

# Conflict and Cooperation in Vertebrate Societies

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**A dissertation submitted to the University of Exeter in application for  
the degree of Doctor of Philosophy in Biological Sciences  
December 2012**

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## Summary

Within animal societies, individuals often differ greatly in their level of investment in cooperative activities. Individuals are predicted to show high cooperative investment if high levels of relatedness lead to large indirect fitness benefits, or if differences in individual characteristics such as age, sex, rank, or body condition increase the direct fitness benefits of helping. However, individual differences often persist after these differences are controlled for; a residual variation that remains unexplained. Understanding the proximate mechanisms underlying variation in behaviour can give novel insights into the selection pressures shaping behavioural differences. This suggests that a research focus onto the proximate mechanisms underpinning cooperative behaviours is needed to further our understanding of why individuals behave differently within social groups. In this thesis, I address this shortfall in understanding by investigating hormonal variation alongside individual differences in cooperative investment in the banded mongoose (*Mungos mungo*). Banded mongooses are a highly social carnivore with two highly conspicuous forms of cooperative offspring care that are easily measurable and show large inter-individual variation. In chapter 3, I demonstrate a negative carry-over effect of investment in offspring care in consecutive breeding attempts. I show that this carry-over effect is mediated by variation in glucocorticoid concentrations, which may be attributable to the energetic costs of helping. Glucocorticoids predict investment in offspring care, suggesting that this mechanism may drive inter-individual variation in cooperative investment. In chapter 4, I find evidence for a testosterone mediated trade-off between offspring care and mating effort, which suggests that inter-individual differences may also be driven by variation in the costs of helping attributable to missed mating opportunities. In chapter 5, I use simulated territorial intrusions to show that there is unlikely to be a trade-off between offspring care and territory defence in banded mongoose societies. However, carers and non-carers show a differential physiological response to territorial intrusion, suggesting that there may be a more subtle behavioural trade-off that occurs post-intrusion. In chapter 6, I find evidence for consistent individual differences in both cooperative and competitive behaviours, which suggests that individual differences in adult behaviour may be determined by early-life effects. Individual differences in cooperative investment are positively correlated, suggesting that individuals are not specialised to different cooperative activities, but are consistently either helpful or selfish. Together, these results give insights into the selection pressures shaping individual differences and highlight endocrine research as a valuable tool in understanding the evolution of cooperative societies.



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## Abbreviations

<b>UWA</b>	Uganda Wildlife Authority
<b>UNCST</b>	Uganda National Council for Science and Technology
<b>UofE</b>	University of Exeter (in Cornwall)
<b>CZEL</b>	Chester Zoo Endocrinology Lab
<b>GC</b>	Glucocorticoid
<b>fGC</b>	Faecal glucocorticoid metabolites
<b>T</b>	Testosterone
<b>fT</b>	Faecal testosterone metabolites
<b>STI</b>	Simulated territorial intrusion
<b>IIR</b>	Intruder inspection rate
<b>LMM</b>	Linear mixed model
<b>GLMM</b>	Generalised linear mixed model
<b>DIC</b>	Deviance information criterion
<b>CI</b>	Credible interval
<b>HPD</b>	Highest posterior density
<b>SE</b>	Standard error
<b>HPA</b>	Hypothalamic-pituitary-adrenal axis



## Author's Declarations

During the research contributing to this thesis, Jennifer Louise Sanderson (JLS) was supported by a PhD studentship provided by the European Social Fund (ESF). All of the chapters in this thesis were written by JLS with comments from Michael Cant, Sarah Hodge, and Andrew Young. The wild population of banded mongooses (*Mungo mungo*) used in this study is part of a long-term project run by Michael Cant from the University of Exeter (Cornwall Campus) with the help of Sarah Hodge and Emma Vitikainen.

Behavioural observations and faecal samples were collected primarily by JLS, assisted by Solomon Kyabulima, Francis Mwanguhya, David Jansen, Kenneth Mwesige, and Robert Businge. Chapters 3, 4, and 6 also use long-term data collected before JLS joined the research project, which were collected by numerous previous researchers (see [bandedmongoose.org](http://bandedmongoose.org) for details). All statistical analyses were carried out by JLS. Chapters 3 – 5 involve hormonal analyses that were carried out in collaboration with Sue Walker at the Chester Zoo Endocrinology Lab (CZEL). Rebecca Purcell, Katie Edwards, and Vicki Norton also contributed to both the validation and running of these assays at the CZEL.

All photographs in this thesis were taken by and are the property of JLS.



## Acknowledgements

First of all, I would like to thank Mike Cant and Sarah Hodge for bringing me under their wings and being my supervisors for this project. Mike and Sarah share an enthusiasm for banded mongooses and behavioural ecology that is incredibly inspiring. They have both been incredibly supportive and helpful throughout my project; I really could not have asked for better supervisors. I would especially like to thank Mike for being patient and welcoming discussion of ideas no matter how difficult I find it to express them, and Sarah for taking me into the field and introducing me to the Ugandan way of life. Andy Young has been a great addition to my supervisory team. His enthusiasm continues to astound me, and my thesis would have looked very different without his insightful ideas. I would also like to thank Emma Vitikainen, who consistently goes beyond the point of call to help me out and has become a formidable companion in discussing the complexities of the Ugandan personality.

My field work was made possible by four invaluable field assistants; Solomon Kyabulima, Francis Mwanguhya, Kenneth Mwesige, and Robert Businge. All four of them happily accepted the task of collecting thousands of faecal samples without a single complaint. Solomon has been my assistant in the field for 3 (long) field seasons, he has meticulously collected data for use in this project, both when I was in the field alongside him and when I returned to the UK. His keen eyes, on watch for elephants, hippos, and leopards, have saved my life on numerous occasions. Francis is an amazing field project manager. His knowledge and experience have been priceless throughout my time in the field. I would also like to thank David Jansen for his help in the field (who also collected hundreds of faecal samples without complaint). He became a good friend and a friendly face, with whom I could discuss ideas and the trials and tribulations of mongoose fieldwork. Thanks to Jennifer Krauser who also helped me to keep me sane and reminded me to paint my toenails every now and again. I'd also like to say thank you to Will Symes for adding to the fun adventures and reminding me never to take up primatology research. Most of all, I would like to thank everyone in Uganda for being so warm and friendly, and welcoming me into Mweya; I'm certain my 'Mweya madness' would have been much greater without you all.

This project would not have been possible without the support of Uganda Wildlife Authority and the Uganda Council for Science and Technology, especially Dr. Margret and Nelson Guma. I would like to thank them for giving me permission to carry out this research and for their support in the field.

The hormone analysis in this project was carried out in collaboration with Sue Walker at Chester Zoo. Sue has been a friendly source of advice and enthusiasm throughout my project, which has led to a fruitful collaboration that I hope to continue into the future. I would also like to extend my thanks to everyone else in the Endocrinology Lab at Chester Zoo; Katie Edwards, Rebecca Purcell, Vicki Norton, and the numerous other volunteers that have spent time with my samples.

My parents, Mike and Diana Sanderson deserve a HUGE thank you for supporting me for the last 26 years. I remember a few years back being told to "Shine on you crazy diamond", I can only hope that I have made them proud. I'm sure parental duties do not normally involve transporting boxes of faeces around the country – thank you! And lastly, I would like to thank them visiting me in Uganda; my time in the field would not have been the same without their visit and I am very happy to have shared that part of my life with them.

I would like to thank all the staff at the Centre for Ecology and Conservation in Cornwall for providing a strong network of support throughout this project. I would especially like to thank Chris Mitchell and Jo Faulks for their help in the lab, and John Hunt for sorting out the oil issue. I would like to thank Sasha Dall and (again) Andy Young for inviting me to join the Kenya field course, it was a great experience and I am very much looking forward to doing it again next year. Then there begins the long list of friends who have helped me along my way while in Cornwall: Matt Perkins, Tom Davies, Lindsay Walker, Dom Cram, Caro Moussy, Fran Tyler, Andy Robertson, Callum Lawson, Damian Smith, Ross Dwyer, Julian Evans, Matt Silk, Alfredo Attisano, Iker Vaquero-Alba, Siobhán O'Brien, Bea Downing, Kim Stokes, Xav Harrison, Ted Glasgow, Rich Woods, and many others - you guys ROCK MY WORLD. Climbing, swimming, surfing, ginning, dancing, adventuring would not be the same without you, and neither would all the science. Alecia (AJ) Carter is amazing; A-MAZING. I would like to give her a huge thank you for listening to all of my rambling rants; both science and non-science related. I hope that one day we can take over the world of science together and implement all of our AWESOME ideas. And last but not least, a big thank you goes to Matt Wright, who unexpectedly became a source of smiles during my write-up and added a sparkle to the light at the end of the tunnel.

# Chapter 1

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# Introduction





The many slight differences which appear in the offspring from the same parents [...] may be called individual differences. [...] I am convinced that the most experienced naturalist would be surprised at the number of the cases of variability, even in important parts of structure, which he could collect on good authority, as I have collected, during a course of years.

C. Darwin, 1872

Phenotypic differences can exist between species, between local populations of the same species, and between individuals within single local populations [...]. The interpretation of differences at the finest scale - between individuals within single populations - is still unresolved.

D. S. Wilson, 1998

...cooperative personalities or individual differences in cooperative type have been almost entirely neglected in research on the evolution and stability of cooperation, though such inter-individual variation may play a central role for the evolution and stability of cooperation.

R. Bergmüller, R. Schürch, & I. Hamilton, 2010



## 1.1 Individual Differences in Cooperative Investment

Individuals within the same species or even the same local population can differ greatly in their level of investment in different behaviours (Dall et al. 2012). Prior to the 1990s it was widely assumed that these differences were non-adaptive 'noise' around an adaptive mean (Wilson 1998). However, evidence that different behavioural strategies can lead to similar fitness outcomes (e.g. Shuster & Wade, 1991) lead to a shift in understanding; individual differences became a potentially adaptive phenomenon. Individuals may behave differently due to individual variation in the costs and benefits of a given behaviour that are attributable to variation in internal state and/or extrinsic environment (Wilson 1998; Dall et al. 2004). For example, large, attractive males benefit greatly from investing in courtship behaviours, while small, unattractive males are more likely to benefit from pursuing sneaky mating tactics. Additionally, similar individuals within the same population may behave differently if the fitness outcomes of two or more different strategies are frequency dependent (Dall et al. 2004). Understanding why individuals behave differently can give insights into the selection pressures associated with different behaviours and facilitate novel hypotheses about the evolution of different behavioural strategies.

If individuals in non-social species are expected to behave differently to match variation in both internal state and extrinsic environment (Dewitt et al. 1998), individuals in social species may be even more likely to behave differently as they modulate their behaviour to the additional variation of their social environment (Bergmüller et al. 2010). This 'social environment' includes both the identity and behaviour of other individuals in the group. For example, the benefits of cooperative vigilance, or sentinel behaviour, are much lower if another individual is already on guard, so individuals may benefit from negotiating and coordinating cooperative behaviours within a group (Bell et al. 2010). Similar to any other type of behaviour, variation in the expression of cooperative behaviours is likely to coincide with differences in the associated costs and benefits. Identifying the sources of variation in the costs and benefits of cooperating will add to our understanding of why individuals behave differently in cooperative societies, and may lead to novel inferences about how species differences in the costs and benefits of cooperation lead to the evolution of sociality in some species but not others.

The potential costs and benefits of any cooperative behaviour are very complex. Hamilton proposed that an individual will invest in a cooperative behaviour if the personal lifetime fitness cost ( $c$ ) is less than the lifetime fitness benefit to the receiver ( $b$ ) multiplied by its relatedness ( $r$ ); i.e.  $c < rb$  (Hamilton 1964). The right-hand side of this equation describes the *indirect* fitness benefits of cooperating, which suggests that individuals can maximise the benefits of cooperating by directing

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cooperative behaviours towards relatives (where the indirect fitness benefits are greatest). Variation in cooperative investment does commonly correlate with variation in relatedness, as exemplified by Trivers & Hare's (1976) observation that hymenopteran helpers bias their investment in offspring care towards the sex to which they are most related. However, results from a meta-analysis suggest that variation in relatedness only describes 10% of variation in the likelihood of helping (Griffin & West 2003), which suggests that there must be other benefits of cooperating that vary between individuals. Furthermore, within-group kin discrimination will only be favoured if there is large within-group variation in relatedness and helpers have a large effects on the breeders' reproductive success (Cornwallis et al. 2009).

Cooperation can have direct benefits if it leads to group augmentation (Kokko et al. 2001; Clutton-Brock 2002), or if individuals 'pay to stay' on a good territory (Kokko et al. 2002). However, the magnitude of these benefits is likely to vary between individuals. For example, competition for resources may lead to intra-group conflict over optimum group size (Gaston 1978; Balshine et al. 2001) and the reward for paying to stay is likely to be greater for individuals with more chance of inheriting a dominant position within the group. Cooperative investment often comes with 'opportunity costs', i.e. costs attributable to opportunities missed when helping (Heinsohn & Legge 1999). For example, there may be a trade-off between indirect benefits of offspring care and direct benefits of reproduction (Clutton-Brock et al. 2000; Cant & Field 2001; Field et al. 2006). Variation in individual quality or age leads to variation in the likelihood of successfully attracting a mate, which itself underpins a variation in the opportunity costs of helping (Hodge 2007). Where individuals within a group form a social hierarchy and queue to gain a dominant breeding position there is systematic variation in both current and future mating opportunities which creates a systematic variation in the costs of helping so that individuals at the top of the queue are less likely to help than those at the bottom (Cant & Field 2005; Field et al. 2006). Previous studies have highlighted a range of factors that describe variation in individual contributions to cooperative offspring care (see list of examples in table 1.1); however, a large amount of residual variation in cooperative investment still remains unexplained.

Table 1.1: Life-history factors that describe variation in individual contributions to offspring care within cooperative breeding societies.

Sex			
Acorn woodpecker	<i>Melanerpes formicivorus</i>	F>M	Mumme et al. 1990; Koenig & Walters 2011b
Apostlebird	<i>Struthidea cinerea</i>	F>M	Woxvold et al. 2006
Banded mongoose	<i>Mungos mungo</i>	M>F	Cant 2003; Hodge 2007; Bell 2010
Brown jay	<i>Cyanocorax mori</i>	F>M	Williams & Hale 2007
Laughing kookaburra	<i>Dacelo novaeguineae</i>	M>F	Legge 2000
Meerkat	<i>Suricata suricatta</i>	F>M	Clutton-Brock et al. 2000; Clutton-Brock et al. 2002; English et al. 2008
Purple gallinule	<i>Porphyryla martinica</i>	F>M	Hunter 1987
Age			
Acorn woodpecker	<i>Melanerpes formicivorus</i>	O>Y	Koenig & Walters 2011a
Apostlebird	<i>Struthidea cinerea</i>	O>Y	Woxvold et al. 2006
Banded mongoose	<i>Mungos mungo</i>	Y <sup>1</sup> >A	Cant 2003; Gilchrist & Russell 2007; Hodge 2007; Bell 2010
Meerkat	<i>Suricata suricatta</i>	A>J	Clutton-Brock et al. 2000; Clutton-Brock et al. 2002
Purple gallinule	<i>Porphyryla martinica</i>	O>Y	Hunter 1987
Western bluebird	<i>Sialia mexicana</i>	O>Y	Dickinson 2004
White-winged Chough	<i>Corcorax melanorhamp</i>	Y<O	Heinsohn & Cockburn 1994
Relatedness/Indirect Fitness Benefits			
Bell miner	<i>Manorina melanophrys</i>	↑R	Wright et al. 2010; McDonald & Wright 2011
Chestnut-crowned Babbler	<i>Pomatostomus ruficeps</i>	↑R	Browning et al. 2012
Long-tailed tit	<i>Aegithalos caudatus</i>	↑R	Nam et al. 2010
Purple-crowned fairy wren	<i>Malurus coronatus</i>	↑R	Kingma et al. 2011
Seychelles warbler	<i>Acrocephalus sechellensis</i>	↑R	Komdeur 1994
White-fronted bee-eater	<i>Merops bullockoides</i>	↑R	Emlen & Wrege 1988
Breeding Status			
Apostlebird	<i>Struthidea cinerea</i>	B>NB	Woxvold et al. 2006
Acorn woodpecker	<i>Melanerpes formicivorus</i>	B>NB	Mumme et al. 1990; Koenig & Walters 2011b
Banded mongoose	<i>Mungos mungo</i>	B>NB	Gilchrist & Russell 2007; Hodge 2007
Laughing kookaburra	<i>Dacelo novaeguineae</i>	B>NB	Legge 2000
Long-tailed tit	<i>Aegithalos caudatus</i>	B>NB	MacColl & Hatchwell 2003
Purple gallinule	<i>Porphyryla martinica</i>	B>NB	Hunter 1987
Western bluebird	<i>Sialia mexicana</i>	B>NB	Dickinson 2004

## 1. Introduction

### Body size or energetic state

Banded mongoose	<i>Mungos mungo</i>	↑ES*	Gilchrist & Russell 2007; Hodge 2007; Bell 2010
Golden lion tamarin	<i>Leontopithecus rosalia</i>	↑BW	Bales et al. 2002
Ground tit	<i>Parus humilis</i>	↑FA*	Lu et al. 2011
Long-tailed tit	<i>Aegithalos caudatus</i>	↑BC	Meade & Hatchwell 2010
Meerkat	<i>Suricata suricatta</i>	↑ES*	Clutton-Brock et al. 2000; Clutton-Brock et al. 2002; Russell et al. 2003
Moorhen	<i>Gallinula chloropus</i>	↑FA*	Eden 1987
Seychelles warbler	<i>Acrocephalus sechellensis</i>	↑BC	Crommenacker et al. 2011

### Group size

Apostlebird	<i>Struthidea cinerea</i>	S>L	Woxvold et al. 2006
Azure-winged magpie	<i>Cyanopica cyanus</i>	L>S <sup>†</sup>	Valencia et al. 2006
Lake Tanganyika cichlid	<i>Neolamprologus pulcher</i>	L>S <sup>†</sup>	Brouwer 2005
Golden lion tamarin	<i>Leontopithecus rosalia</i>	S>L <sup>†</sup>	Bales et al. 2002
Laughing kookaburra	<i>Dacelo novaeguineae</i>	S>L	Legge 2000
Hairy-faced hover wasp	<i>Liostenogaster flavolineata</i>	L>S <sup>‡</sup>	Field et al. 2006
White-winged chough	<i>Corcorax melanorhamp</i>	S>L	Heinsohn & Cockburn 1994

### Brood Size

Golden lion tamarin	<i>Leontopithecus rosalia</i>	L>S	Bales et al. 2002
Laughing kookaburra	<i>Dacelo novaeguineae</i>	L>S	Legge 2000
Purple gallinule	<i>Porphyryla martinica</i>	L>S	Hunter 1987

### Heritable variation

Western bluebird	<i>Sialia mexicana</i>		Charmantier et al. 2007
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### Rank

Hairy-faced hover wasp	<i>Liostenogaster flavolineata</i>	L>H	Field et al. 2006
Naked mole-rat	<i>Heterocephalus glaber</i>	L>H	Clarke & Faulkes 1997
Purple-crowned fairy wren	<i>Malurus coronatus</i>	H>L <sup>°</sup>	Kingma et al. 2011

### Mating opportunities

Banded mongoose	<i>Mungos mungo</i>	↓MO	Cant 2003
White-browed scrubwren	<i>Sericornis frontalis</i>	↓MO	Magrath & Whittingham 1997
White-fronted bee-eater	<i>Merops bullockoides</i>	↓MO	Emlen & Wrege 1988

↑ = positive correlation with investment in cooperative offspring care; Sex: M = males, F = females; Age: O = old, Y = young, Y<sup>1</sup> = yearling, A = adult, J = juvenile; Relatedness/Indirect Fitness Benefits: R = relatedness; Breeding Status: B = breeders, NB = non-breeders; Body Size or Energetic State: ES = energetic state, BW = body weight, FA = food availability, \* = supplementary feeding experiment; Group Size: S = small, L = large, † = effect of helper number on investment by breeders, ‡ = experimentally manipulated group size; Brood Size: S = small, L = large; Rank: L = low, H = high, ° = likelihood of inheriting a breeding position as a proxy for rank (effect only present when variation in relatedness is controlled for); Mating Opportunities: MO = mating opportunities.

## 1.2 The Importance of Considering Mechanisms

There have been large advances in understanding the ultimate mechanisms behind within-group variation in cooperative investment (reviewed in Emlen 1991, see table 1.1); studies concerned with the fitness consequences of a given behaviour which address questions asking 'Why?'. Meanwhile, little attention has been paid to the proximate mechanisms underlying behavioural differences (Soares et al. 2010); studies concerned with the mechanisms underpinning behaviour variation which address questions asking 'How?'. This is perhaps surprising as both Tinbergen and Mayr pointed out that to fully understand any behaviour we must obtain both ultimate and proximate explanations (Tinbergen 1963; Mayr 1974). Understanding the physiological mechanisms that modulate behaviour can give useful insights into the evolution of behavioural strategies. For example, a study of meerkats showed that experimental elevation of circulating oxytocin concentrations leads to increased investment in a suite of cooperative behaviours (Madden & Clutton-Brock 2010). Where different behaviours share a common causal mechanism natural selection may not be strong enough to decouple expression of those behaviours, so these findings may help to understand why helpers seem to invest in both beneficial and costly forms of helping and why individuals vary in their expression of different forms of helping behaviour.

Endocrine systems are good candidate mechanisms for behaviour modulation as they can act in a relatively short timescale (a few minutes) and can modify multiple behaviours simultaneously. Variation in cooperative investment commonly runs alongside variation in investment in other behaviours. For example, individuals may decrease their cooperative investment while increasing investment in mating behaviours; in such a case it is likely that a hormone would inhibit cooperation and promote mating behaviours concurrently. Studies of hormonal modulation of behaviour have repeatedly highlighted two types of steroid hormone as behaviour modulators: testosterone and glucocorticoids (corticosterone/cortisol) (Wingfield et al. 1990; Eens et al. 2007; Dey et al. 2010; Hau et al. 2010). Emerging studies of cooperative systems indicate that these two hormones may also play an important role in the modulation of cooperative investment (e.g. Young et al. 2005; Carlson et al. 2006a), though further work is needed.

The term glucocorticoid (GC) refers to a family of steroid hormones that are released from the adrenal glands in response to secretion of ACTH from the anterior lobe of the pituitary. At low concentrations, GCs preferentially bind to high-affinity mineralcorticoid receptors and regulate physiological processes such as extracellular fluid volume (Rook 1999). At medium and high concentrations, these high-affinity mineralcorticoid receptors become saturated and GCs bind to low-affinity glucocorticoid receptors, mediating responses to predictable seasonal changes in energy

## 1. Introduction

expenditure (allostatic load) and emergency-responses to unpredictable stressful situations, respectively (Wingfield et al. 1998; Sapolsky et al. 2000; Romero 2004; Landys et al. 2006). When investigating the role of GCs in modulating behaviour investment it is important to make a distinction between these three categories of GC function. Individual differences in responses to a stressor such as predation or injury are most likely to be attributable to GC variation at high concentrations. Variation in individual investment in cooperative behaviours such as vigilance, offspring care, or territory defence, is more likely to be attributable to GC variation at medium level concentrations.

Testosterone is a steroid hormone that is secreted predominantly from the testicles of males (and ovaries of females), though it is also secreted from the adrenal glands, and functions through binding with the androgen receptor in the cytoplasm of target cells (Norris 2006). Testosterone is essential for spermatogenesis and promotes secondary sexual characteristics such as increased muscle and bone mass, whilst inhibiting immune function (Folstad & Karter 1992; Lindstrom et al. 2001). It has also been linked to the expression of mating behaviours in a wide variety of taxa, including avian song repertoires (Eens et al. 2007), courtship displays (Gleason & Marler 2010), and territory defence (Wingfield et al. 1990). Circulating concentrations of testosterone tend to be elevated in individuals that are searching for mates, and decreased in males expressing parental care (Wingfield et al. 1990). For example, single men have higher testosterone than men in relationships, and men with children have lower testosterone levels than men without children (Kuzawa et al. 2009). Experimental manipulation of testosterone levels has been seen to alter expression of offspring care and mating behaviours in a range of species (e.g. Marler et al. 1989; Clark & Galef 1999; Duyse et al. 2000; Stoehr & Hill 2000), further supporting the hypothesis that testosterone mediates a trade-off between offspring care and reproduction.

Hormones act through complex pathways; dependent on hormone-hormone interactions (e.g. testosterone dependence on corticosterone, Knapp & Moore 1997), expression of reactors (e.g. aromatase converting testosterone into oestrogens as an alternative aggression/parental care coordinator, Trainor & Marler 2001), and expression of receptors (e.g. site specific oxytocin receptor densities for cooperative behaviours, Olazábal & Young 2006), allowing evolutionary plasticity and multiple potential mechanisms for alternative hormonal controls of behaviour. Hormone profiles differ greatly between species (e.g. Creel et al. 1993; Archer 2006), between individuals within the same species (e.g. Knapp & Moore 1997), and even between individuals within the same social group (e.g. Carlson et al. 2006a; Carlson et al. 2006b). Hormone function may also differ between individuals that are very similar. For example, a study of squirrel monkeys showed that hormone

initiators of aggression and mating behaviour in dominants do not cause the same reaction in subordinates (Winslow & Insel 1991). Winslow & Insel suggest that it would be costly for subordinates to act aggressively or attempt to mate so they have become insensitive to physiological cues. This variation in hormone function seen between individuals suggests that hormones may play a role in mediating other individual differences in behaviour expression. Furthermore, differences in physiological modulation of behaviour expression both within and between species may be the result of different selection pressures. Therefore, understanding the proximate mechanisms underlying modulation of cooperative behaviours can give great insights into how selection pressures for cooperation act differently both within and between species.

But what leads to individual differences in physiology? Moore's "relative plasticity hypothesis" (1998) suggests that there are two stages in endocrinal organisation of phenotype; hormone levels early in development produce fixed alternative phenotypes, while hormone levels later in life govern plastic alternative phenotypes (e.g. onset of mating behaviour); a hypothesis that he tested using tree lizards (*Urosaurus ornatus*) as a model species. External stimuli may affect hormone levels in the short-term, such as aggressive interactions promoting the release of androgens (Wingfield et al. 1990), or in the long-term, such as environment and social interactions during development affecting hormone expression later in life (Sih et al. 2004). For example, studies of humans have shown that expression of certain neuropeptides and the associated social behaviours are dependent on social experience as a child (Fries et al. 2005), while in rodents, increased aggressive encounters and increased circulating testosterone concentrations during puberty lead to increased aggression in adulthood (Wommack et al. 2003). This long-lasting effect of hormonal differences indicates neuroendocrine mechanisms as potential generators of behavioural types or 'syndromes' that could lead to consistent differences in behaviour expression between individuals (Sih et al. 2004; Lessells 2008).

Investment in cooperation tends to involve care of offspring, cooperative vigilance, or aggressive territory defence (in inter-group conflict), and it is likely that the same physiological mechanisms underlie these types of behaviour as in non-cooperative species. Testosterone is seen to promote territory defence in a variety of non-social species (Wingfield et al. 1990), and it seems likely that a similar mechanism promotes territory defence in social species (Soares et al. 2010). The role of testosterone in mediating cooperative offspring care has received more attention and it seems likely that testosterone inhibits offspring care in cooperatively breeding species similar to in non-social species (e.g. Khan et al. 2001; Young et al. 2005). However, more work is needed to verify the extent of this relationship and examine if variation in testosterone is correlated with individual differences

## 1. Introduction

in cooperative investment. The role of GCs in modulating investment in offspring care may also have parallels between cooperative and non-cooperative species (Soares et al. 2010). For example, affectionate contact with infants is associated with elevated GCs in primiparous human mothers (Krpan et al. 2005), and meerkat helpers with high GCs show increased pup feeding rates (Carlson et al. 2006a). Overall, understanding of hormonal mechanisms of cooperative behaviour is currently limited and may be viewed as a starting point in integrating the fields of endocrinology and cooperative behaviour. In order to fully understand the proximate mechanisms behind individual variation in cooperative investment we need to focus on resolving the hormonal mechanisms that underpin these behaviours.

The aim of this thesis is to investigate the ultimate and proximate mechanisms underlying individual differences in behaviour expression within cooperatively breeding societies. I have focussed my research on the banded mongoose (*Mungos mungo*); a species of mongoose that lives in Sub-Saharan Africa in stable cooperatively-breeding groups of up to 40 individuals (further details of the study species are given in Chapter 2). Within banded mongoose societies there is large inter-individual variation in cooperative investment (Cant 2003). Previous studies have highlighted age, sex, breeding status, and body condition as predictors of cooperative investment (Cant 2003; Gilchrist & Russell 2007; Hodge 2007). However, there is still a large residual variation in cooperative investment that is unexplained with some individuals investing several orders of magnitude more in cooperative activities than others.

I will investigate individual investment in a suite of both cooperative and competitive behaviours to test whether individual differences in investment are driven by trade-offs between different behaviours. I will also investigate the roles of glucocorticoids and testosterone as mediators of the trade-offs between both cooperative and non-cooperative investments. I will also take advantage of the long-term nature of the banded mongoose research project to investigate lifetime patterns of cooperative and competitive behaviours to look in more detail at individual differences in behaviour that are not visible in short-term studies. Using a mixed approach, investigating both the ultimate and proximate mechanisms underlying individual differences in cooperative investment in the banded mongoose, will hopefully provide novel insights into why individuals behave differently within social groups, and add to our understanding of the evolution of sociality and cooperatively breeding systems.

### 1.3 Thesis Structure

In Chapter 1, I have given a broad overview of previous research investigating the factors that mediate individual differences in cooperative investment and introduced studying hormonal modulation of behaviour as a key tool in understanding behavioural differences.

In Chapter 2, I give a general introduction to the study species, focussing on the habituated population of banded mongooses that inhabit the Mweya Peninsula, Queen Elizabeth National Park, Uganda on which this study was based. I also outline the general methods used in this thesis, including behavioural observations, collection of faecal samples, hormone assay techniques, and statistical methods.

In Chapter 3, I look for a carry-over effect of investment in cooperative offspring care between consecutive litters to investigate whether variation in helping effort is driven by variation in previous helping effort. I also investigate variation in faecal glucocorticoid metabolite concentrations both between and within individuals, alongside a supplementary feeding experiment, to test the hypothesis that the carry-over effect is modulated by variations in circulating glucocorticoid concentrations attributable to the energetic costs of helping.

In Chapter 4, I investigate whether variation in individual investment in cooperative offspring care is driven by variation in access to mates and the associated opportunity costs of helping. I also look at variation in faecal testosterone metabolite concentrations during the offspring care period both in the presence and absence of mating opportunities to test whether testosterone mediates a trade-off between cooperative offspring care and reproduction in the banded mongoose.

In Chapter 5, I investigate individual differences in response to simulated territorial intrusion. I assess both behavioural and physiological responses to a simulated territorial intrusion between carers and non-carers to determine whether individuals are specialised to roles in care or territory defence.

In Chapter 6, I investigate life-time consistency of individual differences in both cooperative and competitive behaviours. I compare and contrast levels of consistency between different age-rank categories and different behaviours to tease apart the ultimate mechanisms underlying consistent individual differences in banded mongoose societies.

In Chapter 7, I synthesis the findings of this thesis, drawing on the wider implications and making suggestions for future study.

## 1. Introduction

Each chapter is intended to be intelligible as a complete work. Therefore, there is some degree of repetition of methods in each chapter. References from all chapters are collated at the end of the thesis.

# Chapter 2

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# General methods





## 2.1 Study Species

The banded mongoose is a small (< 2 kg) gregarious carnivore belonging to the family *Herpestidae*. It is found in savannah, open forest, and grassland habitats across eastern and central Africa. Banded mongooses are obligate cooperative breeders that live in stable groups of 8 – 40 individuals (Cant 2000). Banded mongooses are not threatened and so are categorised as ‘least concern’ by the International Union for Conservation of Nature (IUCN 2008).

### 2.1.1 Daily activities

Banded mongooses are a diurnal species. They emerge from the den just after sunrise (c. 7am), when they defaecate at a communal latrine site and then rest and groom at the den entrance for up to an hour before embarking on a morning foraging trip. They are opportunistic generalist foragers; their diet mainly consists mainly of invertebrates, but they have been seen to consume a variety of vertebrate prey items (e.g. frogs, small birds, snakes, banded mongoose pups) and human refuse (Rood 1975; Gilchrist & Otali 2002). All individuals remain in close proximity of each other while foraging (< 20 m), using contact calls to communicate both their location and behaviour (Jansen et al. 2012). Each pack returns to their den site late in the morning and rests for several hours in the heat of the day before starting an afternoon foraging session (c. 4pm). They return to den site just before sunset and spend up to an hour engaged in grooming behaviours before retiring inside the den (c. 7pm). Banded mongooses change their den site every 3 – 5 days, preferring to use old termite mounds or clearings within thick undergrowth. They have stable latrine sites throughout their territories and will communally defaecate, urinate, and anal mark multiple sites on each foraging trip (Muller & Manser 2008).

### 2.1.2 Reproduction

In tropical habitats banded mongooses breed all year round and can produce 3 – 4 litters per year. Individuals tend to become sexually mature at one year of age, though females occasionally become pregnant younger than 12 months. Each pack contains a ‘core’ group of breeding adults (1 – 5 males, 3 – 7 females) that reproduce in each breeding attempt, and a subset of younger individuals that only breed occasionally (Nichols et al. 2010). Females usually come into group oestrus 7 – 10 days after giving birth and mate within one week of each other (Cant 2000). During oestrus, males closely guard receptive females, following them at a close distance (< 20 cm) and frequently attempting to

## 2. General Methods

mate with them (Cant 2000). These mate-guards aggressively defend their associated females from attempts to mate by pestering males. Older and heavier males monopolise reproduction within the group by guarding multiple females consecutively and by guarding more successful females (Nichols et al. 2010). Each female gestates 1 – 4 pups, and older females carry larger litters than younger females (Nichols et al. 2010). Females give birth synchronously (usually on the same day; (Hodge et al. 2011) to large litters (1 – 20 pups; Gilchrist 2006), which are then cared for by all individuals within the group (Cant, 2003; Gilchrist & Russell, 2007; Hodge, 2007).

### 2.1.3 Cooperative offspring care

Banded mongoose pups remain in the den until they are around 4 weeks old. During this time, whenever the pack leaves to go on a foraging trip one or more '*babysitters*' remains at the den to care for the pups and protect them from predation and inter-group infanticide (Cant 2003). Babysitting effort is biased towards young males, though all individuals within the group contribute (Hodge 2007). Some individuals invest several orders of magnitude more in babysitting than others and are termed '*super-babysitters*' (Cant 2003). Babysitting is discussed in more detail in Chapters 3 and 5.

Banded mongoose pups emerge from the den at approximately 4 weeks of age, and a few days later they join the pack on foraging trips. Most pups form a stable relationship with a single adult '*escort*' who they closely associate with for the next 5 – 6 weeks until becoming independent (Gilchrist 2004; Hodge 2005; Gilchrist & Russell 2007). Escorts have high investment in pup care; carrying, playing with, feeding, protecting, and grooming their associated pup until it reaches independence. Pups compete aggressively for access to high quality escorts, and escorts tend to follow a 'feed the closest pup' rule, suggesting that the pup-escort relationship is maintained by pups (Gilchrist 2004). Banded mongoose pups use loud and frequent begging calls to elicit help from their escorts (Gilchrist 2004); they have faster growth rates, higher survival rates, and are heavier as adults if they maintain close proximity to their escort (Hodge 2005).

### 2.1.4 Inter-group conflict

Inter-group conflict between neighbouring packs of banded mongooses involves aggressive interactions that can be very intense and lead to serious or fatal injury for some members of the group (Cant et al. 2002). When meeting on a territory boundary, individuals stand on hind legs and

make an alarm call. The subsequent response can then vary from fast retreat to intense fighting for up to an hour. These types of interaction tend to be won by the larger group and commonly lead to shifts in territory boundaries. Individual investment in inter-group aggression tends to be higher in younger males, as older males guard females within the group from mating attempts by members of the other group (Cant et al. 2002). If inter-group interactions occur when there are pups in the group then the pups tend to remain hidden from the conflict with one or two adults. However, they are sometimes found by members of the other group and inter-group infanticide can occur.

### **2.2 Study Population**

All research within this thesis was carried out using a habituated population of banded mongooses that inhabit the Mweya Peninsula, Queen Elizabeth National Park (QENP), Uganda. This population was first studied by Jon Rood in the 1970s, subsequently by Daniela de Luca in the 1990s, and the current research project was started by Michael Cant and Tim Clutton-Brock in 1995. The banded mongoose research project ([www.bandedmongoose.org](http://www.bandedmongoose.org)) is ongoing and incorporates a long-term database that currently (December 2012) holds life-history and behavioural observations for more than 2500 individuals from 42 social groups. The research in this thesis uses a combination of analyses from the long-term database (Chapters 3, 4, & 6) and my own behavioural observations and experimental manipulations (Chapters 3 – 5).

### **2.3 Study Site**

This study was conducted on the Mweya Peninsula, QENP, in western Uganda (0° 12' S, 27° 54' E; figure 2.1). The Mweya Peninsula is a heart-shaped promontory that extends into Lake Edward; it is approximately 4.95 km<sup>2</sup> and connected to the mainland by a narrow isthmus known as the Kanyeswa ridge (figure 2.1c). Mweya was declared part of Queen Elizabeth National Park in 1952. Until recently, it was the home of the Park Headquarters and the Uganda Institute of Ecology, but now the village consists mostly of employees of the Mweya Safari Lodge or Mweya Hostel (c. 400 people).

## 2. General Methods

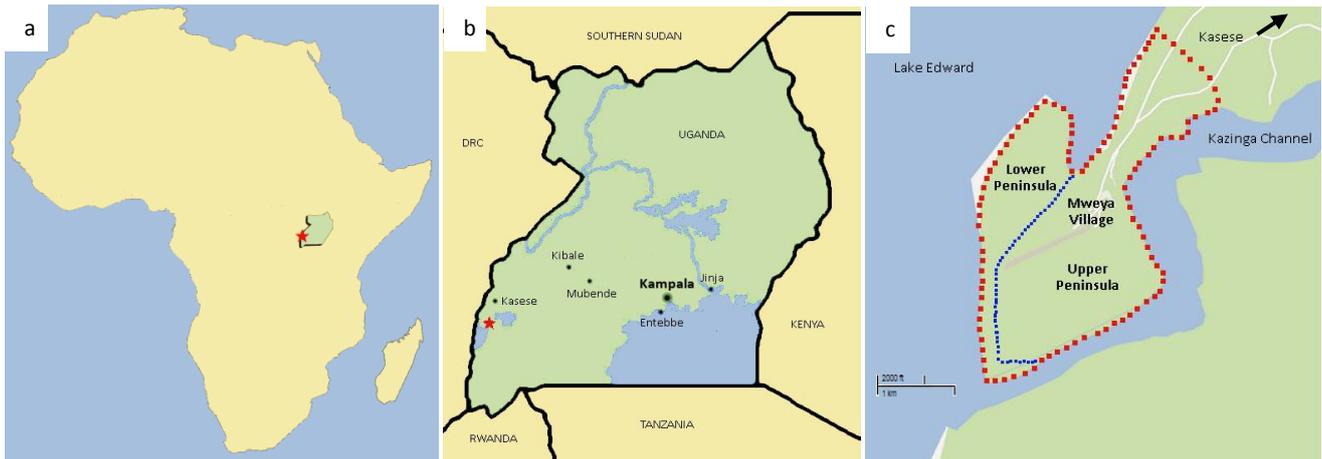


Figure 2.1: Location of the Banded Mongoose Research Project study site. Field site location in (a) Africa and (b) Uganda is indicated with red stars. (c) Map of the Mweya Peninsula; the boundary of the field site is shown with a red dotted line, the grassy verge that separates the upper and Lower Peninsula is shown with blue dots.

### 2.3.1 Habitat

QENP lies on the floor of Africa's Western Rift Valley and is overlooked by the snow-capped Rwenzori Mountains. Most of QENP, including the Mweya Peninsula is open grassland. The vegetation comprises mostly of *Sporobolus pyramidalis*, *Chloris orientalis*, and *Chloris gayana* dotted with *Euphorbia candelabrum* trees and thickets of *Capparis tormentosa* and *Azima tetracantha* (Cant 2000). The Mweya Peninsula is split into upper and lower halves by a 40m high steep grassy slope (figure 2.2c). The shore line (alongside both Lake Edward and the Kazinga Channel) is steep and densely vegetated, and is often impenetrable by foot. The inland area of the Lower Peninsula and the plateau of the Upper Peninsula are comparatively sparsely vegetated, and easy to access both by vehicle and on foot. During the rainy seasons, grasses can reach up to 1 m in height making behavioural observations more difficult.

### 2.3.2 Climate

Daily rainfall and minimum and maximum temperatures from 1999 to present were provided by the UWA staff at the QENP headquarters. These data were collected from a meteorological station within Mweya until 2008, and then at a second site approximately 20 km away until 2012. Annual rainfall was typically around 800 – 900 mm. There are two wet seasons; March – May and September – November, when daily rainfall can commonly reach above 20 mm (figure 2.2a). Mean maximum temperature was 29.6°C and mean minimum temperature was 19.3°C. Temperatures did not fluctuate between the wet and dry seasons (figure 2.2b).

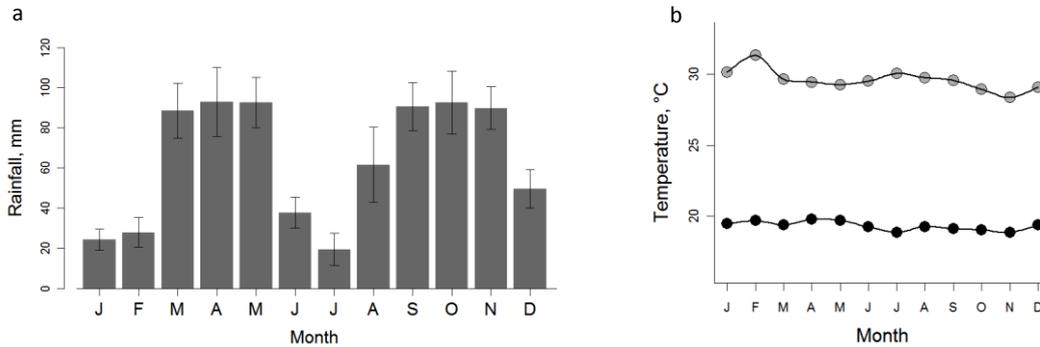


Figure 2.2: Annual meteorological data for Mweya, QENP. (a) Monthly rainfall; means and standard error. (b) Daily maximum and minimum temperature; means. Data from 1999 to 2012 provided by UWA.

### 2.3.3 Fauna

Mweya is home to four of the big five: lion (*Panthera leo*), Cape buffalo (*Syncerus caffer*), leopard (*Panthera pardus*), and African elephant (*Loxodonta africana*) (figure 2.3a-d). The main terrestrial predators of banded mongooses include spotted hyena (*Crocuta crocuta*), lion, leopard, Egyptian mongoose (*Herpestes ichneumon*), African civet (*Civettictis civetta*), and Nile monitor lizards (*Varanus niloticus*). Potential avian predators included martial eagles (*Polemaetus bellicosus*), bateleur eagles (*Terathopius ecaudatus*), black-chested snake eagles (*Circaetus pectoralis*), steppe eagles (*Aquila nipalensis*), and Ayres' hawk eagles (*Hieraetus ayresii*). During the course of this study one adult banded mongoose was witnessed being predated by a leopard (4 other mongooses disappeared within 2 days of this observation and were assumed to have been predated by the same leopard). I also witnessed a failed attempt to take a 6 week old pup by an African fish eagle (*Haliaeetus vocifer*). Marabou storks (*Leptoptilos crumeniferus*; figure 2.3e) have also been seen to feed on banded mongoose pups on a number of occasions.

Mweya is home to four mongoose species; banded, white-tailed, Egyptian, and marsh (*Mungos mungo*, *Ichneumia albicauda*, *Herpestes ichneumon*, *Atilax paludinosus*). Of these four species, banded mongooses are the only ones that are active during the day making the others difficult to observe. One pack of banded mongooses has been observed frequently grooming warthogs (*Phacochoerus africanus*) within the Mweya village (figure 2.4). Subjective observations suggest that this relationship is mutualistic and initiated by both species. Members of this pack have also been seen grooming Cape buffalo on a few occasions.

## 2. General Methods

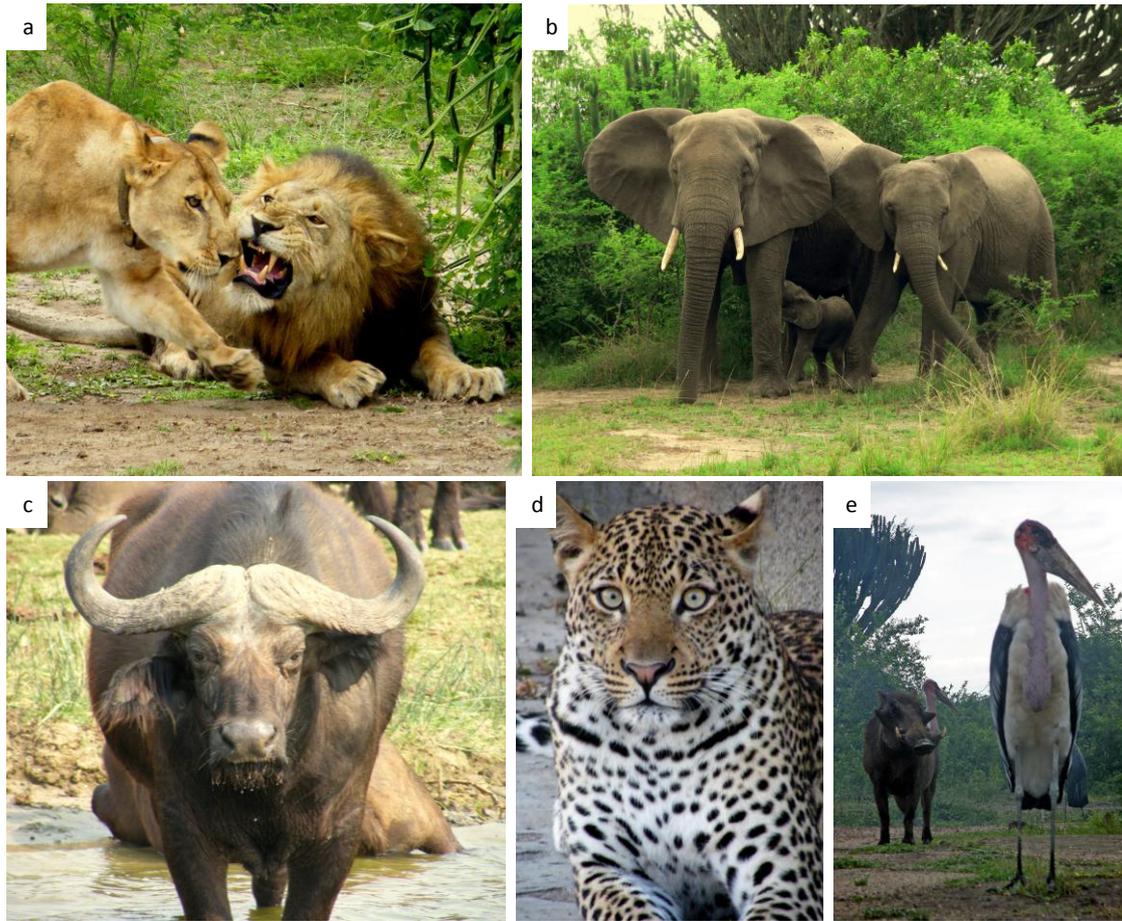


Figure 2.3: Examples of fauna found in Mweya, QENP. (a) Lion, *Panthera leo*; (b) African elephant, *Loxodonta africana*; (c) Cape buffalo, *Syncerus caffer*; (d) leopard, *Panthera pardus*; (e) warthog, *Phacochoerus africanus*; Marabou stork, *Leptoptilos crumeniferus*.

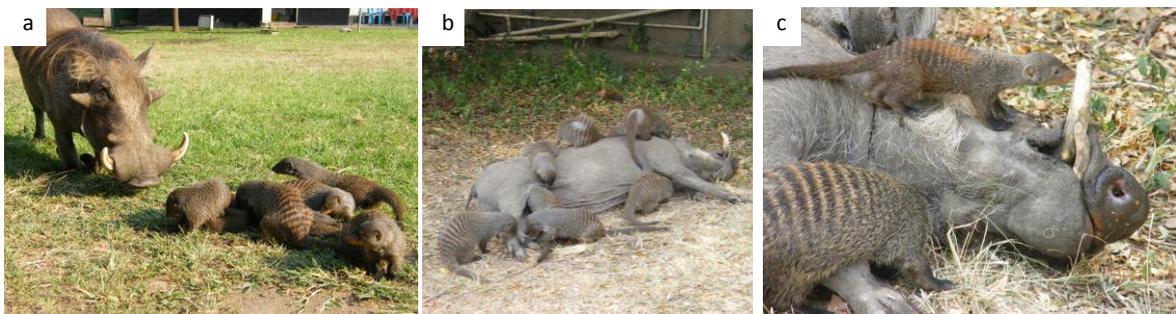


Figure 2.4: Mutualism between banded mongooses (*Mungos mungo*) and warthogs (*Phacochoerus africanus*) in Mweya, QENP. (a) Allo-grooming behaviour seems to be initiated by both species, and (b, c) the banded mongooses climb all over the body of the warthog while grooming.

## 2.4 Data Collection

### 2.4.1 Trapping and habituation

All individuals in the study population were captured within 3 weeks of first emergence and then every 3 – 6 months until they died or dispersed out of the study area. Individuals were trapped using box traps (67 x 23 x 23 cm; Tomahawk Live Trap Co., Tomahawk, WI, USA), baited with a mixture of fish, rice and vegetables available from the Mweya Hostel or Safari Lodge. Following capture, individuals were anaesthetised using either ketamine or isoflurane (details of trapping protocol are given elsewhere; ketamine: Cant 2000; Hodge 2007; isoflurane: Jordan et al. 2010; Jordan et al. 2011). Anaesthetised individuals were marked (see below), ticks on the torso were counted, measurements of head length, head width, and weight were taken, and reproductive status was assessed, including counting foetuses in pregnant females by palpation where appropriate (Cant 2000; Hodge et al. 2011). When individuals were captured for the first time a tail tip was taken for genetic analysis (Nichols et al. 2010) and an identifiable microchip was inserted into the back of their neck. Pups were marked on their backs with a unique combination of patches using blond hair dye (figure 2.5a) and adults were marked either by a unique shave on their back (figure 2.5b, c) or a coloured plastic collar to allow easy identification in the field. One or two individuals within each group were fitted with a radio collar weighing 27 g (Telonics Ltd.) with a 20 cm whip antenna (figure 2.1d). This enabled us to locate packs from a distance of 800 m using a Flexible Yagi (50 Ohm) receiver centred at 152 MHz. All individuals within the study population were habituated to the presence of an observer at 2 – 4 m, enabling the collection detailed behavioural observations without any measurable effect of observer presence.

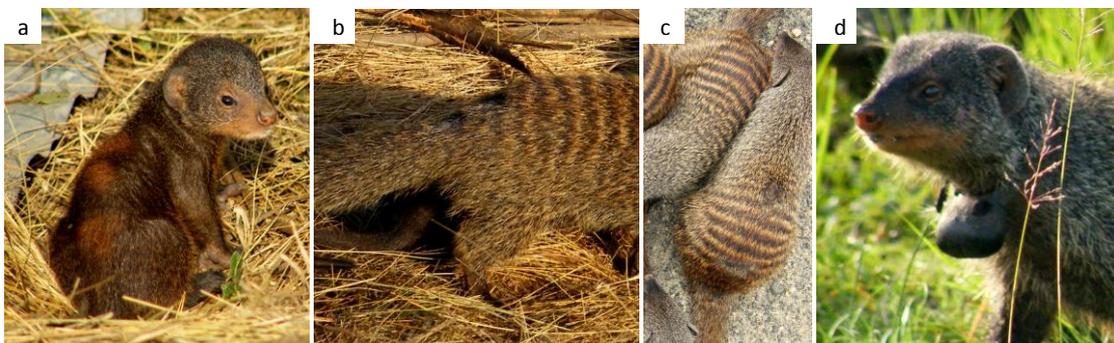


Figure 2.5: Examples of marking procedures. (a) Pups were marked on their backs with patches of blond hair dye. (b, c) Adults were marked with unique shave patches. (d) One or two individuals per group were fitted with a radio collar.

## 2. General Methods

### 2.4.2 Behavioural observations

Each pack of banded mongooses within the study population was visited at least 3 times per week. Typically, observers arrived at group before they got up in the morning and followed them for 3 – 4 hours to collect behavioural data. We then re-located the group in the afternoon (c. 4 pm) by radio-telemetry and followed them until they returned to their den-site in the evening. Packs were visited daily if they were in oestrus, there were dependent pups, or if females were expected to give birth. Most individuals within the study population were trained to step onto a small portable weighing scale using a dilute milk solution as bait (figure 2.6). This method was used to collect ‘morning weights’ before the morning foraging session and ‘evening weights’ at the end of the afternoon foraging session. We collected weights from the same group on three consecutive weighing sessions each week, giving us a weekly estimate of daily weight gain and overnight weight loss for each weighed individual.

Behavioural data were collected using a combination of *ad libitum*, scan sampling, and focal observations (Martin & Bateson 1993). Data was recorded on Psion LZ-64 handheld data loggers (Psion Teklogix Inc., Ontario, Canada) or on paper. Details of behavioural observations used in this study are given in detail in the relevant chapters.

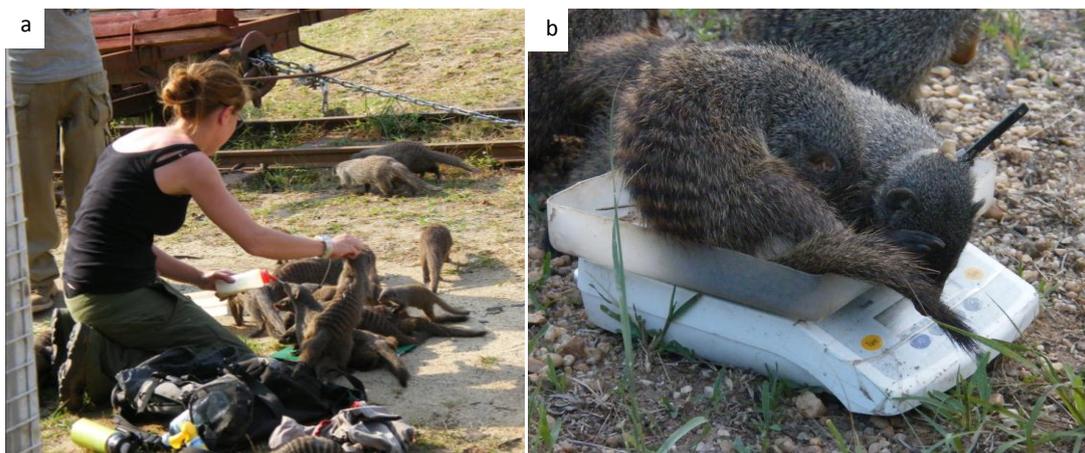


Figure 2.6: Collection of banded mongoose weights in the field. (a) Banded mongooses were trained to step onto a portable scale with a reward of dilute milk solution. (b) They remain on the scale for 1-10 seconds enabling accurate collection of weight data.

### **2.4.3 Experimental data**

Chapters 3 and 5 involved experimental data collection. Chapter 3 involved a supplementary feeding experiment using smoked fish purchased in Katunguru and Chapter 5 involved simulated territorial intrusions. Full details of the experimental protocol are given in the relevant chapters.

### **2.4.4 Collection of faecal samples for hormone sampling**

We collected faecal samples in the field for hormone assay in the UK. Unless otherwise stated, all faecal samples were collected during the morning latrine session when the mongooses emerged from the den. Fresh samples were collected by hand into small plastic bags (4" x 2"), labelled with the mongoose identity, time of defecation, and the date, and placed on ice in a Thermos flask. Samples were then transferred to a -20°C freezer within 5 hours of collection. Over-marking of faeces is common in banded mongooses (Muller & Manser 2008), so samples were only collected if they had been watched continually from defecation and it was known that they hadn't been over-marked. Further to this, only half of each faecal deposit was collected to minimise any resulting interference with scent marking signals.

Mains power supply is unreliable and intermittent in Uganda. To ensure samples remained frozen, we placed a wireless thermometer in the freezer that set off an alarm when the temperature of the freezer rose above -10°C. Upon hearing this alarm a personal diesel generator was used to power the freezer until mains power resumed.

Material transfer permits were obtained from the Uganda Wildlife Authority (UWA) and the Uganda Council for Science and Technology (UNCST), and import permits for the UK were obtained from DEFRA. Frozen faecal samples were transported back to the UK on wet ice in a cool box. Samples were transferred to Entebbe airport by road, flown back to the UK as checked-in luggage, and transported to the University of Exeter in Cornwall (UofE) by train. In total, this journey lasted a maximum of 36 hours and the samples were still chilled when they arrived at their final destination.

## **2.5 Hormone extraction and assay**

Hormones were extracted from faecal samples using a wet-weight shaking extraction adapted from Walker et al. (2002). Details of the extraction protocol are given in the relevant chapters. Faecal

## 2. General Methods

glucocorticoid and/or testosterone metabolites (fGC/FT) concentrations were analysed using modified enzyme immunoassays that have been described previously (EIA; Young et al., 2004, adapted from Munro & Stabenfeldt, 1984). Details of assay modification and validation are given in Appendix 1.

### 2.6 Statistical Analysis

All statistical analyses were carried out using R 2.13.2 (R Development Core Team 2012). Parametric tests were used where possible. Where necessary, data logarithmic transformations were used to achieve normality of error.

Most analyses required the use of multivariate statistics that controlled for repeated measures within social groups, breeding attempts, and individuals. This required the use of linear mixed models (LMMs) or generalised linear mixed models (GLMMs), so that both random and fixed terms could be fitted. I used LMMs when the response variable was normally distributed with a Gaussian error structure, and used GLMMs when the response variable was either proportion (binomial error structure) or count (poisson error structure) data. In mixed models, random terms can be nested to allow for repeated measures within different levels in the population. In short-term analyses, such as those investigating hormonal variation, individuals remained in the same social group throughout so individual was nested within social group. When analyses were carried out on long-term data and stretched over a longer time scale in which individuals changed social groups, individual was not nested within social group. Breeding attempt was nested within social group throughout.

Throughout Chapters 3 – 5 I used a stepwise approach of model simplification. I fitted a maximal model with all terms of interest, and the significance of each term was determined using likelihood ratio tests comparing the deviances of the full model and a model without the term of interest. Fixed terms were sequentially removed from the model by their level of non-significance until a minimum model was found where the removal of any fixed term decreased the fit of the model. Terms that had been removed from the model were then added back into the minimum model to determine their level of non-significance.

In Chapter 6 I used a Bayesian approach to mixed modelling using the MCMCglmm package to allow extraction of variance estimates with credible intervals. Details of this analysis are given in the methods section of Chapter 6 (6.3.3).

# Chapter 3

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## Carry-Over Effects in a Cooperative Breeder:

### The Role of Glucocorticoids and Energetic State





### 3.1 Abstract

Carry-over effects of offspring care occur when individuals are unable to recover from the costs of caring for offspring before the next breeding attempt and are forced to reduce their subsequent investment in offspring care. Though evidence for carry-over effects in non-cooperative species is strong, few studies have looked for carry-over effects of offspring care in cooperatively breeding species or investigated the *proximate* mechanisms mediating these carry-over effects. We demonstrate strong evidence for a carry-over effect of offspring care in a cooperatively breeding mammal; high investment in care leads to a decrease in care investment in subsequent breeding attempts in a long-term study population of banded mongooses (*Mungo mungo*). We suggest that glucocorticoids (GCs) may play a role in mediating this carry-over effect due to evidence that variation in baseline GCs modulates energetically costly behaviours. Using non-invasive faecal GC metabolite (fGC) monitoring and supplementary feeding experiments, we test the hypotheses that (1) GC concentrations modulate individual investment in offspring care and (2) investment in offspring care affects GC concentrations. We find that individuals with low fGC concentrations prior to the helping period invest more heavily in cooperative care. Furthermore, helpers investing heavily in cooperative care show significant fGC elevations both during and after the pup-care period. Supplementary feeding reduces these fGC elevations in top helpers, suggesting that helping leads to an increase in fGC concentrations because of the associated energetic costs. We highlight GC signalling of energetic state as a mechanism mediating cooperative investment within social groups. This, alongside evidence that individuals with high cooperative effort in one breeding attempt have a reduced cooperative effort in subsequent breeding attempts, indicates GC modulation of care effort as a candidate proximate mechanism mediating the carry-over effects of offspring care.

### 3. Carry-Over Effects in a Cooperative Breeder

### 3.2 Introduction

Within cooperatively breeding societies there is striking variation in how much individuals invest in cooperative behaviours, with some individuals investing several orders of magnitude more than others (Komdeur 2006). Theory suggests that this variation may be due to individual differences in the fitness costs of helping because an individual will be more likely to help if the costs are low (Maynard-Smith 1977; Clutton-Brock 1991). Investment in helping behaviours such as provisioning young and cooperative vigilance often lead to a decrease in body mass (Brown et al. 1982; Heinsohn & Cockburn 1994; Hodge 2007) which may affect future survival or reproductive success. The fitness consequences of these energetic costs are likely to be higher in individuals in a low energetic state as they have limited resources available to invest in costly helping behaviours. Individuals experiencing greater costs often show decreased helper effort. For example, Seychelles warbler; *Acrocephalus sechellensis* (Van de Crommenacker et al. 2011), meerkat; *Suricata suricatta* (Russell et al. 2003), and banded mongoose; *Mungos mungo* (Hodge 2007) helpers decrease their provisioning effort when body mass is low prior to helping. Furthermore, energetically costly behaviours often incur a carryover effect due to the time required to recover from the associated decrease in energetic state (Harrison et al. 2011). In non-cooperative species, periods of high reproductive effort can be followed by low reproductive effort in subsequent breeding attempts (Inger et al. 2010). Similarly, if helpers cannot recover from the energetic costs of caring for young before the next breeding attempt they may be less likely to show repetitive high levels of helping investment in consecutive breeding attempts (Russell et al. 2003). Together these findings demonstrate that variation in energetic state is an important modulator of helper investment, and suggest that energetic costs of offspring care may generate carryover effects of helping between consecutive breeding attempts.

Hormones play a central role in modulating parental care (e.g. testosterone [Ketterson & Nolan 1999; Trainor & Marler 2001; Wingfield 2005], prolactin [Schradin & Yuen 2011], oxytocin [Carter et al. 2008], and glucocorticoids [Fleming et al. 1997]), and preliminary evidence suggests that comparable mechanisms may regulate cooperative offspring care (e.g. testosterone [Peters 2002], prolactin [Carlson et al. 2006b], oxytocin [Madden et al. 2010], and glucocorticoids [Carlson et al. 2006a]). For example, affectionate contact with infants is associated with elevated glucocorticoid (GC) concentrations in primiparous human mothers (Fleming et al. 1997), and meerkat helpers with high GC levels show higher pup feeding rates (Carlson et al. 2006a). Studies of solitary and biparental systems have shown that GCs play a key role in energetic homeostasis through increasing foraging effort and decreasing non-essential behaviours when energetic output outweighs available energetic resources (McEwen & Wingfield 2003). For example, wandering albatrosses have increased circulating GC concentrations after bouts of low foraging success which seems to increase locomotor

### 3. Carry-Over Effects in a Cooperative Breeder

activity during subsequent foraging trips (Angelier et al. 2007). Similarly, house sparrow chicks experiencing low provisioning rates show increased GC concentrations alongside increased begging frequency and decreased immune function (Loiseau et al. 2008). Further to this, baseline GC concentrations are negatively correlated with both breeding effort and body weight in house sparrows (*Passer domesticus*; Ouyang et al. 2011) and Galapagos marine iguanas (*Amblyrhynchus cristatus*; Vitousek et al. 2010), suggesting that elevated baseline GC concentrations may also inhibit energetically costly parental behaviours when in a low energetic state. This raises the possibility that elevated GC concentrations may also inhibit helping behaviours in cooperatively-breeding species, and that variation in helper effort both within and between breeding attempts is attributable to GC signalling of energetic state.

If helping is energetically costly, hardworking helpers may experience a corresponding increase in GC concentrations over the period of offspring care. For example, a study of house sparrows found that the most successful breeders have low GC concentrations prior to breeding, but then have high GC concentrations during breeding (Ouyang et al. 2011). Furthermore, in meerkats, individuals that care for young in the den ('babysitting') have low GC concentrations prior to, and high GC concentrations after investment in care, which is likely to be due to their inability to find food while at the den (Carlson et al. 2006b). If GC elevations do signal energetic losses, such as those from heavy investment in helping, then any latency to recover from this energetic cost before the next breeding attempt may leave the focal individual entering the next breeding attempt with elevated GC concentrations, resulting in a negative carry-over effect of helping behaviours in consecutive breeding attempts.

The aim of this study is to investigate if there is a carry-over effect of helper effort in the cooperatively breeding banded mongoose (*Mungos mungo*) and examine variations in energetic state and circulating GC concentrations as a candidate proximate mechanism underlying the carryover effect. Banded mongooses are small diurnal herpestids that live in stable groups of 8 - 40 individuals (Cant 2000). 1 - 10 adult females give birth synchronously (usually on the same day: Hodge et al. 2011) to large litters (1 - 20 pups: Gilchrist 2006), of which 85% are fathered by the 3 oldest males in the group (Nichols et al. 2010). Banded mongooses breed all year round and produce up to 4 litters each year, which provides an opportunity to investigate carry-over effects of helping in cooperatively-breeding societies. Most foraging pups (> 4 weeks after birth) form a stable association with a single adult escort who provisions, carries, plays with, grooms, and protects that pup until it reaches independence at approximately 10 – 11 weeks (Gilchrist 2004). Escorts show no preference to associate with the pups that they have sired (Gilchrist 2004). Escorts provide a level of pup care an order of magnitude greater than non-escorts, creating a dichotomy of investment in

care with individuals of the same age, sex, and dominance status varying greatly in their levels of helper investment (Gilchrist 2004; Hodge 2007). This makes banded mongooses an excellent model system for investigating inter-individual variation in investment in cooperative offspring care.

The unique system of cooperative care displayed in banded mongoose societies also provides an opportunity to investigate the relationship between GC concentrations and offspring care in a social system. Previous research has shown both that supplementary feeding of male escorts increases the rate at which they provision pups and that escorts have decreased weight gain during the escorting period, which suggests that escorting behaviours are associated with changes in energetic state (Hodge 2007). However, differences in weight gain between escorts and non-escorts do not persist after the escorting period (Hodge 2007), which suggests that these energetic losses are unlikely to affect future behaviour. Here, we will investigate changes in GC concentrations during and after the escorting period to see the physiological effects of energetic losses attributable to investment in care. Though there are no differences in weight change in escorts and non-escorts at the end of the care period, if escorts are unable to recover from the associated physiological effects of care this may lead to decreased care effort in subsequent breeding attempts.

Typically the causal link between hormone levels and behaviour is difficult to establish due to problems with experimentally manipulating either behaviour or hormone levels in wild animal populations. Here we address this problem by collecting measures of hormone levels before, during, and after the pup care period to give within-individual measures of hormonal change across the period of pup care. We then follow this with supplementary feeding experiments to test hypotheses of causal links between hormones, behaviour, and energetic state. Specifically we aim to address 4 questions: (1) Are there carry-over effects of investment in helper investment; i.e. do individuals with high investment in pup care have decreased investment in pup care in subsequent breeding attempts? (2) Do baseline fGC concentrations before a breeding attempt predict levels of helper investment? (3) Do individuals with high levels of helper investment show a positive fGC change over the pup care period? (4) Are changes in fGC concentrations over the pup care period due to the energetic costs of helping?

## **3.3 Materials and Methods**

### **3.3.1 Study site and data collection**

We obtained measures of escorting effort in 732 individuals over 122 breeding attempts in 10 social groups between June 2000 and April 2012, on the Mweya Peninsula in Queen Elizabeth National

Park, Uganda (0°12'S, 27°54'E). Details of vegetation and climate are given elsewhere (Cant 2000). All individuals were habituated to the presence of observers at a distance of 2-.

#### *Measuring escorting effort*

During the escorting period (4 – 10 weeks after birth) groups were visited daily and associations between adults and pups were noted. Adults were scored as escorts if they were seen to be within 30cm of the same pup for more than 50% of the observation period (Gilchrist 2004; Gilchrist & Russell 2007). Previous studies have shown that this association score is a reliable summary of quantitative nearest-neighbour data (Gilchrist 2001; Gilchrist & Russell 2007). Individual scores of escorting behaviour per breeding attempt were then calculated as the proportion of days that an individual was recorded as an escort out of the total number of days for that breeding attempt on which escorting was recorded by one or more individuals within the group (7-21 observation days per breeding attempt). This gave a single escorting score for each individual in each escorting period which is representative of individual helping effort in each breeding attempt.

#### *Body condition*

All individuals in this study were trapped every 3 to 12 months using box traps (67x23x23 cm; Tomahawk Live Trap Co., Tomahawk, WI, USA), and anaesthetised using either ketamine or isoflurane (details of trapping protocol are given elsewhere; ketamine: Cant 2000; Hodge 2007; isoflurane: Jordan et al. 2010; Jordan et al. 2011). Measures of body mass ( $\pm 1$  g) and head width ( $\pm 0.1$  mm) were taken from all trapped individuals (850 captured male banded mongooses over 743 trapping sessions [ $4.1 \pm 0.17$  captures per individual; mean  $\pm$  S.E] between February 2000 and March 2012). This capture data was used to calculate a predicted body mass for any given head width by regressing log body mass with log head width. In addition to capture weights, all individuals in this study were trained to step onto a portable weighing scale allowing weights to be recorded daily before the morning foraging trip (Hodge 2007). To assess body condition from weights collected without capture, we compared an individual's body mass with its predicted body mass based on its head width at closest capture date (i.e. observed body mass (collected in the field) / predicted body mass) (following Jakob et al. 1996; Gilchrist et al. 2004).

#### **3.3.2 Statistical Analyses**

All statistical analyses were carried out using R 2.13.2 (R Development Core Team 2012). We used linear mixed models (LMMs) and generalised linear mixed models (GLMMs) to control for repeated measures within social groups, breeding attempts, and individuals. Normally distributed response

variables were analysed with an identity link function, and binomial response variables with logit link function. All possible explanatory variables were initially fitted together in a maximal model and then sequentially dropped from the model in order of least significance until only those variables explaining significant variation ( $p < 0.05$ ) remained. All dropped variables were then put back into the minimal model to reconfirm their non-significance.

#### **3.3.3 Faecal sample collection**

We collected 432 faecal samples from 82 banded mongooses in 5 social groups between April 2010 and April 2012. All samples were collected between 6:30am and 10:00am and immediately placed on ice in a thermos flask. They were then transferred to a  $-20^{\circ}\text{C}$  freezer within 3 hours. Samples were transferred to the UK on ice and again transferred to a  $-20^{\circ}\text{C}$  freezer for storage. As over-marking of faeces is common in banded mongooses (Muller & Manser 2008), samples were only collected if they had been watched continually from defecation and it was known that they hadn't been over-marked. Further to this, only half of each faecal deposit was collected to minimise any resulting interference with scent marking signals. Time of collection and time to freezer were included in all analyses where appropriate but were found to be non-significant predictors of faecal glucocorticoid metabolite (fGC) concentrations throughout.

#### **3.3.4 Hormone extraction and assay**

Hormones were extracted from faecal samples following thawing and manual homogenisation using a wet-weight shaking extraction adapted from Walker et al. (2002). In brief, 0.5 g of faecal material was combined with 90% methanol, shaken overnight at room temperature and centrifuged for 20 minutes at 598 g. The methanol fraction was decanted and evaporated to dryness. Faecal extracts were re-suspended in 1ml methanol and stored at  $-20^{\circ}\text{C}$  until analysis.

Hormone extraction was carried out either at Chester Zoo Endocrinology Lab (CZEL) or at the University of Exeter in Cornwall (UoE). Samples extracted at the UoE were then transferred to CZEL on ice for assay. We extracted a subset of samples ( $n=20$ ) at both CZEL and UoE to ensure the location of extraction did not affect the hormone results. The results from samples extracted in both CZEL and UoE were highly correlated for both the testosterone and glucocorticoids assays (Pearson's correlation: T;  $t_{60} = 2.93$ ,  $p = 0.004$ ; GC;  $t_{45} = 10.66$ ,  $p < 0.001$ ) and there was no significant effect of extraction location on fGC or faecal testosterone (fT) measures (GLMM: T;  $\chi_{12}^{14} = 0.38$ ,

$p=0.83$ : GC;  $\chi_{12}^{14} = 2.72$ ,  $p=0.26$ ), therefore results from both extraction locations were pooled throughout all analyses.

fGC and fT concentrations were analysed using modified protocol from previously described enzyme immunoassays (EIA; Young et al. 2004, adapted from Munro & Stabenfeldt 1984). Details of assay modifications and validations are given in Appendix 1.

### **3.3.5 Are there carry-over effects of investment in pup care in one breeding attempt on investment in pup care in the next breeding attempt?**

To investigate whether there are carry-over effects of helping in one breeding attempt on helping effort in the next breeding attempt we recorded escorting effort in 115 male banded mongooses over 40 pairs of consecutive breeding attempts from 5 social groups. If carry-over effects of helping effort on future helping effort are present we would expect individuals that engaged in high levels of escorting in one breeding attempt to show reduced escorting effort in the subsequent breeding attempt, and this affect to be weaker in breeding attempts with longer inter-birth periods (as individuals would be better placed to recover from the costs of helping from the first breeding attempt). Escorting effort is defined as the proportion of days an individual was seen escorting out of the total number of days that escorting was observed in that breeding attempt.

Analyses of carry-over effects are susceptible to the problem of ‘regression to the mean’ (Kelly & Price 2012); negative correlations between repeated measures from the same individual may be an artefact of the data because individuals with extreme high or low observations in the first measure are likely by chance to be closer to the mean in the second measure. To control for this artefact we calculated adjusted individual change in escorting effort ( $\hat{D}^*$ ) between two consecutive breeding attempts by incorporating the change expected by regression to the mean using the following formulae suggested by Kelly & Price (2012):

$$\hat{D}^* = (X_2 - \bar{X}_2) - \hat{p}(X_1 - \bar{X}_1)$$

and

$$\hat{p} = \frac{2rs_1s_2}{s_1^2 + s_2^2}$$

where  $X_1$  and  $X_2$ , and  $s_1$  and  $s_2$  are the escorting effort and standard deviations of escorting effort from the first and second breeding attempt respectively, and  $r$  is the correlation between escorting effort in the two consecutive breeding attempts.

### 3. Carry-Over Effects in a Cooperative Breeder

To investigate whether individuals that invested heavily in escorting in one breeding attempt reduced investment in the consecutive breeding attempt, the adjusted change in escorting effort between two consecutive breeding attempts was fitted as the response term in a LMM with escorting effort in the first of the two breeding attempts fitted as a continuous fixed predictor term. Inter-birth period was calculated as the number of days between births (median = 83 days, min. = 52 days, max. = 176 days) and fitted as a continuous fixed term. We also included the two-way interaction between previous escorting effort and inter-birth period to test whether increased recovery time affected individuals differently dependent on previous escorting effort. Breeding attempts included in this analysis were limited to those with at least 10 observation days. We also fitted escort age at birth of the second litter (months), group size at emergence of the second litter (number of individuals aged > 1 year), and the number of emerged pups in the second litter as covariates. Social group, breeding attempt, and individual identities were all fitted as random effects to control for repeated measures.

#### **3.3.6 Do baseline fGC concentrations before a breeding attempt predict escorting effort?**

To investigate whether fGC concentrations before a breeding attempt predict escorting effort we observed escorting effort in 28 adult male banded mongooses over 12 breeding attempts in 5 different social groups. We collected 78 faecal samples in the two week period prior to the first observation of escorting behaviour (1 – 4 samples per individual). fGC concentrations from these samples were then averaged for each individual within each breeding attempt to give one pre-escorting fGC value per individual. We fitted escorting effort as a binomial response; the number of days seen escorting (set as the binomial numerator) out of the total numbers of days escorting was observed (set as the binomial denominator), in a GLMM with logit link. Individual mean fGC concentration was fitted as the main predictor of interest. We also fitted age at pup emergence (months), body condition at the start of the escorting period (calculated from mean body mass in the two weeks prior to pup emergence), group size (number of individuals aged > 1 year at pup emergence), and the number of emergent pups as covariates. Social group, breeding attempt, and individual identities were fitted as random terms to control for repeated measures.

### **3.3.7 Does high investment in pup care correlate with an increase in fGC concentrations during the pup care period?**

To investigate whether escorting effort predicted the change in fGC concentrations between the start and end of the escorting period we observed escorting effort in 29 male banded mongooses over 12 breeding attempts in 5 social groups. We collected 166 (1 – 10 per individual) and 266 (1 – 15 per individual) samples in the 30 days prior to, and the 78 days following the first observation of escorting in each breeding attempt, respectively. We took the log of fGC concentrations from both before and during/after escorting to retain normality of errors. We took the mean of log fGC concentrations from samples collected prior to escorting for each individual in each breeding attempt to give one pre-escorting fGC value per individual and breeding attempt. To investigate fGC change over the escorting period we then fitted log fGC concentrations from samples collected during/after escorting as a response in a LMM with individual mean pre-escorting log fGC concentration as a fixed predictor.

Individual escorting effort was calculated as the proportion of days that each individual was observed escorting out of the total number of days that any individual was observed escorting in a given breeding attempt. The day of sample collection was defined as the number of days between the day of sample collection and the first observation of escorting within a breeding attempt. To investigate if escorting effort affected change in fGC concentrations during and after the escorting period we fitted a three-way interaction between escorting effort, day, and mean pre-escorting log fGC concentration. Social group, breeding attempt, and individual identities were included as random effects to control for repeated measures.

### **3.3.8 Are changes in fGC concentrations over the pup care period due to the energetic costs of helping?**

To test whether energetic costs of escorting causally affect fGC concentrations, we provisioned both escorts and non-escorts during the escorting period, and measured changes in fGC concentrations. In doing so, we were able to experimentally reduce the energetic costs of escorting, and investigate the influence of this on fGC concentrations. Specifically, we chose escorts and non-escorts randomly and assigned them to one of four treatment groups: (i) fed escorts (n = 8), (ii) control escorts (n = 6), (iii) fed non-escorts (n = 6), and (iv) control non-escorts (n = 9). Fed escorts and fed non-escorts were both fed 80g of dried fish before the start of the morning foraging trip for 6 consecutive days on the third week of the escorting period. On some occasions, individuals reached saturation in the

morning feeding period and refused to eat the entire portion of fish, in these cases we returned to the group at the beginning of the afternoon foraging session to complete feeding. We collected morning and evening weights from all individuals 0 – 4 days before feeding began (1 – 3 observations per individual), and during feeding (3 – 4 observations per individual) to give a measure of daily weight gain. Faecal samples were collected from all individuals 0 – 4 days before the experiment (1 – 4 samples per individual) and on days 3 – 6 of feeding the experiment (1 – 3 samples per individual) for GC assay.

To test whether supplementary feeding reduced the energetic costs of escorting we used paired t-tests to compare mean daily weight gain for each individual both before and during feeding within each treatment group. Similarly, to test if supplementary feeding reduced fGC concentrations we used paired t-tests to compare mean standardised fGC concentrations for each individual both before and at the end of feeding (days 3 – 6) in each treatment group. Increased food intake increases faecal throughput which can alter hormone measures from faecal samples. To investigate the possibility that changes in fGC concentrations may be due to increased faecal throughput in fed individuals we conducted the same analyses for testosterone concentrations in males; if fGC change was caused directly by increased faecal-throughput we would expect to see the same response for other steroid hormones such as testosterone.

## 3.4 Results

### 3.4.1 Are there carry-over effects of investment in pup care in one breeding attempt on investment in pup care in the next breeding attempt?

Individual adjusted change in escorting effort between consecutive breeding attempts was significantly affected by an interaction between previous escorting effort and inter-birth period (LMM;  $\chi^2_{(1)} = 4.81$ ,  $p = 0.028$ ; figure 3.1); individuals that showed high escorting effort in the first breeding attempt had a more negative change in escorting effort than individuals with low escorting effort in the first breeding attempt, and this effect decreased with a longer inter-birth period. Adjusted change in escorting effort between consecutive breeding attempts was also significantly decreased in larger groups (LMM;  $\chi^2_{(1)} = 13.87$ ,  $p < 0.001$ ) and increased with larger litters (LMM;  $\chi^2_{(1)} = 28.01$ ,  $p < 0.001$ ). Older individuals had a lower adjusted change in escorting effort between consecutive breeding attempts than younger individuals (LMM;  $\chi^2_{(1)} = 8.30$ ,  $p = 0.004$ ).

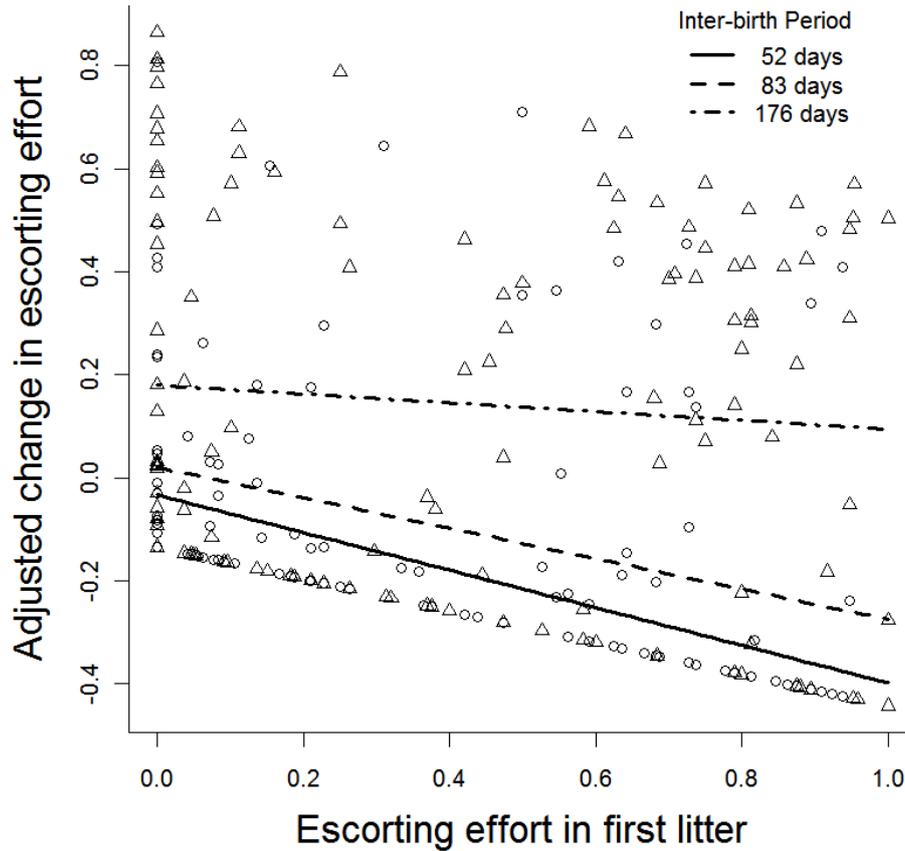


Figure 3.1; The effect of escorting effort in first breeding attempt on adjusted change in escorting effort between two consecutive breeding attempts ( $n_{\text{observations}} = 382$ ). Change in escorting effort is adjusted to control for regression to the mean following Kelly & Price (2012). Lines show predicted estimates from a LMM whilst controlling for significant effects of group size, number of pups, and escort age (LMM; group size:  $\chi^2_{(1)} = 13.87$ ,  $p < 0.001$ ; number of pups:  $\chi^2_{(1)} = 28.01$ ,  $p < 0.001$ ; escort age:  $\chi^2_{(1)} = 8.30$ ,  $p = 0.004$ ). Observations from breeding attempts with an inter-birth period above and below the median inter-birth period (83 days) are shown with triangles and circles respectively. Pack, breeding attempt, and individual identities were included in the analysis as random effects.

### 3.4.2 Do baseline fGCs before a breeding attempt predict escorting effort?

fGC concentrations prior to escorting predicted individual escorting effort with dependent on age (GLMM;  $\chi^2_{(1)} = 37.72$ ,  $p < 0.001$ ; figure 3.2). Further analyses with the data split into young or old individuals (less than or greater than median age) revealed a significant negative correlation between fGC concentrations prior to escorting and individual escorting effort in older individuals but not in younger individuals (GLMM [escorting effort  $\sim$  fGC concentrations prior to escorting]; age 12 – 30 months:  $\chi^2_{(1)} = 0.36$ ,  $p = 0.85$ ; age 31-115 months:  $\chi^2_{(1)} = 15.5$ ,  $p < 0.001$ ). Body condition at the

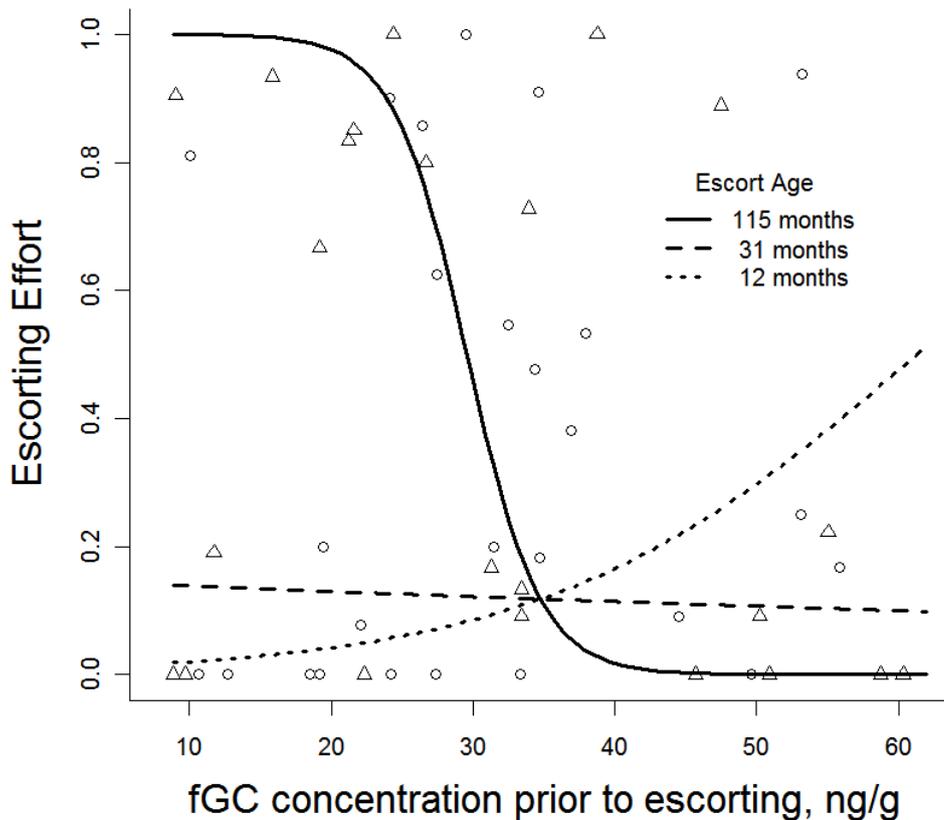


Figure 3.2: The effect of fGC concentration prior to escorting and age at pup emergence on individual escorting effort ( $n_{\text{observations}} = 50$ ). Escorting effort is given as the proportion of days an individual was escorting out of all escorting observation days. Lines show back-transformed (logit link) predicted trends from a GLMM. Points show raw data: escorts above and below median age (31 months) are given by triangles and circles respectively.

start of the escorting period, group size, and the number of pups were not significant predictors of escorting effort (GLMM: condition;  $\chi^2_{(1)} = 0.19$ ,  $p = 0.66$ ; group size;  $\chi^2_{(1)} < 0.001$ ,  $p = 0.99$ ; number of pups;  $\chi^2_{(1)} = 0.85$ ,  $p = 0.36$ ).

### 3.4.3 Does high investment in pup care correlate with an increase in fGC concentrations during the pup care period?

GC concentrations tended to increase over the escorting period (mean fGC level  $\pm$  SE: before escorting;  $35.1 \text{ ng/g} \pm 3.01$ , during/after escorting;  $42.1 \pm 1.7 \text{ ng/g}$ ). There was a tendency for individuals with high escorting effort to show a greater increase in log fGC concentrations during and

### 3. Carry-Over Effects in a Cooperative Breeder

after the escorting period (LMM; escorting effort x day interaction:  $\chi^2_{(1)} = 3.81$ ,  $p = 0.051$ , figure 3.3). When this interaction was removed from the model we found log fGC concentrations were significantly higher both with increasing number of days after the first observation of escorting and in individuals with higher escorting effort (LMM; day:  $\chi^2_{(1)} = 6.85$ ,  $p = 0.009$ ; escorting effort:  $\chi^2_{(1)} = 3.87$ ,  $p = 0.049$ ). We found a significant positive relationship between mean pre-escorting log fGC concentrations and log fGC concentrations during/after escorting (LMM;  $\chi^2_{(1)} = 9.23$ ,  $p = 0.002$ ).

#### 3.4.4 Are changes in fGC concentrations over the pup care period due to the energetic costs of helping?

##### *Daily weight gain*

Supplementary feeding significantly increased daily weight gain in escorts (one-sided paired t-tests:  $t_7 = -2.00$ ,  $p = 0.043$ , figure 3.4) and there was a trend for increased weight gain in non-escorts (one-sided paired t-tests:  $t_4 = -2.03$ ,  $p = 0.056$ , figure 3.4). Control escorts and control non-escorts showed no increase in weight gain over the experiment (one-sided paired t-tests: escorts:  $t_4 = -0.31$ ,  $p = 0.39$ ; non-escorts:  $t_6 = -0.75$ ,  $p = 0.24$ , figure 3.4). One fed non-escort was removed from the analyses because he began escorting during feeding.

##### *fGC concentrations*

Supplementary feeding decreased fGC concentrations in escorts (one-sided paired t-tests:  $t_6 = 2.06$ ,  $p = 0.042$ , figure 3.5), but not in non-escorts (one-sided paired t-tests:  $t_4 = -0.16$ ,  $p = 0.56$ , figure 3.5). Control escorts and control non-escorts both showed no change in fGC concentrations over the experimental period (one-sided paired t-tests; escorts:  $t_5 = 0.69$ ,  $p = 0.26$ ; non-escorts:  $t_6 = 0.3$ ,  $p = 0.39$ , figure 3.5).

There was no change in fT concentrations in any of the treatment groups (one-sided paired t-tests: fed escorts:  $t_3 = -0.019$ ,  $p = 0.57$ ; control escorts:  $t_6 = -0.46$ ,  $p = 0.67$ ; fed non-escorts:  $t_3 = -0.19$ ,  $p = 0.57$ ; control non-escorts:  $t_4 = 0.37$ ,  $p = 0.36$ ).

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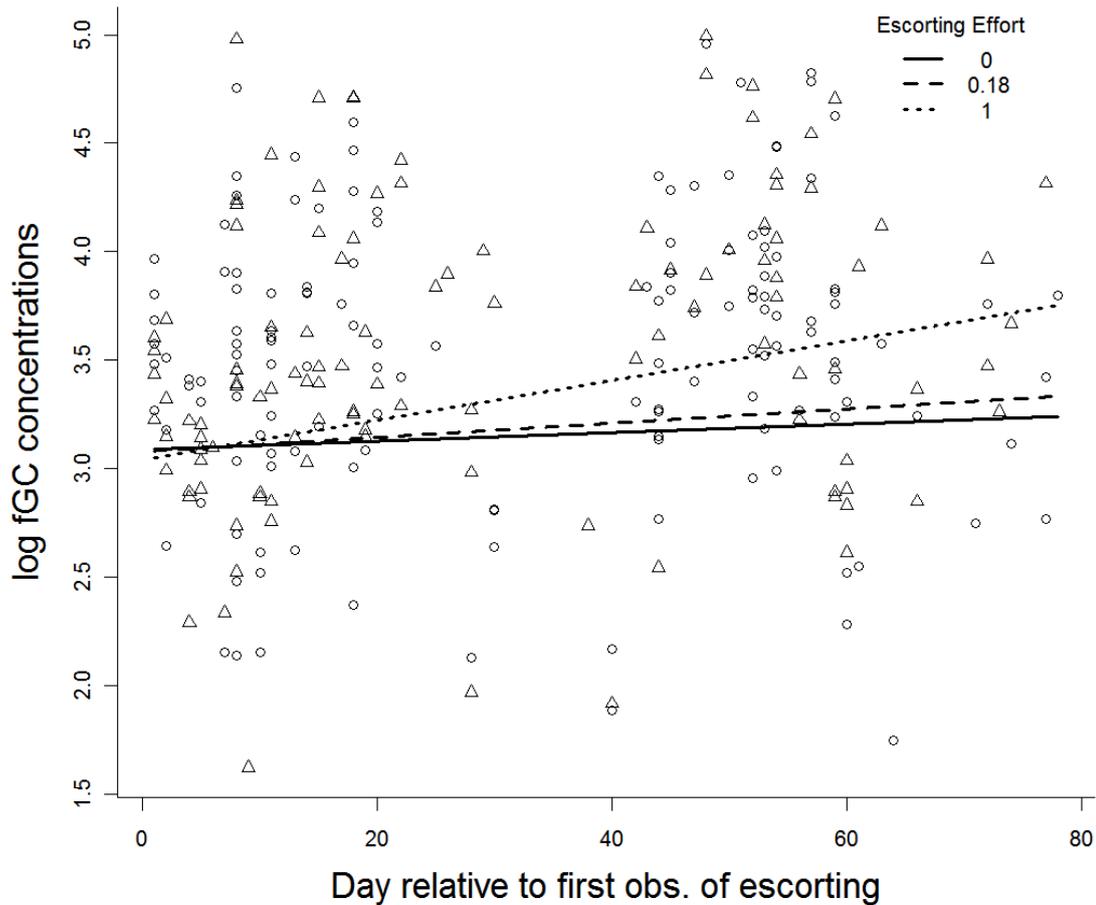


Figure 3.3; The effect of individual escorting effort and day relative to first observation of escorting on log fGC concentrations during the pup care period ( $n_{\text{observations}} = 266$ ). Escorting effort is given as the proportion of days an individual was observed escorting out of the total number of days that escorting was observed. Predicted trends from a linear mixed model (LMM; escorting effort x day interaction:  $\chi^2_{(1)} = 3.81$ ,  $p = 0.051$ ) are shown whilst controlling for a significant effect of individual mean log fGC concentration prior to escorting (LMM;  $\chi^2_{(1)} = 9.23$ ,  $p = 0.002$ ). Observations from individuals with an escorting effort above and below the median escorting effort (0.18) are shown with triangles and circles respectively. Pack, breeding attempt, and individual identities were included in the analysis as random effects.

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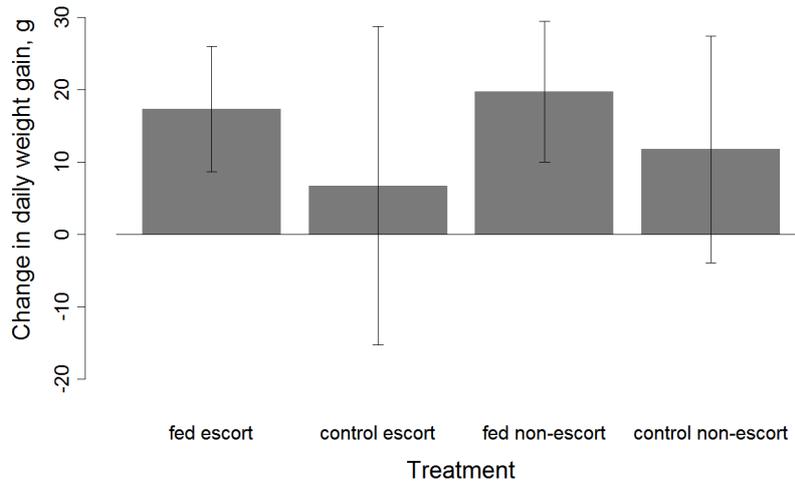


Figure 3.4; Effects of supplementary feeding on mean daily weight gain in fed escorts (n=8), control escorts (n=6), fed non-escorts (n=6), and control non-escorts (n=9). Fed individuals were given 80g dried fish daily for 6 consecutive days during the peak of the escorting period. Mean changes in daily weight gain  $\pm$  SE are shown.

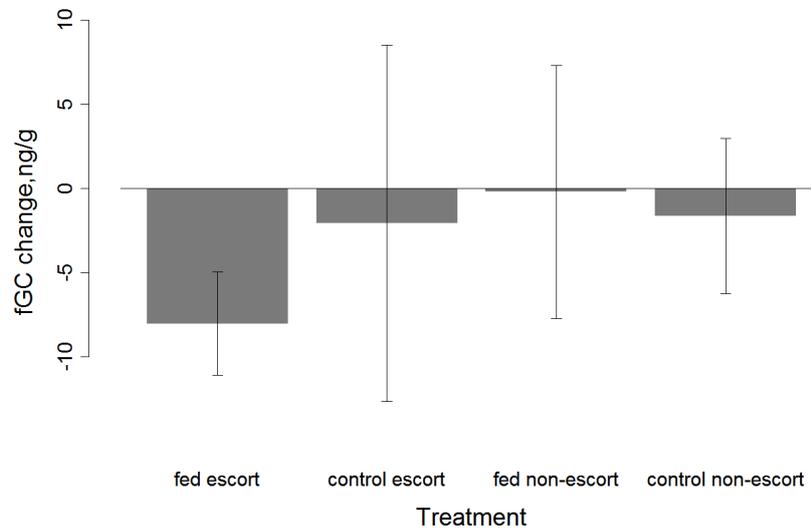


Figure 3.5: Effects of supplementary feeding on faecal GC concentrations in fed escorts (n=8), control escorts (n=6), fed non-escorts (n=6), and control non-escorts (n=9). Fed individuals were given 80g dried fish daily for 6 consecutive days during the escorting period. Mean changes in fGC concentrations  $\pm$  SE are shown.

### 3.5 Discussion

We have demonstrated evidence for a negative carry-over effect of escorting effort in one breeding attempt on escorting effort in the subsequent breeding attempt in the cooperatively breeding banded mongoose. This effect was decreased with increased inter-birth interval, suggesting that given enough time individuals may be able to recover from the costs of cooperative care before the next breeding attempt. We found that low fGC concentrations prior to pup care predict high escorting effort in older individuals, suggesting that their decisions to help may be mediated by GCs. Individuals with high investment in offspring care had higher fGC concentrations during and after the escorting period, which suggests that pup care may cause an increase in fGC concentrations. Supplementary feeding of individuals during the escorting period increased daily weight gain and decreased fGC concentrations in escorts, implying that the elevated fGCs seen in escorts may be due to their decreased daily weight gain when engaged in care. Together, these results provide strong support for a role for GCs in mediating a state-dependent modulation of helping effort and thereby also provide support for GCs as a mechanism for the observed carry over effect between care effort in one breeding attempt and the next.

Previous research on banded mongooses has shown that the decrease in weight gain observed in escorts does not persist after the pup-care period, suggesting that individuals are able to recover from the energetic costs of offspring care before the next breeding attempt (Hodge 2007). However, in the present study we have shown that male banded mongooses with high escorting effort show reduced escorting effort investment in subsequent breeding attempts. This is indicative of a carry-over effect of the costs of helping, suggesting that individuals may *not* be able to recover from the energetic costs of helping before the next pup-care period. Moreover, pup care investment in the second breeding attempt increases with increasing inter-birth period suggesting that individuals are able to recover from the factor(s) driving the reduction in helper effort in consecutive breeding attempts over time. Together these results are highly suggestive of a carry-over effect of investment in pup care similar to that observed in meerkats (Russell et al. 2003).

Banded mongooses that contributed most to escorting had lower fGC concentrations prior to the escorting period. These results suggest that GCs modulate escorting effort in banded mongooses by inhibiting helping in those individuals in low energetic state for which the fitness costs of escorting would be particularly high. Our results contrast with findings that pup feeding rates in meerkats are positively associated with cortisol concentrations prior to the pup provisioning period (Carlson et al. 2006a). In banded mongoose societies, the unique escorting system results in large differences in helper investment between escorts and non-escorts that are stable throughout the care period

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(Gilchrist 2004). In contrast to banded mongooses, in meerkat societies all subordinate adults feed and care for pups within each litter. This suggests that meerkat helpers may be able to modulate their pup care investment throughout the pup care period given daily fluctuations in energetic state, and so hormone measures prior to the pup care period may not be representative of hormone concentrations when the decision to invest in pup care behaviours is being made. For example, babysitting effort (care of young in the den) in meerkats is negatively correlated with GC concentrations when measured daily but not when measured as a long-term contribution (Carlson et al. 2006b). Studies of GC concentrations and pup provisioning behaviours in meerkat societies have so far been limited to GC measures prior to the pup provisioning period (Carlson et al. 2006a) so it is not known if individuals vary their helper effort dependent on their current state while provisioning pups. Though it is possible that banded mongoose escorts alter their levels of pup care on a daily basis similar to that suggested of meerkat helpers, pup-escort relationships tend to be stable throughout the pup care period. This unique system has enabled us to investigate physiological differences in potential helpers when the decision to help is being made (0-2 weeks prior to escorting) in similar individuals that maintain large differences in pup care investment for an extended period of time.

The negative correlation between fGC concentrations prior to escorting and individual escorting effort was only present in older individuals. It has previously been suggested that older males may avoid engaging in pup care in comparison to younger males because they experience a trade-off between offspring care and mating opportunities (Hodge 2007). Previous experimental studies have also shown that older males decrease their provisioning effort when deprived of food, but younger males do not (Bell 2010). If younger males have nothing to lose by engaging in pup care, i.e. because they have no access to mates, it may be beneficial for them to engage in pup care irrespective of their current state and so become insensitive to GC cues. In contrast, older individuals in low energetic state may experience costs of reduced mating opportunities when engaging in pup care due to resource limitations and so could benefit from being sensitive to GC cues. If this is the case we would expect individuals with and without potential access to mates to not have and have GC modulation of offspring care, respectively, which matches the pattern of results found here.

The use of non-invasive faecal sampling allowed us to collect samples repeatedly from the same individuals giving us hormone measures before and during the escorting period as well as after the pups had gained independence. Individuals with high escorting effort showed higher fGC concentrations during and after escorting than individuals with low escorting effort, which suggests that pup care behaviours cause an increase in circulating GC concentrations. Escorts experience significant energetic costs during the escorting period (Hodge 2007), which may lead to GC release

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(McEwen & Wingfield 2003; Angelier et al. 2007). Though it is not possible to infer causality through correlative studies of hormones and behaviours, and it is not possible to experimentally manipulate escorting effort to directly test whether escorting affects GC concentrations, we have used a supplementary feeding experiment to test if reducing the energetic costs of escorting also reduces the circulating GC concentrations in escorts. Though our sample sizes were necessarily small, we found that supplementary feeding reduced fGC concentrations in escorts, providing support for the hypothesis that the elevated fGC concentrations in escorts are due to increased energetic turnover associated with high investment in pup care. These conclusions are strengthened by the finding that supplementary feeding increases daily weight gain in non-escorts but that their GC concentrations remain unaffected. GCs are released when energetic output is greater than energetic resources (McEwen & Wingfield 2003). Supplementary feeding is likely to have balanced the high energetic output seen in escorts due to their investment in pup care and so inhibited GC release. However, as non-escorts have a low energetic output throughout the experiment increasing their energetic resources will have no effect on fGC concentrations.

We have suggested that the elevated fGCs seen in individuals with high escorting effort during the care period in banded mongooses are due to the energetic costs of caring for young. However, an alternative hypothesis based on previous findings in humans and meerkats (Fleming et al. 1997; Carlson et al. 2006a), is that an increase in circulating GC concentrations may be an evolved facultative response to the presence of young which serves to increase care or attentiveness. The higher GC concentrations in escorts found in this study may therefore not be due to the energetic costs of helping, but instead a direct response to the close proximity to begging pups. However, pup begging calls are heard both prior to and throughout the escorting period (personal obs., J. Sanderson). Therefore, if elevated GC concentrations are a response to pup begging calls we would expect escorts to have higher GC concentrations throughout the escorting period, and be reduced when pups reach independence. However, fGC concentrations are similar between escorts and non-escorts at the beginning of the escorting period and are elevated in escorts even when pups are no longer begging.

Without experimental manipulations it is very difficult to infer causality in correlations between hormones and behaviour. A few recent studies have collected hormone measures prior to or after the behaviour observations, providing evidence that physiology predicts behaviour or that behaviour alters hormone concentrations respectively (Thompson & Moore 1992; Trainor & Marler 2001; Carlson et al. 2003; Carlson et al. 2006a). Here, we collected repeated hormone measures from the same individuals before, during, and after behavioural observations, allowing us to look in an unusual level of detail at the interaction between behaviour and physiology. In addition, we have

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used a supplementary feeding experiment to further elucidate our causative hypotheses based on correlative data. In the present study, we have used mixed approaches to illustrate that GC concentrations prior to the care period predict individual investment in offspring care and that high investment in offspring care leads to an increase in GC concentrations. We have also demonstrated strong evidence for a negative carryover effect of cooperative care; individuals with high escorting effort in one breeding attempt have reduced escorting effort in the subsequent breeding attempt. Together, these results highlight GC modulation of offspring care as a candidate proximate mechanism mediating the carryover effect of care effort between consecutive breeding attempts and advocates further investigation into the role of GC concentrations in modulating life history decisions in both cooperative and non-cooperative systems.

# Chapter 4

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## Testosterone Mediation of a Trade-Off between Mating Effort and Offspring Care





## 4.1 Abstract

Investment in offspring care can lead to direct fitness costs due to missed mating opportunities. This suggests that breeding individuals may benefit from reducing their parental investment when there are alternative mating opportunities; a shift in investment that is commonly mediated by testosterone. Current investment in offspring care can also have detrimental effects on future survival and fecundity. Individuals with high expected *future* mating opportunities, such as those near the top of a social hierarchy, are predicted to decrease their investment in offspring care even when there are no available mating opportunities. Here, we examine individual investment in cooperative offspring care in the cooperatively-breeding banded mongoose both in the presence and absence of male mating opportunities to test whether individuals are responding to trade-offs between offspring care and current and/or future reproduction. Further to this, we investigate variations in testosterone concentrations alongside behavioural observations to test the hypothesis that testosterone mediates a trade-off between care and reproduction in social species similar to what has been observed in non-social species. We show that investment in offspring care was lowest during group oestrus, and this effect was greatest in high ranking males. Faecal testosterone metabolite (fT) concentrations were highest during group oestrus, and again this effect was greatest in high ranking males. As only high ranking males have access to females during oestrus, this suggests that testosterone is mediating a trade-off between investments in offspring care and mating behaviours when there are available mating opportunities. This suggestion is strengthened by findings that fT concentrations during group oestrus are positively correlated with rank in individuals that were observed mate-guarding, and short-term investment in offspring care is predicted by low fT concentrations. Our results suggest that male banded mongooses modulate their investment in offspring care dependent on current mating opportunities but not dependent on a trade-off with future expected mating opportunities.

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## 4.2 Introduction

Parental investment can have large direct fitness benefits due to increased survival or fecundity of young (Smith & Fretwell 1974; Clutton-Brock 1991; Lycett et al. 1998). In iteroparous species, the benefits of parental investment commonly coincide with direct fitness costs as the increased work load associated with parental care reduces fecundity and/or survival (Trivers 1974). Variation in the costs of caring for offspring may explain variation in parental investment previously left unexplained by variation in direct fitness benefits; individuals with alternative mating opportunities may be less likely to care due to provide the '*opportunity costs*' of missing these opportunities (Lessells 2012). In males, experimentally increased mating opportunities can lead to a decreased investment in care (e.g. through increasing quality of sexual signals, Qvarnstrom 1997; Nakagawa et al. 2007), suggesting that males can facultatively adjust their investment in offspring care and reproduction dependent on changes in the associated benefits.

Within social species, dominant males often monopolise reproductive opportunities within the group (Cant 2000; Clutton-Brock et al. 2001; Nelson-Flower et al. 2011). The presence of helpers in the group can alleviate the need for breeders to care for current offspring and have a load-lightening effect; allowing dominants to further increase their investment in reproduction (Crick 1992; Hatchwell & Russell 1996). However, where reproduction is not monopolised by a breeding pair, lower ranking individuals may also face a trade-off between care and current reproduction. For example, though within-group mating opportunities in meerkat societies are commonly limited to a dominant pair (Clutton-Brock et al. 2004), subordinate males sometimes prospect for extra-group paternities and these individuals have decreased investment in offspring care (Young et al. 2007). To date, most studies of the trade-off between care and reproduction within animal societies have focussed on systems where breeding is limited to a dominant pair and little is known about how males modulate their investment in care in systems where multiple males compete for multiple mating opportunities within the group.

Increased workload from investment in offspring care can also have direct fitness costs due to reductions in *future* survival and fecundity (Cant & Field 2001; Field et al. 2006). This trade-off between offspring care and future reproduction suggests that individuals benefit from modulating their helper effort dependent on expected future mating opportunities. Higher ranking subordinates are more likely to gain breeding status and so have more to lose from investing in costly care behaviours than low ranking subordinates with little chance of inheriting future reproductive success (Kokko & Johnstone 1999; Cant & Field 2001; Shreeves & Field 2002; Cant & Field 2005). This creates a systematic variation in expected investment in offspring care among subordinates, which

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has been documented in cooperative species with a single dominant breeder (e.g. paper wasps, *Polistes dominulus*; Cant & Field 2001; naked-mole rats, *Heterocephalus glaber* Clarke & Faulkes 1997, though see discussion of alternatives in Cant & Field 2005). Though the trade-offs between investment in offspring care and both current and future reproductive effort each predict that individual investment in offspring care will reduce down the social hierarchy, an important distinction is that the trade-off between offspring care and current reproduction will only be present when there are mating opportunities within the group, while the trade-off between offspring care and future reproduction is omnipresent. Studies of individual investment in offspring care and reproduction both in the presence and absence of mating opportunities are required to elucidate which trade-off is driving hierarchical differences in helper effort in social groups.

Studies of variation in parental investment have been well supported with studies of the proximate mechanisms mediating the trade-off between offspring care and reproductive effort (Wingfield et al. 1990; Ketterson & Nolan 1994; McGlothlin et al. 2007). Elevated testosterone levels are commonly associated with mating behaviours such as territory defence and courtship, while reduced testosterone levels are commonly associated with the expression of offspring care in males (Wingfield et al. 1990; Ketterson & Nolan 1994). Though this relationship has been demonstrated extensively in large range of taxa (e.g. birds: Van Duyse et al. 2002; Peters 2002; mammals: Nunes et al. 2000; Kuzawa et al. 2009; fish: Desjardins et al. 2005; Bender et al. 2008) there are clear exceptions (Lynn 2008). Testosterone may also play a role in mediating a trade-off between mating and offspring care in cooperatively breeding species. For example, experimental testosterone treatment results in an increase in mating displays and decrease in provisioning rates in superb fairy-wrens (*Malurus cyaneus*; Peters et al. 2002; Peters 2002) (though natural variation in testosterone between dominant and subordinate individuals does not correlate with differences in provisioning rates: Peters et al. 2002). Male helpers in meerkat societies show elevated testosterone and reduced investment in offspring care in association with increased prospecting forays (Young et al. 2005), which suggests that testosterone may mediate the trade-off between care-effort and mating-effort in mammal societies.

Banded mongooses (*Mungos mungo*) provide an ideal study system for investigating patterns of offspring care and reproductive effort within animal societies. They are a cooperative breeder with low female reproductive skew, where multiple males compete aggressively for access to receptive females (Cant 2000; Nichols et al. 2010). Banded mongooses live in stable groups of 8 – 40 individuals with 1 – 10 adult females that each enters oestrus within one week of each other. Male mating success is skewed towards older individuals, with 85% of paternities being assigned to the three oldest males in the group. (Nichols et al. 2010). Females give birth synchronously (usually on

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the same day: Gilchrist 2006; Hodge et al. 2011) and all adults within the group are engaged in offspring care. There are large intra-group variations in care investment and helping is male-biased (Cant 2003; Hodge 2007). Banded mongooses breed all year round and females commonly enter oestrus approximately 2 weeks after giving birth (Cant 2000). At this time the pups remain in the den and each day one or more adults remain at the den as '*babysitters*' to care for the pups. Thus, there is an unavoidable trade-off between investment in offspring care (babysitting) and current reproduction (mate-guarding) because receptive females never remain at the den. Furthermore, babysitting is energetically costly (Hodge 2007) and males in better condition are more likely to gain paternities (Nichols et al. 2010) so there may be an energetic trade-off between offspring care and future mating effort.

A previous study observed that when oestrus occurred during the babysitting period, overall babysitting effort was decreased by 68% and all pups died before the end of oestrus (Cant 2003), which is highly suggestive of a trade-off between offspring care and current reproduction. Oestrus commonly occurs two weeks post-partum, but there can also often be an extended period of anoestrus (personal obs. J. Sanderson). In this study we will investigate individual investment in offspring care both in the presence and absence of receptive females to tease apart trade-offs between offspring care and both current and future reproduction. If males face a trade-off between investment in offspring care and current reproduction we expect to see a reduction in care effort in breeding males during oestrus. If males face a trade-off between investment in offspring care and future reproduction we expect to see a reduction in care effort in breeding males throughout the care period. In addition, we will use non-invasive endocrine monitoring to examine variations in faecal testosterone concentrations and investigate testosterone as a candidate proximate mechanism mediating a trade-off between cooperative offspring care and mating effort.

### 4.3 Materials and Methods

#### 4.3.1 Study Site and Data Collection

We measured babysitting effort in 252 male banded mongooses over 227 breeding attempts in 13 social groups between April 2003 and January 2012, inhabiting the Mweya Peninsula in Queen Elizabeth National Park, Uganda. Details of vegetation and climate are given elsewhere (Cant 2000). All individuals are habituated to the presence of observers at a distance of 2 – 4 m (Hodge 2007). The population has been studied continuously for 17 years so the age of all individuals within the population is known through direct observation of births ( $\pm 1$  week). Mating opportunities are

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commonly monopolised by the three oldest males in banded mongoose packs (Nichols et al. 2010). Therefore male age was ranked in all analyses as age-rank is more likely to represent access to mating opportunities than actual age.

##### *Measuring babysitting effort*

During the babysitting period (0 - 5 weeks after birth) packs were visited daily and babysitters were identified. Banded mongooses tend to remain together as a foraging group so babysitters could be identified by elimination as individuals missing from the foraging pack (Cant 2003; Hodge 2007). On occasion a radio-collared individual was babysitting and in these cases the babysitters could be identified by direct observation of their presence at the den. Individual scores of babysitting effort were then calculated as the proportion of days that an individual was recorded as a babysitter out of the total number of days that babysitting was recorded by any individual within the group (1 - 39 observation days per breeding attempt, total 3815 observation days).

##### *Identification of Oestrus*

Banded mongoose females typically enter oestrus within one week of one another, generating a group oestrus that lasts  $5.6 \pm 0.5$  days (mean  $\pm$  S.E., from 108 oestrus events). During group oestrus each female is closely followed and guarded by a single male '*mate-guard*' for periods that last from several hours to several consecutive days. Mate guards defend their associated female from attempts to mate by other males by snapping, lunging, and pouncing towards approaching conspecifics, and as a result gain a high proportion of parentage (Nichols et al. 2010). These mate guarding behaviours are conspicuous and are easy to identify subjectively (Cant 2000). In this study, we defined a single oestrus event as the period between the first and last consecutive days when mate guarding was observed.

Breeding attempts where oestrus occurred during babysitting period were classified as '*oestrous litters*' and breeding attempts where oestrus did not occur during the babysitting period were classified as '*non-oestrous litters*'. Where no mate-guarding behaviours were observed throughout an entire babysitting attempt we checked for visible signs of pregnancy in the subsequent 90 days to ensure that there had not been any fully cryptic mating behaviour.

##### *Body condition*

All individuals in this study were trapped every 3 to 12 months using box traps (67x23x23 cm; Tomahawk Live Trap Co., Tomahawk, WI, USA), and anaesthetised using either ketamine or isoflurane (details of trapping protocol are given elsewhere; ketamine: Cant 2000; Hodge 2007;

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isoflurane: Jordan et al. 2010; Jordan et al. 2011). Measures of body mass ( $\pm 1$  g) and head width ( $\pm 0.1$  mm) were taken from all trapped individuals (850 captured male banded mongooses over 743 trapping sessions [ $4.1 \pm 0.17$  captures per individual; mean  $\pm$  S.E] between February 2000 and March 2012). This capture data was used to calculate a predicted body mass for any given head width by regressing log body mass with log head width. In addition to capture weights, all individuals in this study were trained to step onto a portable weighing scale allowing weights to be recorded daily before the morning foraging trip (Hodge 2007). To assess body condition from weights collected without capture, we compared an individual's body mass with its predicted body mass based on its head width at closest capture date (i.e. observed body mass (collected in the field) / predicted body mass) (following Jakob et al. 1996; Gilchrist et al. 2004).

##### **4.3.2 Faecal sample collection**

We collected 396 faecal samples from 46 male banded mongooses in 5 social groups between April 2010 and April 2012. All samples were collected between 6:30 am and 10:00 am and immediately placed on ice in a thermos flask. They were then transferred to a  $-20^{\circ}\text{C}$  freezer within 3 hours. Samples were transferred to the UK on ice and again transferred to a  $-20^{\circ}\text{C}$  freezer for storage. Over-marking of faeces is common in banded mongooses (Muller & Manser 2008). Hence, we only collected samples that had been watched continually from defecation and had not been over-marked. We collected half of each faeces to preserve any scent marking function of the remaining faeces. Time of collection and time to freezer were included in all analyses where appropriate but were non-significant predictors of variation in hormone metabolite concentrations throughout.

##### **4.3.3 Hormone extraction and assay**

Frozen samples were transported to the UK on wet ice for extraction and assay. Hormones were extracted from faecal samples following thawing and manual homogenisation using a wet-weight shaking extraction adapted from Walker et al. (2002). In brief, 0.5 grams of faecal material was combined with 90 % methanol, shaken overnight at room temperature and centrifuged for 20 minutes at 598 g. The methanol fraction was decanted and evaporated to dryness. Faecal extracts were re-suspended in 1 ml methanol and stored at  $-20^{\circ}\text{C}$  until analysis.

Hormone extraction was carried out either at Chester Zoo Endocrinology Lab (CZEL) or at the University of Exeter in Cornwall (UofE). Samples extracted at the UofE were then transferred to CZEL on dry ice for assay. We extracted a subset of samples ( $n = 20$ ) at both CZEL and UofE to ensure the

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location of extraction did not affect the hormone results. The results from samples extracted in both CZEL and UofE were highly correlated (Pearson's correlation:  $t_{60} = 2.93$ ,  $p = 0.004$ ) and there was no significant effect of extraction location on faecal testosterone metabolite (fT) measures (GLMM:  $\chi_{12}^{14} = 0.38$ ,  $p=0.83$ ), therefore results from both extraction locations were pooled throughout all analyses.

fT concentrations were measured using a modified enzyme immunoassay that has been described previously (EIA; Young et al. 2004, adapted from Munro & Stabenfeldt 1984). Details of assay modification and validation are given in Appendix 1.

#### 4.3.4 Statistical Analyses

All statistical analyses were carried out using R 2.13.2 (R Development Core Team 2012). We used linear mixed models (LMMs) and generalised linear mixed models (GLMMs) to control for repeated measures within social groups, breeding attempts, oestrus events, and individuals. Normally distributed response variables were analysed with an identity link function, and binomial response variables with logit link function. All possible explanatory variables were sequentially dropped from the model until only those variables explaining significant variation ( $p < 0.05$ ) remained. All dropped variables were then put back into the minimal model to determine their level of non-significance.

#### 4.3.5 Do male banded mongooses decrease their investment in offspring care in the presence of mating opportunities?

To investigate variations in babysitting effort in relation to the presence of oestrous females we measured babysitting effort in 155 adult male banded mongooses (> 1 year) in 169 breeding attempts and 8 social groups. Of these breeding attempts 85 were 'oestrous litters' and 84 were 'non-oestrous litters'. Each babysitting period was divided into week-long sessions (1 – 5 weeks per babysitting period) to control for variation in babysitting effort with litter age. Babysitting effort was calculated per week for each individual as the proportion of the total number of babysitting observation days within that week that each individual was observed babysitting. Weeks within oestrous litters were labelled '*oestrous weeks*' if mate-guarding was observed on any day during that week. Weeks within oestrous litters when mate-guarding behaviours were not observed were categorised as '*pre-oestrous weeks*' or '*post-oestrous weeks*' if they were before or after

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observations of mate-guarding, respectively. All weeks in non-oestrous litters were classified as '*non-oestrous weeks*'.

Babysitting effort was fitted as a binomial response variable in a GLMM with week relative to oestrus as a categorical fixed term ('pre-oestrous', 'oestrous', 'post-oestrous', 'no oestrous'). The following terms were also included as fixed terms: week after birth, body condition (calculated from mean body mass in the month prior to birth), age-rank (ranked male age at birth, with ties ranked at mean values), and group size (number of individuals aged > 1 year at litter birth). The availability of mating opportunities may affect individual care effort differently dependent on age-rank, body condition, or group size. To test for this we included all two-way interactions including week relative to oestrus. Social group, breeding attempt, and individual identities were fitted as random effects to control for repeated measures.

#### **4.3.6 Do male banded mongooses show elevated testosterone levels in the presence of mating opportunities?**

We collected 396 faecal samples from 46 adult (aged > 1 year) male banded mongooses over 19 babysitting periods in 5 social groups between March 2010 and February 2012. Day of sample collection was categorised dependent on week relative to oestrus. Samples collected during oestrous litters were labelled '*oestrous samples*' if they were collected during oestrus. Samples collected during oestrous litters outside of oestrus were categorised as '*pre-oestrous samples*' or '*post-oestrous samples*' if they were collected before or after oestrus, respectively. All samples collected in non-oestrous litters were classified as '*non-oestrous samples*'.

To investigate how testosterone levels vary in relation to oestrus we fitted faecal testosterone metabolite (fT) concentrations as a response variable in a LMM with time relative to oestrus as a fixed term ('pre-oestrous', 'oestrous', 'post-oestrous', and 'non-oestrous'). We included time relative to birth, age-rank (ranked male age at birth, with ties ranked at mean values), body condition (residual index calculated from mean body mass  $\pm$  one week of sample collection), and group size (number of individuals aged > 1 year at litter birth) as fixed effects. We also fitted all two-way interactions including time relative to oestrus to investigate if age-rank, body condition, or group size affected individual variation in fT concentrations differently in different time periods. Social group, breeding attempt, and individual identities were included as random effects to control for repeated measures.

#### 4. Testosterone Mediation of a Trade-Off between Mating Effort and Offspring Care

##### **4.3.7 Do male banded mongooses engaged in mate-guarding behaviours have higher testosterone levels than those not engaged in mate-guarding?**

To investigate correlations between testosterone levels and mate-guarding effort we collected 89 faecal samples from adult (> 1 year) males alongside observations of mate guarding from 8 oestrus events in 3 social groups. fT concentrations were fitted as the response variable in a LMM with mate guarding status as the response, where mate guarding status was categorised as 'mate-guard' or 'non-mate-guard' dependent on whether the individual had been observed as a mate guard at any point during an oestrus event. We also included age-rank (ranked male age at birth, with ties ranked at mean values), body condition (from mean body mass at emergence  $\pm$  one week of sample collection, 1-4 observations per individual; residual index), and group size (number of individuals aged > 1 year at litter birth) as fixed effects and all two-way interactions. Social group, oestrus, and individual identities were included as random terms to control for repeated measures.

##### **4.3.8 Do high testosterone levels predict low investment in babysitting?**

To investigate hormonal predictors of babysitting effort we collected 212 faecal samples from 36 adult (> 1 year) male banded mongooses over 17 breeding attempts in 5 social groups between March 2010 and February 2012. Individual babysitting effort was calculated for each sample as the proportion of babysitting observations in the 3 days following sample collection (including the day of sample collection) that the sampled individual was observed babysitting. Babysitting effort was fitted as a binomial response in two separate GLMMs; (1) we tested for direct hormonal predictors of babysitting effort by fitting fT concentrations as the only main effect, and (2) to further investigate the relationship between testosterone and babysitting we fitted fT concentrations as a main effect in the same model alongside age-rank (ranked male age at birth, with ties ranked at mean values), body condition (calculated from mean body mass  $\pm$  one week of sample collection), and group size (number of individuals aged > 1 year at litter birth), as well as all possible two-way interactions. Social group, breeding attempt, and individual identities were fitted as random effects in both models to control for repeated measures.

## 4.4 Results

### 4.4.1 Do male banded mongooses decrease their investment in offspring care in the presence of mating opportunities?

Overall babysitting effort was lowest in oestrous weeks, and this effect was dependent on individual age-rank (GLMM: week relative to oestrus x age-rank;  $\chi^2_{(3)} = 14.73$ ,  $p=0.002$ , figure 4.1). Further analysis revealed that babysitting effort during oestrous weeks was lowest in males of high age-rank (GLMMs; babysitting effort  $\sim$  age-rank + group size; during oestrus:  $\chi^2_{(1)} = 12.91$ ,  $p < 0.001$ ), and there was a tendency for males of high age-rank to have lower babysitting effort before oestrus

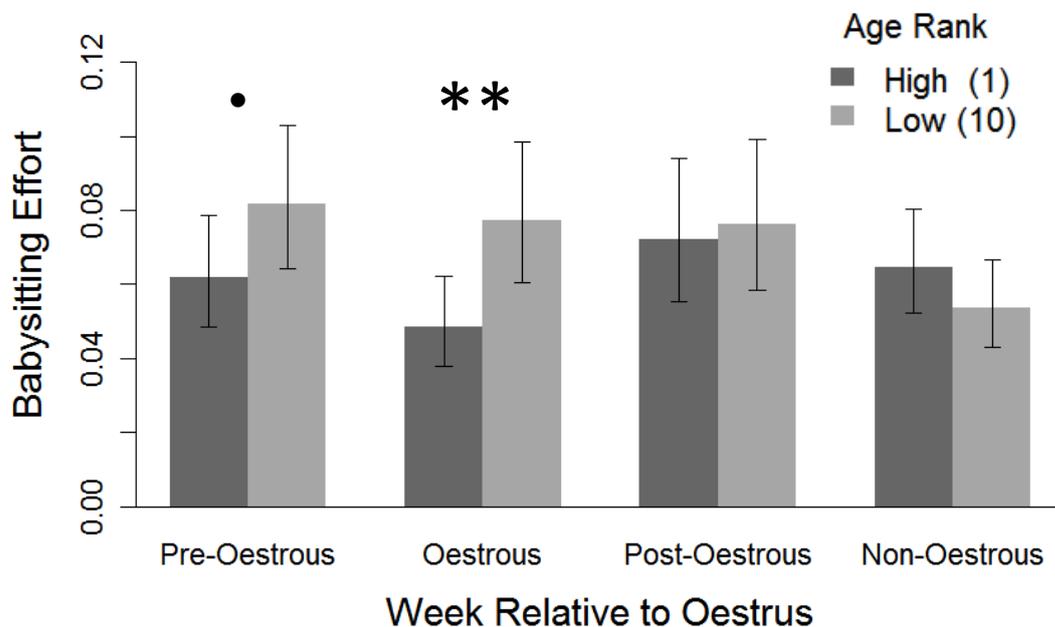


Figure 4.1: Individual babysitting effort in high and low age-ranked males during oestrous litters and non-oestrous litters. Babysitting effort was measured as the number of days an individual was recorded as a babysitter out of the total number of days that babysitting was observed. Weeks in oestrous litters are categorised as pre-, during, and post-oestrous weeks ( $n_{\text{weeks}} = 200, 151, 225$ ). Weeks in non-oestrous litters are classified as non-oestrous weeks ( $n_{\text{weeks}} = 497$ ). Bars show predictions and standard errors for high and low age-ranked individuals (ranks 1 & 10) from a GLMM whilst controlling for a significant negative effect of group size (GLMM;  $\chi^2_{(1)} = 4.90$ ,  $p = 0.027$ ). Social group, breeding attempt, and individual identities were included in the analysis as random factors to control for repeated observations. Stars show significant relationships between age-rank and babysitting effort when data from each time period were analysed separately ( $\bullet = p < 0.10$ ,  $** = p < 0.001$ ).

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(pre-oestrous:  $\chi^2_{(1)} = 1.98$ ,  $p = 0.084$ ). There was no effect of age-rank on babysitting effort either post-oestrous or in non-oestrous litters (post-oestrous:  $\chi^2_{(1)} = 0.48$ ,  $p = 0.49$ ; non-oestrous:  $\chi^2_{(1)} = 0.82$ ,  $p = 0.37$ ).

Individual babysitting effort was lower in larger groups (GLMM;  $\chi^2_{(1)} = 4.90$ ,  $p = 0.027$ ). Neither body condition nor season had significant effects on babysitting effort (GLMM; body condition:  $\chi^2_{(1)} = 2.75$ ,  $p = 0.10$ ; season:  $\chi^2_{(1)} = 0.05$ ,  $p = 0.82$ ).

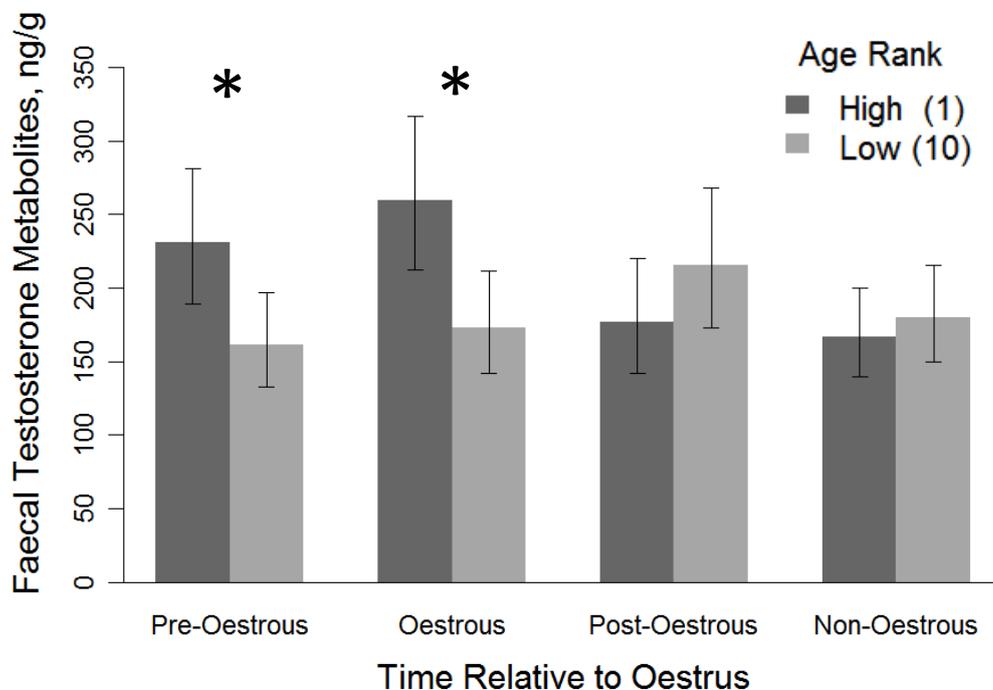


Figure 4.2: Faecal testosterone metabolite (fT) concentrations in high and low age-ranked males during oestrous litters and non-oestrous litters. Samples collected during oestrous litters were categorised as pre-, during, and post-oestrous samples ( $n_{\text{samples}} = 93, 111, 59$ ). Samples collected in non-oestrous litters were categorised as non-oestrous samples ( $n_{\text{samples}} = 121$ ). Bars show prediction estimates and standard errors for high and low age-ranked individuals (ranks 1 & 10) from a LMM. Social group, breeding attempt, and individual identities were included in the analysis as random factors to control for repeated measures. Stars show significant correlations between age-rank and fT when data from each time period were analysed separately (\* =  $p < 0.05$ ).

#### **4.4.2 Do male banded mongooses show elevated testosterone concentrations in the presence of mating opportunities?**

fT concentrations were highest during oestrus, and this effect was dependent on age-rank (LMM:  $\chi^2_{(3)} = 12.72$ ,  $p = 0.005$ , figure 4.2). Further analyses revealed that fT concentrations were positively correlated with age-rank before and during oestrus (GLMMs; fT ~ age-rank; before oestrus:  $\chi^2_{(1)} = 7.46$ ,  $p = 0.006$ ; during oestrus:  $\chi^2_{(1)} = 4.45$ ,  $p = 0.34$ ), but there was no effect of age-rank on fT concentrations after oestrus or in non-oestrous litters (post-oestrous:  $\chi^2_{(1)} = 0.74$ ,  $p = 0.39$ ; non-oestrous:  $\chi^2_{(1)} = 0.33$ ,  $p = 0.56$ ). None of week relative to birth, body condition, or group size had significant effects on fT concentrations (LMM; week:  $\chi^2_{(1)} = 2.11$ ,  $p = 0.15$ ; body condition:  $\chi^2_{(1)} = 3.79$ ,  $p = 0.051$ ; group size:  $\chi^2_{(1)} = 0.53$ ,  $p = 0.47$ ).

#### **4.4.3 Do male banded mongooses engaged in mate-guarding behaviours have higher testosterone levels than those not engaged in mate-guarding?**

Mate-guards had higher fT concentrations during group oestrus than non-mate-guards, and this effect was greatest in individuals of high age-rank (LMM; age-rank x mate-guarding status:  $\chi^2_{(1)} = 5.28$ ,  $p = 0.022$ , figure 4.3). Further analyses showed a significant positive relationship between age rank and fT concentrations in mate-guards but not non-mate-guards (LMM; fT ~ age-rank [mate-guards only]:  $\chi^2_{(1)} = 4.81$ ,  $p = 0.028$ ; fT ~ age-rank [non-mate-guards only]:  $\chi^2_{(1)} = 0.24$ ,  $p = 0.63$ ). Neither body condition nor group size had significant effects on fT concentrations during oestrus (body condition:  $\chi^2_{(1)} = 0.36$ ,  $p = 0.55$ , group size:  $\chi^2_{(1)} = 0.00$ ,  $p = 1.00$ ).

#### **4.4.4 Do high testosterone levels predict low investment in babysitting?**

When analysed alone, high fT concentrations predicted low babysitting effort in the 3 days following sample collection (GLMM;  $\chi^2_{(1)} = 4.90$ ,  $p = 0.027$ ; figure 4.4). When age-rank, group size, and body condition were included in the analyses testosterone levels remained a significant predictor of babysitting effort (GLMM;  $\chi^2_{(1)} = 4.76$ ,  $p = 0.029$ ). Age-rank was found to have a significant effect on babysitting effort; relatively older individuals had lower babysitting effort (GLMM;  $\chi^2_{(1)} = 5.67$ ,  $p = 0.018$ ), whilst neither body condition nor group size had a significant effect on babysitting effort (body condition:  $\chi^2_{(1)} = 2.22$ ,  $p = 0.14$ , group size:  $\chi^2_{(1)} = 0.95$ ,  $p = 0.33$ ).

#### 4. Testosterone Mediation of a Trade-Off between Mating Effort and Offspring Care

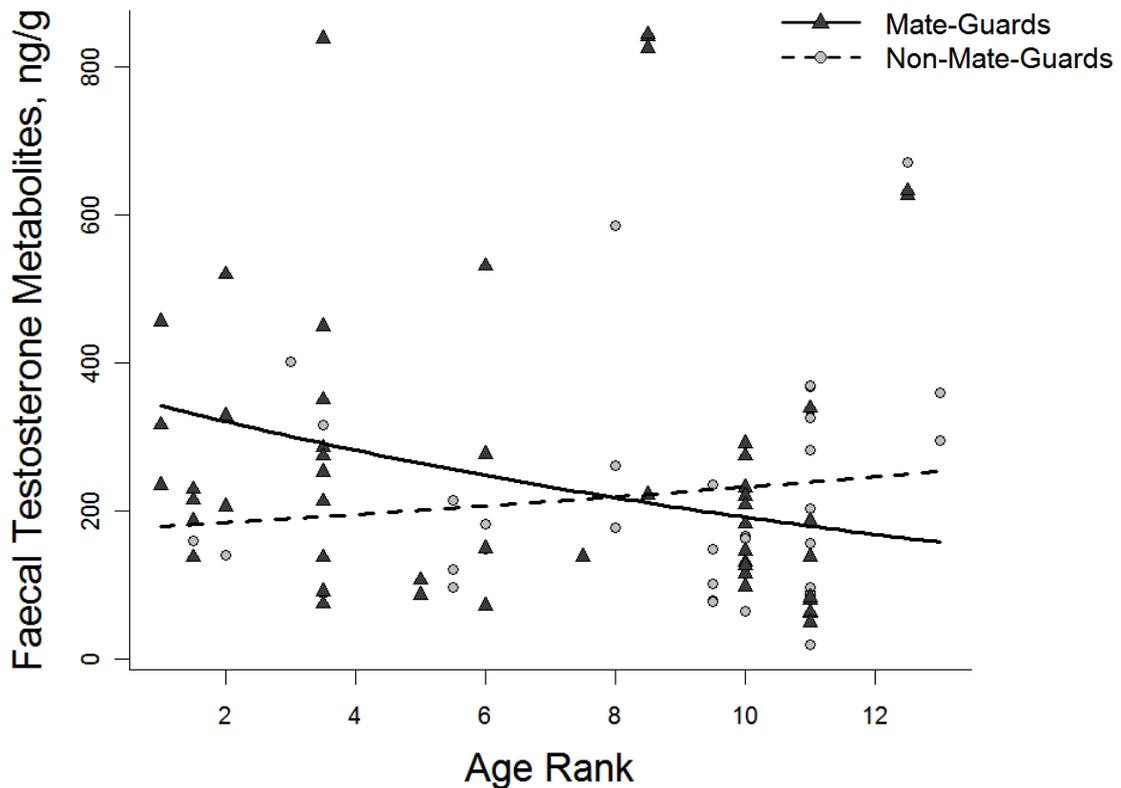


Figure 4.3: Faecal testosterone metabolites (fT) during oestrous across age-ranks in mate guards and non-mate guards during group oestrus. Line shows prediction estimate from a generalised linear mixed model (GLMM). Adult male banded mongooses were classified as 'mate-guards' or 'non-mate-guards' if they were or were not observed guarding a female at any point during oestrus respectively ( $n_{\text{individuals}} = 37, 52$ ). Social group, oestrus, and individual identities were included in the analysis as random effects to control for repeated measures.

#### 4.5 Discussion

Limitations in both time and resources create trade-offs between offspring care and reproduction. Here, we found that males of higher age-rank helped to care for offspring less than lower age-ranked males both before and during oestrus, but no relationship between age-rank and helping effort was found after oestrus or where no oestrus occurred. Dominance status and associated access to mating opportunities is acquired with age-rank in banded mongooses (Nichols et al. 2010) so these results suggest that males shift investment from offspring care to reproductive effort in the presence of mating opportunities. Testosterone levels increased with age-rank both before and during oestrus, but we found no relationship between age-rank and testosterone levels after oestrus or

#### 4. Testosterone Mediation of a Trade-Off between Mating Effort and Offspring Care

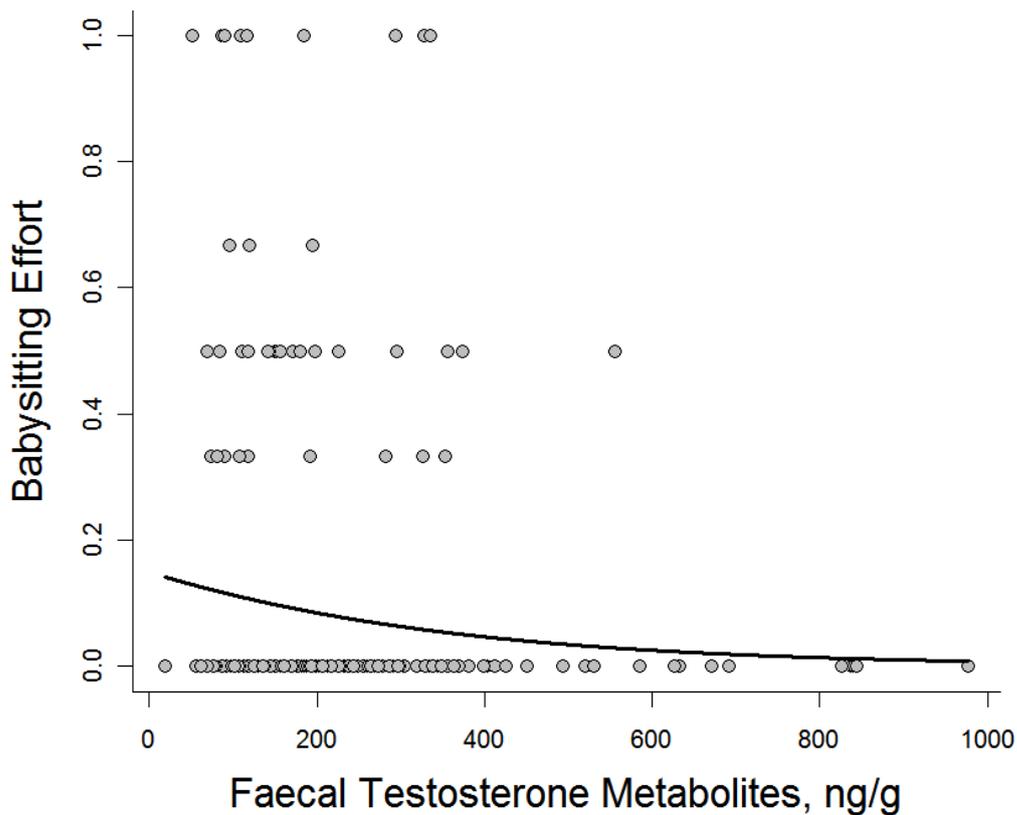


Figure 4.4: Faecal testosterone metabolite (fT) concentrations and individual babysitting effort in the 3 days following sample collection (n = 216). Babysitting effort is measured as the proportion of babysitting observation days that each individual was recorded as a babysitter. Line shows predictions from a generalised linear mixed model (GLMM). This effect is significant both when analysed alone and when age-rank, group size, and body condition are included in the model. Social group, breeding attempt, and individual identities were included in the analysis as random effects in both models to control for repeated measures.

when no oestrus occurred. This parallels patterns of helping effort and suggests that changes in circulating testosterone levels may mediate a trade-off between offspring care and current reproduction. This suggestion was complimented by findings that high age-ranked males that were observed mate-guarding had higher testosterone levels than similarly age-ranked males that were not observed mate-guarding. Further to this, low testosterone levels predicted high babysitting effort in the short term, suggesting that low testosterone levels are required for expression of offspring care in this species.

Where care of current offspring leads to missed mating opportunities we expect individuals experiencing these costs to reduce their investment in offspring care (Lessells 2012). In cooperatively

#### 4. Testosterone Mediation of a Trade-Off between Mating Effort and Offspring Care

breeding groups, where access to mating opportunities is dependent on individual rank, individuals at the top of the queue are expected to invest less in offspring care than those at the bottom as the opportunity costs are greater (Cant & Field 2001; Cant & Field 2005; Field et al. 2006). Using age-ranks to identify a social hierarchy in banded mongoose packs, we found that investment in offspring care was highest in low ranking males both prior to and during oestrus. These results compliment previous findings that babysitting effort is male biased, especially towards young males, presumably due to the opportunity costs associated with offspring care (Hodge 2007). High-ranking males are likely to reduce their care effort *during* oestrus due to a temporal trade-off with mate-guarding. However, the reduction in care effort seen in high-ranking males *prior* to oestrus is likely to be driven by an energetic trade-off as babysitting is energetically expensive (Hodge 2007) and heavier males are more likely to gain paternity (Nichols et al. 2010). Studies of bi-parental species have found that parents increased their investment in care when there are no alternative mating opportunities (Burley 1986; Beissinger 1990). Here, we found that male babysitting effort does not differ between age-ranks when there are no mating opportunities within the group, providing evidence that male banded mongooses make facultative adjustments to their investment in offspring care within each breeding attempt to match variations in the opportunity costs of caring.

When there is a trade-off between offspring care and future reproduction, i.e. through decreased growth or survival, we expect individuals with higher expected future reproductive opportunities to show decreased current investment in offspring care (Cant & Field 2001; Cant & Field 2005; Field et al. 2006). In hierarchical systems, where individuals queue to gain breeding positions, individuals nearer the top of the queue are more likely to gain a breeding position, so we predicted that higher age-ranked banded mongooses may show lower investment in offspring care than lower age-ranked individuals even in the absence of current mating opportunities. However, we found no evidence that this was the case, which suggests that individuals were not modulating their care effort depending on future expected mating opportunities. This contrasts with previous findings in paper wasps (Cant & Field 2001) and naked-mole rats (Clarke & Faulkes 1997) where high ranking individuals with no current access to mates have decreased investment in offspring care. However, it is important to note that there may be other benefits to investment in offspring care that increase with age-rank that could confound the effects due to costs of decreased future fecundity. For example, high age-ranked individuals are more likely to have gained paternity in the previous oestrus so may be more related to the current litter than low age-ranked individuals.

Testosterone is commonly associated with mating effort; promoting behaviours such as courtship and/or territory defence (Wingfield et al. 1990). We found that high age-ranked males had higher testosterone levels than low age-ranked males during oestrus. High age-ranked males also have

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lower investment in babysitting than low age-ranked males during these periods, so these findings correspond with the hypothesis that testosterone mediates a trade-off between investment in offspring care and current reproduction. When we looked at the relationship between testosterone and mating behaviours in more detail, we found that mate guards had higher testosterone than non-mate-guards and that this effect was greater in males of high age-rank. This demonstrates that the high levels of testosterone seen in high ranking males are not just an effect of age-rank, but are associated with the expression of mating behaviours. Though it is difficult to show directions of causality with correlative studies, experimentally increased testosterone has been shown to increase mating behaviours in many species (e.g. mountain spiny lizards, *Sceloporus jarrovi*, Marler et al. 1989; house finches, *Carpodacus mexicanus*, Stoehr & Hill 2000; great tits, *Parus major*, Van Duyse et al. 2002; Mongolian gerbils, *Meriones unguiculatus*, Clark & Galef 1999), which suggests that the variation in testosterone levels seen here may function as the proximate mechanism modulating investment in reproduction in this species.

High age-ranked males also had higher testosterone than low age-ranked males prior to the onset of oestrus. If testosterone inhibits care, elevated testosterone levels in older males may function to mediate a reduction in energetically expensive care behaviours to avoid a future reduction in mating-effort when females become receptive. In social species males compete aggressively for dominance status and the associated mating opportunities (Cowlshaw & Dunbar 1991). If testosterone is linked to aggressive behaviours we expect to see a positive correlation between testosterone and dominance status during times of contest, with high-ranking males showing the largest increase in testosterone levels (Sapolsky 1993). Males may benefit from gaining dominance status prior to the onset of oestrus to avoid a trade-off between between-male aggression and mating-effort during oestrus. Therefore the pattern of testosterone levels seen prior to oestrus may be associated with rank-formation; however, detailed studies of intra-group aggressive interactions in this period are required to test this hypothesis.

If testosterone mediates a trade-off between mating-effort and investment in offspring care we would expect that testosterone would be decreased in individuals expressing high investment in offspring care (Wingfield et al. 1990). In babysitting sessions before and during oestrus we found that low age-ranked individuals have lower testosterone than high age-ranked males. This, alongside findings that low age-ranked males have a relatively higher babysitting effort, suggests that testosterone may be negatively correlated with care. When we examined testosterone levels and short term individual babysitting effort we also found that testosterone negatively predicted babysitting effort. This result remained significant even when other individual characteristics were

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included in the model so it is highly suggestive that low levels of testosterone are required for the expression of offspring care in this species.

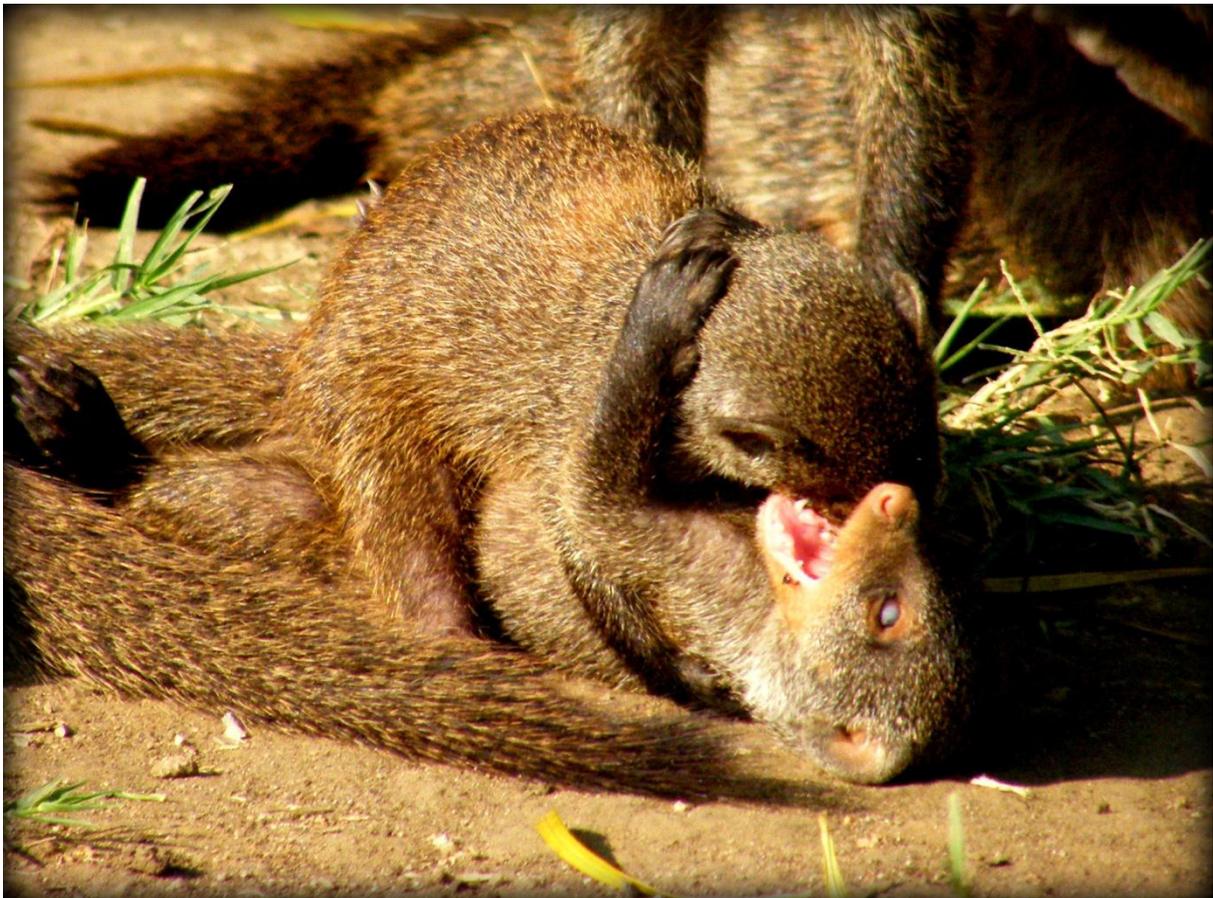
In banded mongoose societies, investment in current reproduction and offspring care are mutually exclusive as they occur at the same time but in different locations. Endocrine signalling is a strong candidate mechanism for mediating behavioural trade-offs as variation in circulating hormone concentrations can modulate multiple behaviours simultaneously. Testosterone has been linked to care and mating in a wide range of biparental species (Wingfield et al. 1990; Nunes et al. 2000; Smorkatcheva et al. 2009), including humans (Kuzawa et al. 2009). However, most studies of cooperative species have investigated systems where within-group breeding opportunities are limited to one dominant individual, and subordinate mating opportunities are limited to prospecting forays and extra-group paternities (e.g Peters 2002; Young et al. 2005). Here we have demonstrated compelling evidence of a testosterone mediated trade-off between care and mating effort in a cooperatively breeding mammal where male mating opportunities are not monopolised by a single individual.

Males vary both in their ability to obtain mates and in their optimum level of investment in mating behaviours (Trivers 1972; Nur & Hasson 1984; Getty 1998). We have shown that, in banded mongooses, older males show high investment in reproduction in the presence of receptive females while younger males show higher investment in care behaviours, adding to evidence that variation in helper effort may be driven by variations in the costs of cooperating. Decreased investment in offspring care by some individuals may lead to within-group conflict. Previous studies have shown that in some species dominant individuals decrease their investment in offspring care when there are helpers at the nest (Crick 1992; Hatchwell & Russell 1996). However, it is not known if lower ranking or non-breeding individuals increase their investment in offspring care in response to a decrease in investment from breeders. If subordinate individuals benefit from increased survival of offspring we may expect them to show a compensatory increase in investment in offspring care in response to a decrease in effort from others. It is important to note that in this study we have focused on male investment in offspring care, and further, more-detailed investigation incorporating variation in female investment is required to fully elucidate mechanisms underlying intra-group conflict over mating-effort and offspring care.

# Chapter 5

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## Behavioural and Physiological Responses to Simulated Territorial Intrusion





## 5.1 Abstract

Behaviours associated with territorial aggression and offspring care commonly trade-off with each other due to functional or energetic constraints. Inter-species comparisons suggest that this trade-off limits expression of aggressive territorial behaviours in species with high levels of offspring care. In social species, the constraints of this trade-off may be avoided if individuals specialise to roles in care or territory defence, similar to the worker and soldier castes in eusocial insects. Testosterone is commonly associated with the expression of territorial behaviours, and increases following territorial intrusion in non-social species with low levels of offspring care. This is in contrast to species with high levels of offspring care, which may not show elevated testosterone levels following territorial intrusion because of inhibitive effects on offspring care. We extend this hypothesis to suggest that in social species, where there are marked inter-individual differences in investment in offspring care, there may also be inter-individual differences in testosterone response to territorial intrusion similar to inter-species differences in non-social systems. We use simulated territorial intrusions to test for inter-individual differences in both behavioural and physiological response to territorial intrusion in the cooperatively breeding banded mongoose (*Mungo mungo*). The escorting system in banded mongooses creates a large inter-individual variation in investment in offspring care, which allows us to test the predictions that both behavioural and physiological response to territorial intrusion will be lower in carers than non-carers. We find that behavioural response to territorial intrusion does not differ between escorts and non-escorts, and suggest that this may be due to the high costs of losing an inter-group conflict and correspondingly high selection for group competitive ability. We show that territorial intrusion induces a significant testosterone increase in non-escorts, but escorts show no change in testosterone despite a similar behavioural response to the stimulus. This differential response to territorial intrusion between escorts and non-escorts is comparative to differences seen in non-social species with moderate and essential offspring care. We find no evidence that baseline testosterone levels modulate individual investment in care or territory defence and suggest that further study is required to elucidate the role of post-intrusion elevations in testosterone levels seen in non-escorts. These findings raise the possibility that banded mongooses exhibit a previously unexplored form of individual specialisation in which non-carers show physiological responses to challenges that leave them better prepared to repel future intruders, while carers do not. While vertebrate societies differ from insect societies in that they lack physical castes, our results provide tentative evidence for more subtle physiologically-based social roles promoting soldier-like and worker-like behaviour in mammals.

## 5. Behavioural and Physiological Response to Simulated Territorial Intrusion

## 5.2 Introduction

Territorial aggression and offspring care commonly trade-off against each other due to the different morphological and physiological optima associated with these behaviours (figure 5.1a; Wingfield et al. 1990). Within cooperative societies, selection acting on individuals with shared interests can promote the evolution of individual specialisation and a division of labour (Modlmeier et al. 2012), especially when the costs of switching between behaviours are high (Duarte et al. 2012). This suggests that social species may overcome the constraints of a trade-off between offspring care and territorial aggression through individual specialisation akin to the worker and soldier castes in eusocial insects (Wilson 1979; Bourke & Franks 1995) and mammals (Jarvis 1981). If individuals are specialised to roles as carers or defenders we would expect carers and non-carers to respond differently to territorial intrusion. A recent study of meerkats (*Suricata suricatta*) found that territory defence lead to a decrease in pup provisioning throughout the group, though the study did not investigate if individuals with low investment in care were more likely to repel intruders (Mares et al. 2012). Variation in the likelihood of repelling prospecting intruders was found to be dependent on the associated costs and benefits for each meerkat, similar to results of an experimental study in cooperatively breeding cichlids (*Neolamprologus pulcher*, Desjardins et al. 2008), which suggests that individuals are not specialised to different roles. However, in cooperatively breeding groups of noisy miners, carers with low provisioning rates are more likely to defend the nest against predators (Arnold et al. 2005), which suggests that individuals may become specialised to roles in aggression and care.

Within vertebrates, individuals with high investment in aggressive and territorial behaviours (e.g. competition for mates, establishment of territories, or competition for social status) often show elevated testosterone levels (Mazur 1985; Wingfield et al. 1990; Mazur & Booth 1998; Young et al. 2005; Hau 2007; Carré & McCormick 2008). Further to this, testosterone can inhibit offspring care (figure 5.1b; Wingfield et al. 1990, though see Trainor & Marler 2001 and Gleason & Marler 2010), so it is a likely candidate mechanism to mediate the trade-off between offspring care and territory defence. In non-social species, individuals often have low testosterone levels during periods of offspring care and elevated testosterone levels during periods of territory establishment (Wingfield et al. 1990). Testosterone also correlates with individual investment in offspring care in some cooperatively-breeding species (Vleck & Brown 1999; Young et al. 2005), which suggests that testosterone may have a similar function in cooperative species as non-cooperative species. However, studies of testosterone modulation of aggression in cooperatively-breeding vertebrates

## 5. Behavioural and Physiological Response to Simulated Territorial Intrusion

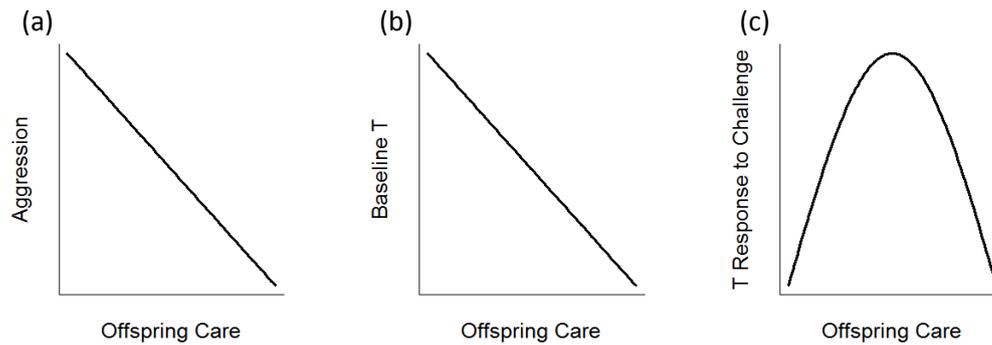


Figure 5.1: Predicted correlations between offspring care and aggression, baseline testosterone (T), and T response to social challenge in the presence of a trade-off between care and aggression. If there is a trade-off between offspring care and aggression we expect that individuals with high levels of offspring care will show low levels of aggression (a; Wingfield et al. 1990). Testosterone mediation of the trade-off between offspring care and aggression predicts that testosterone will be lower in individuals with high levels of offspring care (b; Wingfield et al. 1990). Testosterone response to social challenge is predicted to show a bell-shaped correlation with offspring care as individuals with low levels of offspring care and already primed for conflict and individuals with high levels of offspring care cannot afford to respond to challenge because the potential costs of decreasing care behaviours (c; Lynn et al. 2002; Goymann et al. 2007; Lynn & Wingfield 2008).

have so far concentrated on individual variation in intra-group conflict over foraging or mating opportunities (e.g. Creel et al. 1993; Muller & Wrangham 2004), and it is not known if testosterone mediates individual investment in group territory defence.

When an individual encounters an intruder the likelihood of encountering another intruder in the subsequent time period is increased and they may benefit by increasing their testosterone levels to prime themselves for future conflict (Wingfield et al. 1987; Marler et al. 2005; Wingfield 2005; Fuxjager et al. 2009; Fuxjager et al. 2011). This testosterone response to territorial intrusion is higher in facultative carers than both individuals who provide no care at all and those who provide high levels of offspring care (figure 5.1c; Lynn et al. 2002; Goymann et al. 2007; Lynn & Wingfield 2008). Individuals with little or no investment in offspring care may already be primed for conflict, especially during periods of territory establishment, and so show no change in testosterone in response to intrusion as they are unable to increase aggressive or territorial behaviours above the current level. Moreover, species with essential offspring care may become insensitive to territorial intrusion and show no increase in testosterone as the inhibitive effects on offspring care would be very costly to the survival of young. Evidence backing these hypotheses in non-social species, through comparative studies of both intra- and inter-species differences in response to social conflict, is strong (e.g. Lynn et al. 2005). In social species where *individuals* show relatively high and

## 5. Behavioural and Physiological Response to Simulated Territorial Intrusion

low investment in offspring care they may show similar patterns of response to intrusion to different non-cooperative *species* with relatively high and low investment in offspring care.

In the present study, we look for individual variation in both behavioural and physiological response to inter-group conflict in a cooperatively breeding mammal; the banded mongoose (*Mungos mungo*), to test the hypothesis that *individuals* show differential responses to inter-group conflict that are comparative to *species* differences seen elsewhere (Wingfield et al. 1990; Goymann et al. 2007; Lynn 2008). Banded mongooses (*Mungos mungo*) provide an ideal study system for investigating patterns of care and aggression within animal societies as they are cooperative breeders that commonly engage in intense inter-group interactions that are highly aggressive, and can lead to serious injury or death (Cant et al. 2002; Muller & Bell 2009). Banded mongooses are small diurnal herpestids that live in stable groups of 8 – 40 individuals; 1 – 10 adult females give birth synchronously (usually on the same day: Gilchrist 2006; Hodge et al. 2011) to large litters (1 – 20 pups: Gilchrist 2006), of which 85% are fathered by the 3 oldest males in the group (Nichols et al. 2010). Most foraging pups (> 4 weeks old) form a stable association with a single adult escort who provisions, carries, plays with, grooms, and protects that pup until it reaches independence (Cant 1998; Gilchrist 2004). This creates a large variation in pup care investment within the group, with escorts providing a level of pup care several orders of magnitude greater than non-escorts. Escorts are most commonly adult males, though adult females and sub-adult males also become escorts (Hodge 2007). During inter-group conflicts, subordinate males tend to be the most aggressive (Cant et al. 2002); however, no studies to date have investigated whether escorts and non-escorts vary in their response to inter-group conflict. For example, if escorts are constrained to maintaining high investment in care we may expect that non-escorts have a stronger response to territorial intrusion.

A powerful experimental method to investigate variations in individual response to inter-group conflict is through simulated territorial intrusions (STIs). In non-social species, this has been used to investigate inter-species or seasonal differences in both behavioural and physiological responses to a standardised intruder stimulus (e.g. Lynn et al. 2007; Scriba & Goymann 2008; Scriba & Goymann 2010; Apfelbeck et al. 2011). In cooperative vertebrates this method can help to reveal intra-group differences in responses to territorial intrusion. Here, we will use STIs to test for variations in both behavioural and physiological responses to territorial intrusions in escorts and non-escorts (i.e. carers and non-carers) in a wild population of banded mongooses. Furthermore, we will test for relationships between baseline testosterone concentrations and both escorting effort and behavioural response to territorial intrusion to investigate the role of testosterone in mediating a trade-off between offspring care and inter-group conflict.

## 5.3 Materials and Methods

### 5.3.1 Study site and data collection

We followed 5 packs of banded mongoose (5 – 20 adults per group) inhabiting the Mweya Peninsula in Queen Elizabeth National Park, Uganda between April 2010 and April 2012. Details of vegetation and climate are given elsewhere (Cant 2000). All individuals were habituated to the presence of observers at a distance of 2 – 4 m.

#### *Measuring escorting effort*

During the escorting period (4 – 10 weeks after birth) groups were visited daily and associations between adults and pups were noted (7 – 21 observation days per breeding attempt). Adults were scored as escorts if they were seen to be within 30 cm of the same pup for more than 50% of the observation period (Gilchrist 2004; Hodge 2005; Gilchrist & Russell 2007). Previous studies have shown that this association score is a reliable summary of quantitative nearest-neighbour data (Gilchrist 2001; Gilchrist & Russell 2007). Measures of escorting effort were available from 34 individuals over 13 breeding attempts in 5 social groups between April 2010 and April 2012.

#### *Simulated territorial intrusions*

Previous studies have shown that a suite of stimuli are required to initiate a behavioural and/or physiological response to territorial intrusion (Wingfield & Wada 1989; Narins et al. 2003), and that model intruders may not be sufficient (Scriba & Goymann 2008). To increase the likelihood of initiating a natural response to territorial intrusion we used live intruders from neighbouring packs. Each individual was only used as an intruder for one simulated territorial intrusion (STI) and intrusions were limited to a maximum of ten minutes to minimise stress.

A dominant adult male 'intruder' from a neighbouring group was trapped in a box trap (67 x 23 x 23 cm; Tomahawk Live Trap Co., Tomahawk, WI, USA), covered with a cloth and transported by car to the home range of the focal group. The intruder was then placed 100 m in front of the foraging path of the focal group, and the cloth removed. We placed a white rope in a circle around the intruder at a radius of 0.5 m to allow estimation of the distance of aggressive individuals from the intruder. The experiment began when one mongoose from the focal group approached the intruder to a distance of < 2 m and lasted until the focal group moved away or up to a maximum of 10 minutes. The experiment was filmed from a distance of 8 m with annotation by observers for analysis later on. At the end of the experiment the intruder was again covered with a cloth and then transported back to

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his social group where he was released and given a small food reward (fish and/or rice) to retain habituation. The intruder was then watched for 10 minutes to insure that he was reaccepted into the group. In all cases the intruder was accepted back into the group immediately and showed no signs of injury or stress.

In total, we conducted 7 STIs in 3 social groups. Individuals were scored as 'escorts' or 'non-escorts' prior to the STI by observing the group for 1 hour prior to the experiment. Adult mongooses were individually marked by either a coloured plastic collar or a unique shave on its back (trapping and marking protocol is given elsewhere; see above; Jordan et al. 2010; Jordan et al. 2011). During each experiment we recorded an intruder inspection rate (IIR) for all adult males within the group (> 1 year old; n = 25 individuals; 3 – 15 per experiment). This was calculated as the number of 10 second intervals that the individual was observed within 0.5 m of the intruder out of the total number of 10 second intervals in the experiment (max. 60). Faecal samples were collected the morning of ( $n_{\text{samples}} = 31$ ), and the mornings of one and two days after the intrusion ( $n_{\text{samples}} = 34, 29$ ) for testosterone assay.

### *Body condition*

All individuals in this study were trapped every 3 to 12 months using box traps (67x23x23 cm; Tomahawk Live Trap Co., Tomahawk, WI, USA), and anaesthetised using either ketamine or isoflurane (details of trapping protocol are given elsewhere; ketamine: Cant 2000; Hodge 2007; isoflurane: Jordan et al. 2010; Jordan et al. 2011). Measures of body mass ( $\pm 1$  g) and head width ( $\pm 0.1$  mm) were taken from all trapped individuals (850 captured male banded mongooses over 743 trapping sessions [ $4.1 \pm 0.17$  captures per individual; mean  $\pm$  S.E] between February 2000 and March 2012). This capture data was used to calculate a predicted body mass for any given head width by regressing log body mass with log head width. In addition to capture weights, all individuals in this study were trained to step onto a portable weighing scale allowing weights to be recorded daily before the morning foraging trip (Hodge 2007). To assess body condition from weights collected without capture, we compared an individual's body mass with its predicted body mass based on its head width at closest capture date (i.e. observed body mass (collected in the field) / predicted body mass) (following Jakob et al. 1996; Gilchrist et al. 2004).

### **5.3.2 Faecal sample collection**

Faecal samples were collected from all adult males to be analysed for testosterone metabolite (fT) concentrations. All samples were collected between 6:30am and 10:00am and immediately placed

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on ice in a thermos flask. They were then transferred to a -20°C freezer within 3 hours. Samples were transferred to the UK on ice and again transferred to a -20°C freezer for storage. As over-marking of faeces is common in banded mongooses (Muller & Manser 2008), samples were only collected if they had been watched continually from defecation and it was known that they hadn't been over-marked. Further to this, only half of each faecal sample was collected to minimise any resulting interference with scent marking signals. Time of collection and time to freezer were included in all analyses where appropriate but were found to be non-significant predictors of FT concentrations throughout.

### 5.3.3 Hormone extraction and assay

Samples were collected as soon as possible after defecation or urination and stored at -20°C until hormone analysis. Hormones were extracted from faecal samples following thawing and manual homogenisation using a wet-weight shaking extraction adapted from Walker et al. (2002). In brief, 0.5 grams of faecal material was combined with 90% methanol, shaken overnight at room temperature and centrifuged for 20 minutes at 598 g. The methanol fraction was decanted and evaporated to dryness. Faecal extracts were re-suspended in 1 ml methanol and stored at -20°C until analysis.

Hormone extraction was carried out either at Chester Zoo Endocrinology Lab (CZEL) or at the University of Exeter in Cornwall (UofE). Samples extracted at the UofE were then transferred to CZEL on ice for assay. We extracted a subset of samples (n = 20) at both CZEL and UofE to ensure the location of extraction did not affect the hormone results. The results from samples extracted in both CZEL and UofE were highly correlated (Pearson's correlation:  $t_{60} = 2.93$ ,  $p = 0.004$ ) and there was no significant effect of extraction location on FT measures (GLMM:  $\chi_{12}^{14} = 0.38$ ,  $p = 0.83$ ), therefore results from both extraction locations were pooled throughout all analyses.

Faecal testosterone metabolites were analysed using a modified protocol from a previously described enzyme immunoassay (EIA; Young et al. 2004, adapted from Munro & Stabenfeldt 1984). Details of assay modification and validation are given in Appendix 1.

### 5.3.4 Statistical Analyses

All statistical analyses were carried out using R 2.15.2 (R Development Core Team 2012). We used linear mixed models (LMMs) and generalised mixed models (GLMMs) to control for repeated

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measures within social groups, breeding attempts, experiments, and individuals. Normally distributed response variables were analysed with an identity link function, and binomial response variables with logit link function. All possible explanatory variables were initially fitted together in a maximal model and then sequentially dropped from the model in order of least significance until only those variables explaining significant variation ( $p < 0.05$ ) remained. All dropped variables were then put back into the minimal model to reconfirm their non-significance.

### **5.3.5 Do escorts and non-escorts differ in their behavioural response to territorial intrusion?**

To investigate whether escorts and non-escorts differed in their behavioural response to territorial intrusion we fitted individual intruder inspection rate (IIR) as a binomial response (number of 10 s intervals seen to enter  $< 0.5$  m of intruder out of the total number of 10 s intervals the intruder was present) in a GLMM with logit link and escorting status (escort/non-escort) as the fixed predictor of interest. We fitted this model twice; (1) with escorting status as the only predictor, and (2) also fitting age (months), body condition (from weights collected the morning of the STI), and group size (number of individuals aged  $> 1$  year) as fixed terms, as well as all two-way interactions. This allowed us to test for differential responses to territorial intrusion whilst allowing for the possibility that escorting status levels may be confounded with other factors. Social group, experiment, and individual identities were included as random terms to control for repeated measures.

### **5.3.6 Do escorts and non-escorts differ in their physiological response to territorial intrusion?**

To test whether escorts and non-escorts showed differential testosterone responses to territorial intrusion we fitted fT concentrations ( $n_{\text{samples}} = 94$ ) as the response in a LMM with identity link. We fitted day of sample collection relative to STI (0 – 2) as a continuous fixed term with an interaction with escorting status; if escorts and non-escorts do respond differently we would expect to find a significant interaction. We also fitted age (months), body condition (calculated from weights collected the morning of the STI), and group size (number of individuals aged  $> 1$  year) as fixed terms, as well as all two-way interactions with day relative to STI to test for effects of life-history variation on testosterone response to intrusion. Pack, experiment, and individual identities were fitted as random terms to control for repeated measures.

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### 5.3.7 Do fT concentrations prior to territorial intrusion predict individual investment in territory defence?

To investigate whether fT concentrations prior to territorial intrusion predict individual investment in territory defence we fitted individual IIR as a binomial response (number of 10 s intervals seen to enter < 0.5 m of intruder out of the total number of 10 s intervals the intruder was present) in a GLMM with logit link and fT concentrations as the main predictor of interest ( $n_{\text{samples}} = 31$ ). We fitted this model twice; (1) with fT concentrations as the only predictor, and (2) also fitting escorting status (escort/non-escort), age (months), body condition (calculated from weights collected the morning of the STI), and group size (number of individuals aged > 1 year) as fixed terms, as well as all two-way interactions. This allowed us to test for testosterone modulation territorial aggression whilst allowing for the possibility that testosterone levels may be confounded with other life-history variables. Social group, experiment, and individual identities were included as random terms to control for repeated measures.

### 5.3.8 Do individuals that invest heavily in pup care have low fT concentrations?

To investigate the relationship between escorting effort and testosterone levels we collected 233 faecal samples from 35 male adult (aged > 1 year) mongooses before (0 – 14 days prior to the first observation of escorting;  $n = 54$ ) and during the first six weeks of escorting (up to 42 days after first observation of escorting;  $n = 179$ ) in 13 breeding attempts from 5 social groups. We defined the start of the escorting period as the first day that any adult was recorded as an escort. fT concentrations were fitted as the response variable in a LMM with identity link and escorting effort (proportion of days observed escorting out of total number of days that any individual was observed escorting) fitted as a fixed term.

Day relative to first observation of escorting was fitted as a fixed term in the model as a three level factor to allow for non-linear variations in fT concentrations over the escorting period: pre-escorting (0 – 14 days before first observation of escorting,  $n = 54$ ), early escorting (1 – 14 days after first observation of escorting,  $n = 88$ ), and escorting (15 – 42 days after first observation of escorting,  $n = 91$ ). Group size (number of individuals aged > 1 year), age (months), body condition (calculated from mean weight in two weeks prior to escorting), and the number of pups were fitted as fixed terms, as well as all possible two-way interactions including time period. Pack, breeding attempt, and individual identities were fitted as random terms to control for repeated measures.

## 5.4 Results

### 5.4.1 Do escorts and non-escorts differ in their behavioural response to territorial intrusion?

When escorting status was considered as the only predictor of IIR, there was no significant difference in IIR between escorts and non-escorts (GLMM:  $\chi^2_{(1)} = 0.39$ ,  $p = 0.53$ ). Further to this, escorting status did not predict IIR when other variables were included in the model (GLMM:  $\chi^2_{(1)} = 0.21$ ,  $p = 0.64$ ). IIR was higher in individuals in better body condition (GLMM:  $\chi^2_{(1)} = 7.07$ ,  $p = 0.008$ ; figure 5.2), but there was no significant effect of either group size or age on IIR (group size:  $\chi^2_{(1)} = 2.87$ ,  $p = 0.090$ ; age:  $\chi^2_{(1)} = 0.002$ ,  $p = 0.97$ ).

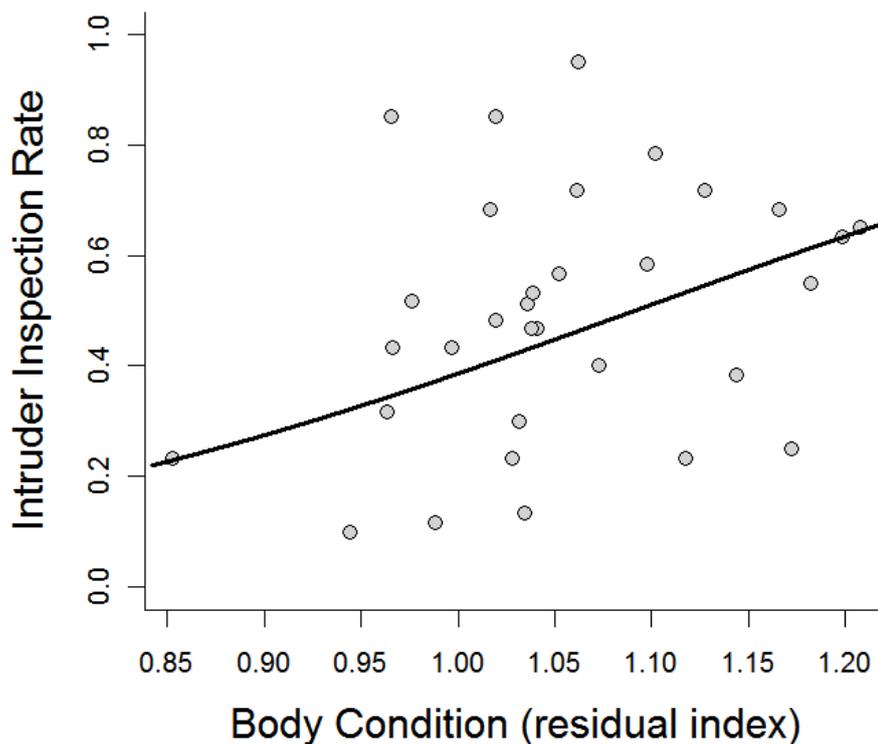


Figure 5.2: Effect of body condition on individual intruder inspection rate. A prediction line is drawn showing the predicted trend from a GLMM and points show the raw data ( $n = 31$ ). Body condition is calculated as observed body mass  $\div$  predicted body mass, where predicted body mass is extracted from a model of  $\log$  body mass  $\sim$   $\log$  head width (6637 observations). Social group, experiment, and individual identities were included in the model as random terms.

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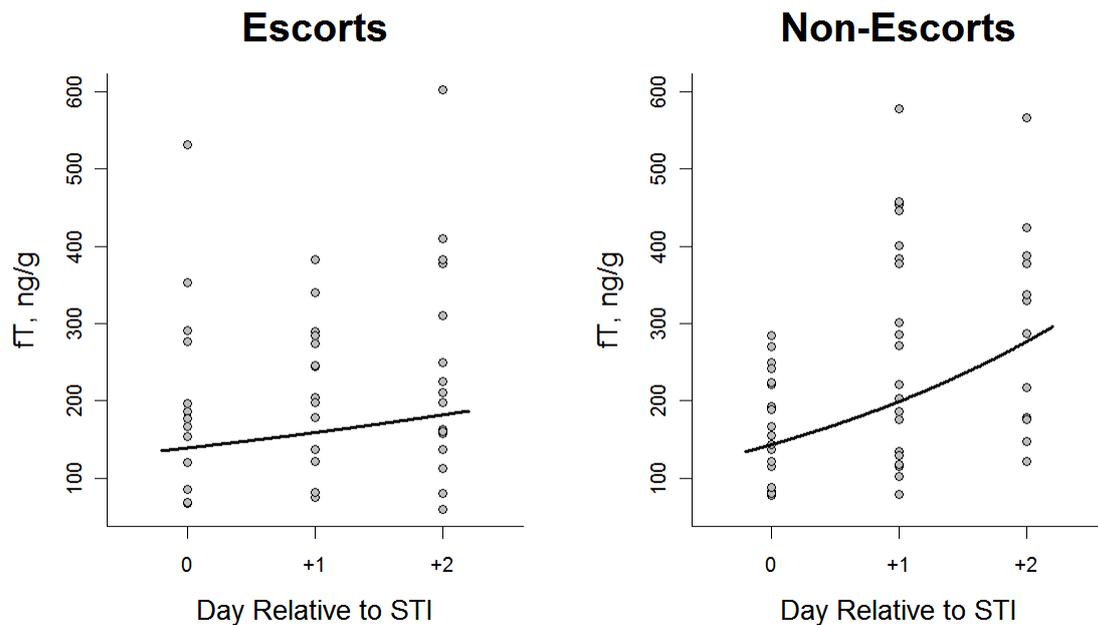


Figure 5.3: Changes in faecal testosterone metabolite (fT) concentrations after the STI for individuals classified as escorts and non-escorts. Predicted trends from a linear mixed model while controlling for the significant effect of body condition (LMM:  $\chi^2_{(9)} = 18.3$ ,  $p < 0.001$ ) are shown by a solid line for escorts ( $n = 21$ ) and dashed line for non-escorts ( $n = 26$ ). Social group, experiment, and individual identities were included in the model as random terms.

### 5.4.2 Do escorts and non-escorts differ in their physiological response to territorial intrusion?

We found a significant interaction between escorting status and day relative to STI (LMM:  $\chi^2_{(1)} = 3.85$ ,  $p = 0.049$ , figure 5.3), so that non-escorts showed a greater increase in fT concentrations following the STI than did escorts. Post-hoc analyses examining the change in fT concentrations in either escorts or non-escorts revealed that there was a significant increase in fT concentrations in non-escorts following the STI but not in escorts (LMM [fT ~ day; non-escorts only]:  $\chi^2_{(1)} = 20.7$ ,  $p < 0.001$ ), but not in escorts (LMM [fT ~ day; escorts only]:  $\chi^2_{(1)} = 2.57$ ,  $p = 0.11$ ).

Individuals in better body condition had lower fT concentrations (LMM:  $\chi^2_{(1)} = 18.3$ ,  $p < 0.001$ ), but there was no significant interaction with day relative to STI ( $\chi^2_{(1)} = 0.06$ ,  $p = 0.81$ ). None of IIR, age, or group size had significant effects on fT concentrations (LMM; IIR:  $\chi^2_{(1)} = 1.37$ ,  $p = 0.24$ ; age:  $\chi^2_{(1)} = 1.69$ ,  $p = 0.19$ ; group size:  $\chi^2_{(1)} = 2.74$ ,  $p = 0.10$ ).

### 5.4.3 Do testosterone levels prior to territorial intrusion predict individual investment in territory defence?

fT concentrations were not significant predictors of IIR either when considered as the only predictor or with other life history variables included in the analysis (GLMM: only predictor:  $\chi^2_{(1)} = 2.01$ ,  $p = 0.16$ ; with other predictors:  $\chi^2_{(1)} = 0.20$ ,  $p = 0.65$ ). After fT concentrations were dropped from the model the remaining results became the same as those reported in 4.4.1.

### 5.4.4 Do individuals that invest heavily in pup care have low fT concentrations?

There was no significant relationship between escorting effort and fT concentrations, either with fT concentrations as a main effect (GLMM:  $\chi^2_{(1)} = 0.34$ ,  $p = 0.56$ ) or in an interaction with time period ( $\chi^2_{(3)} = 0.46$ ,  $p = 0.93$ ). There was a significant effect of session on fT concentrations (GLMM:  $\chi^2_{(2)} = 20.7$ ,  $p = <0.001$ ), so that fT concentrations were decreased in the early escorting period in comparison to pre-and main escorting periods. Age, body condition, group size, and the number of pups all did not significantly affect fT concentrations (GLMM: age:  $\chi^2_{(1)} = 3.36$ ,  $p = 0.067$ ; body condition:  $\chi^2_{(1)} = 2.78$ ,  $p = 0.095$ ; group size:  $\chi^2_{(1)} = 0$ ,  $p = 1$ ; number of pups:  $\chi^2_{(1)} = 0$ ,  $p = 1$ ).

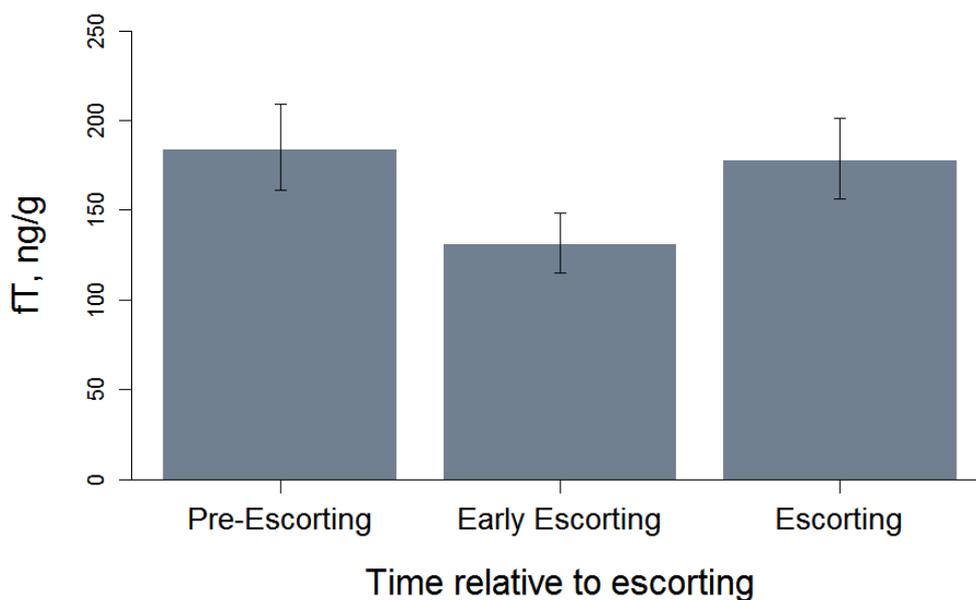


Figure 5.4: Male faecal testosterone metabolite (fT) concentrations before and during the escorting period. Prediction estimates from a GLMM are shown with standard errors. Day was categorised into pre-escorting (0 - 14 days before first observation of escorting,  $n = 54$ ), early escorting (1 - 14 days after first observation of escorting,  $n = 88$ ), and escorting (15 - 42 days after first observation of escorting,  $n = 91$ ). Social group, litter, and individual identities were included in the model as random terms.

### 5.5 Discussion

We used simulated territorial challenges (STIs) to test for individual variations in both behavioural and physiological response to territorial challenge in the cooperatively breeding banded mongoose. In banded mongoose societies there is a clear dichotomy of care effort between escorts and non-escorts, with escorts investing in care several levels of magnitude more than non-escorts (Gilchrist 2004; Hodge 2007). If there was a trade-off between care and territory defence we would expect escorts to show lower response to territorial intrusion than non-escorts. However, we found no evidence that this is the case, as there was no difference in behavioural response to intrusion between escorts and non-escorts. However, escorts and non-escorts did differ in their testosterone response to territorial intrusion (measured over two days following the STI); non-escorts had elevated faecal testosterone metabolite (fT) concentrations following intrusion whilst escorts did not. This corresponds with previous findings that individuals in species with essential parental care have no testosterone response to territorial challenge (Lynn 2008). fT concentrations prior to territorial intrusion did not predict individual aggression level and there was no correlation between escorting effort and baseline fT concentrations. Though this leaves the role of testosterone in modulating aggression and pup care open to interpretation, the differential response to territorial challenge observed between escorts and non-escorts suggests that they may have differential investment in other aggressive behaviours post-intrusion.

We found no evidence that escorting effort predicts individual aggression level in response to territorial intrusion. This suggests that there is no trade-off between care and aggression and that individuals do not specialise to either behaviour. Inter-group interactions between banded mongoose packs lead to changes in territory ownership and can be highly aggressive resulting in serious injury or death to both adults and pups (Cant et al. 2002; Muller & Bell 2009). Synchronous births (Gilchrist 2006; Hodge et al. 2011) create large litters that result in more than half of the adults within a group commonly becoming escorts, each investing highly in pup care. Any reduction in aggressive behaviours by such a large proportion of the adults within a group could have a large effect on group competitive ability, and increase the likelihood of losing inter-group conflicts. This may contrast with other species where only a small subset of individuals is engaged in offspring care or the pressures for group competitiveness are low, and suggests that mechanisms mediating a trade-off between offspring care and aggression may have become decoupled in the banded mongoose to maintain group competitiveness throughout the escorting period.

## 5. Behavioural and Physiological Response to Simulated Territorial Intrusion

We predicted that testosterone response to territorial intrusion would be dependent on the level of offspring care (figure 5.1c; Wingfield et al. 1990; Goymann et al. 2007). Our study found that non-escorts showed a greater increase in fT concentrations than escorts. Individuals with low escorting effort engage in infrequent pup care behaviours so may be comparable to species or individuals engaging in facultative offspring care. Meanwhile, individuals with high escorting effort may be more comparable to species or individuals with essential offspring care because pups have reduced survival and growth rates if they are not escorted (Hodge 2005). Species or individuals with facultative offspring care are predicted to have large testosterone response to territorial intrusion, while species or individuals with essential offspring care strategies are predicted to have reduced or absent testosterone response due to the costs of associated inhibitive effects on offspring care (figure 5.1; Lynn et al. 2005; Goymann et al. 2007). The results of this study suggest that carers in banded mongoose societies are also desensitised to territorial challenge and may lack a testosterone response because any inhibitive effects on the expression of pup care behaviours would be costly.

We have already suggested above that the trade-off between care and behavioural response to territorial challenge may be absent due to pressure for high group competitiveness in inter-group conflicts. However, physiological response to territorial challenge (i.e. in the subsequent day[s]) differs between escorts and non-escorts suggesting that there may be a post-conflict role differentiation between care and aggression in banded mongooses. Previous research has shown that increases in testosterone in response to territorial challenge may not be directly linked to aggressive behaviours during the interaction but promote other persistent aggressive behaviours such as increased vigilance, and aggressive advertising including increased territorial marking after the event (Johnston 1981; Wingfield et al. 1987; Ulibarri & Yahr 1996; Wingfield 2005; Goymann et al. 2007). This suggests that the observed difference in post-STI fT concentrations between escorts and non-escorts may represent specialisations to care or persistent aggressive behaviours after territorial intrusion; a division of labour between care and aggression when the pressure for all individuals to engage in aggressive behaviours is low. Further to this, individuals may only specialise to roles as carers or aggressors given the knowledge that territorial intrusion is likely; following an encounter with an intruder individuals become aware that intruders are around and the chances of another encounter in the subsequent time period are much greater. If this is the case, we may only expect to see a relationship between testosterone levels and aggression level if we were to perform consecutive territorial challenges.

It is important to note that we found no relationship between intruder inspection rate and testosterone response to territorial intrusion. This contrasts with previous findings in other species that change in testosterone correlates with aggression (Ross et al. 2004; Wingfield 2005). Aggressive

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interactions may lead to an increase in glucocorticoids (GCs) due to both the increased energetic demands (Marler et al. 1989; Soto-Gamboa et al. 2005; Valero et al. 2005) and the stressing environment created by social instability (social stress hypothesis; Soto-Gamboa et al. 2005), which can inhibit testosterone release and associated aggressive behaviours (Silverin 1986; Wingfield & Silverin 1986; Tilbrook et al. 2000; Soto-Gamboa et al. 2005). We have previously shown that escorts have increased GC levels compared to non-escorts during the escorting period (Sanderson, in prep.). This suggests that testosterone response to challenge may be inhibited in escorts by their elevated baseline GC levels, which may underlie the variation in testosterone response observed in the present study.

FT concentrations were not negatively correlated with individual escorting effort in the present study. This suggests that baseline testosterone levels cannot readily explain the marked inter-individual difference in care effort observed in this species. Previous studies of meerkats and dwarf mongooses also found that levels of investment in pup care behaviours were independent of testosterone levels (Creel et al. 1993; Carlson et al. 2006a). Similar to dwarf mongooses, banded mongooses are non-seasonal breeders with inter-group copulations that can lead to opportunistic mating opportunities and so any reduction in testosterone levels may have fitness costs due to the risk of 'being caught with your androgens down' and missing out of extra-group mating opportunities (Creel et al. 1993). Testosterone levels are reduced in all males early in the escorting period. This suggests that testosterone may be correlated with other types of care behaviours that are undetected in the current study, e.g. care of young in the den. Alternatively, testosterone may increase over the escorting period to prepare individuals for mate-guarding behaviours in the subsequent oestrus. Banded mongoose packs can have up to 4 communal litters per year, and females typically enter oestrus synchronously 1 - 2 weeks after the birth of a previous litter (Cant 2000). During oestrus, dominant males guard receptive females and engage in aggressive interactions with other members of the pack (Nichols et al. 2010), and as testosterone is often associated with gaining status within social groups (Mazur 1985; Mazur & Booth 1998) it is possible that this increase in testosterone correlates with a build up to the next oestrus event.

Previous studies of testosterone modulation of care and aggression have mostly been limited to seasonal comparisons of non-social species. Here, we have extended the hypotheses surrounding the trade-off between care and aggression to a social species with large intra-group variation in offspring care and intense inter-group conflict. We found no difference in behavioural response to territorial intrusion between carers and non-carers, and proposed that this may be due to the high costs of losing an inter-group conflict, and correspondingly strong selection for group competitive ability. Interestingly, we have shown that testosterone response to territorial intrusion *is* dependent on

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escorting status; only non-carers show a testosterone response to territorial intrusion. This suggests that individuals may differ in their investment in *persistent* aggressive behaviours such as vigilance and aggressive advertising, though more detailed study is needed to confirm these hypotheses. Evidence for role specialisation and a division of labour within social groups is lacking, and indeed may be rare outside of eusocial species such as ants, termites, and mole-rats. Our results suggest that individuals within social groups may have subtle physiological differences and so measurement of obvious behaviours such as care and aggression may not be sufficient to reveal individual specialisation. Future studies looking in depth at subtle behavioural and physiological differences both within and between individuals are needed to elucidate whether divisions of labour exist in cooperatively breeding species and further our understanding of the evolution of animal societies.



# Chapter 6

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## Lifetime Patterns of Cooperation and Competition: Behavioural Syndromes through Time and Across Contexts





## 6.1 Abstract

Individual differences in cooperative investment can be consistent and persist after life-history variation has been controlled for. This suggests that plasticity of cooperative behaviours may be limited, and individuals may be constrained to different behavioural trajectories or behavioural roles within animal societies. We use long-term observations of cooperative offspring care and mate-guarding behaviours in a wild population of banded mongooses (*Mungos mungo*) to investigate lifetime patterns of behavioural consistency in a social context. Moreover, we examine patterns of consistent individual differences through time (in different age-rank categories) and across contexts (between cooperative and competitive behaviours) to investigate what selection pressures may be driving consistent individual differences in this system. We find evidence for consistent individual differences in both cooperative (offspring care; ‘babysitting’ and ‘escorting’) and competitive (mate-guarding) behaviours, though the patterns of consistency differ across different behaviours. We suggest that changes in the costs of offspring care and the availability of mates that occur through time may drive variation in behavioural consistency seen between different age-ranks. Individual differences in two forms of pup care are correlated, suggesting that individuals are not specialised to different cooperative activities, rather they may be specialised as helpful and selfish individuals. We find no evidence of correlation between individual differences in investment in offspring care and mate-guarding behaviours, suggesting that individuals do not show life time specialisations to roles as carers and breeders. This is one of the first studies to test lifetime consistency of behaviours in the wild, and may be the first to investigate consistencies in cooperative and competitive behaviours concurrently. Evidence for lifetime consistency of individual differences is suggestive of lifetime behavioural trajectories and advocates further study into early-life effects to determine the factors that lead to different individual trajectories.



## 6.2 Introduction

Within animal societies there is striking variation in how much individuals invest in cooperative activities (Komdeur 2006). Research has repeatedly demonstrated that individual variation in cooperative investment can be explained by differences in individual characteristics such as energetic state (e.g. Arabian babblers, *Turdoides squamiceps*; Wright et al. 2001), sex (e.g. meerkats, *Suricatta suricata*; Clutton-Brock et al. 2002), and age (e.g. banded mongooses, *Mungos mungo*; Hodge 2007). However, recent evidence indicates that individual differences in cooperative investment can be consistent and persist after this variation has been controlled for (Bergmüller & Taborsky 2007; Bergmüller et al. 2010; Le Vin et al. 2011). For example, a recent study of meerkats found consistent individual differences in investment in two types of cooperative pup care (English et al. 2010). Though the causes and consequences of consistent individual differences in cooperative investment have received growing attention in recent years (Wright 2007; McNamara et al. 2009; Bergmüller et al. 2010; Dall et al. 2012), results are still equivocal, and there is need for further empirical studies of consistent individual differences in cooperative investment.

Behavioural consistency may be costly if the benefits of exhibiting a behavioural trait vary in different contexts. For example, high levels of aggression can be beneficial when competing over resources, but can have costly spill-over effects in non-competitive contexts (e.g. aggression in parental care: Wingfield et al. 1990; excessive sexual cannibalism: Johnson & Sih 2005). This suggests that there can be large benefits of behavioural plasticity in varying environments (Sih et al. 2004). The benefits of maintaining behavioural plasticity to allow behaviour to be modified according to changes in both the intrinsic and extrinsic environment (Dewitt et al. 1998) are likely to be even greater in social species as they must also adjust to fluctuations in their social environment. However, to date, few studies have investigated the extent of behavioural plasticity among individuals in a social context (Bergmüller et al. 2010).

So why are individuals consistent in their behaviour if they could benefit from behavioural plasticity? Behavioural plasticity may be limited due to inaccurate judgement of the current environment (Dewitt et al. 1998), either due to costs of accurate assessment or the lack of reliable cues (Cohen 1967; Moran 1992; Sih 1992; Getty 1996). In animal societies this may be the case if individuals are unable to accurately assess the behaviour or intention of other members of the group. Alternatively, individuals may show behavioural inflexibility due to ‘absolute’ or ‘functional’ constraints in the underlying anatomical structures or physiological pathways (e.g. connectivity of brain tissues or density of hormone receptors) (Duckworth 2010). It is important to note, however, that evidence for consistency does not necessarily suggest limited plasticity; if individuals find themselves in a

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consistent environment we would expect them to behave consistently even though an eventual shift in environment will lead to changes in behaviour (Sih & Bell 2008).

It has been hypothesised that consistent investment in the same behaviour may itself be beneficial and adaptive (Dall et al. 2004). Firstly, ‘individual status quo’ selection (Sih & Bell 2008) suggests that if individuals can gain efficiency through repeatedly engaging in the same behaviour they may be more likely to re-invest in that behaviour in the future than naïve individuals who are less practised. This type of selection may be particularly evident in animal societies if task partitioning and role specialisation increase group productivity (e.g. cooperative hunting: Gazda et al. 2005; cooperative breeding: Ridley & Raihani 2008). McNamara *et al.* (2009) recently developed a model that suggests consistency in cooperative investment can serve as a signal in social interactions. If individuals are sensitive to changes in cooperative investment from others then we expect them to be consistent in their cooperative investment. Though this model is based on a two-player game, it may have implications for cooperatively breeding groups where individuals pay-to-stay (Gaston 1978), or where social decisions are based on previous observations of cooperative investment (i.e. social prestige theory; Zahavi 1972; Zahavi 1990; Wright 2007).

There may be no selection to maintain behavioural plasticity if early life conditions constrain life-history decisions (Biro & Stamps 2008). For example, in a social context, individuals born in conditions of limited resource availability may be less likely to breed and so may benefit from investing more in helping (Russell et al. 2007). Where this is the case, we expect to find consistent individual differences in adult behaviour that are attributable to natal or early-life effects. Evidence for early-life effects is expanding (Bateson et al. 2004; Gluckman et al. 2005; Monaghan 2008), but it is difficult to determine the importance of early-life effects without first demonstrating behavioural consistencies or limited plasticity; if individuals are fully plastic in adult life then early-life effects are likely to be of little importance. Only a handful of studies have so far investigated behavioural consistency in cooperative breeders (see examples in Bergmüller et al. 2010), and to our knowledge only one study has looked at lifetime consistency (English et al. 2010), so the importance of early-life effects in cooperative breeders is not yet known.

The potential mechanisms leading to consistent individual differences discussed above are by no means an exhaustive list (see Dall et al. 2004; Sih & Bell 2008; Bergmüller et al. 2010), and it is difficult to elucidate the causes of individuality with short-term measures of individual consistency. However, studies of Namibian rock agamas (*Agama planiceps*) and three-spined sticklebacks (*Gasterosteus aculeatus*) have demonstrated that comparisons of individual differences across different ‘situations’ (defined as ‘a given set of conditions at one point in time’; Sih et al. 2004) can

help to identify the underlying mechanisms promoting consistent individual differences (Bell 2005; Bell & Sih 2007; Carter et al. 2012). For example, if individuals behave consistently across multiple situations it is unlikely to be an artefact of consistent environment or state and may indicate limited plasticity. Further to this, if individuals behave consistently across an entire lifetime then consistent individual differences are likely to be determined by environmental factors during development (e.g. pre-natal stress effects; Kaiser & Sachser 2005). Contrastingly, if individuals become specialised due to learned efficiencies in different behaviours (i.e. ‘individual status quo’ selection; Sih & Bell 2008) we may expect individuals to become more consistent through time.

Banded mongooses are cooperative breeders with large intra-group variation in two highly conspicuous forms of offspring care (*‘babysitting’* and *‘escorting’*; Cant 2003; Gilchrist 2004; Hodge 2007), making them an ideal model species for investigating consistent individual differences in cooperative investment. Banded mongooses also exhibit large intra-group variation in mating behaviours, and males compete aggressively for access to mating opportunities, allowing us to also test for consistency of competitive behaviours. The banded mongoose population inhabiting Mweya Peninsula, Queen Elizabeth National Park (QENP), Uganda has been studied in detail for over 17 years and so we have access to long-term behavioural observations for >1500 individuals. In contrast to laboratory or short-term wild studies, this dataset will enable us to look at *lifetime* consistency of *natural* behaviours, allowing us to investigate in detail the consistency of individual differences across different *situations*.

Comparisons of individual differences across different *‘contexts’* (defined as ‘a functional behavioural category’; Sih et al. 2004) may also help to elucidate the cause of behavioural consistencies. For example, young meerkats that show high investment in care of pups in the den also show high investment in pup-feeding outside of the den (English et al. 2010). This positive correlation across different cooperative contexts is suggestive of a society with individuals that differ intrinsically in their level of helpfulness. If comparisons across cooperative contexts reveal negative correlations between different behaviours it may be suggestive of individual specialisations and task-partitioning within animal societies (Bergmüller et al. 2010), similar to the division of labour seen in insect societies (Bourke & Franks 1995; Dall et al. 2012). Moreover, examining individual differences across different categories of behaviour, such as cooperation, dispersal, and mating effort, may help to reveal divergent developmental trajectories and associated fitness consequences. Here, we will test for individual specialisation and task-partitioning by looking for consistency of individual differences across different behavioural contexts. For example, if banded mongoose helpers become specialised to different roles we may expect to see a negative correlation between lifetime levels of investment in the two types of pup care. However, if individuals take on roles as carers and breeders

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we would expect to find a positive correlation between the two pup care behaviours and a negative correlation between lifetime levels of investment in pup care and mating behaviours.

In summary, first we will use long-term observations of two forms of cooperative behaviour (babysitting and escorting) and one form of competitive behaviour (mate-guarding) to investigate consistency of individual differences in both cooperative and competitive investment in the banded mongoose. We will then examine the consistency of individual differences in further detail by investigating correlations of individual differences in different situations and behavioural contexts.

### 6.3 Methods

#### 6.3.1 Study system

Banded mongooses are highly social diurnal herpestids that live in large stable groups of 8 – 40 individuals (Cant 2000). All adult females within the group give birth synchronously (usually on the same day; Hodge et al. 2011) to large litters (1 – 20 pups; Gilchrist 2006) that are then cared for by all individuals within the group. There are two highly conspicuous forms of pup care: (i) '*Babysitting*' occurs when one or more individuals remain at the den to care for the pre-emergent pups (ages 0 – 4 weeks) whilst the remainder of the group are foraging (Cant 2003), and (ii) '*Escorting*', whereby each post-emergence pup (aged 5 – 10 weeks) forms a stable relationship with a single adult *escort* who then feeds, plays with, protects, grooms, and generally cares for the associated pup until it reaches independence (Gilchrist 2004).

Banded mongooses have high reproductive skew among males, with male mating success monopolised by the three oldest males in the group (Nichols et al. 2010). Females tend to enter oestrus synchronously (Cant 2000) and older males closely guard oestrous females to defend them against younger males and increase their chances of gaining paternity (Nichols et al. 2010). These mate-guarding behaviours are conspicuous and easy to identify subjectively (Cant 2000; Nichols et al. 2010). Females enter group oestrus soon after giving birth (c. 2 weeks post-partum), so mating opportunities overlap with periods of pup care generating a trade-off between offspring care and mating effort (Cant 2000; Cant 2003).

We took behavioural observations from 771 male banded mongooses over 509 breeding attempts and 207 oestrus events in 15 social groups inhabiting the Mweya Peninsula in QENP, Uganda between April 2003 and January 2012. Details of vegetation and climate are given elsewhere (Cant 2000). All individuals are habituated to the presence of observers at a distance of 2 – 4 m. The

population has been studied continuously for 17 years so the age of all individuals within the population is known through direct observation of births ( $\pm 1$  week).

### **6.3.2 Behavioural observations**

#### *Babysitting effort*

During the babysitting period (0 – 5 weeks after birth) groups were visited daily and babysitters were identified as individuals present at the den when the rest of the group was foraging. Babysitters could be identified either by direct observation or through elimination as individuals missing from the foraging group (Cant 2003; Hodge 2007). Individual scores of babysitting effort within each breeding attempt were then calculated as the proportion of days that an individual was observed as a babysitter out of the total number of days that babysitting was recorded by any individual within the group (1 – 39 observation days per breeding attempt, total 3762 observation days). This gave a single babysitting score for each individual in each babysitting period which is representative of individual helping effort in each breeding attempt.

#### *Escorting effort*

During the escorting period (4 – 10 weeks after birth) groups were visited daily and associations between adults and pups were noted. Adults were scored as escorts if they were seen to be within 30cm of the same pup for more than 50% of the observation period (Gilchrist 2004; Gilchrist & Russell 2007). Previous studies have shown that this association score is a reliable summary of quantitative nearest-neighbour data (Gilchrist 2001; Gilchrist & Russell 2007). Individual scores of escorting behaviour per breeding attempt were then calculated as the proportion of days that an individual was recorded as an escort out of the total number of days for that breeding attempt on which escorting was recorded by one or more individuals within the group (7 – 21 observation days per breeding attempt, total 6231 observation days). This gave a single escorting score for each individual in each escorting period which is representative of individual helping effort in each breeding attempt.

#### *Mating Effort*

Banded mongoose females tend to come into oestrus synchronously, with a group oestrus period that lasts  $5.3 \pm 0.6$  days (mean  $\pm$  SE, from 207 oestrus periods). When banded mongoose females enter oestrus each female is closely followed and guarded by a single male for periods that last from

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several hours to several consecutive days. Mate-guards defend their associated female from the mating attempts of other males by snapping and lunging at approaching conspecifics, which results in them gaining a high proportion of parentage (Nichols et al. 2010). Mate-guarding effort was calculated as the number of observation days that each individual was observed mate-guarding out of the total number of days within an oestrus period that mate-guarding was observed (1 – 12 observation days per oestrus period, total 696 observation days). This gave a single mate-guarding score for each individual in each oestrus which is representative of individual reproductive effort in each breeding attempt.

### *Body condition*

All individuals in this study were trapped every 3 to 12 months using box traps (67x23x23 cm; Tomahawk Live Trap Co., Tomahawk, WI, USA), and anaesthetised using either ketamine or isoflurane (details of trapping protocol are given elsewhere; ketamine: Cant 2000; Hodge 2007; isoflurane: Jordan et al. 2010; Jordan et al. 2011). Measures of body mass ( $\pm 1$  g) and head width ( $\pm 0.1$  mm) were taken from all trapped individuals (850 captured male banded mongooses over 743 trapping sessions [ $4.1 \pm 0.17$  captures per individual; mean  $\pm$  S.E.] between February 2000 and March 2012). This capture data was used to calculate a predicted body mass for any given head width by regressing log body mass with log head width. In addition to capture weights, all individuals in this study were trained to step onto a portable weighing scale allowing weights to be recorded daily before the morning foraging trip (Hodge 2007). To assess body condition from weights collected without capture, we compared an individual's body mass with its predicted body mass based on its head width at closest capture date (i.e. observed body mass (collected in the field) / predicted body mass) (following Jakob et al. 1996; Gilchrist et al. 2004).

### **6.3.3 Statistical analyses**

All statistical analyses were carried out using R 2.13.2 (R Development Core Team 2012) with the MCMCglmm package (Hadfield 2010).

#### *Are there consistent individual differences in cooperative and competitive investment?*

Consistent individual differences in any phenotypic trait are indicated by a high '*repeatability*' (*R*) or intra-class correlation coefficient (ICC; Lessells & Boag 1987) of that trait (the proportion of phenotypic variation that is attributable to between-subject variation).

Formally, this is calculated as;

$$R = \frac{\sigma_{\alpha}^2}{\sigma_{\alpha}^2 + \sigma_{\varepsilon}^2}$$

Where  $\sigma_{\alpha}^2$  is the between-subject variance and  $\sigma_{\varepsilon}^2$  is the within-subject variance (or residual variance), so that the total observed phenotypic variance is equal to the sum of  $\sigma_{\alpha}^2$  and  $\sigma_{\varepsilon}^2$ . Behavioural variation often coincides with variation in intrinsic and extrinsic variables such as age-rank, body condition, and group size. When calculating repeatability estimates we can control for these differences by including them in the model and calculating *adjusted repeatabilities* (Nakagawa & Schielzeth 2010). Life-history variables are included in the model as fixed terms and group-level factors such as social group are included as random terms. This controls for residual variance ( $\sigma_{\varepsilon}^2$ ) which is due to underlying differences in life-history and gives a more accurate estimate of repeatability as if all individuals were measured under the same conditions.

We have used Bayesian modelling with MCMCglmm (Hadfield 2010), analysing data with an information-theoretic approach and comparing density distributions rather than p-values. This allowed us to extract the posterior mode and highest posterior density (HPD) of both between-subject ( $\sigma_{\alpha}^2$ ) and within-subject ( $\sigma_{\varepsilon}^2$ ) variances to calculate an estimate of repeatability and associated credible intervals (Nakagawa & Schielzeth 2010). These values are constrained to being positive so the error boundaries will never cross zero and we are unable to suggest phenotypic repeatability by subjective analysis of error boundaries. We classified behaviours as ‘repeatably’ if the difference between deviance information criterion values (DIC) from models with and without individual included as a random effect was less than 10. DIC values can be analysed similarly to Akaike’s information criterion (AIC) values (Spiegelhalter et al. 2002; Ward 2008); if including an individual level term in the model decreases the DIC value by >10 it is suggested to increase the model fit and we are justified in including it in the model (Burnham & Anderson 2002; Symonds & Moussalli 2010). Pack and birth litter identities were included as random effects in all models. We were interested in individual variance whilst controlling for pack and litter differences, so the variance components attributable to pack and birth litter were not incorporated when calculating individual repeatability values (Nakagawa & Schielzeth 2010).

To investigate individual repeatability of babysitting, escorting, and mating efforts we fitted individual investment in each behaviour per breeding attempt/oestrus event as a binomial response in a MCMCglmm with a binomial error structure and logit link. MCMCglmm binomial models are fitted with additive over-dispersion (Hadfield 2010), and so the residual variance ( $\sigma_{\varepsilon}^2$ ) becomes equal to  $\sigma_{\varepsilon}^2 + \pi^2/3$  (where  $\sigma_{\varepsilon}^2$  is the estimate of within-individual variance from the model; Nakagawa &

Schielzeth 2010). Therefore, individual repeatability of traits with binomial error structure can be estimated as;

$$R_{logit} = \frac{\sigma_{\alpha}^2}{\sigma_{\alpha}^2 + \sigma_{\epsilon}^2 + \pi^2/3}$$

Detailed break-down of the fixed effects included in each model are given in tables 6.1-6.3. We are aware of suggestions to calculate repeatability estimates for different classes of individual as *conditional repeatabilities* (i.e. through including interactions between fixed effect covariates and random factors; Nakagawa & Schielzeth 2010). However, difficulties in modelling heteroscedastic variance structures in MCMCglmm models with binomial errors have limited us to comparing repeatability estimates from separate models for each category of individual (Personal communication, Nakagawa, S.). As we are analysing repeatability of behaviours we only analysed data for individuals for which we had at least two observations. Access to mates is monopolised by the three oldest males in banded mongoose societies (Nichols et al. 2010). This suggests that changes in age-rank are more likely to predict change in circumstance and associated behavioural optima than changes in age, so we included age as age-rank (ranked male age in years with tied ages assigned a mean age-rank) in all analyses.

Firstly, to estimate the *lifetime* consistency of investment in each behaviour we calculated repeatability estimates from models including observations from all age-ranks. Secondly, to look in more detail at changes in behavioural consistency over time we calculated repeatability estimates from models restricted to data collected from individuals at high (rank 1 – 3) and low (rank 4 +) age-ranks. This allowed us to determine whether consistent individual differences become more or less pronounced through time.

*Is investment in cooperative or competitive behaviours correlated between early adulthood and later life; i.e. do consistent individual differences correlate across different situations?*

To further investigate whether individuals are consistent in their investment in cooperative and competitive behaviours throughout their lifetimes, we extracted best linear unbiased predictor (BLUP) values for each individual when at both low and high age-rank. BLUPs give a value of the trait of interest for each individual relative to the mean of the population given expected variation for other factors that are incorporated in the model (Kruuk 2004). Within the MCMCglmm (Hadfield 2010) error structure they are extracted from the model as the posterior mode of the random effect distributions. This gave us a value of individual investment in each behaviour when at both low and high age-rank. We tested for correlations between these values using Pearson's correlation measures (escorting effort was not analysed in this way because escorting effort was not found to be

repeatable within high age-rank individuals; see below) and corrected for the use of multiple test using the Bonferroni correction (Rice 1989).

*Are there role specialisations or helpful phenotypes; i.e. do consistent individual differences correlate across different behavioural contexts?*

To investigate whether individual differences correlate across different behavioural contexts, we again extracted BLUP values for each behaviour. We tested for individual specialisation in different types of pup care by correlating BLUP scores for babysitting and escorting within each age-rank category with Pearson's correlation tests (high age-rank scores were not analysed because escorting effort was not found to be repeatable within high age-rank individuals; see below). Secondly, we tested for specialisations as breeders and carers by correlating BLUP scores for both cooperative behaviours and mating effort within each age-rank category. We corrected for the use of multiple tests using the Bonferroni correction (Rice 1989).

## 6.4 Results

### 6.4.1 Do individuals show consistent differences in cooperative investment?

#### *Babysitting*

Individual investment in babysitting was repeatable overall ( $R = 0.11$ , 95% CI: 0.07 – 0.16,  $\Delta\text{DIC} = 96.43$ , figure 6.1a), and in both high and low age-ranks (high age-ranks:  $R = 0.06$ , 95% CI: 0.02 – 0.14,  $\Delta\text{DIC} = 14.53$ ; low age-ranks:  $R = 0.12$ , 95% CI: 0.08 – 0.18,  $\Delta\text{DIC} = 68.27$ , figure 6.1a).

Individual differences in babysitting effort were found to be correlated across age-rank categories; there was a significant positive correlation between the individual's low and high age-rank BLUP estimates in babysitting effort (Pearson's  $R = 0.56$ , 95% CI = 0.28 – 0.75,  $t_{33} = 3.88$ ,  $p < 0.001$ ; figure 6.2a). This result remained significant when using the Bonferroni correction to control for multiple tests ( $p < 0.017$ ).

#### *Escorting*

Individual investment in escorting was also repeatable overall ( $R = 0.26$ , 95% CI: 0.18 – 0.38,  $\Delta\text{DIC} = 11.99$ , figure 6.1b), and in low age-ranks ( $R = 0.28$ , 95% CI: 0.18 – 0.42,  $\Delta\text{DIC} = 10.96$ , figure 6.1b). However, individual investment in escorting was not repeatable in high age-ranks ( $R = 0.16$ , 95% CI: 0.00 – 0.31,  $\Delta\text{DIC} = -1.13$ , figure 6.1b).

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Babysitting														
Age/rank category	Fixed Effects (HPD interval)													
	Intercept		Age-rank		Group Size		Body Condition		Oestrus (no overlap)		Season (dry)		Rainfall	
	lower	upper	lower	upper	lower	upper	lower	upper	lower	upper	lower	upper	lower	upper
All	<b>-3.75</b>	<b>-1.65</b>	-0.01	0.05	<b>-0.06</b>	<b>-0.01</b>	-0.43	1.28	-0.30	0.18	-0.15	0.31	<b>0.02</b>	<b>0.19</b>
High Age-rank	<b>-4.96</b>	<b>-1.42</b>	-0.25	0.13	-0.05	0.01	-0.65	2.53	-0.27	0.37	-0.33	0.33	-0.02	0.23
Low Age-rank	<b>-3.97</b>	<b>-1.48</b>	-0.04	0.02	-0.05	0.00	-0.51	1.61	-0.25	0.27	-0.12	0.38	<b>0.00</b>	<b>0.20</b>

Table 6.1: Credible intervals of factors included in model to estimate adjusted repeatabilities of babysitting effort within 3 age-rank categories; all age-ranks ( $n_{\text{individuals}} = 123$ ), high age-rank (ranks 1 – 3;  $n_{\text{individuals}} = 45$ ), and low age-ranks (ranks 4 +;  $n_{\text{individuals}} = 112$ ). Age-rank was calculated from male age in years within a pack where individuals of the same age in years shared a mean rank. Group size was the total number of individuals within the pack aged over 1 year at birth. Body condition was calculated as a mean relative body weight to that expected from head width (from measures of body weight collected during the babysitting period). We classified babysitting period as having an overlapping oestrus if mate-guarding was observed during the babysitting period. Litters born between December – February and June – August were classified as dry season litters and rainfall was calculated as the mean daily rainfall during the babysitting period. Credible intervals from a mixed model MCMCglmm with pack, litter, and individual as random effects, with a burn-in period of 30000, thinning interval of 100, and sample size of 1000 (credible intervals that do not overlap with zero are shown in bold).

Escorting														
Age/rank category	Fixed Effects (HPD interval)													
	Intercept		Age-rank		Group Size		Body Condition		Number of Pups		Season		Rainfall	
	lower	upper	lower	upper	lower	upper	lower	upper	lower	upper	lower	upper	lower	upper
All	<b>-6.82</b>	<b>-0.43</b>	-0.05	0.12	<b>-0.23</b>	<b>-0.09</b>	-2.09	3.65	<b>0.31</b>	<b>0.48</b>	-0.42	0.92	-0.08	0.48
High Age-rank	-8.63	7.41	-1.01	0.44	<b>-0.34</b>	<b>-0.12</b>	-9.46	6.40	<b>0.29</b>	<b>0.60</b>	-0.53	1.84	-0.07	0.90
Low Age-rank	<b>-7.80</b>	<b>-0.41</b>	-0.14	0.08	<b>-0.17</b>	<b>-0.01</b>	-1.68	4.48	<b>0.30</b>	<b>0.44</b>	-0.30	0.93	-0.08	0.44

Table 6.2: Credible intervals of factors included in model to estimate adjusted repeatabilities of escorting effort within 3 age-rank categories; all age-ranks ( $n_{\text{individuals}} = 105$ ), high age-rank (ranks 1-3;  $n_{\text{individuals}} = 36$ ), and low age-ranks (ranks 4+;  $n_{\text{individuals}} = 81$ ). Age-rank was calculated from male age in years within a pack where individuals of the same age in years shared a mean rank. Group size was the total number of individuals within the pack aged over 1 year at pup emergence. Body condition was calculated as a mean relative body weight to that expected from head width (from measures of body weight collected during the first two weeks of escorting). The number of pups is the number of pups seen to emerge from the den. Litters that emerged between December – February and June – August were classified as dry season litters and rainfall was calculated as the mean daily rainfall during the escorting period. Credible intervals from a mixed model MCMCglmm with pack, litter, and individual as random effects, with a burn-in period of 30000, thinning interval of 100, and sample size of 1000 (credible intervals that do not overlap with zero are shown in bold).

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Mate-guarding														
Age/rank category	Fixed Effects (HPD interval)													
	Intercept		Age-rank		Number of Males		Number of Females		Body Condition		Season		Rainfall	
	lower	upper	lower	upper	lower	upper	lower	upper	lower	upper	lower	upper	lower	upper
All	-2.41	0.18	<b>-0.35</b>	<b>-0.26</b>	-0.04	0.03	-0.09	0.02	<b>0.28</b>	<b>2.66</b>	<b>0.06</b>	<b>0.53</b>	-0.12	0.03
High Age-rank	<b>-5.14</b>	<b>-0.03</b>	-0.62	0.00	-0.09	0.02	-0.12	0.07	<b>0.48</b>	<b>5.54</b>	-0.02	0.73	-0.17	0.08
Low Age-rank	-1.31	1.80	<b>-0.33</b>	<b>-0.23</b>	-0.05	0.05	-0.08	0.08	<b>1.35</b>	<b>1.29</b>	<b>0.05</b>	<b>0.69</b>	-0.14	0.04

Table 6.3: Credible intervals of factors included in model to estimate adjusted repeatabilities of mate-guarding effort within 3 age-rank categories; all age-ranks ( $n_{\text{individuals}} = 123$ ), high age-rank (ranks 1-3;  $n_{\text{individuals}} = 46$ ), and low age-ranks (ranks 4+;  $n_{\text{individuals}} = 112$ ). Age-rank was calculated from male age in years within a pack where individuals of the same age in years shared a mean rank. Number of males and number of females were the total number of males and females within the pack aged over 1 year at the start of oestrus respectively. Body condition was calculated as a mean relative body weight to that expected from head width (from measures of body weight collected within one week of observations of mate-guarding). Oestrus events occurring between December – February and June – August were classified as dry season oestrus events and rainfall was calculated as the mean daily rainfall during the oestrus event. Credible intervals from a mixed model MCMCglmm with pack, oestrus, and individual as random effects, with a burn-in period of 30000, thinning interval of 100, and sample size of 1000 (credible intervals that do not overlap with zero are shown in bold).

We did not carry out correlative tests between BLUP scores of escorting effort in high and low age-ranks because individual investment in escorting was not repeatable in high age-ranks.

#### **6.4.2 Do individuals show consistent differences in competitive investment?**

##### *Mate-guarding*

Individual investment in mate-guarding was repeatable overall ( $R = 0.10$ , 95% CI: 0.05 – 0.14,  $\Delta\text{DIC} = 47.55$ , figure 6.1c), and in both high and low age-ranks (high age-ranks:  $R = 0.15$ , 95% CI: 0.08 – 0.28,  $\Delta\text{DIC} = 21.8$ ; low age-ranks:  $R = 0.07$ , 95% CI: 0.03 – 0.13,  $\Delta\text{DIC} = 26.96$ , figure 6.1c).

Individual differences in mate-guarding effort were not found to be consistent across age-rank categories. However, there was a tendency for a positive correlation between the low and high age-rank BLUP estimates in mate-guarding effort (Pearson's  $R = 0.30$ , 95% CI = -0.04 – 0.57,  $t_{33} = 1.78$ ,  $p = 0.08$ ; figure 6.2b)

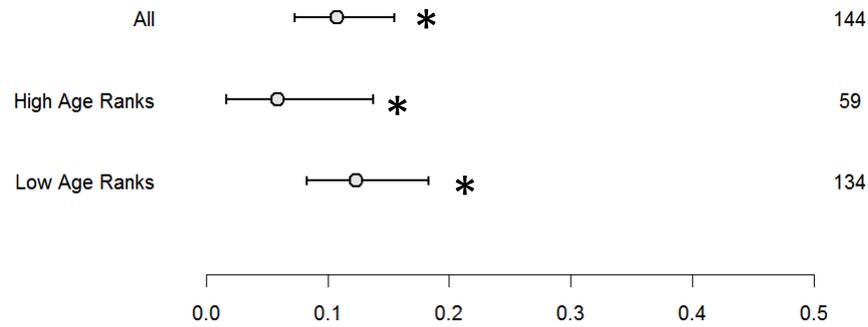
#### **6.4.3 Are there role specialisations or helpful phenotypes; i.e. do consistent individual differences correlate across different behavioural contexts?**

BLUP estimates for babysitting and escorting effort were positively correlated in low age-ranked individuals, but not when data from all age-ranks were pooled (table 6.2; this result remained significant when using the Bonferroni correction to control for multiple tests;  $p < 0.005$ ). We found no significant correlations between BLUP estimates of mate-guarding effort and either babysitting or escorting in any age-rank categories (table 6.2). Correlations including BLUP values for escorting effort in high age-rank males were not analysed because we did not find evidence for consistent individual differences in this category (see above; figure 6.1b; table 6.1).

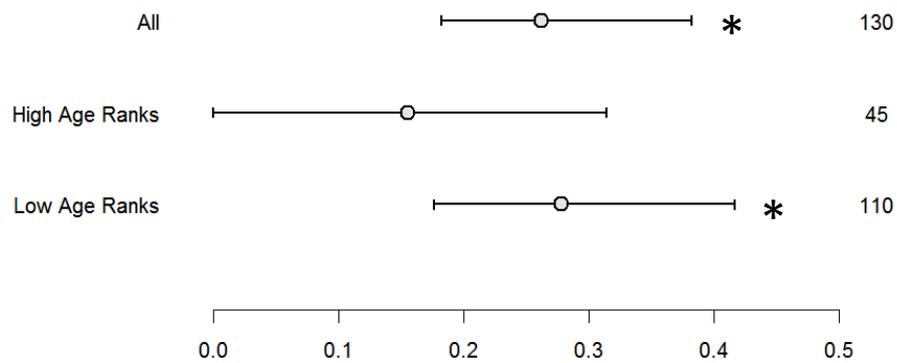
## **6.5 Discussion**

We have used long-term observations of two forms of cooperative offspring care (babysitting and escorting) and one form of competitive behaviour (mate-guarding) in banded mongooses to investigate if individuals differ consistently in their cooperative and competitive investment. We found evidence for consistent individual differences in all three behaviours, suggesting that individuals do consistently differ in their investment in both cooperative and competitive

(a) **Babysitting Effort**



(b) **Escorting Effort**



(c) **Mate Guarding Effort**

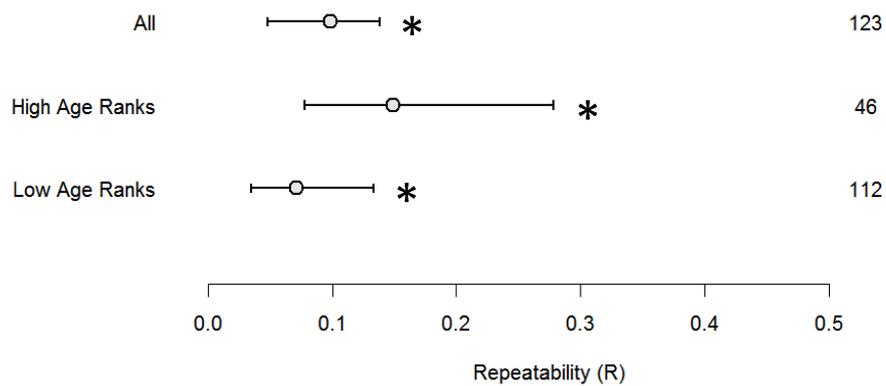


Figure 6.1: Estimates of repeatability and associated 95% credible intervals for (a) babysitting, (b) escorting, and (c) mate-guarding effort across three subsets of age-rank. Sample sizes (number of individuals) are shown to the right. Repeatable behaviours (where including individual as a random effect decreased the DIC estimate by > 10) are noted by \*.

Behaviour	Age-rank Category	Between-Individual Variance $\sigma_{\alpha}^2$	Within-Individual Variance $\sigma_e^2$	DIC	
				With Individual	Without Individual
Babysitting Effort	All	0.45	0.20	<b>12015.19</b>	<b>12111.62</b>
	High Age-ranks	0.21	0.15	<b>3602.57</b>	<b>3617.10</b>
	Low Age-ranks	0.45	0.21	<b>8911.31</b>	<b>8979.58</b>
Escorting Effort	All	2.88	4.10	<b>5622.39</b>	<b>5634.38</b>
	High Age-ranks	1.92	6.36	1571.95	1570.82
	Low Age-ranks	3.09	3.92	<b>3937.57</b>	<b>3948.53</b>
Mating Effort	All	0.45	0.83	<b>4100.69</b>	<b>4148.24</b>
	High Age-ranks	0.83	1.05	<b>1806.42</b>	<b>1828.22</b>
	Low Age-ranks	0.28	0.25	<b>2615.94</b>	<b>2642.90</b>

Table 6.4: Estimate of variance components used to calculate repeatabilities of babysitting, escorting, and mating effort in 3 age-rank categories; all individuals, high age-ranks (rank 1 – 3), and low age-ranks (rank 4 +). Estimates are from mixed model MCMCglms with pack, litter, and individual as random effects, with a burn-in period of 30000, thinning interval of 100, and sample size of 1000. DIC estimates are given from comparative models with and without individual included as a random effect. Where removing individual from the model decreases the DIC value by > 10 the behaviour is considered repeatable; these cases are shown in bold. Details of fixed terms included in each model are described in tables 6.1 – 6.3.

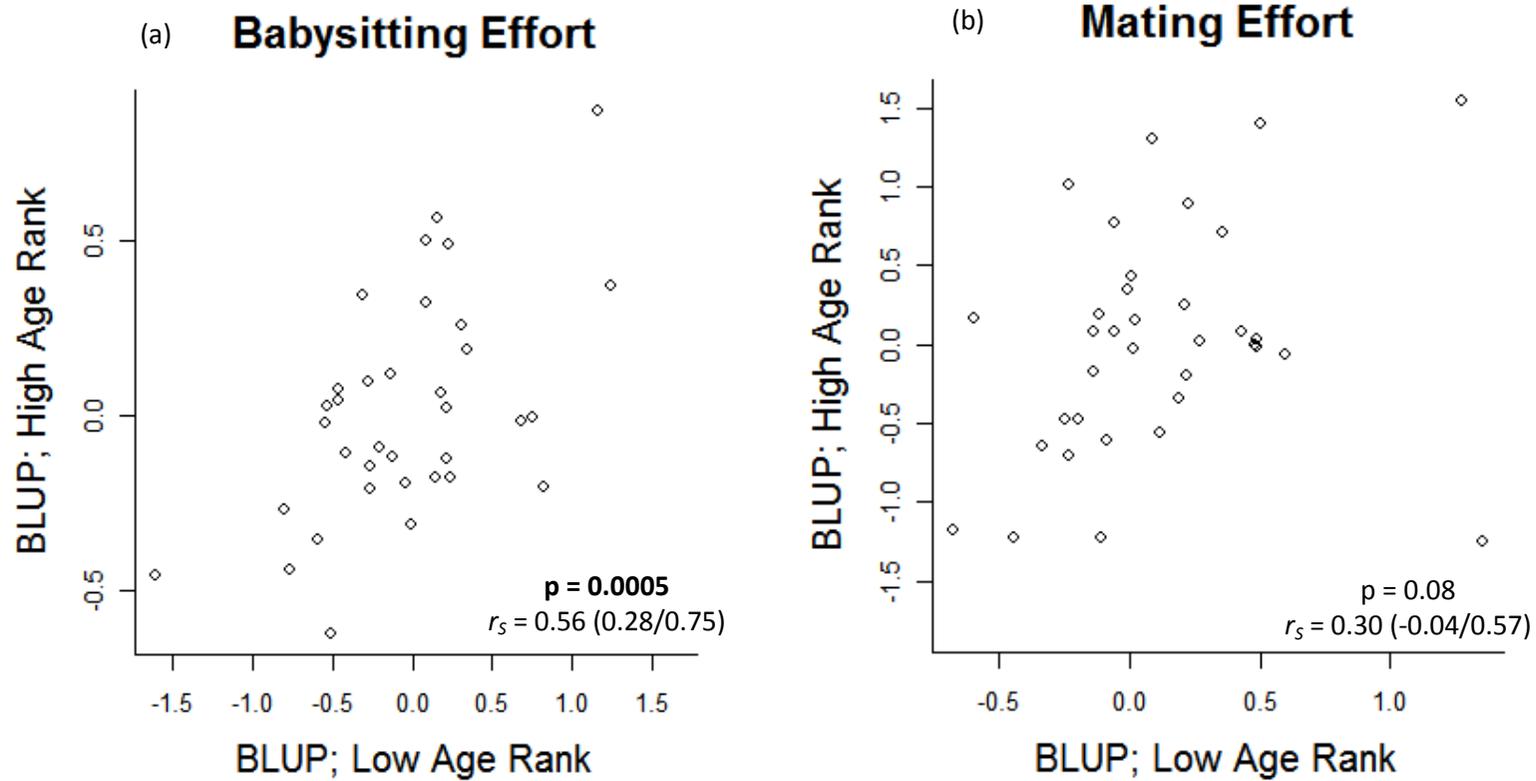


Figure 6.2: Within-individual correlations of (a) babysitting ( $n_{\text{individuals}} = 35$ ), and (b) mate-guarding ( $n_{\text{individuals}} = 35$ ) effort from models limited to high (age-rank 1 – 3) and low (age-rank 4 +) age-ranks. Results from Pearson’s moment correlation tests are inset; p values and  $r_s$  estimates (95% confidence intervals). Results that are significant are shown in bold ( $p < 0.017$ ; using Bonferroni correction).

Behaviours	Age-rank Category	$r_s$ estimate (95% CI)	$t$	d. f.	$p$
<b>Babysitting - Escorting</b>	All	0.10 (-0.09/ 0.29)	1.04	99	0.30
	High Age-rank	NA			
	<b>Low Age-rank</b>	<b>0.34 (0.12/ 0.53)</b>	<b>3.05</b>	<b>71</b>	<b>0.003</b>
Mate-guarding - Babysitting	All	-0.08 (-0.26/ 0.11)	-0.86	111	0.39
	High Age-rank	-0.21 (-0.49/ 0.11)	-1.31	38	0.20
	Low Age-rank	0.02 (-0.18/ 0.22)	0.23	97	0.82
Mate-guarding - Escorting	All	0.18 (-0.02/ 0.36)	1.76	96	0.08
	High Age-rank	NA			
	Low Age-rank	0.02 (-0.21/ 0.25)	0.17	69	0.87

Table 6.5: Pearson's product moment correlation test results: correlations between individual BLUP estimates from models of babysitting, escorting, and mate-guarding effort in three subsets of age-rank: all age-ranks, high age-ranks (rank 1 – 3), low age-ranks (rank 4 +). Significant correlations are shown in bold ( $p < 0.0056$ ; using Bonferroni correction).

behaviours. Previous studies have highlighted that differences in the level of consistency across situations can be used to elucidate the mechanisms underlying consistent individual differences in behaviour (Bell 2005; Bell & Sih 2007; Carter et al. 2012). We investigated patterns of consistency across different age-ranks and found that the pattern of individual consistency was different for each type of behaviour. These differences suggest that consistency in each behavioural context could be driven by a different evolutionary mechanism and we go on to discuss each of these in more detail below. We also analysed correlations between BLUP scores for high and low age-ranked individuals to test if individual differences carry across different situations. Individual investment in babysitting was correlated across high and low age-ranks, but investment in mate-guarding was not. In similar analyses, to test if individual differences carry across behavioural contexts, we found that individual investment in babysitting and escorting was correlated (though only in individuals of low age-rank) but individual investment in cooperative and competitive behaviours was not. This suggests that individuals do not specialise in different types of cooperation, or to roles as breeders and carers; rather they show a consistent level of helpfulness similar to that seen in meerkats (Clutton-Brock et al. 2003; English et al. 2010). Together these results provide strong evidence for consistent individual differences in cooperative and competitive behaviours and are suggestive of a level of individuality in a social environment that carries across both situations and behavioural contexts.

All of the repeatability estimates in the current study were calculated whilst controlling for intrinsic and extrinsic environmental factors that are expected to affect behaviour expression (e.g. social group, age-rank: Nichols et al. 2010; body condition: Hodge 2007; group size: Gilchrist 2004; and rainfall: Nichols et al. 2012; see tables 6.1-6.3 for an exhaustive list). This, alongside the fact that repeatability estimates were calculated from a long-term data set that spans several generations, strongly suggests that the observed consistency is not an artefact of a consistent environment. It has previously been suggested that intra-specific differences in behaviour could simply be non-adaptive variation around adaptive optima. However, if behaviours are costly (see costs of babysitting and escorting in Hodge 2007) we would expect high selection pressure to remove such maladaptive variation. This suggests that the consistent individual differences we have observed in banded mongooses may not be maladaptive noise but are more likely to be an adaptive trait. We will now review patterns of consistency for each behaviour in turn to discuss the possible underlying evolutionary mechanisms.

We found evidence for consistent individual differences in babysitting effort. The level of consistency in babysitting effort was similar both when data from all age-ranks was pooled and when it was separated into classes of high and low age-ranks. Further to this, BLUP scores for babysitting were positively correlated between high and low age-ranks. Together, these results

suggest that individuals are consistent in their babysitting effort throughout their lifetime. Early-life conditions can propel individuals along life-time trajectories (Russell et al. 2007). The lifetime consistency of differences between individuals seen here concurs with the hypothesis that variation in life-history trajectories mediates consistent individual differences in cooperation (Dall et al. 2004; Wolf et al. 2007). However, we found no correlation between BLUP scores for babysitting and mate-guarding, which does not support the idea that males are on trajectories to become helpers or breeders. Babysitting is energetically costly (Hodge 2007) and is likely to involve risk-taking as babysitters are seen to defend the pups both from potential predators and raids from neighbouring groups (Cant et al. 2001; Cant 2003). Previous studies of both rats and rhesus monkeys have shown that adult expression of care and risk-taking behaviours and the associated variation in stress-reactivity are dependent on the level of care received when young (Suomi 1997; Meaney 2001). This suggests that individuals may show consistent variation in babysitting effort due to variation in stress-reactivity attributable to environmental variation when young.

We found evidence for consistent individual differences in escorting effort across the lifetime of male banded mongooses. However, when the analysis was split into categories of high and low age-ranks, we found that escorting effort was only consistent in low age-ranks. Previous study has shown that older individuals decrease their provisioning effort when deprived of food but young individuals do not (Bell 2010). Further to this, we have previously shown that escorting effort is dependent on glucocorticoid levels prior to the pup care period in old males but not young males (Chapter 3). These results suggest that older males may be sensitive to GC cues because they benefit from decreasing their pup care effort when in a low energetic state. In contrast, young males are insensitive to GC cues because the costs of investment in pup care even when in a low energetic state are still low. If this is the case we would expect escorting effort to be plastic in older individuals and consistent individual differences only in young individuals, which matches the findings of this study.

Investment in mate-guarding showed consistent individual differences. When age-rank categories were analysed separately, repeatability of mate-guarding was higher in high age-ranked males than low age-ranked males. ‘Individual status quo’ selection suggests that individuals will behave more consistently as they gain efficiencies in certain behaviours (Sih & Bell 2008). The pattern of consistency in mate-guarding effort across age-ranks in the current study matches this hypothesis. High-quality males may become better mate-guards as they age and so become more efficient at monopolising available females and show more consistent high investment in mating. Low-quality males do not have the opportunity to become more efficient and are likely to remain as non-mate-guarding individuals. If this were the case, we would expect increased efficiency and associated high

investment in mate-guarding to only occur in practised individuals that showed high investment in mate-guarding when young. However, we found no significant correlation between BLUP scores of mate-guarding in low and high age-ranks, so it is unlikely that this is the case.

As mentioned previously, male mating opportunities in banded mongooses are monopolised by the oldest three males in the group (Nichols et al. 2010). This means that low age-ranked males will show opportunistic mating behaviours, with low-ranking but high-quality males occasionally mate-guarding if an opportunity arises. This means that there are unlikely to be consistent individual differences in mate-guarding effort in low age-ranked males. Males at high age-rank have more consistent access to mates, so individual differences in mate-guarding effort in high age-ranked individuals are likely to be driven by differences in male-quality. For example, high-quality males are likely to always have access to mates and show consistent high effort in mate-guarding, while low-quality males are more likely to have no access to mates and show consistent low effort in mate-guarding. This matches the patterns observed in the current study as we would expect individuals in high age-rank to show more consistent investment in mate-guarding than individuals in low age-ranks irrespective of past experience.

We tested for individual specialisations to roles as carers or breeders by correlating individual BLUP scores for offspring care and mating behaviours. If individuals become specialised to these roles we would expect to see a negative correlation between BLUP scores for investment in offspring care and mating behaviours. However, we found no evidence that this is the case, which suggests there is no lifetime trade-off between cooperative and competitive investment. We looked for individual specialisations in different cooperative behaviours by correlating individual BLUP scores for babysitting and escorting within different age-rank categories. If individuals are specialised to different helping activities within a society we would expect a negative correlation between different types of cooperative behaviour. We found no evidence of a negative correlation between babysitting and escorting efforts in any age-rank category, which suggests that individuals do not become specialised to different forms of cooperation. However, BLUP scores for babysitting and escorting effort were positively correlated in low age-ranked individuals, suggesting that young individuals may specialise more widely as 'helpers' and 'non-helpers', similar to in meerkats (Clutton-Brock et al. 2003; English et al. 2010). The cooperative activities studied here are by no means an exhaustive list of those exhibited by banded mongooses (e.g. inter-group conflict: Cant et al. 2002; anti-predatory response: Rood 1983), so there remains the possibility that individuals are specialised to wider categories of cooperation (e.g. offspring orientated and non-offspring orientated). Though we have identified babysitting and escorting as two different forms of cooperative care, they are likely to have the same cues (e.g. pup begging calls; Gilchrist 2004) and involve the same behaviours

(e.g. grooming and carrying pups). Therefore, individuals suited to one form of cooperative care are likely to also be well suited to other forms of pup care, whilst other individuals may be more suited to aggressive roles in inter-group conflict or anti-predatory response.

We have used long term observations of both cooperative and competitive behaviours to investigate patterns of consistent individual differences within a social context. We found consistent individual differences in three different behaviours, though the patterns of consistency differed within each context. We suggest that consistent individual differences seen in babysitting, escorting, and mate-guarding effort are driven by (i) variation in life-history trajectories, (ii) variation in the costs of investment, and (iii) variation in the consistency of the environment (access to mating opportunities), respectively. Consistent individual differences carried across cooperative contexts but not between cooperative and competitive contexts, suggesting that while there may be helping phenotypes, these do not have lifetime trade-offs with mating success. We have used a mixed approach to investigate patterns of consistent individual differences, which has enabled us to make valuable inferences about the evolution of consistent cooperative investment. Previous studies have investigated consistent individual differences in cooperative care (e.g. Bergmüller & Taborsky 2007; English et al. 2010). However, this is the first study to investigate consistent individual differences in both cooperative and competitive behaviours simultaneously over whole lifetimes. We have found compelling evidence for individuality in a social system, but both the ultimate and proximate mechanisms underlying this variation remains speculative. Individual differences may be due to a multitude of factors, including both genetic and environmental differences. Detailed and perhaps experimental or manipulative studies of the factors preceding individual differences are required to fully understand why individuals consistently differ in their behaviour.



# Chapter 7

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# Discussion





## 7.1 Overview

The study of cooperative societies has historically focused on asking why individuals cooperate; for example, why should individuals remain in their natal group to assist in rearing the offspring of others rather than dispersing to pursue their own mating opportunities? These questions have been answered with observations that indirect fitness benefits, ecological constraints, inheritance, reciprocity, and benefits of group augmentation may all lead to cooperation (Hamilton 1964; Hatchwell & Komdeur 2000; Kokko et al. 2001; Kokko et al. 2002; Wright 2007). However, many fundamental questions remain. In particular, why do individuals within the same social group behave differently and have very different levels of cooperative investment? Research is now focussed on understanding why there are such individual differences within animal societies (e.g. Bales et al. 2002; McNamara et al. 2004; Komdeur 2006; Riechert & Jones 2008; Le Vin et al. 2011; Mares et al. 2012). Understanding the adaptive reasoning for these individual differences requires research into both the *ultimate* and *proximate* underlying mechanisms; ultimate questions that ask 'Why?', and proximate questions that ask 'How?'. While the ultimate mechanisms behind individual differences in cooperative behaviour have received much attention (e.g. Cant & Field 2001; Dall et al. 2004; reviewed in Emlen 1991; Heinsohn & Legge 1999), but empirical tests in natural populations are often lacking, and research into the proximate mechanisms is lagging far behind (Soares et al. 2010).

In this thesis, I have addressed this shortfall in understanding by using both observational and experimental approaches to investigate the ultimate and proximate mechanisms behind inter-individual variation in cooperative investment in the banded mongoose (*Mungos mungo*). Throughout this thesis, I have discussed the implications of my results within each chapter. In this discussion I will bring together these results and discuss their wider implications. In the first section I will synthesise my findings associated with ultimate and proximate mechanisms separately. I will then discuss these findings together, and consider the advances that can be made by investigating both the proximate and ultimate factors underlying behavioural strategies in the same study. I will finish by discussing the wider implications and suggesting further study both in the banded mongoose study system and in other areas of behavioural ecology and endocrinology research.

## 7.2 Individual Differences in Cooperative Investment:

### The Ultimate Factors

Within cooperatively-breeding animal societies, subordinate helpers care for young that are not their own. The extent to which each individual invests in cooperative offspring care is expected to correlate with the associated fitness costs and benefits of helping (Clutton-Brock 2002). For example, individuals may invest more in cooperation towards relatives to increase their associated indirect fitness benefits. However, only 10% of variation in the likelihood of helping is described by variation in relatedness (Griffin & West 2003), and individuals may not be expected to show kin discrimination average relatedness within groups is high with low variation (Cornwallis et al. 2009). More recently, it has been posited that individuals may vary in their helper investment due to variation in the *costs* of helping. Costs of cooperating may arise due to behavioural trade-offs; when limitations in time or resources mean that investment in one behaviour leads to a concurrent decrease in another behaviour (Stiver & Alonzo 2009). For example, there may be trade-offs between cooperative offspring care and future fecundity or survival. In this thesis I investigated if variation in helper effort could be due to variation in fitness costs attributable to energetic load or missed mating opportunities when helping.

In Chapter 3, I built on previous findings that offspring care in banded mongooses is energetically costly (Hodge 2007), and showed that high investment in offspring care had a negative carry-over effect on offspring care in subsequent litters. This carry-over effect decreased over time, but it was still present even when there is a gap of several months between care periods. It has been shown in a range of species that increased investment in care of current offspring can have detrimental carry-over effects on reproductive success in subsequent breeding attempts (Harrison et al. 2011). Though this study was limited to looking at carry-over effects on care behaviours, it seems likely that the energetic costs of offspring care would also affect future investment in other behaviours such as mating effort. In chapter 4, I found that higher age-ranked males, who are likely to have access to mating opportunities, had lower investment in offspring care than lower age-ranked males both prior to and during oestrus. This is in contrast to care periods with no oestrus overlap when high and low age-ranked males had similar investments in offspring care. These results suggest that males are able to make facultative adjustments to their investment in offspring care dependent on available mating opportunities and that variation in cooperative investment may be driven by a trade-off between offspring care and reproduction.

Investment in offspring care may also be costly due to functional or morphological constraints that prevent individuals switching between different behaviours (Huang & Robinson 1996). For example,

individuals engaged in offspring care may not be able to respond effectively to territorial intrusion if the costs of switch between care and aggression are high (Wingfield et al. 1990). However, in social species, these costs may be avoided if individuals take on different roles or specialisations as carers and guarders (Duarte et al. 2012), as is seen in the eusocial insects (Wilson 1979; Bourke & Franks 1995). In Chapter 5, I showed that carers and non-carers both exhibit the same aggressive response to a simulated territorial intrusion. This suggests individuals can easily switch between care and aggression and are not specialised to roles as carers and guarders in banded mongoose societies. In Chapter 6, I investigated the possibility that individuals may become specialised to roles as carers or breeders by testing for consistent individual differences in investment in offspring care and mate-guarding behaviours. I found evidence for consistent individual differences in both types of behaviour, but as these differences were not correlated it is unlikely that individuals are specialised to roles in care and breeding. Rather, I speculate that these consistent differences in cooperative effort may be due to differences in early-life experience that affect the costs of offspring care later in life.

### **7.3 Individual Differences in Cooperative Investment:**

#### **The Proximate Factors**

Hormonal systems are good candidates for the proximate mechanisms behind individual differences in behaviour as they act over a relatively short time scale (a few minutes) and can modify multiple behaviours simultaneously (Norris 2006). Glucocorticoids (GCs) modulate investment in energetically expensive behaviours to match variation in resource availability (McEwen & Wingfield 2003). In Chapter 3, I showed that individuals with low faecal glucocorticoid metabolite (fGC) concentrations prior to the pup care period are more likely to subsequently invest heavily in offspring care. I also demonstrated that fGC concentrations increased in carers over the care period, and that this is likely to be due to the energetic costs of helping. This result highlighted variations in GC levels as a likely modulator of the carry-over effect of investment in offspring care seen between consecutive breeding attempts. These results conflict with previous findings that GCs promote offspring care in other mammalian systems (e.g. Fleming et al. 1997; Carlson et al. 2006). I suggest that this may be because the current study measured fGC concentrations prior to the pup care period, when the decision of whether to care or not is being made, which is in contrast to other studies have looked at GC concentrations during the care period where GCs may modulate care effort in response to daily fluctuations in individual state.

## 7. Discussion

Testosterone commonly mediates a trade-off between offspring care and mating behaviours (e.g. birds: Van Duyse et al. 2002; Peters 2002; mammals: Nunes et al. 2000; Kuzawa et al. 2009; fish: Desjardins et al. 2005; Bender et al. 2008). Within non-social species, individuals tend to have high testosterone during mating periods and low testosterone during care periods as testosterone promotes and inhibits mating and care behaviours, respectively (Wingfield et al. 1990; Peters 2002; Lynn 2008). Within cooperatively breeding species, individuals show a similar trade-off between care and mating behaviours (e.g. Young et al. 2005; Hodge 2007) so it is likely that testosterone plays a similar role in modulating investment in offspring care and reproduction. In Chapter 4, I showed that individuals with high investment in mating behaviours or offspring care had high and low faecal testosterone metabolite (fT) concentrations respectively. Moreover, I showed that high concentrations of fT predicted low investment in offspring care. These results suggest that testosterone may mediate a trade-off between offspring care and reproduction in banded mongooses similar to that seen in meerkats (Young et al. 2005). In Chapter 5, I used simulated territorial intrusions to investigate the possibility that testosterone may also mediate a trade-off between investment in offspring care and territorial aggression, as is seen in a wide variety of non-social species (Wingfield et al. 1990). I found no evidence for a testosterone mediated trade-off. However, carers and non-carers did not differ in their behavioural response so it may be that there was in fact no trade-off to mediate. Carers and non-carers did differ in their testosterone response to territorial intrusion; non-carers had elevated fT concentrations after the intrusion, whilst carers did not. This is comparable to the pattern seen between species with different levels of offspring care and suggests that testosterone may mediate a trade-off between offspring care and aggressive behaviours post-intrusion.

### **7.4 Ultimate and Proximate Explanations for Individual Differences;**

#### **What Can They Tell Us Together?**

In this thesis I have investigated both the ultimate and proximate mechanisms underlying variation in individual investment in cooperative offspring care, territory defence, and mating behaviours. Looking at individual variation from both perspectives has enabled me to make conclusions that would not have been possible if I had only taken one approach. In Chapter 3, I demonstrated that GC modulation of investment in offspring care was age-dependent, and GC variation is attributable to variation in energetic state. Together, these results strongly suggest that the age-dependent variation in offspring care seen in banded mongooses (Hodge 2007; Bell 2010) is a result of tighter resource limitation in older individuals, presumably because mating behaviours are also

energetically demanding. In Chapter 5, I found that carers and non-carers had the same behavioural response to territorial intrusion but had differential physiological responses. This highlights the possibility of post-intrusion specialisations that would have remained hidden if I had only investigated the immediate behavioural response.

Evolutionary ecologists are primarily interested in the costs and benefits associated with different behaviours and strategies, and the constraints that may prevent individuals from reaching theoretical optima. Hormonal mechanisms of control are often perceived as constraining mechanisms (Ketterson & Nolan 1999; Duckworth 2010). For example, if testosterone promotes aggression as well as inhibiting offspring care then individuals may be constrained to investing in *either* aggression or offspring care within a life-history stage. In Chapter 4, I showed that testosterone is positively correlated with mating behaviours and negatively correlated with offspring care; mate-guarding individuals have high fT concentrations, while individuals engaged in offspring care have low fT concentrations. It is easy to suggest that this is the result of a constraint due to testosterone function. However, it becomes more interesting if we think of hormones as the mechanisms that mediate behavioural trade-offs given functional constraints; i.e. functional constraints exist due to limitations in time or resources, and hormonal mechanisms evolve to mediate optimum allocation of resources among competing functions. In this case the functional constraint is likely to be due to time limitation as mate-guarding and offspring care occur at the same time but in different locations. Simultaneous modulation of both offspring care and mate-guarding by the same hormonal modulator suggests that individuals can benefit from switching investment from offspring care to mate-guarding when there are receptive females within the group.

Hormonal modulation of behaviour may be different in different life-history stages if two behaviours are under correlated selection in one life-history stage but not in another. For example, territory defence during the mating season is commonly promoted by testosterone as it coincides with mating behaviours, whilst territory defence outside of the mating season is commonly independent of testosterone to avoid the associated immunosuppressive and energetic costs ((Wingfield et al. 2001). Understanding variation in hormonal modulation of multiple behaviours over different life-history stages can help to identify different behavioural trade-offs and selection pressures that occur over the lifetime of an individual. In Chapter 5, I tested for a testosterone mediated trade-off between care and territorial aggression, and found no evidence for testosterone modulation of either behaviour. However, perhaps this is not surprising given that these experiments were done when there were no available mating opportunities. It may be interesting to carry out a similar experiment in the presence of receptive females. Within a reproductive life-history stage we may

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expect elevated testosterone levels to promote both mating behaviours and territorial aggression which is associated with defending females from inter-group copulations.

In Chapter 6, I found evidence for lifetime consistent individual differences in investment in both cooperative offspring care and mating behaviours. Given that I have also shown hormonal modulation of both of these types of behaviours it seems reasonable to predict that these consistent individual differences may be associated with consistent individual differences in hormone profiles. However, it has been suggested that variations in circulating hormone concentrations are unlikely candidates for mediating long-term consistency of traits because hormones respond quickly to environmental changes, making them more suitable mechanisms to mediate flexible phenotypes (Duckworth 2010) (though individual variation in hormone receptor density may underlie consistent individual differences in behavioural response to hormonal changes e.g. HPA activity, (Liu et al. 1997; Plotsky et al. 2005)). I found that investment in offspring care was more consistent in lower age-ranked individuals. This result coincides with results of Chapter 3 where I found that only high age-ranked individuals reduced their care effort in response to GC cues. Hormonal mechanisms have maintenance costs (Lessells 2008), so where a behaviour is more often expressed than not expressed it may be beneficial for a hormonal mechanisms to switch off the behaviour rather than switch it on. Our results suggest that individuals may be 'hard-wired' to express a certain level of investment in offspring care, but then may become sensitive to GC cues in later life-history stages that switch off offspring care when the associated costs become greater. Further study of hormonal mechanisms over multiple life stages in wild animal populations are needed to further elucidate the complexity of hormonal mechanisms that may mediate individual investment in different behaviours.

### 7.5 Wider Implications and Future Study

Hormone function may be altered through varying the number of receptors in different tissues, restricting the active form of the hormone to certain target-cells by the use of specific target-binding proteins in the blood, transforming hormone molecules from their inactive form to an active form at the target site, or synthesising them locally at the target site (Wingfield et al. 2001; Hau 2007; Lessells 2008). This makes the potential array of hormonal mechanisms perhaps as broad as the selection pressures that act on different behaviours or strategies. Hormonal mechanisms can vary both between and within individuals, and here I have discussed how hormonal differences may underlie behavioural differences both between and within individuals in a long-term study population of banded mongooses. Many studies of hormone and behaviour take a short-term view,

sometimes only collecting one hormone and one behaviour measure per individual before concluding that hormonal modulation of behaviour is present or absent. As evidence for differences in hormone function both within and between individuals increases, it becomes increasingly apparent that simple, short-term studies may not be sufficient to understand the true relationship between hormones and behaviour.

Major insights into the hormonal mediation of behavioural trade-offs have been made through inter-species comparisons. For example, inter-species comparisons of testosterone and mating strategies were integral to Wingfield's seminal paper introducing the 'Challenge Hypothesis' (1990). Hormonal mechanisms may be conserved between distantly related species (Lynn 2008). This suggests that asking why hormonal mechanisms differ between similar species (especially where there are similarities in behaviour) could give great insights into the selection pressures acting on the associated behaviours. Banded mongooses share many similarities with the closely related meerkat (*Suricata suricatta*). The behavioural ecology of these two species differs mainly in that meerkats have high reproductive skew within a single breeding pair (Clutton-Brock et al. 2001), subordinate males in meerkat societies prospect for extra-group mating opportunities (Young et al. 2007), and adult meerkats indiscriminately feed pups (Russell et al. 2003) rather than forming the one-to-one escorting relationship seen in banded mongooses (Gilchrist 2004). Comparison of long-term behavioural data from these two closely related social species has led to fruitful insights into the evolution of cooperative breeding under different selection pressures (e.g. Russell et al. 2003; Hodge 2007). Further collaborations between the two projects; comparing the function of hormonal mechanisms, may add further insight into the evolutionary mechanisms that have shaped behavioural differences between the two closely related species.

Similar to genes, hormones can have both pleiotropic and epistatic effects; they can affect multiple traits simultaneously and affect the actions of other hormones (McGlothlin & Ketterson 2008). Each chapter within this thesis was limited to investigating one hormone alongside one or two behaviours. It is likely that some of the residual variation seen in the relationships between hormones and behaviours may be attributable to other hormones that also influence the behaviour(s) of interest. When one hormone promotes the release of another, circulating concentrations of different hormones will be correlated, which makes it sometimes easy to attribute variation in behaviour to the wrong hormone. For example, a study in meerkats suggested that investment in pup care was attributable to variation in prolactin, but further investigation incorporating GCs showed that variation in cortisol concentrations actually described a larger proportion of variation in pup care behaviours than did prolactin (Carlson et al. 2006b). Further work, investigating multiple hormones simultaneously, may provide a much greater insight into the

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proximate mechanisms underlying behaviour variation. In this study I used enzyme immunoassays and hormone extracts from faecal samples. Enzyme immunoassays are time-consuming and require a different assay for each hormone. Further to this, the use of faecal samples limits the range of hormones that you can measure as peptide hormones are broken down in the gut and so are no longer measurable. Recent development of liquid chromatography-mass spectrometry (LC-MS) from blood plasma enables measurement of multiple hormones concentrations in the same assay (Nelson et al. 2004; Westerlund & Hoffmann 2004). This, alongside measures of multiple behaviours and use of multivariate statistics will enable investigations of complete hormone and behaviour profiles to fully elucidate the role hormones in mediating behaviour trade-offs.

### 7.6 Concluding Remarks

The number of signalling molecules within the endocrine system of any individual is limited, so pleiotropic effects of hormonal signals are unavoidable. Similar to how morphological traits may be constrained by coordinated development (Maynard-Smith et al. 1985), behavioural traits may be constrained by pleiotropic effects of hormonal signalling (Lessells 2008; Duckworth 2010). However, no hormone-behaviour link is ubiquitous across taxa (e.g. testosterone modulation of care effort: reviewed in Hau 2007), and hormone function can differ even between closely related species (e.g. glucocorticoid modulation of care effort; Carlson et al., 2006; current study), suggesting that hormone function may not be a constraining factor. Moreover, hormones act through complex pathways, creating multiple levels where selection can act to decouple a hormone from its pleiotropic effects (Lessells 2008). Hence, it is reasonable to suggest that hormonal mechanisms do not constrain the evolution of behaviour expression. However, any individual at a given time is itself likely to experience behavioural constraints due to its current organisation of hormone receptors and associated signalling pathways that are attributable to previous selection pressures.

Where there is evolution of a constraining mechanism, such as the coupling of multiple behaviours to the same hormonal modulator, the expression of these behaviours is likely to be under correlated selection. Understanding how and where hormone behaviour links are de-coupled, both between species and between different life-history stages in the same species, can thus give great insights into how selection acts on suites of behaviours. Time and resource limitations mean that the expression of any behaviour is involved in a trade-off with one or many other behaviours or traits. Therefore, understanding how selection acts on suites of behaviours is imperative to understanding the evolution of any behaviour or strategy, and there may be much to gain through further integrating endocrinology into the fields of evolutionary and behavioural ecology.





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## Appendix 1: Corticosterone and Testosterone Assay; Methods and Validation

Each EIA utilised an antibody (polyclonal corticosterone CJM006 or testosterone R156/7 antiserum supplied by CJ Munro, University of California, Davis, CA), horseradish peroxidase conjugated label (corticosterone or testosterone; prepared according to Munro and Stabenfeldt, 1984) and standards (corticosterone or testosterone; Sigma-Aldrich, UK). The modified assay procedures for the corticosterone EIA were as follows: i) antiserum was diluted at 1:15,000 in coating buffer (0.05 M NaHCO<sub>3</sub>, pH 9.6), loaded 50 µl / well on a 96-well Nunc-Immuno Maxisorp microtiter plate (Thermo-Fisher Scientific), and covered with a plate sealer and left overnight at 4°C; ii) plates were washed five times (0.15 M NaCl, 0.05% Tween 20); iii) standards (corticosterone, 3.9 – 1000 pg/well) or samples diluted 1:20 in EIA buffer (0.1 M NaPO<sub>4</sub>, 0.149 M NaCl, 0.1% bovine serum albumin, pH 7.0) were loaded 50 µl / well; and iii) the horseradish peroxidase conjugate was diluted in EIA buffer to 1:70,000 and added 50 µl / well. The modified assay procedures for the testosterone EIA were as follows: i) non-specific goat anti-rabbit gamma globulin (IgG; Sigma, R2004) was diluted in coating buffer and loaded 1.0 µg in 250 µl / well on Nunc-Immuno Maxisorp microtiter plates and left overnight at room temperature (RT). The nonspecific IgG was then discarded and 300 µl / well of Tris blocking buffer (0.02 M Trizma, 0.300 M NaCl, 1.0% BSA, pH 7.5) was added and incubated for a minimum of 2 hours at RT; ii) plates were washed five times; iii) EIA buffer was loaded 50 µl / well and iv) standards (testosterone, 2.3 – 600 pg / well) or samples (diluted 1:100 in EIA buffer) were loaded 50 µl / well; v) the horseradish peroxidase conjugate diluted in EIA buffer to 1:40,000 and was added 50 µl / well, and vi) antiserum diluted in EIA buffer at 1:25,000 for testosterone was added 50 µl / well. For both the corticosterone and testosterone assay following incubation in the dark for 2 hours at RT, plates were washed 5 times and incubated with 100 µl / well of RT substrate (0.4 mM 2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) diammonium salt, 1.6 mM H<sub>2</sub>O<sub>2</sub>, 0.05 M citrate, pH 4.0) and left to develop at RT in the dark and measured at 405 nm at optical density 0.8 to 1.0.

The corticosterone antiserum CJM006 was found to cross-react with Corticosterone 100%, Desoxycorticosterone 14.25%, Progesterone 2.65%, Tetrahydrocorticosterone 0.90%, Testosterone 0.64%, Cortisol 0.23%, Prednisolone 0.07%, 11-desoxycortisol 0.03%, Prednisone < 0.01%, Cortisone < 0.01% and Estradiol < 0.01% (Watson et al., 2013) and the testosterone R156/7 antiserum cross-reacted with Testosterone 100%, 5α-Dihydrotestosterone 57.37%, Androstenedione 0.27%, Androsterone 0.04%, DHEA 0.04%, Cholesterol 0.03B-Estradiol 0.02%, Progesterone < 0.02%, Pregnenolone < 0.02%, Hydrocortisone < 0.02%, Cholic Acid < 0.02%, Chenodeoxycholic Acid < 0.02%, Cholic Acid Methyl Ester < 0.02%, Dehydrocholic Acid < 0.02%, Deoxycholic Acid < 0.02%,

Lithocholic Acid < 0.02%, Glycholic Acid < 0.02%, Taurodeoxycholic Acid < 0.02%, Taurocholic Acid < 0.02%, Taurochenodeoxycholic Acid < 0.02 and Glycochenodeoxycholic Acid < 0.02% (Coralie Munro personal communication). The intra- and inter-assay coefficients of variation for the corticosterone assay were 7.52 and 6.33% (C1 and C2) and 8.66 and 10.47% (C1 and C2) respectively. The intra- and inter-assay coefficients of variation for the testosterone assay were 6.18 and 14.69% (C1 and C2) and 13.20 and 14.51% (C1 and C2) respectively.

The corticosterone and testosterone assays were validated for measuring corticosterone or testosterone metabolites in female and male banded mongoose faeces by parallelism, accuracy check, and ACTH challenge. Serial dilutions of male banded mongoose faecal extract yielded a displacement curve parallel to the standard curve (corticosterone: sample % binding =  $11.713 + 0.651$  (standard % binding),  $R^2 = 0.9957$ ,  $F_{1,7} = 1631.26$ ,  $p < 0.001$ ; testosterone: sample % binding =  $11.713 + 0.651$  (standard % binding),  $R^2 = 0.9547$ ,  $F_{1,7} = 147.58$ ,  $p = 0.101$ ). There was no evidence of matrix interference in male corticosterone and testosterone assays, as addition of diluted faecal extract to standards did not alter the amount expected (corticosterone: Observed =  $31.074 + 1.522$  (Expected),  $R^2 = 0.997$ ,  $F_{1,7} = 2342$ ,  $p = 0.06$ ; testosterone: Observed =  $31.074 + 1.522$  (Expected),  $R^2 = 0.9989$ ,  $F_{1,7} = 6139.60$ ,  $p = 0.004$ ). Serial dilutions of female banded mongoose faecal extract yielded a displacement curve parallel to the standard curve (corticosterone: sample % binding =  $11.713 + 0.651$  (standard % binding),  $R^2 = 0.969$ ,  $F_{1,7} = 218.64$ ,  $p = 0.683$ ). There was no evidence of matrix interference in assays of female corticosterone, as addition of diluted faecal extract to standards did not alter the amount expected (corticosterone: Observed =  $31.074 + 1.522$  (Expected),  $R^2 = 0.9954$ ,  $F_{1,7} = 1512.316$ ,  $p = 0.4329$ ). The physiological validity of using this EIA assay technique to measure faecal glucocorticoid (fGC) concentrations in banded mongoose (*Mungos mungo*) samples was established by demonstrating a cause-and-effect relationship between exogenous administration of corticotrophin (ACTH; one intramuscular injection of 13  $\mu$ l of 1 mg / ml synthetic ACTH [Tetracosactide; Synacthen],  $n = 3$  males and 3 females) and the subsequent excretion of fGC metabolites in the faeces. fGC levels were higher in the two days following injection than the two days prior to injection (Mann-Whitney;  $n_{\text{samples}} = 26$  and 25,  $p < 0.001$ ; figure A1; N.B. one female failed to show an increase in fGC concentration, though this may be due to missed samples). Peak fGC elevation occurred  $6.67 \pm 0.21$  hours (mean  $\pm$  SE,  $n_{\text{individuals}} = 5$ ) after ACTH administration.

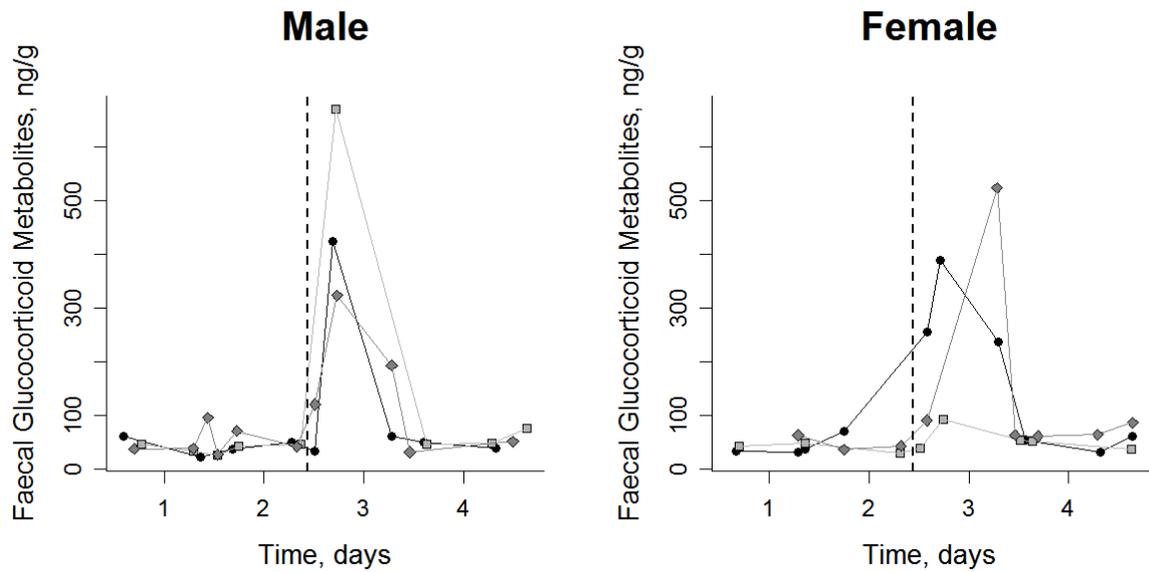


Figure A1: Faecal glucocorticoid metabolite (fGC) concentrations from male and female banded mongooses before and after administration of synthetic ACTH (Tetracosactide; Synacthen). Each solid line represents a single individual. The vertical dashed line represents time of ACTH administration.

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