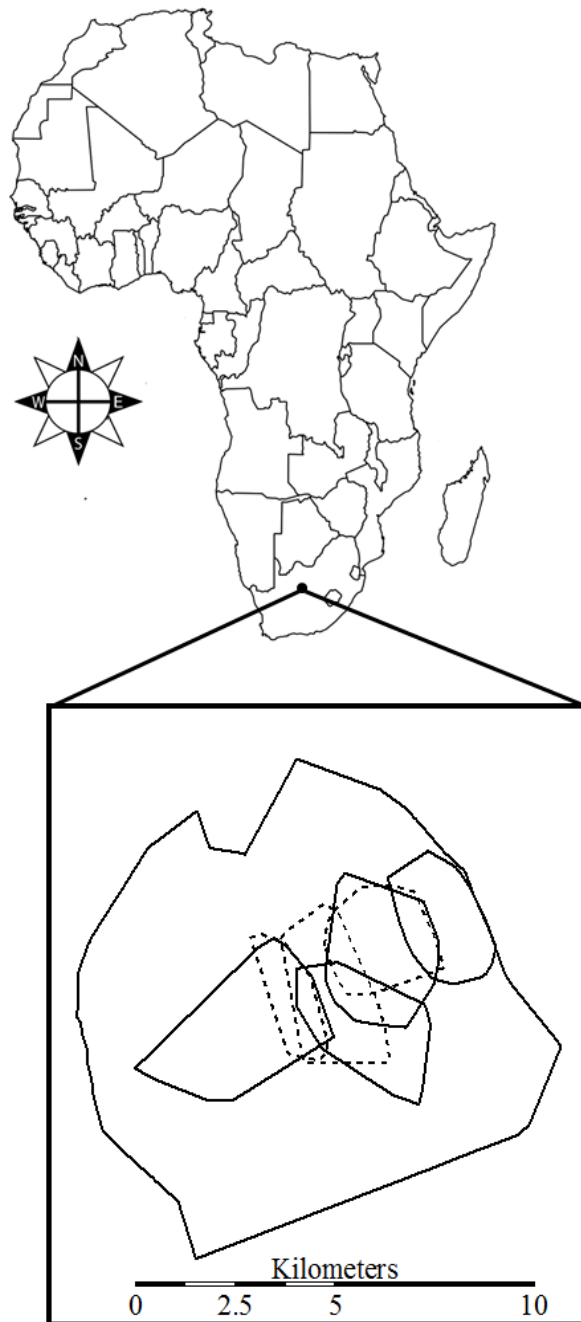


Fig. 3.1 Geographic location of Benfontein Nature Reserve, enclosed in the square box, situated approximately 10kms south-east of Kimberley, Northern Cape Province, South Africa. Male aardwolf annual home ranges are represented by solid lines, while female annual home ranges are represented by dashed lines. Home ranges were delineated by 100% Minimum Convex Polygons (MCPs).

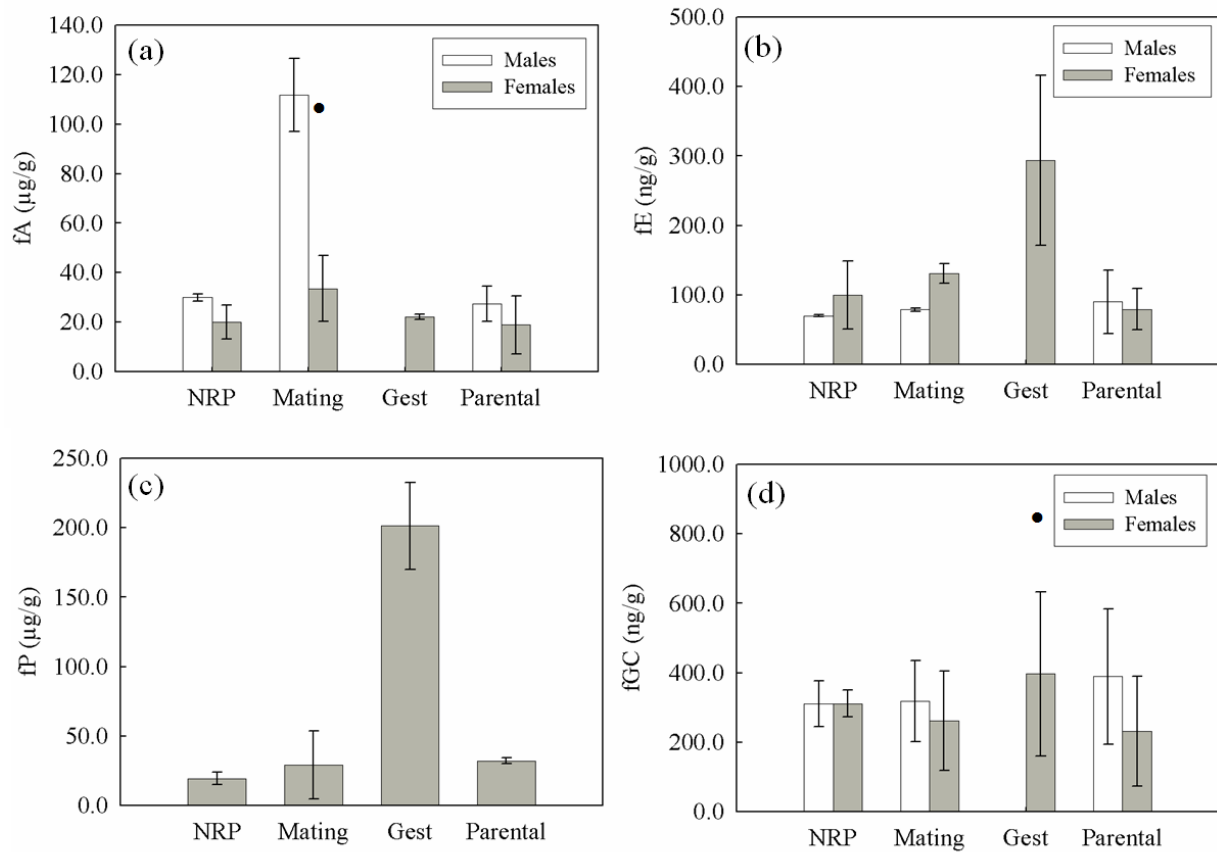


Fig. 3.2 Seasonal and sex-specific fA (a), fE (b) and fGC (d) hormone metabolite concentrations for male and female wild aardwolves. Seasonal inter-individual means and variances for female fP (c) are also shown. Data was averaged across years for each individual and represented as means across all individuals within sex \pm SE of inter-individual differences in these mean concentrations. Data are expressed as $\mu\text{g/g}$ or ng/g dry organic material. NRP = non-reproductive season, Gest = gestation season, Parental = parental care season. ● = outlier in fA and fGC for females.

CHAPTER 4

Behavioural correlates to the aardwolf mating system

4.1 Abstract

Detailed knowledge regarding behavioural regulation of reproductive activity may produce important insights into the maintenance of mating systems. Inter-sexual discrepancies in the regulation of reproductive success have amounted to the majority of mammal species having a polygynous mating system, with only 5% of mammals being socially monogamous. Generally, sexual symmetry in behaviour has been found for monogamous species while sexual asymmetry has been found for polygamous species. In monogamous species there is often a discrepancy between social mating bonds and sexual mating activities. In the socially monogamous aardwolf a number of studies have observed a high frequency of extra pair copulations (EPCs) and a recent study linked sex variation in den use to polygamous mating. In this study, we test if sex-specific seasonal patterns in home range size and utilisation, rates of scent marking and foraging, and the spatial distribution of these two behaviours relate to predictions based on monogamous or polygamous mating. We found sex differences in home range size, home range utilisation, scent marking rates and foraging rates, but no sex differences in the spatial distribution of scent marking or foraging behaviours. However, despite the observed sex differences we suggest that our results relate to social monogamy more closely than to polygamous mating. Such an interpretation would contradict earlier observations of EPCs and polygamous mating patterns in wild aardwolves. This contradiction could potentially be explained by temporal variation in mating activities where sometimes males attempt to optimise mating opportunities (polygamy) while at other times they are more

involved in paternal care of resident offspring (obligate monogamy). An alternate explanation could be that these observed behaviours may not be directly regulated by processes related to mating competition.

Key words: *Aardwolf, Forage, Home range, Monogamy, Polygamy, Scent mark, Space use*

4.2 Introduction

Mating systems are broad classifications of individual strategies aimed at maximising fitness (Emlen & Oring 1977; Clutton-Brock 1989; Davies 1991). Mating systems could be described to have two components; one describing the social mating associations while the other describes the genetic outcomes of actual mating activities (Kappeler & van schaik 2002). There is often a discrepancy between these mating system components which have been highlighted by a large body of research on extra-pair copulations (EPCs) in birds (Griffith et al. 2002). In mammals, the distribution of extra-pair paternities (EPPs) resulting from EPCs is considered to be low (Lukas & Clutton-Brock 2012). However, EPPs have been found in numerous mammalian groups, including the Carnivora (Clutton-Brock & Isvaran 2006; Cohas & Allaine 2009).

In mammals there is a sex bias in the regulation of reproductive success, where females are regulated by the availability of resources and males are regulated by the number of successful mating opportunities (Trivers 1972). This has resulted in the majority of mammals being polygynous with only 5% exhibiting social monogamy (Clutton-Brock 1989). It is not clear why a male restricts himself to breed with just a single female during a reproductive period (Clutton-Brock 1989), but two hypotheses have been put forward for the evolution of monogamy. The first hypothesis stresses the importance of bi-parental care for reproductive

success (i.e., obligate monogamy: Kleiman 1977; Wynne-Edwards 1987; Clutton-Brock 1989; Gubernick et al. 1993). The second hypothesis focuses on males' inability to monopolise more than one partner if females are widely dispersed (facultative monogamy: Emlen & Oring 1977; Rutberg 1983; van Schaik & van Hooff 1983; Barlow 1988; Clutton-Brock 1989; FitzGibbon 1997). Although males and females of both obligate and facultative monogamous mammals have been found to engage in EPCs (Richardson 1987; Agren et al. 1989; Reichard 1995; Cohas & Allaine 2009), there are lower degrees of EPPs for mammals with obligate compared to facultative monogamous mating systems (Clutton-Brock & Isvaran 2006; Cohas & Allaine 2009). These observations correspond to a predicted lower mating competition for obligate monogamous species.

The degree of male mating competition and mate fidelity can lead to a range of sex differences in behaviours. For example, in red deer (*Cervus elaphus*) there is intense inter-sexual asymmetry in behaviour caused by a strongly polygamous mating pattern and subsequently very high male mating competition (Clutton-Brock et al. 1982; Clutton-Brock & Albon 1989). Within socially monogamous pairs, on the other hand, there is often inter-sexual symmetry in behaviour. This symmetry can partly be caused by communal sharing of duties within the social pair bond (e.g., jackdaw [*Corvus monedula*], Henderson et al. 2000; sardinian warbler [*Sylvia melanocephala*], Bas et al. 2005; three-toed woodpeckers [*Picoides tridactylus*], Pechacek et al. 2005) or by little differences in the intensity of mating competition between males and females (e.g., golden jackals [*Canis aureus*] and black-backed jackal [*Canis mesomelas*], Fuller et al. 1989; sardinian warbler, Bas et al. 2005). Therefore, in socially monogamous pairs we would expect similarities between males and females in behaviours such as home range size and space use (e.g., Geffen & Macdonald 1992; Roberts & Dunbar 2000; Jácomo et al. 2009). Contrastingly, sex differences in behaviours such as home range size, space use and territorial defense may suggest differences in mating competition between males

and females which resonate more closely with polygamous mating (Clutton-Brock et al. 1982; Clutton-Brock 1989; Carranza et al. 1990).

The aardwolf is an extreme resource specialist that feeds almost exclusively on a single genus of termites (*Trinervitermes*: Richardson 1987a; Koehler & Richardson 1990). This resource fluctuates seasonally, with significant decreases during the dry months (Koehler & Richardson 1990). In addition, *Trinervitermes* are energetically sub-optimal as prey because of their small individual body size. Females are therefore required to forage more intensively during lactation than carnivores feeding on larger prey. Subsequently, they need to be away from the natal den which generates an increased need for paternal care (Richardson 1987a; Richardson & Coetzee 1988). Aardwolves are therefore described as obligate socially monogamous (Richardson 1985; Koehler & Richardson 1990), which is supported by endocrine data (Chapter 3). However, aardwolves engage in a high frequency of EPCs (60-70% of all observed matings: Richardson 1987b; Sliwa 1996), and behavioural observations further support promiscuity in wild aardwolves (Kotze et al. 2012). The aim of this study was to test how sex-specific seasonal variation in home range size, space use, the rate of scent marking and foraging, and the spatial distribution of behaviours relate to predictions from social monogamy.

4.3 Materials and methods

4.3.1. Study area and study animals

This study was conducted on Benfontein Nature Reserve (Fig. 3.1, 28°50'S; 24°50'E). The reserve has a distinct cold and dry winter period and a hot and wet summer period. The mean annual rainfall for the Kimberley area is 431±127 mm (Weather Bureau in Pretoria) and the temperature in winter usually drops below freezing at night.

This chapter uses data from seven aardwolves (four males, three females; Table 3.1) that were captured and radio-tagged on Benfontein Nature Reserve between November 2008 and May 2010. The aardwolves were fitted with very high frequency radio collars (VHF; Sirtrack Ltd, Havelock North, New Zealand: weight $68.25\text{g} \pm 8\text{g}$, mean $\pm 1\text{sd}$) for relocation, and after habituation followed from a vehicle at distances ranging from no more than 20 metres. A detailed description of the study area as well as the capture process is given in Chapter 3.

4.3.2. Spatial and behavioural data collection

Between March 2009 and November 2011 radio-tagged aardwolves were relocated and observed from a 4x4 vehicle to record home range size and utilisation as well as spatially explicit scent marking and foraging behaviour. We located each animal at its day time den, and once it had emerged we followed it for approximately two hours (range = 0.16-6.8 hours, mean = 1.47 hours) with a GPS that continuously tracked the aardwolf's movements while being followed. Each of these two hour periods were defined as a single spatial track. While active, scent marking behaviours were recorded as the action of an aardwolf straddling a grass stalk, rapidly squatting while everting the anal pouch and wiping a smear of secretion onto the grass (Kruuk & Sands 1972; Richardson 1985; Sliwa 1996; Sliwa & Richardson 1998). Foraging behaviours were recorded as the action of an aardwolf lowering its head to ground level and proceeding to lick travelling termites off the soil or mound surface into its mouth (Koehler & Richardson 1990). These behaviours were recorded using a handheld PDA loaded with the CyberTracker™ software, and a GPS coordinate was stored for each logged behaviour. This period of recording scent mark and foraging was defined as a single behavioural session. We attempted to follow two aardwolves per night. We recorded 269 spatial tracks and 157 behavioural sessions for the seven collared aardwolves at varied seasonal frequencies for each individual (Table 4.1).

4.3.3. Revised definition of seasons

The definitions of season for this Chapter are different from those in Chapter 3 (pages 56 & 57). The reason for this discrepancy between chapters was that the resolution in the aardwolf behavioural data (Table 4.1) was much lower than that of the physiological data (Table 3.1). Therefore, we only had enough data to define two seasons for this Chapter: (1) the wet season is the six hot and rainy months from September to February and (2) the dry season is the six cold and dry months from March to August. The mating season described in Chapter 3 falls within the dry season for this chapter. The gestation season as described in Chapter 3 corresponds to the end of the dry season and beginning of the wet season for this chapter. The weaning/parental care season described in Chapter 3 falls within the wet season for this Chapter. The non-mating season described in Chapter 3 corresponds to the end of the wet season and beginning of the dry season for this chapter.

4.3.4. Estimation of home range size, utilisation and evenness

We used 95% Minimum Convex Polygons (MCPs: Mohr 1947) to estimate seasonal home range sizes for each animal. We based the MCP's on all pooled relocation data, including both data recorded during spatial data collection and opportunistic sightings. We used MCP's to characterise home range size because they are relatively robust to possible temporal autocorrelation among data (Swihart & Slade 1985). Home range sizes were calculated using Hawth's Tools (<http://www.spatial ecology.com/htools>).

We used a novel method for estimating home range utilisation based on line density, which were based on continuous tracks delineating aardwolf movement as opposed to fixed relocation points. We created line density isopleths which represents the density of linear features in the neighbourhood of a pre-determined raster cell, measured as the unit of length of lines per unit of area. We used a 20m X 20m raster cell size as we typically were not more than

20m from the animal being observed. The area was defined as a circle drawn around each raster cell using a user-defined search radius. We defined the search radius as the mean distance from 10 000 random points to the closest line segment within each aardwolf's annual home range.

The function to calculate line densities is:

$$\text{Line density} = \frac{\sum_1^K L_i}{a}$$

where K is the total number of segments within a given search radius, L_i is the length of segment i and a is the area within the search radius.

We used a normalised Shannon spatial diversity index (Payne 1997; Payne et al. 2005) to estimate the evenness of home range use in aardwolves. The index was calculated on the cell values of line densities according to the following function:

$$\text{Shannon spatial diversity index} = -1 \frac{\sum_1^R P_i \cdot \log P_i}{\log(R)}$$

where R is the number of pixels within each home range and P_i is the relative abundance of the linear features within a raster cell. An index value of 0 indicates complete unevenness in space use while a value of 1 indicates complete evenness. This analysis was not conducted seasonally due to low resolution in seasonal track data (Table 4.1).

4.3.5. Temporal and spatial patterns in scent marking and time spent foraging

We calculated the number of scent markings and foraging bouts per 10 minutes for each behavioural session. We also quantified the proportion of time spent foraging for each session. We used two separate methods to quantify the spatial distribution of scent marking and foraging within individual home ranges for males and females. First, we intersected spatial coordinates of scent marking and foraging behaviours with the home range utilisation estimate and extracted the utilisation intensity (i.e. the density of movement tracks) for locations used for scent marking and foraging behaviours. Second, we calculated the distance between each

scent mark and foraging bout to the nearest home range border as delineated from the 95% MCP's to estimate where within the home ranges the different behaviours were conducted.

4.3.6. Statistical analyses

We used linear mixed-effects (LME) models to evaluate the effect of sex and season on home range size, scent marking rates, foraging rates, proportion of time spent foraging, utilisation intensity of scent marks, distance to border of scent marks, utilisation intensity of foraging and distance to border effects of foraging bouts. In all models sex, season and their two way interaction were fitted as fixed effect predictors. To account for heteroscedasticity we used a variance power function in the models on home range size, scent marking rates and foraging rates (Pinheiro & Bates 2000) and an arcsine square root transformation in the model on proportion of time spent foraging. In the model on home range size we fitted animal identity as a random effect, whereas in the other models we fitted observation day nested within each individual as the random effects structure. In all models, except the one on home range size, we fitted a spatial autocorrelation function to control for the spatial structure of our data points (Venables & Ripley 1997). Fixed effects were evaluated by conditional F-tests in all models (Pinheiro & Bates 2000). We used a Welch two sample t-test to test if there was a significant difference in the Shannon space use index between males and females.

Statistical significance was set to 0.05 with all tests two tailed. Statistical analyses were performed with the software R, version 2.15.1 for windows (<http://www.R-project.org>), using functions in the user contributed packages nlme (Pinheiro et al. 2012) and ramps (Venables & Ripley 1997). We used ArcGIS version 9.3.2 with the spatial analyst tool to create the line density isopleths and extracted raster values for each logged behaviour using Geospatial Modeling Environment (GME) program. Data represented in text and figures are mean \pm SE, unless otherwise stated.

4.4 Results

4.4.1. *Effects of sex on home range size and utilisation*

Sex had a significant effect on home range size ($F_{1,5} = 8.28$, $p = 0.03$), where males had significantly larger home ranges than females (Figure 4.1a). Season did not have an effect on home range size for either males or females ($F_{1,5} = 3.76$, $p = 0.11$; Fig. 4.1a), nor was there a significant interaction of sex and season ($F_{1,5} = 0.18$, $p = 0.69$). Males used their home ranges significantly more evenly than females ($t_{(4,94)} = 4.13$, $p = 0.01$; Fig. 4.1b), although both males and females had Shannon Index values close to complete evenness (Shannon index values: males = 0.93, females = 0.90).

4.4.2. *Effects of sex and season on rate of scent marking and foraging*

Sex had a significant effect on scent marking rate ($F_{1,5} = 7.84$, $p = 0.04$), with males scent marking more frequently than females (Fig. 4.2a). Season also had a significant effect on scent marking rate ($F_{1,145} = 10.97$, $p < 0.01$), with both males and females scent marking more frequently in the wet compared to the dry season (Fig. 4.2a). However, the interaction of sex and season did not have a significant effect on scent mark rate in aardwolves ($F_{1,145} = 1.25$, $p = 0.26$).

Sex had a significant effect on foraging rate ($F_{1,5} = 8.89$, $p = 0.03$), with females foraging more frequently than males (Fig. 4.2b). Season did not have a significant effect on foraging rate ($F_{1,145} = 2.87$, $p = 0.09$), nor was there a significant interaction effect of sex and season ($F_{1,145} = 0.0001$, $p = 0.99$). Neither sex ($F_{1,5} = 6.04$, $p = 0.06$), season ($F_{1,145} = 3.26$, $p = 0.07$) nor the interaction of sex and season ($F_{1,145} = 0.06$, $p = 0.80$) had a significant effect on proportion of time spent foraging (Fig. 4.2c).

4.4.3. *Effects of sex and seasons on the spatial distribution of scent marking and foraging*

Neither sex ($F_{1,5} = 0.74$, $p = 0.43$), season ($F_{1,3709} = 0.06$, $p = 0.80$) nor the interaction of sex and season ($F_{1,3709} = 0.16$, $p = 0.69$) had a significant effect on either the utilisation rate of locations used for scent marking (Fig. 4.3a) or the distance of scent marking locations to nearest home range border (sex: $F_{1,5} = 5.19$, $p = 0.07$; season: $F_{1,3709} = 0.25$, $p = 0.61$; sex x season : $F_{1,3709} = 0.28$, $p = 0.60$; Fig. 4.3b).

Similarly, neither sex ($F_{1,5} = 2.41$, $p = 0.18$), season ($F_{1,3003} = 0.52$, $p = 0.47$) nor the interaction of sex and season ($F_{1,3003} = 0.01$, $p = 0.93$) had a significant effect on the utilisation intensity of foraging locations (Fig. 4.3c). However, there was a significant interaction effect of sex and season on the distance of foraging locations to nearest home range border ($F_{1,3003} = 1272.93$, $p < 0.01$), where females foraged further from the home range borders in the wet season compared to the dry season while there were little seasonal differences for males (Fig. 4.3d).

4.5 Discussion

We found sex differences both in home range size and space use in this study. However, these discrepancies between males and females do not seem to have been related to optimisation of male mating opportunities. If male aardwolves were trying to continuously maximise extra mating opportunities we would expect their home ranges to be orders of magnitude larger than females' to include as many females as possible within their home range (Clutton-Brock et al. 1982; Clutton-Brock & Albon 1989; Ferguson et al. 2009; Loveridge et al. 2010). In addition, males utilised their range more evenly than females. This result suggests that their activity was not concentrated to the periphery of the home range which could have been predicted since this is where extra-pair mating partners would be located. Numerous studies have correlated

increased testosterone to an increase in roaming behaviour (Chandler et al. 1994) and we suggest that the larger home range size and more even movement patterns in males compared to females could have been the result of non-adaptive space use and movement caused by high testosterone levels in males.

We found sex differences also in scent marking behaviour in aardwolves. Scent marks of urine (Rich & Hurst 1998; Palagi et al. 2005), faeces (Kruuk 1992; Brashares & Arcese 1999a, b) and glandular secretions (Rossell et al. 1998; Rossell & Sundsdal 2001; Burgener et al. 2009) function as a way of advertising ownership of a territory (Gosling & Roberts 2001). However, if male aardwolves' scent mark as a way of mate guarding we would expect scent marking rates to increase during the dry season (Gorman & Mills 1984; Gorman 1990), as well as being more concentrated along the periphery of their ranges to ward off potential rivals (Gorman & Mills 1984; Gorman 1990; Sliwa & Richardson 1998; Caspers & Voigt 2009; Lledo-Ferrer et al. 2011). Our data instead showed that both male and female aardwolves scent marked more during the wet season and that neither sex concentrated their scent marks at the border of their ranges. The wet season is a period of intense offspring dependency at natal dens (Koehler & Richardson 1990; Anderson & Richardson 2005). We therefore suggest that scent marking may function as a way of protecting the territory from intruders to secure enough food resources to successfully raise offspring. The observation of higher scent marking in males compared to females may be attributed to sex differences in behaviour within a monogamous pair where males could be more involved in the protection of resources.

The lack of sex and seasonal differences in the time spent foraging suggests no cost of mating activities in terms of foraging time. Therefore, we suggest that the observed sex differences in the frequency of foraging bouts are reflecting non-mating related differences in male and female foraging behaviour. For females, we further found a seasonal variation in the spatial distribution of foraging locations where they foraged further from home range borders

in the wet compared to the dry season. This may be caused by a central tendency towards maternal dens when offspring are present (Joshi et al. 1999; Boydston et al. 2003; Kotze et al. 2012). Females are required to forage more intensively during the wet season when they are lactating (Richardson & Coetzee 1988). Therefore, female aardwolves have to balance the demands of foraging sufficiently for themselves in addition to foraging enough to provide an adequate amount of milk for offspring at natal dens (Richardson & Coetzee 1988). Alternatively, the observed seasonal variation in foraging locations could be explained by decreased food availability around foraging dens immediately after a dry season as predicted from central foraging theory (Orians & Pearson 1979; Schoener 1979; Daniel et al. 2008; Burke & Montecchi 2009). This entails that during the dry season and immediately thereafter, females are required to forage further from the dens and closer to home range borders to meet this energetic demand. During the dry season in the Northern Cape Province food resource availability of termites is low due to a seasonal decrease in temperature (Richardson 1987a; Koehler & Richardson 1990). The lack of a seasonal variation in the spatial location of foraging sites for males could be caused by a less pronounced fidelity to natal dens than females.

A general problem for most carnivore field studies is acquiring sample sizes large enough to allow for robust statistical testing. This problem is connected to logistical difficulties in studying carnivores as they often occur at low densities, move over large areas, are cryptic and often nocturnal and solitary (Bekoff et al. 1984; Karanth et al. 2010). Despite our low sample size of individuals and the frequency of mating events, we used intensive individual-resolution data on wild aardwolves to quantify clear sex-specific differences in behaviours related to space use, territoriality and foraging. We therefore believe that this study provides an important contribution and highlight that detailed behavioural observations can generate important insights even if statistically rigid sample sizes may not have been met.

4.5.1. Conclusions

Despite the observed sex differences in home range size, space use, scent marking rates and rates of foraging bouts, we suggest that our results are more likely related to social monogamy than to polygamous mating. Therefore our study, others that recorded EPCs (Richardson 1987b; Sliwa 1996) and a more recent study that recorded temporal variation in den use (Kotze et al. 2012) show how different behavioural traits correspond to different predictions based on social monogamy versus polygamous mating. This discrepancy between different traits may be due to a high degree of plasticity in the mating system of wild aardwolves, or that different physiological and behavioural traits have contrasting responses to social behaviour versus actual mating activities. We therefore suggest two possible, but not mutually exclusive, explanations for this contradiction. Firstly, it is possible that there were no, or at least substantially lower, EPCs during our study than during Richardson (1987b) and Sliwa (1996). An alternate explanation could be that the behaviours observed here are not directly regulated by processes related to mating competition.

4.6 References

- Agren, G., Zhou, Q. & Zhong, W. 1989. Ecology and social behavior of Mongolian gerbils, *Meriones unguiculatus*, at Xilinhot, Inner Mongolia, China. *Animal Behaviour* 37: 11-27
- Anderson, M. D. & Richardson, P. R. K. 2005. The physical and thermal characteristics of aardwolf dens. *South African Journal of Wildlife Research* 35: 147-153
- Barlow, G. W. 1988. Monogamy in relation to resources. In: Slobodchikoff, C. N. S. (Ed.), *The Ecology of Social Behavior*. London: Academic Press, London, UK, pp. 55-79

- Bas, J. M., Pons, P. & Gomez, C. 2005. Home range and territory of the Sardinian warbler *Sylvia melanocephala* in Mediterranean shrubland: Capsule singing territories were well separated. *Bird Study* 52: 137-144
- Bekoff, M., Daniels, T. J. & Gittleman, J. L. 1984. Life history patterns and the comparative social ecology of carnivores. *Annual Review of Ecology and Systematics* 15: 191-232
- Boydston, E. E., Kapheim, K. M., Szykman, M. & Holekamp, K. E. 2003. Individual variation in space use by female spotted hyenas. *Journal of Mammology* 84: 1006-1018
- Brashares, J. S. & Arcese, P. 1999a. Scent marking in a territorial African antelope: I. The maintenance of borders between male oribi. *Animal Behaviour* 57: 1-10
- Brashares, J. S. & Arcese, P. 1999b. Scent marking in a territorial African antelope: II. The economics of marking with faeces. *Animal Behaviour* 57: 11-17
- Burgener, N., Dehnhard, M., Hofer, H. & East, M. L. 2009. Does anal gland scent signal identity in the spotted hyaena? *Animal Behaviour* 77: 707-715
- Burke, C. M. & Montevocchi, W. A. 2009. The foraging decisions of a central place foraging seabird in response to fluctuations in local prey conditions. *Journal of Zoology* 278: 354-361
- Carranza, J., Alvarez, F. & Redondo, T. 1990. Territoriality as a mating strategy in red deer. *Animal Behaviour* 40: 79-88
- Caspers, B. A. & Voigt, C. C. 2009. Temporal and spatial distribution of male scent marks in the polygynous greater sac-winged bat. *Ethology* 115: 713-720
- Chandler, C. R., Ketterson, E. D., Nolan, V. & Ziegenfus, C. 1994. Effects of testosterone on spatial activity in free ranging male dark-eyed juncos, *Junco hyemalis*. *Animal Behaviour* 47: 1445-1455
- Clutton-Brock, T. H. 1989. Mammalian mating systems. *Proceedings of the Royal Society of London, B: Biological Sciences* 236: 339-372

- Clutton-Brock, T. H. & Albon, S. D. 1989. *Red deer in the Highlands*. BSP Professional Books, Oxford, UK
- Clutton-Brock, T. H. & Isvaran, K. 2006. Paternity loss in contrasting mammalian societies. *Biology Letters* 2: 513-516
- Clutton-Brock, T. H., Guinness, F. E. & Albon, S. D. 1982. *Red deer: Behaviour and ecology of two sexes*. Edinburgh University Press, Edinburgh, Scotland
- Cohas, A. & Allaine, D. 2009. Social structure influences extra-pair paternity in socially monogamous mammals. *Biology Letters* 5: 313-316
- Daniel, S., Korine, C. & Pinshow, B. 2008. Central-place foraging in nursing, arthropod-gleaning bats. *Canadian Journal of Zoology* 86: 623-626
- Davies, N. B. 1991. Mating systems. In: Krebs, J. R. & Davies, N. B. (Eds.), *Behavioural Ecology: An evolutionary approach, 3rd Edition*. Blackwell Scientific Publications, Oxford, UK, pp. 263-294
- Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection and the evolution of mating systems. *Science* 197: 215-223
- Ferguson, A. W., Currit, N. A. & Weckerly, F. W. 2009. Isometric scaling in home-range size of male and female bobcats (*Lynx rufus*). *Canadian Journal of Zoology* 87: 1052-1060
- FitzGibbon, C. 1997. The adaptive significance of monogamy in the golden-rumped elephant-shrew. *Journal of Zoology* 242:167-177
- Fuller, T. K., Biknevicius, A. R., Kat, P. W., Van Valkenburgh, B. & Wayne, R. K. 1989. The ecology of three sympatric jackal species in the Rift Valley of Kenya. *African Journal of Ecology* 27: 313-323
- Geffen, E. & Macdonald, D. W. 1992. Small size and monogamy: spatial organization of Blanford's foxes, *Vulpes cana*. *Animal Behaviour* 44:1123-1130

- Gorman, M. L. & Mills, M. G. L. 1984. Scent marking strategies in hyaenas (Mammalia). *Journal of Zoology* 202: 535-547
- Gorman, M. L. 1990. Scent marking strategies in mammals. *Revue Suisse de Zoology* 97: 3-29
- Gosling, L. M. & Roberts, S. C. 2001. Scent-marking by male mammals: Cheat-proof signals to competitors and mates. *Advances in the Study of Behavior* 30: 169-217
- Griffith, S. C., Owens, I. P. F. & Thuman, K. A. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology* 11: 2195-2212
- Gubernick, D., Wright, S. & Brown, R. 1993. The significance of father's presence for offspring survival in the monogamous California mouse, *Peromyscus californicus*. *Animal Behaviour* 46: 539-546
- Henderson, I. G., Hart, P. J. B. & Burke, T. 2000. Strict monogamy in a semi-colonial passerine: the jackdraw *Corvus monedula*. *Journal of Avian Biology* 31: 177-182
- Jácomo, A. T. de A., Kashivakura, C. K., Ferro, C., Furtado, M. M., Astete, S. P., Tôrres, N. M., Sollmann, R. & Silveira, L. 2009. Home range and spatial organization of maned wolves in the Brazilian Grasslands. *Journal of Mammalogy* 90: 150-157
- Joshi, A. R., Smith, J. L. D. & Garshelis, D. L. 1999. Sociobiology of the myrmecophagous sloth bear in Nepal. *Canadian Journal of Zoology* 77: 1690-1704
- Kappeler, P. M. & van Schaik, C. P. 2002. Evolution of primate social systems. *International Journal of Primatology* 23: 707-740
- Karanth, K. U., Funston, P. & Sanderson, E. 2010. Many ways of skinning a cat: tools and techniques for studying wild felids. In: Macdonald, D. W. & Loveridge, A. J. (Eds.), *Biology and Conservation of wild felids*. Oxford University Press, Oxford, UK, pp. 197-216
- Kleiman, D. G. 1977. Monogamy in mammals. *Quarterly Review of Biology* 52: 39-69
- Koehler, C. E. & Richardson, P. R. K. 1990. *Proteles cristatus*. *Mammalian Species* 363: 1-6

- Kotze, R., Bennett, N., Cameron, E., De Vries, L., Marneweck, D., Pirk, C. & Dalerum, F. 2012. Temporal patterns of den use suggest polygamous mating patterns in the socially monogamous aardwolf. *Animal Behaviour* 84: 1573-1578
- Kruuk, H. 1992. Scent marking by otters (*Lutra lutra*): signalling the use of resources. *Behavioral Ecology* 3: 133-140
- Kruuk, H. & Sands, W. A. 1972. The aardwolf (*Proteles cristatus*, Sparrman 1783) as a predator of termites. *East African Wildlife Journal* 10: 211-227
- Lledo-Ferrer, Y., Peláez, F. & Heymann, E. W. 2011. The equivocal relationship between territoriality and scent marking in wild saddleback tamarins (*Saguinus fuscicollis*). *International Journal of Primatology* 32: 974-991
- Loveridge, A. J., Hemson, G., Davidson, Z. & Macdonald, D. W. 2010. African lions on the edge: reserve boundaries as 'attractive sinks'. In: Macdonald, D. W. & Loveridge, A. J. (Eds.), *Biology and Conservation of wild felids*. Oxford University Press, Oxford, UK, pp. 283-304
- Lukas, D. & Clutton-Brock, T. 2012. Cooperative breeding and monogamy in mammalian societies. *Proceedings of the Royal Society, B: Biological Sciences* 279: 2151-2156
- Mohr, C. O. 1947. Table of equivalent populations of North American small mammals. *American Midland Naturalist* 37: 223-249
- Orians, G. H. & Pearson, N. E. 1979. On the theory of central place foraging. In: Horn, D. J., Mitchell, R. D. & Stairs, G. R. (Eds.), *Analyses of ecological systems*. Ohio State University Press, Ohio, USA, pp.154-177
- Palagi, E., Dapporto, L. & Borgognini Tarli, S. 2005. The neglected scent: on the marking function of urine in Lemur catta. *Behavioral Ecology and Sociobiology* 58: 437-445
- Payne, L. X. 1997. Dispersion patterns of sandpipers migrating through the Eastern United States. MSc thesis, University of Wisconsin, USA

- Payne, L. X., Schindler, D. E., Parrish, J. K. & Temple, S. A. 2005. Quantifying spatial pattern with evenness indices. *Ecological Applications* 15: 507-520
- Pechacek, P., Michalek, K. G., Winkler, H. & Blomqvist, D. 2005. Monogamy with exceptions: social and genetic mating system in a bird species with high paternal investment. *Behaviour* 142: 1093-1114
- Pinheiro, J. C. & Bates, D. M. 2000. *Mixed effect models in S and S-plus*. Springer-Verlag, New York, USA
- Pinheiro, J. C., Bates, D. M., DebRoy, S. & Sarkar, D. 2012. *Nlme: linear and nonlinear mixed effects models*. R package version 3.1-104
- Reichard, U. 1995. Extra-pair copulations in a monogamous gibbon *Hylobates lar*. *Ethology* 100: 99-112
- Rich, T. J. & Hurst, J. L. 1998: Scent marks as reliable signals of the competitive ability of mates. *Animal Behaviour* 56: 727-735
- Richardson, P. R. K. 1985. The social behaviour and ecology of the aardwolf, *Proteles cristatus*, (Sparrman, 1783) in relation to its food resources. Ph.D. thesis, University of Oxford, UK
- Richardson, P. R. K. 1987. Aardwolf mating system: overt cuckoldry in an apparently monogamous mammal. *South African Journal of Science* 83:405-410
- Richardson, P. R. K. 1987a. Aardwolf mating system: overt cuckoldry in an apparently monogamous mammal. *South African Journal of Science* 83: 405-410
- Richardson, P. R. K. 1987b. Food consumption and seasonal variation in the diet of the aardwolf *Proteles cristatus* in Southern Africa. *Zeitschrift für Säugetierkunde* 52: 307-325

- Richardson, P. R. K. & Coetzee, M. 1988. Mate desertion in response to female promiscuity in the socially monogamous aardwolf *Proteles cristatus*. *South African Journal of Zoology* 23: 306-308
- Roberts, S. C. & Dunbar, R. I. M. 2000. Female territoriality and the function of scent-marking in a monogamous antelope (*Oreotragus oreotragus*). *Behavioral Ecology and Sociobiology* 47: 417-423
- Rossell, F. & Sundsdal, L. J. 2001. Odorant source used in Eurasian beaver territory marking. *Journal of Chemical Ecology* 27: 2471-2491
- Rossell, F., Bergan, F. & Parker, H. 1998. Scent-marking in the Eurasian beaver (*Castor fiber*) as a means of territory defence. *Journal of Chemical Ecology* 24: 207-219
- Rutberg, A. T. 1983. The evolution of monogamy in primates. *Journal of Theoretical Biology* 104: 93-112
- Schoener, T. W. 1979. Generality of the size-distance relation in models of optimal foraging. *American Naturalist* 114: 902-914
- Sliwa, A. 1996. A functional analysis of scent marking and mating behaviour in the aardwolf, *Proteles cristatus* (Sparrman, 1783). Ph.D. thesis, University of Pretoria, RSA
- Sliwa, A. & Richardson, P. R. K. 1998. Responses of aardwolves, *Proteles cristatus*, Sparrman 1783, to translocated scent marks. *Animal Behaviour* 56: 137-146
- Swihart, R. K. & Slade, N. A. 1985. Testing for independence of observations in animal movements. *Ecology* 66:1176-1184
- Trivers, R. L. 1972. Parental investment and sexual selection. In: Campbell, B. (Ed.), *Sexual selection and the descent of man*. Aldine, Chicago, USA, pp. 136-179
- van Schaik, C. P. & van Hooff, J. A. R. A. M. 1983. On the ultimate causes of primate social systems. *Behaviour* 85: 91-117

Venables, W. N. & Ripley, B. D. 1997. *Modern Applied Statistics with S-plus*, 2nd Edition.

Springer-Verlag, New York, USA

Wynne-Edwards, K. 1987. Evidence for obligate monogamy in the Djungarian hamster,

Phodopus campbelli: pup survival under different parenting conditions. *Behavioral*

Ecology and Sociobiology 20: 427-437

Table 4.1. Number of spatial tracks and sessions where behavioural data was recorded for each of the seven aardwolves on Benfontein Nature Reserve. Behavioural sessions ranged between 2-316 minutes and figures within brackets are the number of tracks/behavioural sessions.

ID	Sex	Behavioural follow period	Time (min) of tracks			Time (min) of behavioural sessions		
			Wet season	Dry season	Total	Wet season	Dry season	Total
BWF08006	Female	May 2010 - Dec 2010	1991 (27)	606 (14)	2597 (41)	785 (11)	475 (8)	1260 (19)
BWF09009	Female	May 2010 - July 2011	1861 (24)	3256 (32)	5117 (56)	1036 (12)	2505 (23)	3541 (35)
BWF10015	Female	July 2010 - April 2011	1560 (13)	396 (6)	1956 (19)	649 (8)	283 (4)	932 (12)
BWM08002	Male	May 2010 - June 2011	2121 (19)	867 (14)	2988 (33)	434 (6)	646 (9)	1080 (15)
BWM09008	Male	May 2010 - April 2011	1756 (20)	735 (13)	2491 (33)	333 (4)	641 (10)	974 (14)
BWM10012	Male	May 2010 - June 2011	2026 (22)	1236 (19)	3262 (41)	669 (8)	1023 (15)	1692 (23)
BWM10014	Male	May 2010 - July 2011	986 (10)	3604 (36)	4591 (46)	931 (9)	2733 (30)	3664 (39)
			Total		23 002 (269)		Total	13 143 (157)

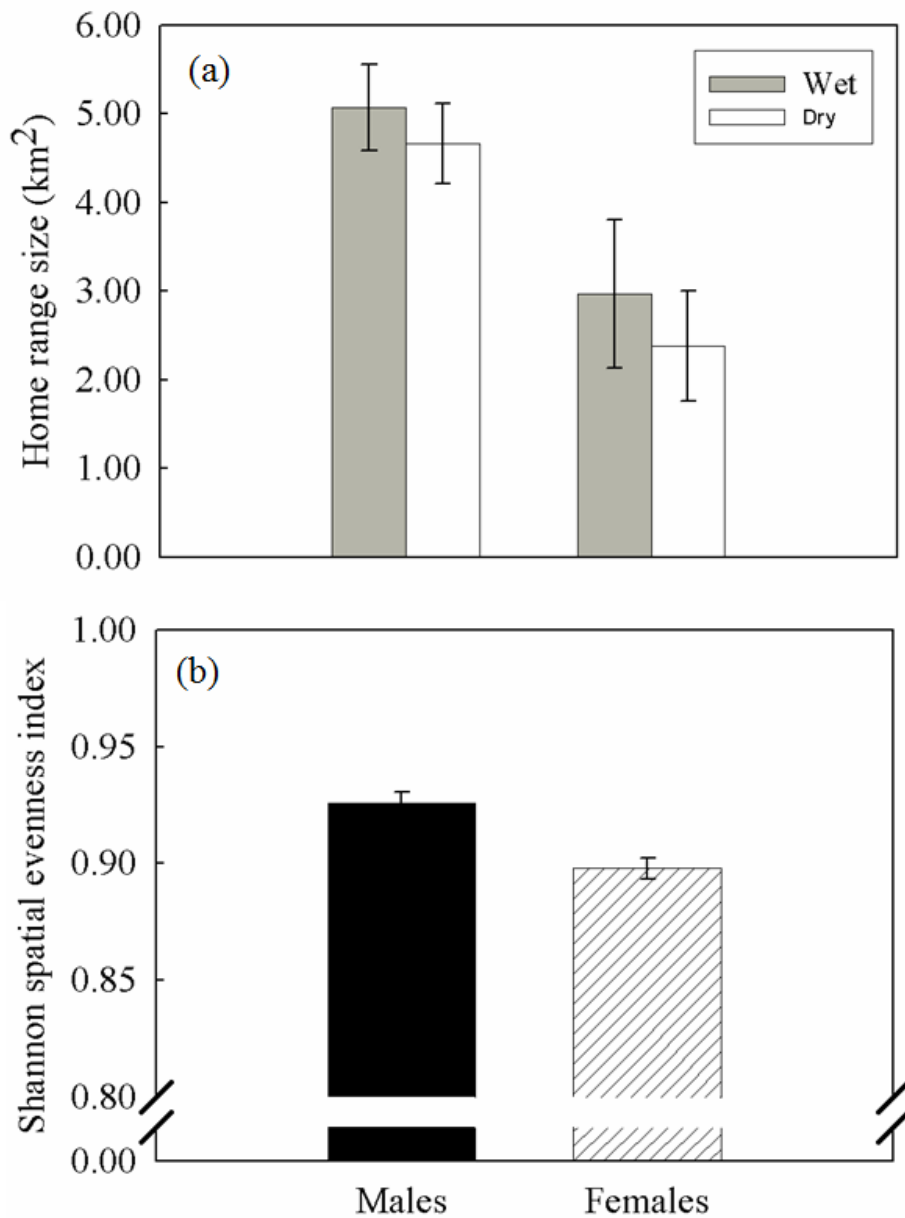


Fig. 4.1 Seasonal home range sizes (a) and spatial evenness indices (b) for male (n=4) and female (n=3) aardwolves on Benfontein. Home range sizes were delineated using the 95% MCP method and the evenness of spatial utilisation was calculated with the Shannon spatial diversity index. Data are represented as a mean of individual means \pm SE.

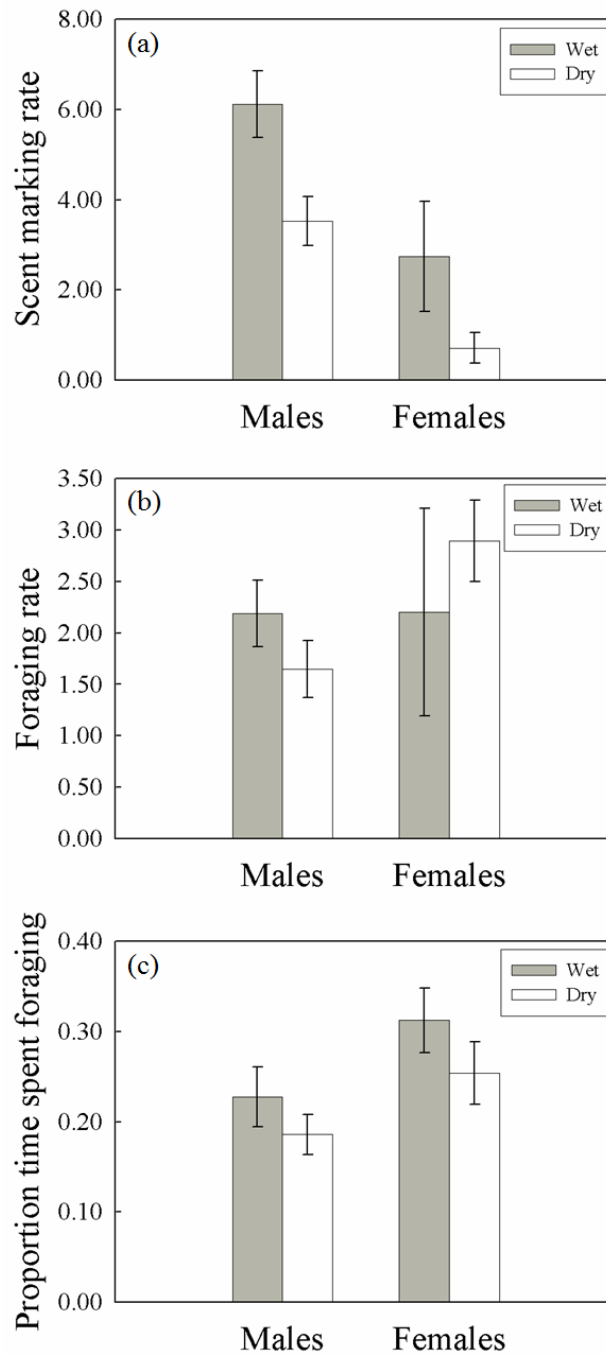


Fig. 4.2 Seasonal scent marking rates (scent marks / 10 minutes: a), foraging rates (foraging bouts / 10 minutes: b) and time spent foraging (proportion of observed time: c) for male (n=4) and female (n=3) aardwolves. Data are represented as individual means \pm SE.

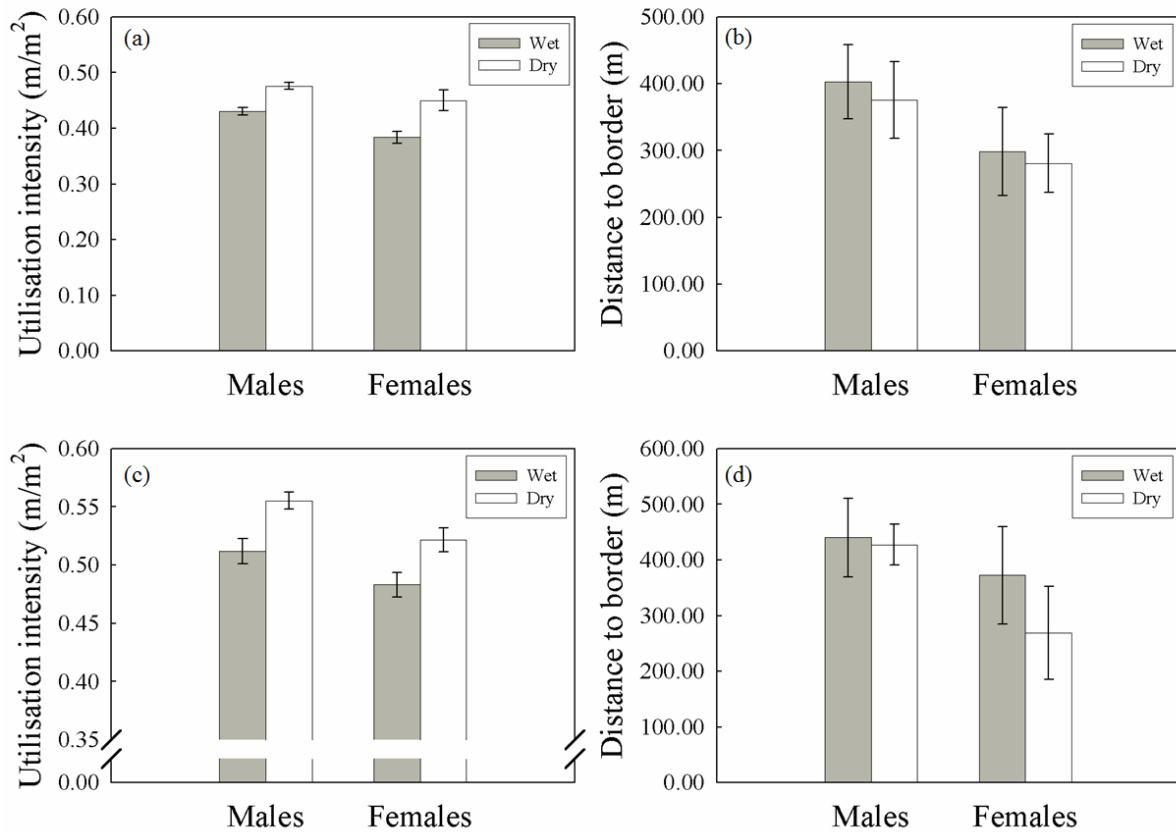


Fig. 4.3 Spatial utilisation intensity (metres of movement tracks per square metre of area) and distance to nearest home range border of locations used for scent marking (a, b) and for foraging (c, d). Utilisation intensity was determined from the intersection of spatial coordinates of scent marking and foraging with the home range utilisation estimate. Home range borders were delineated from 95% MCP's. Data are represented as individual means \pm SE.

CHAPTER 5

Conclusions

Chapters 2 and 3 found support for reproductive seasonality in both zoo-housed and wild aardwolves. The ultimate cause for seasonal breeding strategies is linked to temporal variation in resource abundance (Emlen & Oring 1977; Clutton-Brock & Harvey 1977; Greenwood 1980; Clutton-Brock 1989; Bronson & Heideman 1994). Aardwolves are extreme resource specialists that feed almost exclusively on a single genus of termites, *Trinervitermes* spp. (Smithers 1971; Kruuk & Sands 1972; Richardson 1987a; Koehler & Richardson 1990; Matsebula et al. 2009; De Vries et al. 2011). These termite species fluctuate seasonally in activity, which results in peak abundance in the wet season and very low abundance during the dry season (Richardson 1987a; Koehler & Richardson 1990). These seasonal variations in termite activity patterns are closely linked to rainfall and temperature (Richardson 1987a, c; Koehler & Richardson 1990). Aardwolves' strong dietary dependence on this temporally fluctuating food resource leads to periods of nutritional stress during the season with low termite abundance (Richardson 1987c; Koehler & Richardson 1990). It is therefore important for aardwolves to time periods of high physiological investment in reproduction, primarily related to gestation and lactation, with high seasonal abundance of these termites. Aardwolves appear to do this by mating during the resource-sparse dry season, to allow for parturition to occur at an appropriate time in relation to seasonal fluctuations in termite abundance. We therefore suggest that the seasonal breeding strategy in aardwolves is a direct result of their dietary dependence of a temporally fluctuating resource.

Data on animals in zoo-housed conditions suggested that the social environment appeared to modify the physiological responses of aardwolves to physical environmental

conditions (Chapter 2). We suggest that it may be adaptive for aardwolves to retain social receptivity even if resource distributions cause these animals to forage alone. Aardwolves also exhibit strict seasonal breeding and time their mating activities to optimise food abundance during lactation and offspring rearing (Richardson 1987c; Koehler & Richardson 1990). Individual females are therefore likely to be constrained in their receptivity period to closely achieve optimal reproductive timing, which combined with their largely solitary behaviour would imply that males need to be dynamic in their reproductive activity to closely match locally receptive females (Haigh 1983; Fadem 1985; Scott 1986; Bronson & Heideman 1994; Kraaijeveld-Smit et al. 2002). Additionally, it is not energetically optimal for females to become receptive in the absence of a suitable male to mate with (Bertram 1975; Brown 1985; Bronson & Perrigo 1987; Fanjul & Zenuto 2008), which could explain why we found indications that the social environment also seem to influence reproductive physiology in females.

We found that physiological (Chapter 3) and behavioural (Chapter 4) traits correspond better to predictions based on social monogamy than polygamous mating in a population of wild aardwolves. Our results, previous observations of extra-pair copulations (EPCs: Richardson 1987b; Sliwa 1996) and the recently recorded temporal variation in den use (Kotze et al. 2012) in the same study area show how some traits relate more to social monogamy while others relate more to polygamous mating. We identify two possible explanations for this discrepancy between recorded traits. First, the discrepancy could be caused by temporal variation in the mating activities of aardwolves, which could have resulted in none or few occurrences of EPCs during our study period. However, the study by Kotze et al. (2012) was concurrent to, and conducted on the same individuals as this study. In addition, we did observe one of the males successfully mating with two of the females during the same mating season (Chapter 3). Therefore, this explanation does not seem likely. A second explanation could be

that the social mating system components regulate the observed endocrine and behavioural parameters more than actual mating patterns. This would suggest that the social mating system components in aardwolves pose a stronger selective pressure on physiology and behaviour than sexual mating patterns. Such an interpretation suggests that the fitness effect of male mating competition is low despite the observed occurrence of EPCs. However, we cannot truly resolve these two possible explanations because we did not specifically record nor quantify copulation behaviour in this population of wild aardwolves.

Our behavioural and physiological data relate more to predictions based on social monogamy in aardwolves, while other studies found traits more related to polygamous mating patterns in the same study area (Richardson 1987b; Sliwa 1996; Kotze et al. 2012). Therefore, aardwolves do not seem to fit discretely into the current mating system classification (e.g., Clutton-Brock 1989; Davies 1991), and the evolutionary causes for the potentially conflicting mating strategies suggested for the species, as well as the fitness benefits of these strategies, needs to be further evaluated. To achieve this, we recommend that future projects are set up as long-term investigations in order to encompass potential temporal variation in mating activities and aim to use genetic methods to assess paternities. This would enable quantifications of paternities arising from within social pair bonds versus paternities arising from EPCs. Also, we recommend that projects aim to investigate how variation in the physical environment affects mating tactics in aardwolves, and how such mating tactic variations influence fitness. Finally, since the majority of aardwolf studies, including this one, have been carried out at the same study site we suggest further studies to be placed at other sites to assess inter-population differences in mating patterns of wild aardwolves. This would enable us to evaluate how this extreme resource specialist adapts to local environmental conditions.

5.1 References

- Bertram, B. C. R. 1975. Social factors influencing reproduction in wild lions. *Journal Zoology* 177: 463–482
- Bothma, J. P. & Nel, J. A. J. 1980. Winter food and foraging behaviour of the aardwolf *Proteles cristatus* in the Namib-Naukluft Park. *Madoqua* 12: 141–145
- Bronson, F. H. & Perrigo, G. 1987. Seasonal regulation of reproduction in muroid rodents. *American Zoologist* 27: 929–940
- Bronson, F. H. & Heideman, P. D. 1994. Seasonal regulation of reproduction in mammals. In: Knobil, E., Neill, J. D., Greenwald, G. S., Markert, C. L. & Pfaff, D. W (Eds.), *The Physiology of Reproduction: Volume 1*. Raven Press, New York, pp. 541–584
- Brown, R. E. 1985. The rodents I: effects of odours on reproductive physiology (primer effects). In: Brown, R. E. & Macdonald, D. W. (Eds.), *Social odours in mammals: Volume 1*. Clarendon Press, Oxford, UK, pp. 245–344
- Clutton-Brock, T. H. 1989. Mammalian mating systems. *Proceedings of the Royal Society of London, B: Biological Sciences* 236: 339–372
- Clutton-Brock, T. H. & Harvey, P. H. 1977. Primate ecology and social organisation. *Journal of Zoology* 18: 1–39
- Clutton-Brock, T. & Isvaran, K. 2006. Paternity loss in contrasting mammalian societies. *Biology Letters* 2: 513–516
- Cohas, A. & Allaine, D. 2009. Social structure influences extra-pair paternity in socially monogamous mammals. *Biology Letters* 5: 313–316

- Davies, N. B. 1991. Mating systems. In: Krebs, J. R. & Davies, N. B. (Eds.), *Behavioural Ecology: An evolutionary approach*, 3rd Edition. Blackwell Scientific Publications, Oxford, pp. 263–294
- De Vries, J. L., Pirk, C. W. W., Bateman, P. W., Cameron, E. Z. & Dalerum, F. 2011. Extension of the diet of an extreme diet specialist, the aardwolf *Proteles cristata*. *African Zoology* 16: 194–196
- Fadem, B. H. 1985. Evidence for the activation of female reproduction by males in a marsupial, the gray short-tailed opossum (*Monodelphis domestica*). *Biology of Reproduction* 3: 112–116
- Fanjul, M. S. & Zenuto, R. R. 2008. Female reproductive responses to photoperiod and male odours in the subterranean rodent *Ctenomys talarum*. *Acta Theriologica* 53: 73–85
- Greenwood, P. J. 1980. Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour* 28: 1140–1162
- Haigh, G. R. 1983. Effects of inbreeding and social factors on the reproduction of young female *Peromyscus maniculatus bairdii*. *Journal of Mammalogy* 64: 48–54
- Knöthig, J. 2005. Biology of the aardvark (*Orycteropus afer*). MSc. thesis, University of Heidelberg, Germany
- Koehler, C. E. & Richardson, P. R. K. 1990. *Proteles cristatus*. *Mammalian Species* 363: 1-6
- Kotze, R., Bennett, N., Cameron, E., De Vries, L., Marneweck, D., Pirk, C. & Dalerum, F. 2012. Temporal patterns of den use suggest polygamous mating patterns in the socially monogamous aardwolf. *Animal Behaviour* 84: 1573–1578
- Kraaijeveld-Smit, F. J. L., Ward, S. J., Temple-Smith, P. D. & Paetkau, D. 2002. Factors influencing paternity success in *Antechinus agilis*: last-male sperm precedence, timing of mating and genetic compatibility. *Journal of Evolutionary Biology* 15: 100–107

- Kruuk, H. 1972. *The spotted hyena: a study of predation and social behaviour*. University of Chicago Press, Chicago, USA
- Kruuk, H. & Sands, W. A. 1972. The aardwolf (*Proteles cristatus*, Sparrman 1783) as a predator of termites. *East African Wildlife Journal* 10: 211–227
- Lukas, D. & Clutton-Brock, T. 2012. Cooperative breeding and monogamy in mammalian societies. *Proceedings of the Royal Society, B: Biological Sciences* 279: 2151–2156
- Macdonald, D. 1984. *The Encyclopedia of Mammals*. Facts on File Publishing, New York, USA
- Matsebula, S. N., Monadjem, A., Roques, K. G. & Garcelon, D. K. 2009. The diet of the aardwolf, *Proteles cristatus* at Malolotja Nature Reserve, western Swaziland. *African Journal of Ecology* 47: 448–451
- Mills, M. G. L. 1982. The mating system of the brown hyaena, *Hyaena brunnea*, in the Southern Kalahari. *Behavioral Ecology and Sociobiology* 10: 131–136
- Mills, M. G. L. 1989. The comparative behavioral ecology of hyenas: the importance of diet and food dispersion. In: Gittleman, J. L. (Ed.), *Carnivore Behavior, Ecology and Evolution. Vol. 1*. Cornell University Press, Ithaca, New York, USA, pp. 125–142
- Richardson, P. R. K. 1985. The social behaviour and ecology of the aardwolf, *Proteles cristatus*, (Sparrman, 1783) in relation to its food resources. PhD thesis, University of Oxford, Oxford, UK
- Richardson, P. R. K. 1987a. Aardwolf mating system: overt cuckoldry in an apparently monogamous mammal. *South African Journal of Science* 83: 405–410
- Richardson, P. R. K. 1987b. Food-consumption and seasonal-variation in the diet of the aardwolf *Proteles cristatus* in Southern Africa. *Zeitschrift für Säugetierkunde* 52: 307–325

- Richardson, P. R. K. 1987c. Aardwolf: the most highly specialized myrmecophagous mammal?
South African Journal of Science 83: 643–646
- Richardson, P. R. K. & Coetzee, M. 1988. Mate desertion in response to female promiscuity in the socially monogamous aardwolf *Proteles cristatus*. *South African Journal of Zoology* 23: 306–308
- Scott, M. P. 1986. The timing and synchrony of seasonal breeding in the marsupial, *Antechinus stuartii*: interaction of environmental and social cues. *Journal of Mammology* 67: 551–560
- Sliwa, A. 1996. A functional analysis of scent marking and mating behaviour in the aardwolf, *Proteles cristatus* (Sparrman, 1783). Ph.D. thesis, University of Pretoria
- Smithers, R. H. N. 1971. *The mammals of Botswana*. Trustees of the National Museum, Rhodesia, Salisbury, pp. 340
- Stewart, N. C., Vanepé, C., Sprent, J., Morrow, G. & Andersen, N. A. 2011. Spatial ecology of a ubiquitous Australian anteater, the short-beaked echidna (*Tachyglossus aculeatus*). *Journal of Mammology* 92: 101–110
- Taylor, W.A. 2005. Order Tubulidentata. In: Skinner, J. D. & Chimimba, C. T. (Eds.), *The mammals of the southern African subregion*. University Press, Cambridge, UK, pp. 35–40
- Wagner, A. P., Frank, L. G. & Creel, S. 2008. Spatial grouping in behaviourally solitary striped hyaenas, *Hyaena hyaena*. *Animal Behaviour* 75: 1131–1142

Appendix

Common and scientific names of species cited in text

Mammals

Order Carnivora

Hyaenidae

aardwolf	<i>Proteles cristata</i>
striped hyena	<i>Hyaena hyaena</i>
spotted hyena	<i>Crocuta crocuta</i>

Ursidae

brown bear	<i>Ursus arctos</i>
polar bear	<i>Ursus maritimus</i>
giant panda	<i>Ailuropoda melanoleuca</i>
sloth bear	<i>Melursus ursinus</i>

Herpestidae

banded mongoose	<i>Mungos mungo</i>
dwarf mongoose	<i>Helogale parvula</i>
suricate	<i>Suricata suricata</i>

Mustelidae

Eurasian badger	<i>Meles meles</i>
river otter	<i>Lontra canadensis</i>

Felidae

African lion	<i>Panthera leo</i>
Canada lynx	<i>Lynx canadensis</i>
caracal	<i>Caracal caracal</i>

Canidae

grey wolf	<i>Canis lupus</i>
African wild dog	<i>Lycaon pictus</i>
bat-eared fox	<i>Otocyon megalotis</i>
black-backed jackal	<i>Canis mesomelas</i>
golden jackal	<i>Canis aureus</i>
coyote	<i>Canis latrans</i>
swift fox	<i>Vulpes velox</i>

Order Rodentia

Pedetidae

porcupine	<i>Hystrix africaeaustralis</i>
springhare	<i>Pedetes capensis</i>

Order Tubulidentata

Orycteropodidae

	aardvark	<i>Orycteropus afer</i>
Order Artiodactyla		
Suidae		
	warthog	<i>Phacochoerus aethiopicus</i>
Bovidae		
	bushbuck	<i>Tragelaphus scriptus</i>
	African buffalo	<i>Syncerus caffer</i>
Hippopotamidae		
	hippopotamus	<i>Hippopotamus amphibius</i>
Cervidae		
	red deer	<i>Cervus elaphus</i>
Order Perissodactyla		
Rhinocerotidae		
	white rhinoceros	<i>Ceratotherium simum</i>
Order Proboscidea		
Elephantidae		
	African elephant	<i>Loxodonta africana</i>
Order Primata		
Lepilemuridae		
	red-tailed sportive lemur	<i>Lepilemur ruficaudatus</i>
Lemuridae		
	red-fronted lemur	<i>Eulemur fulvus rufus</i>
Hominidae		
	mountain gorilla	<i>Gorilla gorilla beringei</i>
Ray-fined fishes, spiny rayed fishes		
Order Perciformes		
Pomacentridae		
	Red Sea damselfish	<i>Dascyllus aruanus</i>
Birds		
Order Strigiformes		
Strigidae		
	giant eagle owl	<i>Bubo lacteus</i>
	spotted eagle owl	<i>Bubo africanus</i>
Order Falconiformes		
Accipitridae		
	martial eagle	<i>Polemaetus bellicosus</i>
Order Passeriformes		
Emberizidae		
	dark-eyed junco	<i>Junco hemalis</i>
Corvidae		
	jackdaw	<i>Corvus monedula</i>
Sylviidae		
	sardinian warbler	<i>Sylvia melanocephala</i>
Order Piciformes		

	Picidae		
		three-toed woodpecker	<i>Picoides tridactylu</i>
Insects			
Order Isoptera			
	Termitidae		
		harvester termite	<i>Trinervitermes trinervoides</i>
		east African nasute termite	<i>Trinervitermes bettonianus</i>
Plants			
Order Fabales			
	Fabaceae		
		camel thorn	<i>Acacia erioloba</i>