# THE REPRODUCTIVE TRAJECTORIES OF BACHELOR GELADAS

by

David J. Pappano

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Anthropology)
in The University of Michigan
2013

Doctoral Committee:

Professor Jacinta C. Beehner, Chair
Professor Abigail W. Bigham
Professor John C. Mitani
Professor Elizabeth A. Tibbetts

© David J. Pappano
All rights reserved
2013

# DEDICATION

*For my parents, Teresa and Donald Pappano*

*For indulging a child's curiosity in bats, snakes, and other animals described in Golden Guides to Nature. Most parents would have just read fairy tales to their children.*

*And my best friend and partner, Ashley*

*For your patience, kindness, and love in years past and those yet to come.*

# ACKNOWLEDGEMENTS

Over the past six years, I have been fortunate to have the support of friends, family, colleagues, and funding agencies that have made this dissertation possible. I am grateful for their input at all levels of this endeavor—from conception to completion. My time at the University of Michigan has been among the most formative and memorable experiences of my life, and I hope that many of the friends and colleagues I have met over these past years will continue to be a part of my life in the years to come.

This research would not have been possible without the generous support of several government and non-profit funding agencies. A Graduate Research Fellowship from the National Science Foundation and the James Spuhler Fellowship through the Department of Anthropology largely funded my studies at Michigan. I am also grateful to the National Science Foundation for support through a Doctoral Dissertation Improvement Grant. The U.S. Fulbright Program and a Leakey Foundation General Research Grant supported my fieldwork. I also thank the Rackham Graduate School for additional support while writing the final parts of this dissertation.

My dissertation chair, Dr. Jacinta Beehner, deserves much praise for my research trajectory. Jacinta has shown me how to develop a project and see it to completion all while balancing her duties as director of the Core Assay Facility, co-director of the University of Michigan Gelada Research Project, and mother to her two small children. I met her and Dr. Thore Bergman the last week of December 2006 at Princeton University. I had recently graduated college and was teaching high school biology in Connecticut. They had just started their jobs at the University of Michigan and

were preparing for a trip to Ethiopia in January to set up the gelada project. They needed graduate students to study geladas in the formative years of the project, and I was applying to fulfill that need. Against Thore's warnings that "ice hockey goalies are crazy" and probably her better judgment as well, she took a risk on me as one of their first graduate students. For that, I am grateful.

I am also thankful for the guidance of my committee: Dr. John Mitani, Dr. Abigail Bingham, and Dr. Elizabeth Tibbetts. I had the opportunity to teach two classes under John while at the University of Michigan. In this lecture hall, John taught me how to teach through splicing information and humor with thought-provoking questions. In my early years of graduate school, I would attend the Behavioral Ecology Group organized in part by Liz Tibbetts. This seminar provided me with weekly reminders that behavioral ecology did not start and end with geladas (or primates for that matter). To Abby—the only member of my committee that has fewer years at Michigan than I do—I am happy to have had your input in these final steps of my dissertation. Finally, while he was not on my committee, I am indebted to Thore Bergman for giving me the opportunity to conduct research on the geladas of the Simien Mountains.

I owe a debt of gratitude to my friends at the University of Michigan and in Ethiopia. My cohort in biological anthropology—Zachary Cofran, Dana Begun, and Caroline VanSickle—have made this journey special. Also, to the newly minted Drs. Noah Snyder-Mackler and Eila Roberts, your culinary skills in Ethiopia and help with analyses at home have kept me sane. To the upstarts of the project—Marcela Benitez, Elizabeth Johnson, Morgan Gustison, and Caitlin Barale—I wish the best of luck in their future projects. Finally, to the camp managers and Ethiopian field workers of the gelada project—Clay Wilton, Julie Jarvey, and Ambaye Fanta—your tireless efforts keeping the campsite organized and functioning were greatly appreciated.

Of the full-time project assistants, Esheti Jejaw deserves special recognition for his role in this project. I met Esheti in 2008 during my first summer in Ethiopia. I hired Esheti to be a full-time bachelor expert, which required him to find and name bachelors that were associating with the project's main study band. Because neither of us could speak each other's native language very well, we accomplished our goals that first summer mostly through miming gelada behavior and speaking a pigeon "Amharenglish". By the time I returned my second summer, Esheti was using a Palm pilot to record data and had begun to collect *ad libitum* data on dominance interactions and grooming within bachelor groups. During my long haul of fieldwork in 2010-11, Esheti's training came full circle. He collected fecal samples for genetic and hormonal data, and was recording focal animal samples everyday. Even though our communication has converged on a mutual understanding of many Amharic-English phrases, I don't know if I will ever be able to convey just how grateful I am for his help in completing this work.

To my mother and father that fostered my love of science at an early age, this is for your countless hours spent with me at the library or watching PBS Nature programs at night. And to my brothers and sister, thank you for understanding in planning holidays and events around my field schedule. Lastly, this thesis is for my best friend and partner of the last seven years, Ashley. She agreed to marry me, now if I can only persuade her to proofread this thesis.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# ABSTRACT

Sexual selection strongly influences patterns of male behavior. Fertilizations cannot be shared, therefore male-male relationships tend to be agonistic rather than affiliative. Despite this fact, males in many species form all-male groups that vary in composition and stability. All-male groups have been observed in many mammalian taxa including cetaceans, ungulates, and primates. Because few studies have examined these groups directly, we know little of how they form, the social relationships between the males that comprise them, or if such relationships influence future reproductive success. Geladas (*Theropithecus gelada*) are an Old World monkey species where males disperse from their natal units and join all-male groups prior to acquiring reproductive access to females. In this study, I examine the behavioral, hormonal, and genetic factors that shape the reproductive trajectories of bachelor geladas living in all-male groups.

This study has three major components. First, I address what factors, such as age or kinship, influence the composition of all-male groups. I demonstrate that relatedness influences all-male group formation, although only few pairs of males were considered close kin. Second, I ask how bachelors interact within their groups. I show that bachelors form stratified social bonds within their all-male groups. These bonds were strongest between males that were closely related and similar in age. Third, I attempt to answer how bachelors become dominant “leader” males. Males that eventually became dominant had higher testosterone levels during the bachelor period. I draw on extended field-based observations of wild geladas (2006-2011) as well as hormonal and genetic data to answer my study questions. These findings represent the

first study of gelada all-male groups and one of the few studies on such groups in related species.

# CHAPTER ONE

# INTRODUCTION

> *A dozen millennia ago or so, an adventurous soul managed to lop off a surly bull's testicles and thus invented behavioral endocrinology. It is unclear from the historical records whether this individual received either a grant or tenure as a result of this experiment, but it certainly generated an influential finding—something or other comes out of the testes that helps to make males such aggressive pains in the ass.*
> *-The Trouble with Testosterone by Robert Sapolsky*
>
> *Precisely what controls the level of aggression in the all-male groups is not clear, but the relatively closed nature of these groups suggests that strong inter-personal bonds among the members may be a contributing factor*
> *-Social Dynamics of Gelada Baboons by Robin and Patsy Dunbar*
>
> *A man's friendships are one of the best measures of his worth.*
> *-attributed to Charles Darwin*

Sexual selection strongly influences male behavior. As fertilizations cannot be shared, male-male relationships are expected to be largely competitive rather than affiliative (Andersson 1994). Aggressive relationships are commonplace between males across all taxa. However, in some species males engage in surprising levels of cooperation and coordination with both kin and non-kin (Dugatkin 1997; Sachs et al. 2004). Primate males may build temporary coalitions (Widdig et al. 2000) or long-lasting bonds (Schülke et al. 2010; Mitani 2009) to survive and reproduce. Although strong male-male bonds are common in male-philopatric primates (Van Hooff & Van Schaik 1994) such as chimpanzees (*Pan troglodytes*) (Mitani 2009), evidence for male bonds in male-dispersal species is rare (but see: Hill 1994; Silk 1994; Schülke et al. 2010). Geladas (*Theropithecus gelada*) are an ideal species to study how male bonds may influence male reproductive trajectories in a female-philopatric species. Geladas are an Old World monkey species where individuals live in modular social groups composed of multiple core groups of females and non-natal males (called “reproductive units”) and all-male groups (Dunbar & Dunbar 1975; Kawai 1979). Only dominant “leader” males can mate with females within a reproductive unit (Snyder-Mackler et al. 2012). Prior to acquiring reproductive access to females, bachelor geladas live in cohesive all-male groups (Dunbar & Dunbar 1975; Dunbar 1984; Kawai 1979). Eventually, males graduate from these all-male groups to become leader males. Although the social system of geladas was first described 40 years ago (Crook 1966; Dunbar & Dunbar 1975; Kawai 1979), we nevertheless still do not know why some bachelors become leaders and others fail. Additionally, bachelors within the same all-male group have been observed to ‘cooperate’ in attempts to overthrow leader males – yet only one male eventually reaps the reproductive benefits. I propose that social bonds combined with other behavioral and hormonal factors play a role in the reproductive decisions of bachelor geladas. In this dissertation, I investigate the behavioral and hormonal factors that shape the reproductive trajectory of males as they move from a non-reproductive bachelor to a leader of reproductive unit.

## GELADA (*THEROPITHECUS GELADA*)

Commonly referred to as gelada “baboons,” the gelada (*Theropithecus gelada*, Geoffroy 1843) is a species of Old World monkey (Family: Cercopithecidae, Subfamily: Cercopithecinae, Tribe: Papionini) (Jablonski 1993). The name gelada “baboon” is a misnomer as geladas are sister taxa to true baboons (*Papio* spp.) (Page et al. 1999). The genera *Papio* and *Theropithecus* diverged approximately 3-5 million years ago, and geladas are the sole remaining species within their genus (Jolly 2001; Zinner et al. 2009). Although *Theropithecus* species once ranged across Africa, southwest Asia, and the

Mediterranean, extant geladas are restricted to the highlands of Ethiopia (Jablonski 1993).

Geladas differ from their baboon relatives in their diet, social system, and physiology. First, although baboons are dietary generalists (Barton et al. 1996; Altmann et al. 1993), geladas are dietary specialists foraging nearly entirely on grass (> 90% of their diet) and the occasional small invertebrate (Fashing et al. 2010; Dunbar & Bose 1991; Hunter 2001). This diet likely facilitates their extremely large social aggregations (called *herds*) of greater than 1000 individuals (Kawai et al. 1983).

Second, although most baboons (except hamadryas baboons, *Papio hamadryas*, [Colmenares 1992; Kummer 1968; Schreier & Swedell 2009] and possibly Guinea baboons, *Papio papio*, [Galat-Luong et al. 2006]) live in mixed-sex groups with stable membership, geladas live in a modular society with strong fission-fusion dynamics (Dunbar & Dunbar 1975; Kawai 1979). This society can be organized in to 4 levels: (1) the *reproductive unit* (also called a “one-male” unit, Dunbar & Dunbar 1975; Kawai et al. 1983): a core group of related females (1-12) with their dependent offspring, a dominant “leader” male, and possibly 0-4 subordinate “follower” males that are found together 100% of the time; (2) the *team*: two to three units that spend at least 90% of their time together; (3) the *band*: a collection of 5-30 units that spend at least 50% of their time together; and (4) the *community*: a collection of all the units that share a home range (>100 units) and spend at least some time together (Kawai et al. 1983; Snyder-Mackler et al. 2012). Temporary aggregations of multiple units are referred to as *herds* (Kawai et al. 1983). Herds consist of reproductive units and *all-male groups* from multiple bands and may persist for a few hours to several days (Kawai et al. 1983). Both units and all-male groups are considered to be closed groups, with the majority of social interactions occurring within the unit or all-male group (Dunbar & Dunbar 1975; Dunbar 1983; Mori 1979).

Finally, although similar in body size and appearance to baboons, male and female geladas possess an hourglass-shaped bare patch of skin the chest and neck (Bergman & Beehner 2008). Female chest patch color varies from a dull pink to bright red and is lined with beaded vesicles (Dunbar & Dunbar 1974). The swollenness of the vesicles and color of the chest are hypothesized to indicate changes in reproductive cycle, similar to the swellings of female baboons (Dunbar & Dunbar 1974). Among male geladas, chest patch color is thought to be a sexually selected signal (Bergman et al. 2009). Dominant males possess the reddest chests, although it is unknown if redness is associated with male-male competition, female choice, or both (Bergman et al. 2009).

### *Gelada all-male groups*

Similar to their baboon relatives, female geladas remain in the natal units and males disperse. Prior to male dispersal, juvenile male and female geladas are often form temporary play groups (Dunbar & Dunbar 1975; Kawai 1979). During this time, juvenile males and females will play with each other during the day, but sleep with their natal units at night. In the latter juvenile stages (ages 3-5), males and females begin to segregate (Kawai 1979). Females remain in their unit during the day, and males form sex-segregated peer groups with kin and non-kin males. Peer groups of subadult males loosely associate with all-male groups of adult bachelors. Specifically, these subadult males will occasionally groom adult bachelors shortly after rising from their sleeping cliffs, but tend to forage during the day and sleep apart from the adult bachelors.

Gelada all-male groups consist of adult bachelor males (Dunbar & Dunbar 1975; Mori 1979), and are similar in size to reproductive units (mean size = 6.5, range 2-15, N = 28, Dunbar 1984). Although much is known of the nature of social relationships within reproductive units (le Roux et al. 2011; Dunbar 1979; Mori 1979; Dunbar 1983; Dunbar 1983), very little is known of social relationships of bachelors living within all-male groups. Only two brief studies have been conducted on bachelor male social relationships (Dunbar & Dunbar 1975; Mori 1979). Both of these studies qualitatively described relationships among bachelors as largely affiliative and lacking overt aggression within their groups (Dunbar & Dunbar 1975; Mori 1979). Within their all-male groups, adult bachelors were unable to be ranked in a linear dominance hierarchy, although older males may control group movements and command grooming from younger males (Dunbar & Dunbar 1975; Mori 1979). Despite the prosocial tendencies of bachelors within groups, interactions between bachelors and other males (leaders and followers, as well as extra-group bachelors) are largely hostile (Dunbar & Dunbar 1975; Mori 1979).

### *Gelada male reproductive competition*

To acquire reproductive success, a bachelor gelada must become a leader male. To do this, he must enter a reproductive unit by directly challenging and overthrowing a dominant leader male (i.e., “takeover”) or submissively entering as a follower (Dunbar 1984). Challenges for reproductive access to females for geladas generally take the form of ritualized chases (also called “yelping chases,” Dunbar & Dunbar 1975) that occasionally escalate to fights for dominance within a reproductive unit. When a chase escalates to fighting, the victorious bachelor will replace the leader male as the dominant animal in his reproductive unit (Dunbar & Dunbar 1975; Dunbar 1984). If a bachelor does not directly overthrow and replace a male as leader of a given unit, he may submissively enter a unit as a follower male or remain a bachelor (Dunbar 1984). If he enters as a follower, he may attempt to form an incipient unit by strengthening social bonds with young females (Dunbar 1984), as observed in hamadryas baboons (*P. hamadryas*) (Kummer 1968), although this has never been directly observed in our study population.

## STUDY SITE AND SUBJECTS

I conducted my dissertation research as part of the University of Michigan Gelada Research Project (UMGRP). In 2005, Dr. Jacinta Beehner and Dr. Thore Bergman established the UMGRP and habituated a population of wild geladas living in the Simiens Mountains National Park (SMNP), Ethiopia. The Simien Mountains is home to the largest community of geladas in Ethiopia, with a total population size estimated at 2460 geladas living within the park boundary (Beehner et al. 2007). The UMGRP actively studies approximately 200 individually recognized geladas at any given time. Individuals are habituated to observers on foot and have been under nearly daily observation since 2006. This gelada population ranges near the Sankaber region of the park, where the UMGRP research station is located. The majority of my thesis focuses on adult bachelors (N=58 bachelors, N=10 all-male groups) within our study community between 2007-2011.

The Simien Mountains National Park is located in northern Ethiopia and is listed as a UNESCO World Heritage Site (1978). The park is home to several endemic animal species including the Ethiopian wolf (*Canis simensis*), Walia ibex (*Capra ibex walie*), and the gelada (Beehner et al. 2007). It encompasses an area of Afroalpine habitat (170 km$^2$, 3200-4500 m.a.s.l.) including open grassland plateau and a few remnant forests. The region experiences pronounced “wet” (80% of annual rainfall) and “dry” seasons each year (Beehner & McCann 2008; Hunter 2001). The wet and dry seasons are variable, but generally occur from June-September and October-May, respectively (Beehner & McCann 2008). Temperatures in the Simien Mountains can approach freezing at night, but generally range from 8-17 ºC.

## RESEARCH QUESTIONS

In framing this thesis, I focus on two aspects of male gelada behavior that likely influence their respective reproductive trajectories: cooperation and competition. Compared to other Old World monkeys ( 1987), gelada males are peculiar in their high degree of tolerance for other males. Not only do bachelor geladas form stable all-male groups, but also unit males (leaders and followers) are regularly found in close proximity with extra-group males (Snyder-Mackler et al. 2012; Pappano et al. 2012). Leader males that tolerate the presence of followers within their unit achieve increased tenure length as dominant males (Snyder-Mackler et al. 2012). Additionally, these followers gain a fraction of reproduction, presumably in exchange for unit defense from bachelors (Snyder-Mackler et al. 2012). Taken together, cooperation among non-kin (and possibly kin) likely plays a significant role in the evolution of gelada male behavior.

In this dissertation, I answer four questions about the nature of male gelada social relationships. Specifically, I focus on the ‘cooperative’ interactions between bachelor geladas living in all-male groups, and the ‘competitive’ interactions between bachelors and leader males for access to reproductive units. My questions track the life of an adult bachelor from entry into an all-male group to his eventual graduation to a reproductive unit. I briefly describe each question and predictions below:

### *Question 1: What are the patterns of kinship and age structure within all-male groups?*

Previous research on geladas suggests that all-male groups form through the gradual inclusion of males that disperse solitarily from their natal groups (Dunbar 1984). This early research predates our ability to noninvasively understand how relatedness influences all-male group formation. Given that young males born in the same reproductive unit are likely members of a paternal age cohort (with an occasional case of full siblings as well), these males may disperse and join an all-male group together

(Altmann 1979) as a mechanism for ensuring a high degree of relatedness within all-male groups. If this is how all-male groups are formed, then I expect within all-male group relatedness to be higher than between all-male group relatedness. In agreement with previous studies, I expect all-male groups will be stable, with males only leaving to join reproductive units. Bachelors of all age classes will be found within all-male groups. I also predict that majority of males will be young adults and early prime-aged males as these males will soon compete directly for access to reproductive units (Bergman et al. 2009; Beehner et al. 2009). Finally, given the possible cohort effect of all-male group formation, I expect individuals within the same age class to be the most closely related.

### *Question 2: What are the social dynamics among bachelors within all-male groups?*

In their earliest research on geladas, Dunbar and Dunbar (1975) noted that bachelors formed “strong inter-personal bonds” and exhibited low levels of within group aggression. However, they did not quantify these relationships. I predict that bachelors will exhibit low levels of aggression and high levels of affiliation within their all-male groups. I do not expect bachelors to exhibit a dominance hierarchy, however given that older males may serve as “central” animals and command grooming from younger males (Dunbar & Dunbar 1975), I predict dominance will be associated with age, e.g. older males will outrank younger males, but males of the same age class will not differ in rank. I also predict bachelors will form preferential social bonds within their all-male groups and these bonds will be more likely between individuals of similar age and/or closely related individuals.

### *Question 3: How do bachelors interact with reproductive units?*

Bachelor males represent a threat to the reproductive success of both leader males and adult females. If a bachelor replaces a leader male within a reproductive unit, he not only ends the leader’s tenure, but he is likely to kill dependent offspring of lactating females (Beehner & Bergman 2008; Mori et al. 1997; Roberts et al. 2012). It stands to reason that both males and females within reproductive units should attempt to dilute the cost of associate with the potentially infanticidal bachelors. Through applying the “selfish herd” theory to gelada aggregations, I predict that reproductive individuals should “clump” together in response to bachelor encroachment. In other words, as bachelors approach adult males and females, these individuals, in turn, should move closer together.

*Question 4: What are the social and ecological factors that influence testosterone secretion in male geladas?*

For a male gelada, reproductive success depends on his ability to takeover a reproductive unit (Dunbar 1984). Previous research has shown that testosterone levels of male geladas peak sharply during the years they are mostly likely to be dominant leader males (Beehner et al. 2009). However it is unknown what social and ecological factors influence testosterone secretion over a year. Based on findings in other non-seasonally breeding primates (Gesquiere et al. 2011; Girard-Buttoz et al. 2009), I predict that status, age, rainfall, and temperature will influence gelada male testosterone. I expect that leader males will possess the highest testosterone levels. Similar to mandrills (*Mandrillus sphinx*) (Wickings & Dixson 1992) and chacma baboons (*Papio ursinus*) (Beehner et al. 2006), I expect testosterone will predict the trajectory of males. That is, bachelors that possess the highest testosterone levels will be most likely to takeover units.

## SUMMARY OF CHAPTERS

In this dissertation, I address these questions in five separate chapters that are either published in peer-reviewed journals [Chapter 2 (Pappano et al. 2010) and Chapter 5 (Pappano et al. 2012)] or in the process of being submitted for peer-review (Chapters

3, 4, & 6). Each chapter contributes new data to the understanding of gelada behavioral biology. Although I am the first author (or lead coauthor) on all chapters, each chapter is a collaborative effort with several coauthors. Notably, the genetic component of this research was completed in collaboration with my colleague, Dr. Noah Snyder-Mackler. Additionally, several members of the project collected field data used in this thesis, and individuals are acknowledged in each respective chapter. In the spirit of this collaborative effort, I use the pronoun "we" instead of "I" in all chapters (except in this first chapter where I summarize my individual contributions). Below I outline each chapter, and summarize the relevant findings.

Prior to beginning my fieldwork, I completed a series of methods experiments to optimize our lab group's fecal hormone collection procedure (Pappano et al. 2010). I summarize the results of these experiments in **Chapter 2** of this thesis. Four experiments were conducted to test the storage and extraction parameters of a previously described fecal hormone method (Beehner & Whitten 2004; Beehner & McCann 2008). Experiments were conducted under the supervision of my committee chair—Dr. Jacinta Beehner—in the Core Assay Facility at the University of Michigan. To simulate field conditions in a laboratory setting, frozen fecal samples from geladas living in the Bronx Zoo were used. Each experiment tested one aspect of our project's collection and storage protocol, e.g. time spent at ambient temperature or number of freeze/thaw cycles. Fecal samples were assayed for both testosterone and glucocorticoid metabolites in each experiment. This chapter was published in the *American Journal of Primatology* (Pappano et al. 2010), however we have recently conducted additional experiments that challenge our initial results. As a result I have included an addendum to these results in this chapter.

The remaining chapters of this thesis are derived from long-term data (2006-2011) from the UMGRP and my three field seasons in the Simien Mountains (May-July of 2008 and 2009, and approximately 11 months between 2010-11). In **Chapter 3**, I present a description of demography and social behavior bachelor gelada all-male groups associating with the UMGRP main study bands. Although previous research qualitatively described bachelor gelada social behavior (Dunbar & Dunbar 1975; Mori 1979), this is the first quantitative description of bachelor gelada demography and behavior. In this chapter, I answer several questions. First, what demographic factors (age, kinship, or both) influence all-male group formation? Second, do bachelors form stratified social relationships within all-male groups? In other words, do bachelors form social dominance relationships? My results indicate that relatedness alone predicts coresidence within an all-male group. Additionally, bachelors are extremely affiliative within their all-male groups, and dedicate more time to grooming than adult females. Finally, bachelors lacked strong social dominance relationships, and likely do not form linear dominance hierarchies within their all-male groups. I anticipate submitting this chapter to either the *American Journal of Primatology* or the *International Journal of Primatology.*

In **Chapter 4**, I build on my findings in Chapter 3 and discuss what factors contribute to social bond formation among bachelor geladas. Given that bachelors dedicate a considerable amount of their time to grooming, I hypothesized that they might form preferential social bonds with each other similar to that of male chimpanzees (Mitani 2009) and female baboons (Silk et al. 2003; Silk et al. 2006; Silk et al. 2006). In this chapter, I present evidence for male bonding in a male-dispersal primate species. Both age and relatedness influence bond formation among bachelor geladas. As expected, dominance rank did not influence bond formation. Bachelors that were both similar in age and closely related exhibited the strongest bonds. Moreover, bachelors with the strongest social bonds (*social bond strength*) also had very equal grooming relationships (*social bond equality*). Given these data, I suggest social bonds among bachelor geladas likely develop early in life possibly among paternal siblings. I also suggest that bonds may convey fitness benefits to male geladas, either through stress reduction or coalition formation against leader males. I hope to submit this chapter to either the *American Journal of Primatology* or the *International Journal of Primatology*.

In the final chapters of my thesis, I shift focus to broader patterns of social organization and male-male competition in geladas. To understand how all-male groups influence patterns of gelada social organization, I applied Hamilton's selfish herd theory (1971) to the spatial relationships among geladas in **Chapter 5**. Selfish herd theory predicts that animals aggregate in social groups to reduce their individual risk of predation (Hamilton 1971). Under this model, prey animals are expected to clump together as predators approach. Because bachelors threaten the reproductive success of unit individuals, I hypothesized that bachelors too might exert a "selfish herd" effect on reproductive male and female geladas. In this chapter, I ask whether or not the number and proximity of bachelor geladas influences herding (group size and fission-fusion dynamics) or spacing (inter-individual distances) of reproductive units. I discovered that both the presence and proximity of bachelors influences both the grouping and spacing pattern of reproductive male and female geladas (Pappano et al. 2012). I am a lead coauthor (with N. Snyder-Mackler) of this manuscript that was published in *Animal Behavior* (Pappano et al. 2012).

In **Chapter 6**, I apply the "challenge hypothesis" (Wingfield et al. 1990) to male gelada reproductive competition and testosterone secretion. The "challenge hypothesis" predicts testosterone is associated with aggression in the context of reproductive competition. Using both my own and long-term data from the UMGRP, I discovered that dominant leader males exhibit the highest levels of testosterone overall, however during periods of increased male replacement (or "takeover") bachelor testosterone rises higher than that of leader males. These takeovers tend to occur at the end of the dry season and exhibit a seasonal pattern. Moreover, I discovered that bachelors with high testosterone are more likely to become dominant males than bachelors with low testosterone. These findings suggest that testosterone secretion in geladas predicts a male's reproductive trajectory similar to male chacma baboons (Beehner et al. 2006) and mandrills (Wickings & Dixson 1992). I am in the process of submitting this chapter to *Hormones and Behavior*.

I summarize and present my findings of my thesis in my final chapter—**Chapter 7**. I discuss the relevance of these findings to broader studies of primatology, human evolution, and broader animal behavioral ecology. I also address future avenues of research concerning gelada male social bonds and reproductive trajectories.

# CHAPTER TWO

# TESTING EXTRACTION AND STORAGE PARAMETERS FOR A FECAL HORMONE METHOD

## ABSTRACT

Four experiments were conducted to test different aspects of a “field-friendly” fecal hormone extraction method that utilizes methanol extraction in the field followed by storage on C18 solid-phase extraction cartridges. Fecal samples were collected from geladas (*Theropithecus gelada*) housed at the Bronx Zoo, and experiments were conducted in a laboratory setting to ensure maximum control. Experiments were designed to either simulate conditions fecal samples are subject to during fieldwork or improve upon an existing protocol. Experiments tested the relationship between fecal hormone metabolite preservation/recovery and: (1) the amount of time samples were stored at ambient temperature; (2) the number of freeze/thaw cycles a sample undergoes; (3) the effectiveness of different extraction solutions; and (4) the effectiveness of different cartridge washes. For each experiment, samples were assayed by radioimmunoassay for fecal glucocorticoids and testosterone metabolites. Results for each of the experiments were as follows. First, storage at ambient temperature did not affect hormone levels until 4 weeks of storage; with significant increases for both glucocorticoid (GC) and testosterone (T) metabolites at 4 weeks. Second, hormone levels significantly decreased in samples after 2 freeze/thaw cycles for GCs and 6 freeze/thaws cycles for T. Third, for both GCs and T, hormone extraction using various methanol solutions was significantly higher than using 100% ethanol. Finally, using a

20% methanol solution to wash cartridges significantly increased GC levels but had no effect on T levels. These results suggest that, when utilizing C18 cartridges for fecal steroid storage, researchers should consider several methodological options to optimize hormone preservation and recovery from fecal samples.

## INTRODUCTION

With the arrival of non-invasive hormone sampling, the past decade has yielded a multitude of studies documenting steroid hormone profiles across a wide array of vertebrate taxa (see reviews: Schwarzenberger et al. 1996; Whitten et al. 1998; Wasser et al. 2000; Keay et al. 2006). Our ability to extract steroids from urine and feces ("excreta") has opened up a physiological window into hormone-behavior relationships that was previously unavailable. However, only rarely are these methods able to directly measure the hormone in question. Most steroid hormones are metabolized within the liver and excreted (via bile) into the gastrointestinal tract and (via the kidney) into the urine. Additionally, some of the hormone metabolites in the gastrointestinal tract are reabsorbed and transported back into the bloodstream. As a result, steroid hormones such as cortisol and testosterone are virtually absent in urine and feces, and methods that measure these hormones from urine and feces, are actually measuring one to several downstream products of steroid metabolism.

Consequently, any method that extracts steroid hormones from "excreta" must be properly validated for each new species prior to reporting hormone measures (Touma & Palme 2005; Palme 2005). In addition to analytical and physiological validation, however, other methodological concerns must be considered, such as how hormones are extracted from excreta and how and when samples are stored at sub-zero temperatures prior to assay. This is of particular concern for field primatologists who are limited in their storage and transport capabilities of fecal samples (Ziegler & Wittwer 2005). For example, most studies that have examined the effects of variables such as storage techniques (Khan et al. 2002; Lynch et al. 2003), environmental conditions to which samples are exposed (Millspaugh & Washburn 2004), within-sample hormone variation (Millspaugh & Washburn 2003), and dietary variation (Wasser et al. 1993) have found significant effects.

Here we test four different aspects of a “field-friendly” fecal hormone extraction method (Beehner & McCann 2008; Beehner & Whitten 2004) to test several methodological concerns. Using fecal samples collected from geladas (*Theropithecus gelada*) housed at the Bronx Zoo, we designed experiments to either simulate conditions fecal samples are subject to during fieldwork or improve upon an existing protocol. Two primary questions for field biologists who collect fecal hormone samples are: (1) how long can samples be stored at ambient temperatures prior to freezing? And (2) how many times can samples be thawed and refrozen? For example, we store samples at ambient temperatures at our fieldsite for variable time periods (from 1 day to 2 weeks) until they are transported to a freezer (located 1 hour away in a nearby town). Furthermore, this freezer is subject to infrequent power outages that could cause our samples to thaw. Therefore, we are particularly interested in how these two parameters affect hormone content. Additionally, we wanted to examine the effectiveness of two parameters of our current protocol – mainly the solutions used for extraction and preservation – by comparing them to alternatives. For example, ethanol is much more widely available in developing countries than methanol and acetone. Therefore, we were interested to see if ethanol could be used as a suitable replacement. In this study, we test the relationship between fecal hormone metabolite preservation/recovery and: (a) the time samples were stored at ambient temperature; (b) the number of freeze/thaw cycles a sample undergoes; (c) the effectiveness of different extraction solutions; and (d) the effectiveness of different cartridge wash solutions. We examine each of these parameters with respect to both fecal glucocorticoid (GC) and testosterone (T) metabolites.

## MATERIALS AND METHODS

### *Sample collection*

All fecal samples analyzed in this study were collected from captive geladas living at the Bronx Zoo (New York, NY). This research was approved by the Wildlife Conservation Society's Institutional Animal Care and Use Committee (IACUC). Fecal samples were collected from two adult males and two adult females over a period of two weeks. All samples were frozen immediately (-20 °C) at the Bronx Zoo and transported on dry ice to the Core Assay Facility in the Department of Psychology at the University of Michigan. Steroid hormone metabolites (GCs and T) were later extracted from fecal samples using an extraction method and radioimmunoassay (RIA) that has been analytically and physiologically validated for use in geladas (Beehner & McCann 2008; Beehner et al. 2009). The protocol for fecal steroid extraction was identical for each experiment in this study. However, for each experiment we modified one parameter of the protocol and compared results to the unmodified protocol, the "control" (see specific experiments below for details). Although we refer to the unmodified protocol as a "control", we recognize that it is not a true control (with known hormone content) but rather a relative control (from which variation will be measured). The complete fecal extraction protocol can be found elsewhere (Beehner & McCann 2008; Beehner et al. 2009), but we summarize it here.

### *Fecal steroid extraction*

Frozen fecal samples were incubated at room temperature until thawed and then mixed thoroughly using a metal spatula. An aliquot (~0.5 ml) of wet fecal material was

added to 3 ml of a methanol:acetone solution (8:2). Once in solution, samples were vortexed for 40 sec and then centrifuged at 3000 rpm for 10 minutes.
Immediately following centrifugation, 2.5 ml of fecal homogenate was filtered using a polytetrafluorethylene (PTFE) syringeless filter (0.2 $\mu$m; Whatman, Clifton, NJ). The filter was then washed with 0.7 ml of the methanol:acetone solution. The filtered homogenate was diluted with distilled water (di$H_2O$, 7.0 ml), capped tightly, and mixed. Tubes containing the original fecal matrix were then set aside to air dry under a hood. Once completely dry, the fecal matrix for each sample was weighed (±0.001 g) and recorded.

After priming Sep-Pak Plus C18 cartridges (Waters Associates, Milford, MA) according to the manufacture's instructions (2.0 ml 100% methanol followed by 5.0 ml of di$H_2O$), the sample filtrate was loaded by hand onto the cartridge at a steady rate (~ 0.2 ml/s) using a syringe. Cartridges were then washed with 2.0 ml of a 0.1% sodium azide solution (a preservative), placed in a sterile Whirl-pak bag with ~2.0 g of silica beads (a desiccant), and immediately frozen (-20 °C).

After cartridges were stored frozen for at least two weeks, they were incubated at room temperature for 1 hour. Steroids were eluted by hand with 2.5 ml of methanol (100%) using a syringe. After elution, samples were frozen for at least one week until the time of RIA. Samples were reconstituted in working buffer prior to RIA (buffer varied depending on the hormone assayed). For RIAs, all standards were run in triplicate and all samples were run in duplicate.

*Radioimmunoassays*

All samples were assayed for GC and T metabolites using commercially available RIA kits (Corticosterone $^{125}$I RIA Kit, MP Biomedicals; Testosterone $^{125}$I RIA kit, Diagnostics Systems Laboratories). The primary antibody from the corticosterone kit cross-reacts 100% with corticosterone, 0.34 % with desoxycorticosterone, 0.10% with testosterone, and 0.05% with cortisol (cross reactivity of the antiserum with other specific steroids is less than 0.03%). The primary antibody from the T kit cross-reacts 100% with testosterone, 6.6% with 5α-dihydrotestosterone, 2.2% with 5-androstane-3β, 17β-diol, 1.8% with 11-oxotestosterone, 0.9% with androstenedione, and 0.6% with 5β-dihydrotestosterone (cross reactivity of the antiserum with other steroids is less than 0.5%). Primary antibodies from both kits have been previously validated for use in geladas and were shown to be parallel, precise, and accurate (Beehner & McCann 2008; Beehner et al. 2009). For the GC assay, inter-assay coefficients of variation for a high (20% binding), middle (50% binding), and low (80% binding) fecal pool were 18.84%, 13.82%, and 7.02%, respectively (N=4). The intra-assay coefficient of variation for a middle fecal pool was 7.50% (N=10). For the T assay, inter-assay coefficients of variation for a high, middle, and low fecal pool were 7.01%, 10.79%, and 14.81%, respectively (N=3). The intra-assay coefficient for a middle fecal pool of variation was 6.72% (N=10).

### *Experiment 1 - Testing how long samples can be stored at ambient temperature*

Fifty aliquots ("samples") from a homogenized fecal pool (i.e., several fecal samples combined) were extracted as described above. After loading samples onto the cartridges, samples in the control group (N=10) were frozen immediately. Samples in the test groups (N=10, for each group) were stored at ambient temperature (23˚C) for 1-4 weeks in a dark location (Table 2.1). Each test group was subsequently frozen after 1, 2, 3, and 4 weeks of storage at ambient temperatures. After all cartridges were frozen for at least 2 weeks, samples from all groups were eluted and assayed together.

### *Experiment 2 - Testing how many times samples can be thawed and refrozen*

Fifty aliquots ("samples") from a homogenized fecal pool were extracted as described above. After loading samples onto the cartridges, all 50 samples were frozen immediately. Samples in the control group (N=10) remained frozen until the time of assay. Samples in the test groups (N=10 for each group) were thawed (incubated at 23˚C for 24 h) and subsequently refrozen 1-6 times (Table 2.1). We conservatively chose 24 h for the incubation period because this time period probably represents the maximum time that samples might be "thawed" under field conditions (if an outage occurs, power is generally restored within a day). After all freeze/thaw cycles were complete, all groups were eluted and assayed together. Note that all samples must be thawed to be eluted. For this experiment we only count the number of times samples were *refrozen* (i.e., 0 times for the control, and 1, 2, 4, and 6 times for the test groups).

### *Experiment 3 – Testing the effectiveness of different extraction solutions*

Forty aliquots ("samples") from a homogenized fecal pool were divided into four groups. Samples from the control group (N=10) were homogenized in a MeOH:acetone (8:2) solution as described above. Samples from the remaining three groups (N=10 per group) were homogenized in solutions of MeOH:$H_2O$ (9:1), MeOH:$H_2O$ (8:2), or EtOH, respectively. The rest of the extraction protocol was as described for all four groups. After loading samples onto the cartridges, samples were immediately frozen for two weeks, and all groups were eluted and assayed together.

### *Experiment 4 – Testing the effectiveness of different wash solutions*

Twenty aliquots ("samples") from a homogenized fecal pool were extracted as described above with one exception. After loading samples onto the cartridges, samples in the control group (N=10) were washed as usual with a 0.1% sodium azide solution (2 ml). Samples in the test group (N=10) were washed with a 20% MeOH solution (2 ml). All samples were immediately frozen for two weeks, and both groups were eluted and assayed together.

### *Data analysis*

Due to the possibility of uneven distribution of steroid hormone throughout the fecal pool even after homogenization{, we excluded all outliers (+/- 2SD) from our analyses (N=8 for GC and N=5 for T). For experiments 1 and 2, percent change was calculated as *$((a_n-x)/x)$,* where $a_n$ is the $n^{\text{th}}$ sample value in each experimental group and $x$ is the control mean. Nonparametric statistics were used in all analyses due to small sample sizes for each group. Specifically, Mann-Whitney U tests were used to determine which groups were significantly different from the control. Statistical significance was set at $\alpha=0.05$, and all analyses were conducted using SPSS 17.0.

## RESULTS

### *Experiment 1- Testing how long samples can be stored on C18 cartridges at ambient temperature*

Samples stored at ambient temperature remained stable for up to 3 weeks. However, samples kept at ambient temperature for 4 weeks had significantly higher hormone content for both GC (Mann-Whitney U test: U=17.0, $p<0.05$) and T metabolites (Mann-Whitney U test: U=19.0, $p<0.05$; Fig. 2.1a-b).

### *Experiment 2 - Testing how many times samples stored on C18 cartridges can be thawed and refrozen*

Samples exposed to repeated freeze/thaw cycles exhibited decreased levels of hormone content for both GC and T metabolites. This effect was more pronounced for GC metabolites. Glucocorticoids from samples exposed to 2 freeze/thaw cycles exhibited significantly less hormone content than controls (Mann-Whitney U test: U=14.0, $p<0.05$; Fig 2.2a). An even greater decrease in hormone content was observed in GC samples exposed to 4 (Mann-Whitney U test: U=10.0, $p<0.01$) and 6 freeze/thaws

(Mann-Whitney U test: U=10.0, $p<0.01$). For T metabolites, a significant decrease in hormone content was observed only for samples exposed to 6 freeze/thaw cycles (Mann-Whitney U test: U=14.0, $p<0.05$; Fig 2.2a-b).

*Experiment 3 – Testing the effectiveness of different extraction solutions*

Extraction recovery was significantly different across the solutions tested for both GC and T metabolites. The MeOH:$H_2O$ (8:2) solution extracted significantly more GC metabolites (Mann-Whitney U test: U=18.0, $p<0.05$) and less T metabolites (Mann-Whitney U test: U=13.0, $p<0.01$) than the control solution (MeOH:acetone). Additionally, 100% EtOH extracted significantly less GC (Mann-Whitney U test: U=0.0, $p<0.001$) and T metabolites (Mann-Whitney U test: U=0.0, $p<0.001$; Fig. 2.3a-b).

*Experiment 4 – Testing the effectiveness of different cartridge wash solutions*

For GC metabolites, cartridges washed with different solutions demonstrated significant differences in hormone content with the 20% MeOH solution resulting in more GC metabolites than the control solution (0.1% sodium azide; Mann-Whitney U test: U=17.00, $p<0.05$). No differences were observed between the different wash solutions for T metabolites (Mann-Whitney U test: U=37.00, $p=0.326$).

## DISCUSSION

The first two experiments aimed to simulate field storage conditions in a controlled laboratory setting. Other researchers report that extracted hormones are stable at ambient temperatures for up to 2 weeks if stored in 100% ethanol (Khan et al. 2002). However, if samples could be stored for longer periods time at ambient temperature then researchers would not need to more than necessary trips to urban areas to ship or store samples. Furthermore, due to the potential for power-outages (particularly in developing countries) and the necessity of shipping samples from a fieldsite to a laboratory, samples can be subject to repeated freeze/thaw cycles. Therefore, the first two experiments sought to address these concerns.

With respect to storage at ambient temperature, our results demonstrated that fecal hormone metabolites (GCs and T) were stable at ambient temperature for up to 3 weeks, but by 4 weeks of storage both GC and T levels were significantly higher than the control group (Fig. 2.1). A previous study using the same method also reported that time spent at ambient temperature affected hormone metabolites across a similar time-frame (Beehner & Whitten 2004). However, the results from this previous study differed from the current one in two ways. First, only GCs were observed to change across storage time (not T); and second, GCs were observed to *decrease* with time spent at ambient temperature while in the current study both GC and T metabolites were observed to *increase*. We suspect that these differences might be due to differences in methodology (a correlation study versus a controlled study) and location (field versus laboratory). Although our sample size was small (N=10 per group), all aliquots ("samples") in the current study were derived from a single homogenized fecal pool. By contrast, the previous study (with a much larger sample size, N=728) reported whether there was a trend towards increasing or decreasing hormone content based on samples derived from different individuals (Beehner & Whitten 2004). Additionally, the extraction protocol used in the current study was slightly modified from the previous one to increase hormone recovery (the new protocol increased recovery by an average of 34%; Beehner, unpublished data). Finally, the ambient temperature across our experiments was always 22˚C, while ambient temperature for the previous study ranged from approximately 22-35˚C (Beehner, unpublished data).

At present, we are uncertain why hormone content increased (rather than decreased) after 4 weeks of storage. It is highly unlikely that contamination caused the increased hormone content, as all samples were stored individually in Whirl-pak bags.

One possibility is that samples stored longer at ambient temperatures suffer increased hormone degradation that actually result in more metabolites cross-reacting with antibodies from the RIA kits. Resolving this issue will require high performance liquid chromatograph (HPLC) to determine which metabolites are present at the various storage times. A second possibility is that “drier” samples (i.e., those stored longer at ambient temperature with the silica) yield higher hormone recoveries at the time of elution. We have some data to suggest that semi-“wet” samples (i.e., samples that are immediately frozen) do not elute properly from the cartridges (Beehner, unpublished data), and this could be largely attributed to the aqueous wash solution. Importantly, however, hormones stored at ambient temperatures for 1 day to 3 weeks exhibited no differences from the control group. A third possibility is that both gelada GC and T metabolites are conjugated when they are excreted in fecal samples. If prolonged storage at ambient temperature breaks steroid conjugates, then our control may be pseudo-reduced with respect to overall hormone content (Ziegler & Wittwer 2005).

With respect to freezing and thawing, our results suggest that samples can be exposed to at least one freeze/thaw cycle for GCs and up to 4 freeze/thaw cycles for T metabolites before hormone content is significantly different from control groups. Taken together, this suggests that T metabolites might be more stable on C18 cartridges than GCs are. Note however that we allowed all samples to incubate (“thaw”) for 24 hours, which may over represent how long samples in the field are left to thaw. It only takes about 1-2 hours for samples to fully thaw, thus, a shorter thaw period may result in less of a difference from the control. Although one freeze/thaw cycle does not seem to affect hormone content, we nevertheless recommend that researchers using C18 cartridges to store fecal extracted hormones take pains to avoid freeze/thaw cycles altogether.

The last two experiments tested the effectiveness of different extraction/wash solutions to optimize hormone recovery and/or capitalize on chemicals that are more easily obtained in developing countries (mainly, EtOH). The first of these (*Experiment 3*) tested how well different organic solutions extracted steroid hormones from the fecal matrix. We found no difference between the 100% organic control solution (MeOH:acetone; 8:2) and the 10% aqueous solution (MeOH:$H_2O$; 9:1). By contrast, the 20% aqueous solution (MeOH:$H_2O$; 8:2) extracted significantly *more* GC metabolites and significantly *less* T metabolites than the control. We suspect that the more aqueous extraction solution (MeOH:$H_2O$; 8:2) is better at extracting more polar metabolites (such as GCs or conjugated metabolites (Ziegler & Wittwer 2005), while the more organic solutions are better at extracting less polar metabolites (such as T and unconjugated metabolites). Surprisingly, 100% EtOH extracted significantly less hormone metabolites for both GC and T when compared to the control solution, possibly due to the stronger polarity of methanol (δP=12.3) compared to ethanol (δP=8.8). By contrast, a previous study of fecal GC metabolite extraction in Belding's ground squirrels (*Spermophilus beldingi*) demonstrated no difference in extraction efficiency between MeOH (80% and 100%) and EtOH (80% and 100%) solutions (Mateo & Cavigelli 2005). We suspect that the "best" extraction solution is probably both species- and hormone-specific, and depends on the percentage of conjugated to unconjugated hormones present in excreta for a given species. For example, while most laboratories use at least a 30% solvent (ethanol or methanol), many use a mixture of solvent and aqueous solution to maximize extraction of both unconjugated steroids and conjugated steroids (Ziegler & Wittwer 2005). Although we do not yet know the percentage of unconjugated to conjugated hormones (GCs or T) for geladas, for baboons (*Papio cynocephalus*), a sister-taxa to geladas, Wasser and colleagues (2000) found that less than 20% of the cortisol metabolites found in fecal excreta consisted of hydrolyzable conjugates.

Lastly, we examined the effect of two different wash solutions on hormone preservation (*Experiment 4*). Although there was no difference between a 100%

aqueous sodium azide solution and an 80% aqueous methanol solution for T metabolites, we found that GC metabolites increased when the wash solution was less aqueous (80% aqueous). This difference might be due to the more polar structure of GCs that might get partially eluted from the cartridge during a 100% aqueous wash. It is also possible that GC are degraded more due to the absence of sodium azide (a known hormone preservative).

Fecal hormone values will never be “absolute” measures of circulating hormone levels, but rather are downstream products of steroid hormone metabolism that must be considered as relative hormone measures. Nevertheless, relative hormone measures still provide researchers with a mechanism for testing physiological hypotheses in wild animals. Therefore, perhaps the most important methodological consideration with such non-invasive methods is consistency across samples and across time to keep such relative measures comparable. With respect to chemical extractions and dry storage on C18 cartridges, we recommend the following: (1) experiment with different percentages of organic to aqueous solutions to maximize hormone extraction, and (2) for long-term preservation in the field use a 100% aqueous, sodium azide solution for the wash. (3) Freeze samples 1-3 weeks after collection (allowing samples to sit for at least a few days with silica to allow the cartridge to dry), and (4) minimize freeze/thaw cycles to one cycle (at most) prior to the final thaw before eluting samples from the cartridges. We are currently exploring whether different HPLC-separated fractions of steroid hormone metabolites are differentially affected by freezing-and-thawing or prolonged ambient temperature storage.

It is important to note that all methodological considerations should take into account the expected biological patterns for the species in question. In other words, if a researcher expects large differences between study groups (e.g., differences between sexes, reproductive stages, or times of the year), then biological patterns might easily override any methodological effects. However, if researchers are interested in subtle differences (e.g., differences in basal levels across same-sex, same reproductive stage individuals), then methodological concerns will be of primary importance.

## ADDENDUM

Although this chapter has already been published (Pappano et al. 2010), we did not fully understand the relationship between wash (20% MeOH and sodium azide) and hormone storage (see Experiment 4). As noted, sodium azide appeared to influence glucocorticoid storage more so than testosterone metabolite storage. Indeed, our latter experiments demonstrate that when cartridges are not washed sodium azide, the linearity of our glucocorticoid assay is disrupted. This does not affect testosterone metabolites, but impacted several goals of my dissertation research including examining the relationship between stress and social bonds.

**Table 2.1.** A list of experiments conducted under controlled laboratory settings. Numbers indicate sample sizes for each hormone metabolite assayed

| Experiment 1 | | |
|---|---|---|
| Time stored at ambient temperature | T | GC |
| Control (frozen immediately) | 10 | 10 |
| 1 week | 10 | 10 |
| 2 weeks | 10 | 9 |
| 3 weeks | 10 | 10 |
| 4 weeks | 9 | 7 |
| **Experiment 2** | | |
| Number of freeze/thaw cycles | T | GC |
| Control (never frozen and thawed) | 9 | 9 |
| 1 freeze/thaw | 10 | 10 |
| 2 freeze/ thaws | 10 | 10 |
| 4 freeze/thaws | 10 | 10 |
| 6 freeze/thaws | 9 | 9 |
| **Experiment 3** | | |
| Extraction solution | T | GC |
| Control (MeOH:Acetone, 8:2) | 9 | 10 |
| MeOH:H2O (9:1) | 10 | 10 |
| MeOH:H2O (8:2) | 10 | 9 |
| EtOH | 10 | 10 |
| **Experiment 4** | | |
| Cartridge wash | T | GC |
| Control (0.1% Sodium Azide solution) | 10 | 10 |
| 20% MeOH | 9 | 9 |

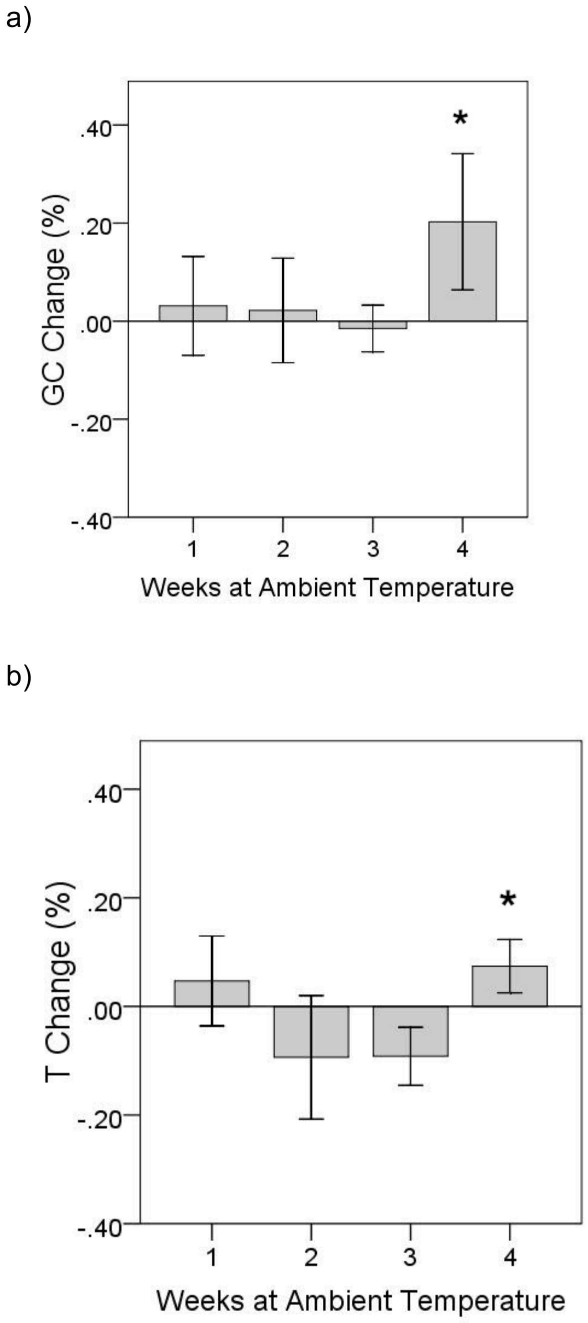

**Figure 2.1.** Change in hormone content from control group (% ± 2 SEM) for samples stored at ambient temperature (22°C) for 1-4 weeks for (a) fecal glucocorticoid (GC) metabolites and (b) fecal testosterone (T) metabolites. (* P<0.05).

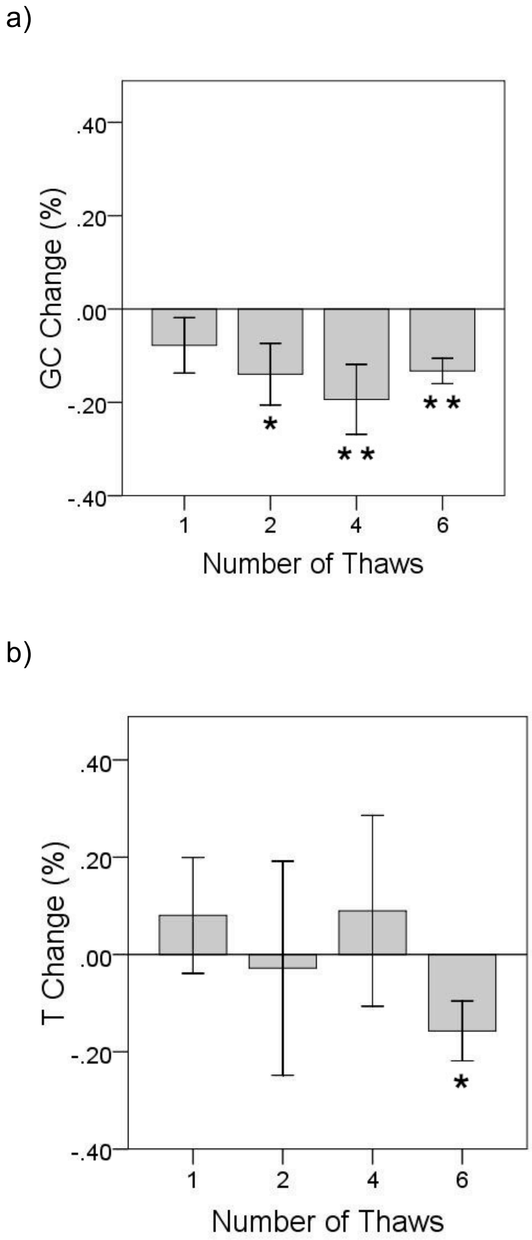

**Figure 2.2.** Change in hormone content from control group (% ± 2 SEM) for samples undergoing 1-6 freeze/thaw cycles for (a) fecal glucocorticoid metabolites (GC) and (b) fecal testosterone metabolites (T) (* P<0.05; ** P<0.01)

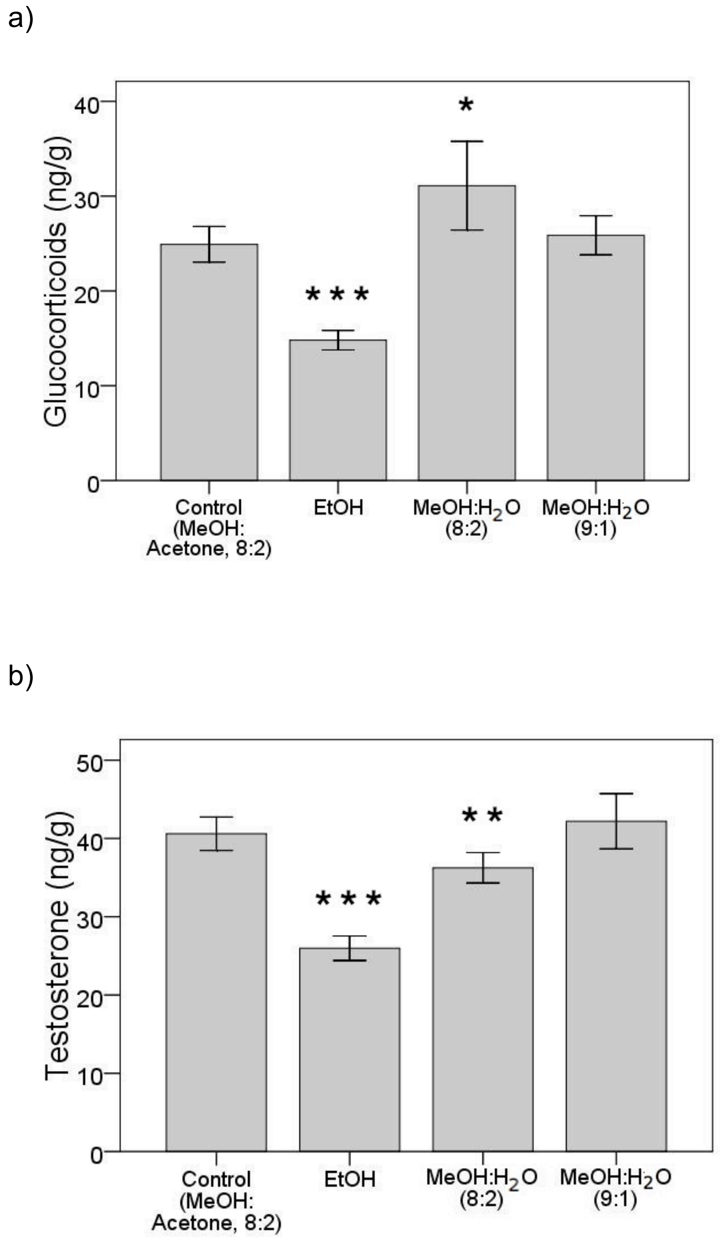

**Figure 2.3.** Mean values (± 2 SEM) for samples extracted using four different solutions for (a) fecal glucocorticoid metabolites and (b) fecal testosterone metabolites (* $P<0.05$; ** $P<0.01$; *** $P<0.001$).

# CHAPTER THREE

# DEMOGRAPHY AND SOCIAL BEHAVIOR OF BACHELOR GELADAS LIVING IN ALL-MALE GROUPS

## ABSTRACT

Affiliative relationships are rare among male mammals. Although affiliative relationships are more common among male primates, few species exhibit exclusive subgroups of bachelor males (called all-male groups or all-male bands). Unlike mixed-sex groups, little is known about how all-male groups form or the nature of social relationships between bachelors comprising them. After dispersing from their natal units, male geladas (*Theropithecus gelada*) join all-male bachelor groups. Here we present demographic, behavioral, and genetic data to describe the formation and social structure of gelada all-male groups. Data were collected from a population of bachelor geladas (N=58 males, 10 all-male groups) living in the Simien Mountains National Park, Ethiopia. In agreement with earlier studies, males of all ages live in all-male groups suggesting they form through gradual inclusion of young males as older males emigrate or die. Transfer between groups was rare, but occurred when a single group member remained after the death or disappearance of group mates. Additionally, relatedness predicted coresidence within an all-male group indicating males possibly disperse together from their natal units or bands. Within all-male groups, bachelors exhibited high levels of affiliative behavior and lacked age-based dominance relationships. We suggest affiliative relationships among bachelor geladas may convey fitness benefits such as predator

avoidance, reduced stress levels, or coalitionary support during aggression with dominant males.

## INTRODUCTION

Behavioral ecological theory predicts that individuals will form social groups when the net benefits of association outweigh the costs (Silk 2007). Group living confers benefits to individuals such as predator protection (Hamilton 1971), mate acquisition (Andersson 1994), cooperative foraging/hunting (Packer & Ruttan 1988; Wenzel & Pickering 1991), communal defense (Port et al. 2011), and information sharing (Byrne et al. 2004). However, group living also places individuals within close proximity, increasing the likelihood they will compete for scarce resources (Kappeler & van Schaik 2002; Clutton-Brock et al. 2006). For example, males living in social groups must compete with each other for mating opportunities (Andersson 1994). As fertilizations cannot be shared, sexual selection has shaped male behavior to be competitive rather than affiliative (Van Hooff & Van Schaik 1994; Kappeler 2000). In species where dominant males can exclude rivals from mixed-sex groups, males may form "bachelor" groups that vary in their stability, structure, and social dynamics (Yamagiwa 1987; Clapham et al. 1992; Waterman 1997; Chiyo et al. 2011; Heitor & Vicente 2010). Given the focus of many behavioral ecology studies on male-female or female-female relationships, these all-male groups (also called "all-male bands" (Ross 1993), or "bachelor groups" (Heitor & Vicente 2010) are less understood compared to mixed-sex groups of the same species. Here, we describe the demography and social dynamics of a population of bachelor geladas (*Theropithecus gelada*), a gregarious Old World monkey, living in all-male groups.

All-male groups are predicted to convey various fitness benefits to males that comprise them. These groups vary in their persistence with some all-male associations being only temporary (e.g., elephants, Chiyo et al. 2011) and others being stable and associated with a bachelor life-history stage (e.g., geladas, Dunbar & Dunbar 1975; Kawai 1979). Several mammalian species possess all-male groups including: humpback whales (*Megaptera novaeangliae*) (Clapham et al. 1992), sperm whales (*Physeter macrocephalus* (Lettevall et al. 2002), Cape ground squirrels (*Xerus inauris*) (Waterman 1997), plains zebra (*Equus burchelli*) (Fischhoff et al. 2009), horses (*Equus ferus caballus*) (Tilson et al. 1988; Heitor & Vicente 2010), and elephants (*Loxodonta africana*) (Chiyo et al. 2011). Males of these species may associate with each other for a variety of reasons including information sharing, predator protection, or coalitionary support against other males. For example, among African elephants older males occupied a central role in their social network suggesting older males are sources of ecological and social information for younger males (Chiyo et al. 2011). Additionally, association with other males may increase protection against predators through mobbing (Ross 1993) or against harassment from extra-group males (Steenbeck et al. 2000). Life in a bachelor group may also be a low-cost alternative for maturing males rather than facing harassment from dominant males within a mixed-sex group (Yamagiwa 1987; Robbins 1996). Males may also benefit from association through practicing aggressive skills with familiar individuals (Chiyo et al. 2011), or mutual aggression against dominant males (Levréro et al. 2006; Rajpurohit 1997), both of which may improve a bachelor's chances of acquiring future mating opportunities.

In primates, all-male groups are often semi-stable associations of bachelors, consisting mostly of young pre-reproductive males (Dunbar & Dunbar 1975; Rajpurohit 1995; Steenbeck et al. 2000; Yamagiwa 1987). All-male groups are observed in all major primate taxa including New World monkeys: red uakari (*Cacajao calvus ucayalii*) (Bowler & Bodmer 2009); Old World monkeys: gelada (*Theropithecus gelada*) (Dunbar & Dunbar 1975; Kawai 1979), Hanuman langurs (*Semnopithecus entellus*) (Rajpurohit 1995;

Rajpurohit 1997; Schülke 2001), Thomas's langurs (*Presbytis thomasi*) (Steenbeck et al. 2000), Mona monkeys (*Cercopithecus mona*) (Glenn et al. 2002), snub-nosed monkeys (*Rhinopithecus* spp.) (Grueter & van Schaik 2010), proboscis monkeys (*Nasalis larvatus*) (Murai 2004); and apes: mountain gorilla (*Gorilla gorilla beringei*) (Yamagiwa 1987; Yamagiwa 1992; Robbins 1996; Watts 2000). Although, all-male groups have been reported in all of these species, few studies have focused exclusively on all-male groups (but see Rajpurohit 1995; Rajpurohit 1997; Yamagiwa 1987; Yamagiwa 1992; Robbins 1996) hence little is known of the factors that contribute to group formation or of the social relationships among males within all-male groups.

In this study, we examine the nature of all-male group formation and social dynamics among bachelor geladas. Geladas are a sister taxon to the more common baboons (*Papio* spp.), but differ markedly in both social structure and ecology (Dunbar & Dunbar 1975; Jablonski 1993; Kawai 1979). For example, baboons live in mixed-sex groups with stable membership, where as geladas live in extremely large (> 1000 individuals) modular societies with flexible membership (Kawai et al. 1983; Snyder-Mackler et al. 2012). This modular (or multilevel) society consists of four levels: (1) the reproductive unit (or "one-male" unit, Kawai et al. 1983; Dunbar & Dunbar 1975): a mixed-sex core group of related females, a dominant "leader" male, and possibly subordinate "follower" males that spend 100% of their time together; (2) the team: 2-3 units that spend > 90% of their time together; (3) the band: 5 - 30 units that spend > 50% of their time together; and (4) the community: all the units that share a common home range (Snyder-Mackler et al. 2012). Juvenile geladas form peer groups that segregate according to sex, with males eventually dispersing from their natal units (Dunbar & Dunbar 1975; Kawai 1979). Adult males that are excluded from reproductive units form all-male "bachelor" groups (Dunbar & Dunbar 1975; Mori 1979). All-male groups are similar in size to reproductive units and considered to be closed groups, i.e. the majority of social interactions occur within the all-male group (Dunbar & Dunbar 1975). Bachelor groups are hypothesized to exhibit stable membership, with young males joining a group shortly after dispersing from their natal unit (Dunbar & Dunbar 1975; Dunbar 1984). These males remain in their all-male group until they takeover a unit of their own (Dunbar & Dunbar 1975; Dunbar 1984). Temporary aggregations of geladas are called herds, and may consist of units from multiple bands and all-male groups (Kawai et al. 1983; Snyder-Mackler et al. 2012). Units and all-male groups may fission and fuse over the course of day, or remain together for several weeks, creating the flexible patterns of association observed in gelada herding (Kawai et al. 1983; Snyder-Mackler et al. 2012).

Geladas are female philopatric, and females within reproductive units form stratified social relationships with each other (le Roux et al. 2011; Dunbar 1979). Less is known of how all-male groups form or of the social relationships among bachelors within them. Dunbar (1984) hypothesized that all-male groups arise from either the gradual inclusion of young males after they disperse from their natal units, or through the maturation of an entire group of young males that have previously associated with each other (i.e. members of the same peer group). He suggests the first hypothesis is more likely, as all-male groups exhibit greater age diversity than peer groups (Dunbar 1984). Therefore, it is unlikely that similarity in age plays a strong role in all-male group formation (Dunbar 1984). However, it is unknown what role, if any, kinship plays in all-male group formation. Because gelada males are the dispersing sex, it is unlikely that they recognize paternal kin outside of a common rearing environment (i.e. within the same unit) (Widdig 2007). It is possible that male siblings from the same unit may disperse together if they are both similar in age (Altmann 1979). This pattern of parallel dispersal among males is common in litter-born species, such as lions (*Panthera leo*) where male siblings associate into adulthood (Packer & Pusey 1982). Therefore, it is probable that all-male groups include close kin if these kin are also similar in age and dispersed from their natal unit together.

Although much is known of social relationships within mixed-sex units of geladas (Dunbar 1979; Mori 1979; Dunbar 1983; Dunbar 1983), few studies have examined the nature of social relationships within all-male groups (Dunbar & Dunbar 1975; Mori 1979). In a brief study of all-male groups, Dunbar and Dunbar (1975) suggested that prime-aged bachelors act as “control’ animals and command the majority of grooming within a group, particularly from younger males. However, adult bachelors could not be ranked in accordance with a linear and transitive dominance hierarchy (Dunbar & Dunbar 1975). In a corroborating study, Mori (1979) determined that within all-male groups, bachelors lacked strong and readily apparent dominant relationships. Both studies concluded that the majority of social interactions among bachelors are affiliative, not agonistic (Dunbar & Dunbar 1975; Mori 1979). However, neither study quantified the degree to which bachelors are affiliative with the exception of a single all-male group (Dunbar & Dunbar 1975). Therefore, these observations may not reflect the variation in bachelor social dynamics within or between groups.

We sought to answer two main questions regarding the demography and social dynamics of all-male groups. First, what are the demographic and genetic factors that are associated with the formation of gelada all-male groups? We predict that both kinship and age influence all-male group formation. Because males may disperse together, we expect within all-male group relatedness to be higher than between all-male group relatedness. All-male groups will be stable, with males only leaving to join reproductive units. Based on earlier studies (Dunbar 1984; Dunbar & Dunbar 1975; Mori 1979), we expect bachelors of all age classes to be found within all-male groups. However, we predict that majority of males will be young adults and early prime-aged males as these males will soon compete directly for access to reproductive units

(Bergman et al. 2009; Beehner et al. 2009). We also predict that within all-male groups, individuals of the same age class will be closely related. Second, do bachelors form stratified social relationships within their all-male group? Again, based on previous research (Dunbar & Dunbar 1975; Mori 1979), we expect bachelors to exhibit low levels of aggression and high levels of affiliation within their all-male groups. We do not expect bachelors to exhibit a dominance hierarchy, however given that older males may serve as "control" animals and command grooming from younger males (Dunbar & Dunbar 1975), we predict dominance will be associated with age, e.g. older males will outrank younger males, but males of the same age class will not differ in rank. Finally, we predict that bachelors will be very affiliative within their all-male group. To quantify this relationship we will compare grooming budgets between bachelors and adult unit females, and we expect bachelors will groom as frequently as adult females.

## METHODS

### *Study site and subjects*

Data were collected over an 18-month period (Jul 2010 – Dec 2011) from a habituated population of wild geladas living in the Simien Mountains National Park, Ethiopia (13°18'N, 38°06'E, 3200-4500 m.a.s.l.) as part of the long-term University of Michigan Gelada Research Project. Demographic, behavioral, and genetic data were collected from 58 individually recognized adult bachelors living in 10 all-male groups. Given the male-biased dispersal pattern of geladas and the limited duration of current demographic records (< 6 years), we do not have known birthdates for most of the adult males in the population. Therefore, the ages of all males were estimated to the nearest half year using secondary sexual characteristics such as canine eruption, tooth wear, pelage coloration, and cape length (Bergman et al. 2009; Beehner et al. 2009).

### *Composition of all-male groups*

For each all-male group, we recorded the initial group size and the estimated age for all adult bachelors. Because all-male groups may change in composition, we recorded the dates for all immigration and emigration events, as well as any known deaths for bachelors in all-male groups. As subadult males loosely associate with fully adult bachelors (Dunbar & Dunbar 1975; Kawai 1979), we added recently matured males to an all-male group once they were considered to be “young adults” (6.5 years old), i.e. exhibited full canine eruption and adult stature (Bergman et al. 2009; Beehner et al. 2009). We used these demographic events (immigration, emigration, maturation, and death) as a natural way to partition group membership rather than choosing arbitrary periods such as months or years. These periods of stable group composition between demographic events (hereafter, *stable periods*) were used to calculate rates of behavior (see below). Because bachelors eventually emigrate from their all-male groups and entered reproductive units to become dominant (leader) or subordinate (follower) males, this method allowed us to easily account for differences in sociality among bachelors attributable to changes in group size or composition.

Whenever a bachelor was observed to be missing from his all-male group, we checked all reproductive units within the gelada herd to determine if he had emigrated to a reproductive unit or transferred to another all-male group. Due to scavenger activity and the inaccessible geography of gelada habitat, it was often impossible to determine if missing bachelors were indeed dead or simply joined an unhabituated all-male group or unit. However, we declared bachelors deceased if they were not observed for 6 weeks and if their former group mates were regularly observed during that time period.

### *Genetics*

To estimate bachelor relatedness, we collected fecal samples from all bachelors (1-2 samples per male) and stored them in RNA later for subsequent DNA extraction and genotyping (see (Snyder-Mackler et al. 2012) for full description of methods and extraction). In brief, we genotyped extracted DNA using polymerase chain reactions at 23 human derived MapPairs microsatellite loci (20 described in (Snyder-Mackler et al. 2012), as well as D4s243, D11s2002, D10s1432 in Tinsley Johnson et al., accepted), which were found to be variable in this gelada population (average number of alleles/locus = 5.91). We successfully genotyped 33 of the 58 bachelors at 90% of the loci. We used Wang's pairwise relatedness estimator ($r_w$) in all analyses (Wang 2002) to estimate relatedness because it provided the best estimate of relatedness between individuals of known pedigree in our population (e.g., for paternal half-siblings $r_w \sim 0.25$). Although Wang's pairwise relatedness estimator ($r_w$) is associated with error for individuals within unknown or shallow pedigrees, previous research has found it to be robust in our study population for known and unknown relationships (Tinsley Johnson et al., accepted).

### *Bachelor dominance relationships*

Dominance interactions among geladas are rare (Dunbar & Bose 1991; Fashing et al. 2010), therefore we combined behavioral data from both focal animal and *ad libitum* sampling to measure social dominance relationships among bachelors. We applied a previously established ethogram of dominance interactions from female geladas that included both vocalized and non-vocalized behaviors and postures of dominance and submission (le Roux et al. 2011)(Tinsley Johnson et al., accepted). We denoted a bachelor the "winner" of a dominance interaction if they displayed stereotypical behaviors (displacement, vocalized threats, contact aggression) associated with dominance, and the other bachelor a “loser” if they displayed behaviors associated with submission (fear grimace, cower, fear bark) (le Roux et al. 2011).

We used an Elo-rating system to assign dominance ranks to bachelors (Elo 1978; Neumann et al. 2011). Elo-ratings are a progressive evaluation method commonly used to rank competitive chess players (Elo 1978) that has recently been applied to studies of animal social dominance (Rusu & Krackow 2004; Pörschmann et al. 2010; Albers & de Vries 2001; Neumann et al. 2011)(Tinsley Johnson et al., accepted). Unlike other matrix-based dominance ranking methods (e.g. David’s scores (David 1987; Gammell et al. 2003), Clutton-Brock’s index (Clutton-Brock et al. 1982), and others: see (de VRIES 1998) for review and (Bayly et al. 2006) for a comparison of methods), Elo-ratings are not sensitive to group size or composition (Albers & de Vries 2001; Neumann et al. 2011). Thus, they are ideal for bachelor geladas that live in small social groups (often < 5 individuals) that may change in composition.

The main difference between Elo-rating and other methods is that an individual’s Elo-rating is based on the sequence in which dominance interactions occur (Elo 1978; Neumann et al. 2011). Individuals are assigned an initial starting value when they enter a social group and adjusting their score by a constant (k) modified by the probability of winning a dominance interaction (Albers & de Vries 2001; Neumann et al. 2011). For each interaction, “winners” gain an adjusted value of points, where as “losers” have an adjusted value subtracted from their score. Therefore, Elo-rating can be used to determine an individual’s dominance rank on any given day or their rank trajectory over time (Albers & de Vries 2001; Neumann et al. 2011).

Due to variation in dominance styles across species, Elo-rating parameters should be modified for each species (Neumann et al. 2011). We use Tinsley Johnson et al. (submitted) parameters for female geladas and set each bachelor’s starting value to 1000 and k=100. As Elo-ratings are a sequential ranking method they require a “start-up” time of approximately 9 interactions to reflect the true ranks of individuals (Neumann et al. 2011). Therefore we eliminated all individuals with less than 9 interactions from our analysis.

### *Bachelor grooming relationships*

To assess grooming relationships among bachelors, we compared the proportion of time bachelors dedicate to grooming to previously published data on adult female gelada grooming (Tinsley Johnson et al., accepted). We used 15-minute focal animal sampling (N = 453 hours, ~4 hours per male, range 1-13) to record all affiliative behavior including grooming bouts and duration. For each pair of males living in the same all-male group at the same time (hereafter *coresident dyad*), we calculated a proportion of grooming time ($G_{ab}$). This value ignores directionality between coresident dyad, and is directly comparably to adult female grooming data (N=656 dyads, Tinsley Johnson et al., accepted).

### *Data analysis*

To determine if age or relatedness influenced all-male group formation we used a generalized linear mixed model (GLMM) fit with a binomial error structure. Our outcome variable was coresidence (*coresident v. not coresident*) in the same all-male group for a given unique dyad (N=542). We included relatedness ($r_w$) and whether or not the individuals belonged to the same age class (*same v. different*) as fixed effects in the each model. Dyads were considered to be in the same age class if their estimated age difference was less than 1.5 years (Bergman et al. 2009; Beehner et al. 2009). Due to differing group sizes, individuals appear in different frequencies in our models. Therefore, we included the identity of each member of a unique dyad as random effects in our model. We compared two univariate (*age class only* and *relatedness only*) models to two multivariate models (an *additive model* including both age class and relatedness, and an *interactive model* including age class, relatedness, and an interaction between age class and relatedness) using Akaike Information Criterion (AIC) (Akaike 1973). We considered the model with the lowest AIC value to be the best fit for our data set (Garamszegi et al. 2009; Symonds & Moussalli 2011). If the difference in AIC between two or more models less than 2, we considered them all as equally good fits for our data (Symonds & Moussalli 2011). All GLMM analysis was conducted using the R package “lme4” and the function “lmer” (Sarkar & Bates 2009; R Core Development Team 2011).

To understand the relationship between dominance and age in bachelor groups, we used an Elo-rating system. First, we modified the R code from Neumann et al. (2011) to calculate Elo-ratings for all bachelors (N=45: 12 young adults, 21 early-prime males, 8 mid prime males, and 4 late-prime males) that fit our criteria (> 9 interactions). To determine if dominance rank is associated with age class, we calculated a mean Elo-rating for each individual and grouped individuals according to their age class. This mean Elo-rating did not include the first 9 interactions for each individual, as variation in these values is due more to the method than to actual dominance (Neumann et al. 2011). Mean Elo-ratings were normally distributed (One sample T test: T=0.0016, df=44,P=0.999). Therefore, we used parametric tests (one-way ANOVA and Tukey’s post-hoc, if significant) to determine if mean Elo-rating was associated with age class among bachelors.

To quantify affiliative relationships among bachelors we compared the proportion of grooming time ($G_{ab}$) of bachelors to previous published data on adult females (Tinsley Johnson et al., accepted) For bachelor and female coresident dyads, $G_{ab}$ was not normally distributed (One sample T-test: T=9.02, df=868, P<0.001); therefore we log-transformed $G_{ab}$ to approximate a normal distribution. After log-transformation, $G_{ab}$ was normally distributed (One sample T-test: T=0.702, df=868, P=0.48), therefore we used parametric statistics to compare bachelor to adult female grooming time. All tests were two-tailed and alpha set to 0.05 for all tests. All statistical analyses were conducted with R (version 2.15.1) statistical software (R Core Development Team 2011).

## RESULTS

### *Composition of all-male groups*

All-male groups exhibited stable membership and consisted of bachelors of all age classes. The mean bachelor age in the population was estimated to be 9.1 +/- 1.4 years, or males of "prime-age" (Beehner et al. 2009; Bergman et al. 2009). The average all-male group size was 4.7 +/- 1.6 (N=21 stable periods). Bachelors were mostly early prime-aged males, but all adult male age classes were present (Fig. 3.1). Bachelors exhibited diverse age classes within each all-male group (Table 3.3). Only one male transferred between groups (DIK). This was due to the death of the only remaining adult bachelor in his previous group (Table 3.3). Several males (N=4; IGO, MAY, MUS, SMA) returned to their all-male groups after failed attempts to emigrate and enter reproductive units (Table 3.3). Additionally, a former leader male (HOO) entered a bachelor group after the last remaining female in his unit died (Tables 3.2-3.3). Given the brevity of the study, we cannot be certain of an adult bachelor's tenure length in a given all-male group. However, if we consider the age for dispersal of young males with known birthdates (mean = 5.4 years, N=15, C. Barale, pers. com.) and the estimated age for bachelor that takeover units (mean = 8.8 +/- 0.8 years, N=47), then adult bachelors spend at least 3 years in an all-male group. Considering at least 6 bachelors were "late prime"-aged in our sample, some males may spend as many as 5 years in an all-male group.

### *Relatedness of all-male groups*

Relatedness rather than age class similarity was associated with coresidence in an all-male group (Fig. 3.2). Overall, most bachelors are unrelated (N=351, $r_w < 0$), but some pairs of bachelors are distant kin (N=171, $0.25 > r_w > 0$), and a few pairs are close kin (N=20, $r_w > 0.25$). For pairs of males not living in the same all-male group most were unrelated (N=304) or distant kin (N=146), and less were close kin (N=12). Similar patterns were observed for males within the same all-male group. Most pairs were unrelated (N=47) or distant kin (N=25), and few were considered to be close kin (N=8). The univariate model of relatedness and both multivariate models (additive and interactive) were equally good fits for our data (all $\Delta AIC < 2$). These three models were better fits than the age class only model (all $\Delta AIC > 6$). Relatedness was the only significant fixed effect associated with coresidence in an all-male group in each model (relatedness only model: $\beta = 2.41$ +/- 0.78, $Z = 3.12$, $P < 0.01$; additive model: $\beta = 2.40$ +/- 0.78, $Z = 3.09$, $P < 0.01$). Age class similarity was not associated with coresidence in an all-male group (additive model: $\beta = -0.27$ +/- 0.29, $Z = -0.93$, $P = 0.35$; interactive model: $\beta = -0.22$ +/- 0.29, $Z = -0.77$, $P = 0.44$), nor was the interaction between age class similarity and relatedness ($\beta = 1.71$ +/- 1.54, $Z = 1.11$, $P = 0.27$).

*Bachelor dominance relationships*

Overall, bachelors were very affiliative and engaged in little aggression within their groups. We observed a total of 286 dominance interactions (mean = 28.5 per group, range 2-91) during the study period. A visual comparison of both bachelor and adult female dominance ranks suggests that bachelors lack clearly defined dominance relationships (Fig. 3.3). Although some bachelors are consistently high ranking and others are consistently low ranking, overall bachelors are unable to be ranked in a linear and transitive dominance hierarchy (Fig. 3.3a). This contrasts sharply with females that form linear hierarchies (Fig. 3.3b).

Among the bachelors (N=45) that we were able to calculated Elo-ratings, the mean (+/- S.E.) Elo-rating for each age class was: young adult (mean = 927.5 +/- 30.9, N=12) early prime (mean = 1013.0 +/- 35.4, N=21) mid prime (mean = 984.7 +/- 58.9, N=8), and late prime (mean = 907.3 +/- 70.0, N=4). Age class was not associated with Elo-rating, however a weak inverse U-shaped relationship between male age and dominance rank is apparent (Fig. 3.4; ANOVA: F=1.157, df=3,41, P=0.338).

### *Bachelor affiliative relationships*

Within their all-male groups, bachelors are extremely affiliative. During our study period, bachelors had 1.81 +/- 1.10 grooming partners (range 1-4) and spent 3.41 +/- 1.82 minutes per focal hour grooming (range 0.38-10.78 min/hr). Bachelor dyads spend more time grooming each other than do adult females (Fig. 3.5; Two sample t-test: T=11.13, P < 0.001).

## DISCUSSION

Overall, our findings support previous research on gelada all-male groups (Dunbar 1984; Dunbar & Dunbar 1975), and provide new data on how groups form and the social relationships among bachelors within them. Relatedness influences all-male group formation, however only several pairs of close kin are found within all-male groups. Additionally, bachelors engage in little agonism and high degrees of affiliation within their groups. We expect that these patterns of social behavior may have fitness consequences for bachelors living in all-male groups.

### *Relatedness and all-male group formation*

Our results indicate that relatedness, not age, influences the formation of gelada all-male groups. Similar to gorillas (Levréro et al. 2006), young males (e.g. early prime-aged) are overrepresented within gelada all-male groups. Early prime males are most likely to compete with leaders for dominance within reproductive units (Bergman et al. 2009; Dunbar 1984), thus successful males would leave the bachelor group during, or shortly thereafter reaching, this age class. This may explain the lower number of mid and late-prime bachelors (Fig.1), however we do not currently know if it is common for males to return to bachelor groups later in life if they are evicted from their reproductive unit. We have observed several males (N=4) return to their former all-male groups, but each of these males spent less than four months in mixed-sex units before being evicted by another male (Table 3.3). Given the prevalence of older follower males in our population (Snyder-Mackler et al. 2012), it is most likely that males remain within a reproductive unit after being replaced as a dominant male. Thus older bachelors (late prime) probably represent males that never entered reproductive units, or returned to an all-male group after quickly being overthrown by a rival male.

Given the lack of relationship between age class similarity, relatedness and coresidence within an all-male group, groups are not strictly male siblings that disperse and mature together. The majority of bachelors are unrelated or distant kin within their respective all-male groups, a few pairs (N=8) of bachelors were considered to be close kin. This supports an observed instance of young sibling males (N=4) within our population forming a bachelor group with an older unrelated male (C. Barale, pers. obs.). Most young males probably join all-male groups alone after dispersal with a few males dispersing from their natal group and joining together. Moreover, considering the low relatedness patterns both within and between all-male groups, it is also likely that bachelors from different bands contribute to their formation (Fig. 3.2). Association patterns between all-male groups and units support this hypothesis, as all-male groups vary considerable in their association with bands (range 10%-100% association) (Kawai et al. 1983; Pappano et al. 2012). Our research project is currently investigating the ontogeny of male social relationships from the juvenile period through dispersal. We expect that those males with the closest relationships eventually disperse and join all-male groups together.

### *Aggression and affiliation within all-male groups*

Within their respective all-male groups bachelors were highly affiliative and lacked age-based dominance relationships (Fig. 3.3-3.5). This supports previous findings in geladas (Dunbar & Dunbar 1975; Mori 1979), and is in agreement with other studies of both captive (Stoinski et al. 2002; Butovskaya & Kozintsev 1996) and wild primate all-male groups (Yamagiwa 1987; Yamagiwa 1992; Robbins 1996). In a captive all-male group of stumptail macaques (*Macaca arctoides*), males lacked linear dominance relationships and also were more affiliative than males housed in mixed-sex groups (Butovskaya & Kozintsev 1996). Additionally, among wild mountain gorillas (*Gorilla g. beringei*), coresident silverbacks in an all-male group also lacked dominant and subordinate relationships (Yamagiwa 1987). Bachelor male gorillas also suffered less injury and had more affiliative interactions with other males, compared to those males living in mixed-sex groups (Yamagiwa 1987). Similar to other bachelor primates, bachelor geladas are highly affiliative and lack overt agonism. The lack of agonism may be even more pronounced in geladas, who also have low levels of feeding competition due to their diet (>90% grass) (Hunter 2001).

Although we were unable to detect a significant association between dominance and age class we believe bachelors may have weak “rank” trajectories within their all-male groups that are based on age. Given the weak inverse U-pattern between age and Elo-Rating, an individual bachelor may rise in rank until he considered “early-prime” (Fig. 3.4). It is around this time bachelors begin to enter reproductive units and become leader males. Thus, rank and age might serve as a proxy for what males are likely to become future leaders. Similar patterns are observed in Hanuman langurs, where the dominant male within an all-male group is able to exclude his former bachelor group mates during reproductive competition (Rajpurohit 1997). Additionally, mid and late prime males possess considerably more variation in their Elo-Ratings compared to young adults and early prime males (Fig. 3.4). This variation may be due to a relaxing of social dominance interactions among older bachelors. Indeed, many older bachelors are quite asocial and do not groom as frequently as younger males (pers. obs.). Taken together, dominance may only be meaningful for young bachelors prior to their “window of opportunity” to takeover a reproductive unit. We anticipate that with more longitudinal data on younger males as they enter bachelor groups we will be able to answer this question.

Compared to previously published data on female geladas (Tinsley Johnson et al., accepted), bachelor dyads spend more time grooming each other than do adult females. All-male groups are similar in size to reproductive units (all-male group mean: 4.7 +/- 1.6 S.E.; reproductive unit: 5.24 +/- 0.09 S.E., Tinsley Johnson et al., accepted), but bachelors have slightly less grooming partners than adult females (bachelors: 1.81 +/- 1.10 S.E.; females 3.6 +/- 0.15 S.E.). This suggests that bachelors may even be more choosey in their social partners than unit females and may develop preferential social bonds within their bachelor group. Social bonding among male primates is rare, and even more so for males in female philopatric species (but see (Hill 1994; Silk 1994; Schülke et al. 2010). As geladas already from stable all-male groups, they have the opportunity to form preferential social bonds with each other. In addition to fitness benefits derived from living within an all-male group, we expect that bachelors may derive fitness benefits through forming strong social bonds with each other such as increased longevity (Silk et al. 2010) or lower stress (i.e. glucocorticoid) levels (Shutt et al. 2007; Crockford et al. 2008; Wittig et al. 2008). For example, captive Western lowland gorillas (*Gorilla g. gorilla*) housed in all-male groups had lower stress levels than males housed solitarily (Stoinski et al. 2002).

Finally, bachelors may form all-male groups to practice aggressive skills with known individuals (Chiyo et al. 2011), or establish coalitions to overthrow dominant males (Widdig et al. 2000). Given the low-levels of aggression among adult bachelors it is unlikely they practice fighting ("sparring") like elephants or horses (Chiyo et al. 2011; Heitor & Vicente 2010), although "play fights" are common among subadult male geladas (Dunbar & Dunbar 1975; Kawai 1979). Aggression among adult bachelors is largely directed at leader males (Dunbar 1984). It is possible that bachelors form coalitions prior to engaging in mutual chases of leader males ("yelping chases," Dunbar & Dunbar 1975) that occasionally escalate to contact aggression. Fights that escalate from chases often result in challenges for dominant status within a reproductive unit, and may be times when bachelors 'cooperate' to wear down a dominant leader male. However, within these putative coalitions, only one bachelor can become the next leader of a reproductive unit. This considered a bachelor might parasitize the efforts of his fellow males to gain access to a reproductive unit (Nunn & Lewis 2001; Kitchen & Beehner 2007). Through a more complete understanding of the social dynamics of all-male groups, we hope to gain a better understanding of the factors that influence gelada male reproductive decisions.

### *Conclusion*

Reproductive competition among males leads to exclusion of some males from mixed-sex groups. In these cases, males may form all-male bachelor groups to acquire benefits of group living. These groups may be structured according to age, kinship, or both. In our study of bachelor geladas, we demonstrate that kinship structures all-male group formation. We also show that within their all-male groups, bachelors engage in little agonism and high degrees of affiliation. We suggest that group living for bachelors may convey fitness benefits with respect to predator protection or harassment from extra-group males.

**Table 3.1**. A description of gelada male age class. Taken from (Beehner et al. 2009; Bergman et al. 2009)

| Age Category | Estimated ages (years) | Description |
|---|---|---|
| Young Adult | 6.5-8.0 | Adult body size and stature, but not weight. Complete canine eruption. Cape hair light in color, extending just past shoulders. Cheek tufts present but not extending below chin. Ears highly visible. Surrounding fur around chest patch gray-brown in color. |
| Early-Prime Adult | 8.0-9.5 | Heavier in appearance than young adults. Cape hair light in appearance and extending to elbows. Cheek tufts extend to chin or just below. Dorsoventral V of dark hair down center of crown (crown V) forming on head. Ears somewhat visible. Surrounding fur around chest patch white. |
| Mid-Prime Adult | 9.5-11.0 | Heaviest stage for an adult male. Cape hair dark and extending past elbows. Cheek tufts extend well below chin. Prominent crown V on head. Ears not visible. Canines show signs of yellowing. Surrounding fur around chest patch white. |
| Late-Prime Adult | 11.0-13.0 | Same size as or slightly smaller than mid-prime males. Cape hair and cheek tufts begin to recede. Crown V beginning to fade. Ears not visible. Obvious canine yellowing and wear. Surrounding fur around chest patch gray. |
| Old Adult | >13.0 | Same size as young adult males. Collapsed look to back and shoulders. Cape receded back to shoulders and cheek tufts receded back to chin line. Crown V no longer present. Ears somewhat visible to highly visible. Worn or missing canines. Hair dull in color and uneven in places. Surrounding fur around chest patch gray-brown. |

**Table 3.2.** Demography of all-male groups. All-male groups in study population (2010-11). Age classes estimated using secondary sexual characters (Bergman et al. 2009, Beehner et al., 2009). *Joined AMG 7 after the disappearance of JEF (8/28/10). **Former leader male joined AMG 7 after the death of last remaining female in his unit (5/10/11). ✝Only 2 bachelors in this group were individually recognized from prior field seasons (2008, 2009).

| Group | ID | Age Class | Relatedness Data (Y/N) |
|---|---|---|---|
| AMG 1 | DIK* | Early Prime | Y |
| | JEF | Early Prime | N |
| AMG 2 | OUP | Early Prime | Y |
| | VOD | Young Adult | Y |
| | OUZ | Young Adult | Y |
| | SYR | Early Prime | Y |
| | TEQ | Early Prime | Y |
| | WHI | Late Prime | Y |
| | RUM | Late Prime | Y |
| | DGU | Mid Prime | Y |
| | ATE | Early Prime | Y |
| | RHI | Early Prime | N |
| AMG 3 | ONE | Early Prime | Y |
| | GHE | Late Prime | Y |
| | KHA | Early Prime | Y |
| | ATT | Early Prime | Y |
| AMG 4 | SMA | Young Adult | Y |
| | DUM | Mid Prime | Y |
| | SHO | Young Adult | Y |
| | KRI | Early Prime | Y |
| | KRO | Early Prime | Y |
| | NSF | Mid Prime | N |
| | FUL | Early Prime | N |
| | POP | Early Prime | N |
| AMG 5 | ZET | Late Prime | Y |
| | REL | Young Adult | N |
| | ONI | Young Adult | N |
| | MUS | Early Prime | N |
| | SAU | Early Prime | N |
| | KET | Early Prime | N |
| AMG 6 | DYE | Mid Prime | Y |
| | MAY | Mid Prime | Y |
| | EAG | Young Adult | Y |
| | EGG | Early Prime | Y |
| | EDG | Early Prime | Y |
| | ERG | Early Prime | Y |
| | ASL | Late Prime | Y |
| AMG 7 | DIK* | Early Prime | Y |
| | QUA | Early Prime | Y |
| | DRA | Early Prime | Y |
| | MEA | Young Adult | Y |
| | HOO** | Early Prime | N |
| | MUM | Young Adult | N |
| | ZOM | Young Adult | N |
| | MOB | Mid Prime | N |
| | WOL | Young Adult | N |
| | IGO | Young Adult | N |
| AMG 8 | HAS | Young Adult | Y |
| | HEX | Early Prime | N |
| | RAN | Early Prime | Y |
| AMG 9 | ELA | Early Prime | N |
| | NYA | Early Prime | N |
| | BUS | Mid Prime | N |
| | LES | Mid Prime | N |
| | ASH | Mid Prime | N |
| AMG 10✝ | PAS | Mid Prime | Y |
| | MOT | Mid Prime | N |

**Table 3.3.** Stable Periods of all-male groups. Group membership for each all-male group (2010-2011). A change in membership through the maturation to adulthood, death, emigration, or immigration of a bachelor indicates a change in stable periods.

| Group | Stable Periods | Bachelors |
|---|---|---|
| AMG 1 | Jul 2010- Aug 2010 | DIK, JEF |
| AMG 2 | Aug 2010- Mar 2011 | ATE, DGU, OUP, OUZ, RUM, SYR, TEQ, VOD, WHI |
| | Apr 2011- Oct 2011 | ATE, DGU, OUP, OUZ, RHI, VOD, WHI |
| AMG 3 | Nov 2010- Feb 2011 | ATT, GHE, KHA, ONE |
| AMG 4 | Oct 2010- Dec 2010 | DUM, KRI, KRO, SHO, SMA |
| | Jan 2011- Mar 2011 | DUM, KRO, SHO, SMA, YOD |
| | Apr 2011- May 2011 | FUL, KRO, NSF, POP, SMA |
| AMG 5 | Mar 2011- May 2011 | KET, MUS, ONI, REL, SAU, ZET |
| | Jun 2011- Jul 2011 | KET, ONI, REL, SAU, ZET |
| | Aug 2011- Oct 2011 | KET, MUS, ONI, REL, SAU |
| AMG 6 | Aug 2010- Feb 2011 | MAY, ASL, DYE, EAG, EDG, EGG, ERG |
| | Mar 2011- Apr 2011 | ASL, DYE, EAG, EGG, ERG |
| | May 2011- Dec 2011 | MAY, ASL, DYE, EGG, ERG |
| AMG 7 | Oct 2010- Jan 2011 | DIK, DRA, IGO, MEA, QUA |
| | Feb 2011- Mar 2011 | DRA, IGO, MEA, QUA |
| | Apr 2011- Jun 2011 | DRA, HOO, IGO, MEA, MUM, WOL, ZOM |
| | Jul 2011- Aug 2011 | HOO, IGO, MEA, MUM, WOL, ZOM |
| | Sep 2011- Dec 2011 | DRA, IGO, MEA, MOB, MUM, WOL, ZOM |
| AMG 8 | Feb 2011- Nov 2011 | HAS, HEX, RAN |
| AMG 9 | Aug 2011- Sep 2011 | ASH, ELA, LES, NYA |
| | Oct 2011- Nov 2011 | ASH, BUS, ELA, LES, NYA |
| AMG 10 | Jul 2010- Apr 2011 | MOT, PAS |

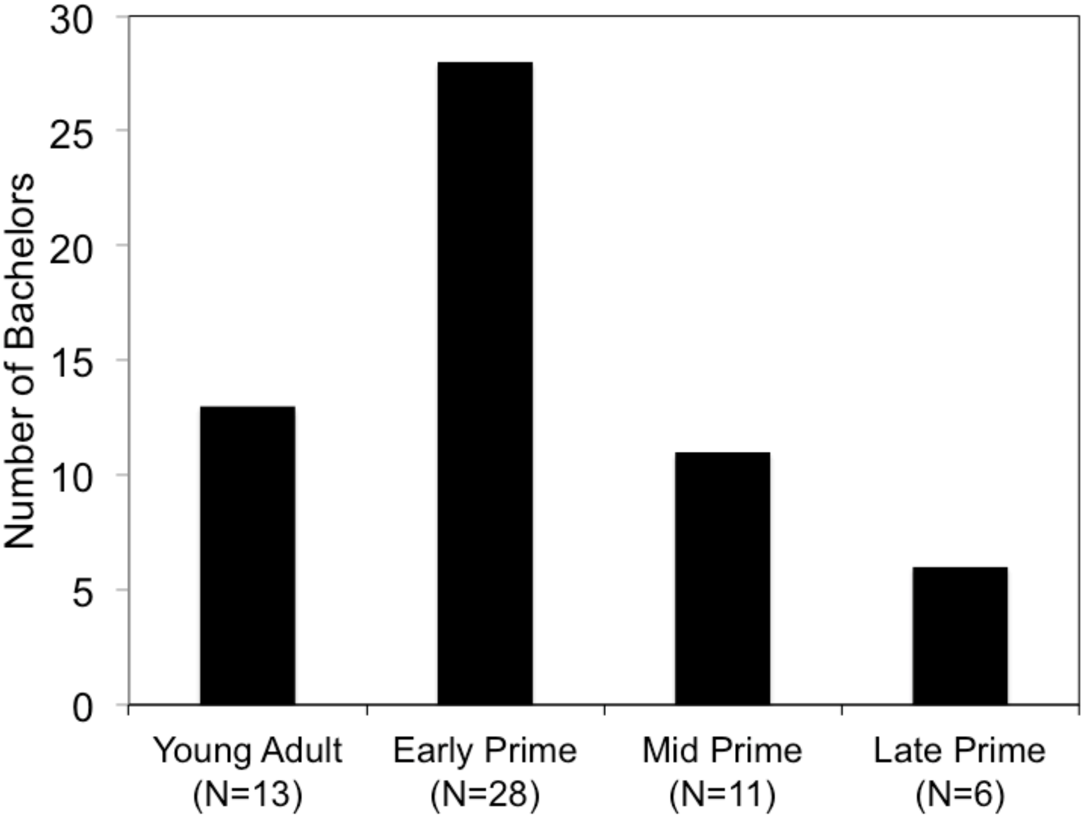

**Figure 3.1.** Age class distribution for all bachelors. Nearly half the bachelors living in all-male groups were considered to be early prime males (8.0-9.5 years in age). Young adults (6.5-8.0 years in age) and mid prime males (9.5-11.0 years in age) were similar in frequency. Only a few bachelors were considered to be late prime (11.0-13.0 years in age) males. All ages were estimated based on tooth wear, cape length, and pelage coloration (see (Bergman et al. 2009; Beehner et al. 2009) for full list of criteria).

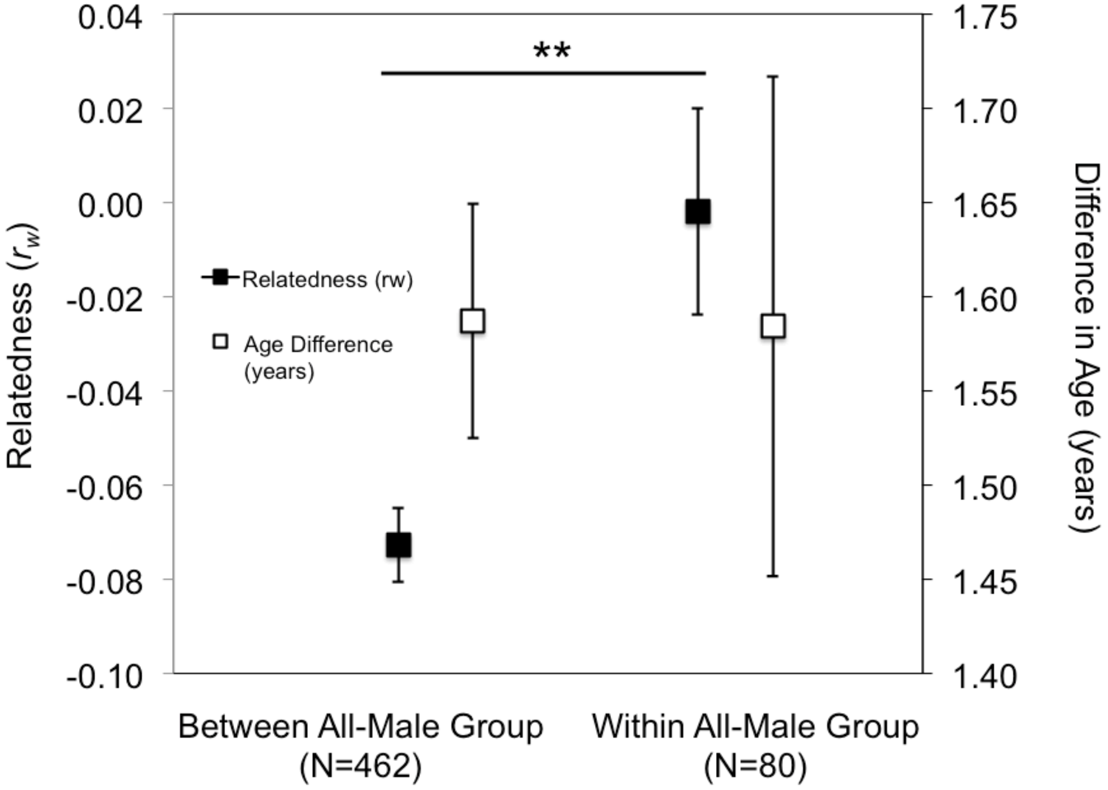

**Figure 3.2.** Relatedness, age difference, and coresidence within an all-male group. Squares indicate mean relatedness ($r_w$) and mean age difference (in years) (+/- S.E.) between two pairs of bachelors. Results from our generalized linear mixed model (GLMM) suggest relatedness alone is associated with coresidence within an all-male group. Males within the same all-male group are more closely related than are males between all-male groups (** $P < 0.01$). Age class similarity was unassociated with coresidence within an all-male group ($P = 0.35$). We visualize age class similarity here using the difference in estimated ages between a pair of bachelors, however in our GLMM males were listed as either being in the same age class (< 1.5 years apart in age) or different age class (> 1.5 years apart in age).

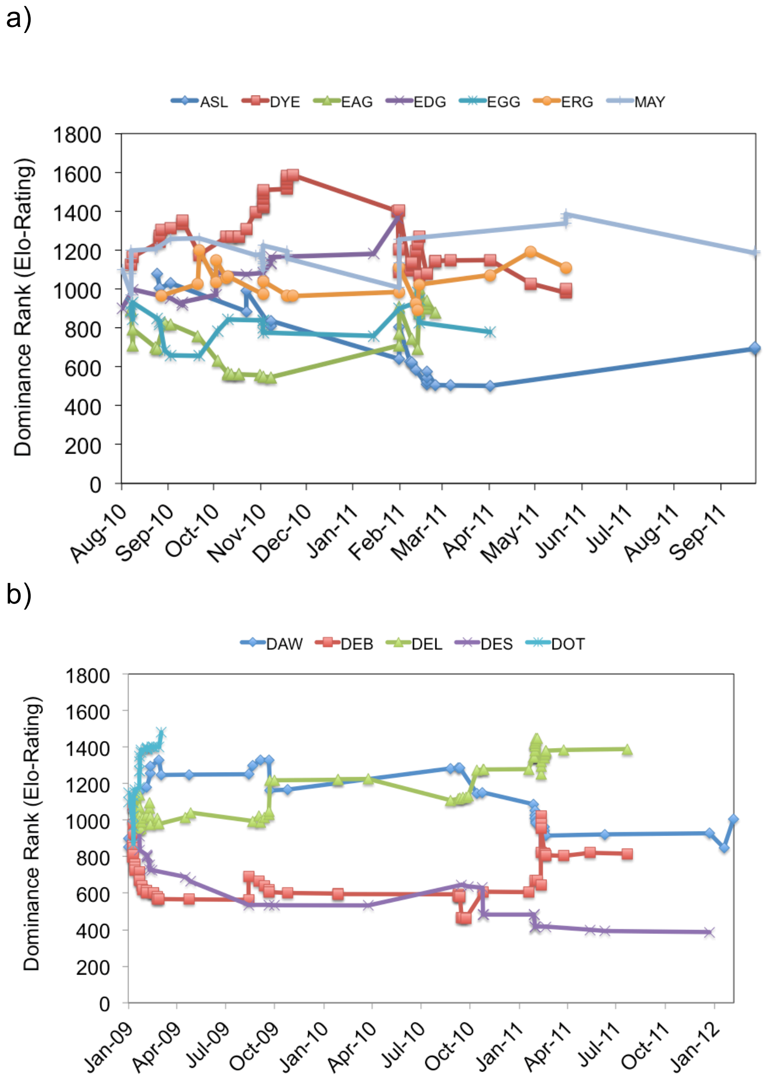

**Figure 3.3.** Elo-ratings for an all-male group compared to females within a reproductive unit. Parallel horizontal lines indicate rank stability and each mark denotes a dominance interaction. (a) Bachelors within an all-male group lack consistent dominance ranks as indicated by “noise” of Elo-rating persisting throughout study period. Although DYE is generally high ranking and ASL is generally low ranking, all bachelors in between these two males are unable to be reliably ordered. Similar patterns are observed in all other all-male groups. (b) Alternatively, females ranking becomes clearly delineated early using the Elo-rating system as female geladas have linear and transitive dominance hierarchies (le Roux et al. 2011). Female Elo-rating data from Tinsley Johnson et al. (accepted).

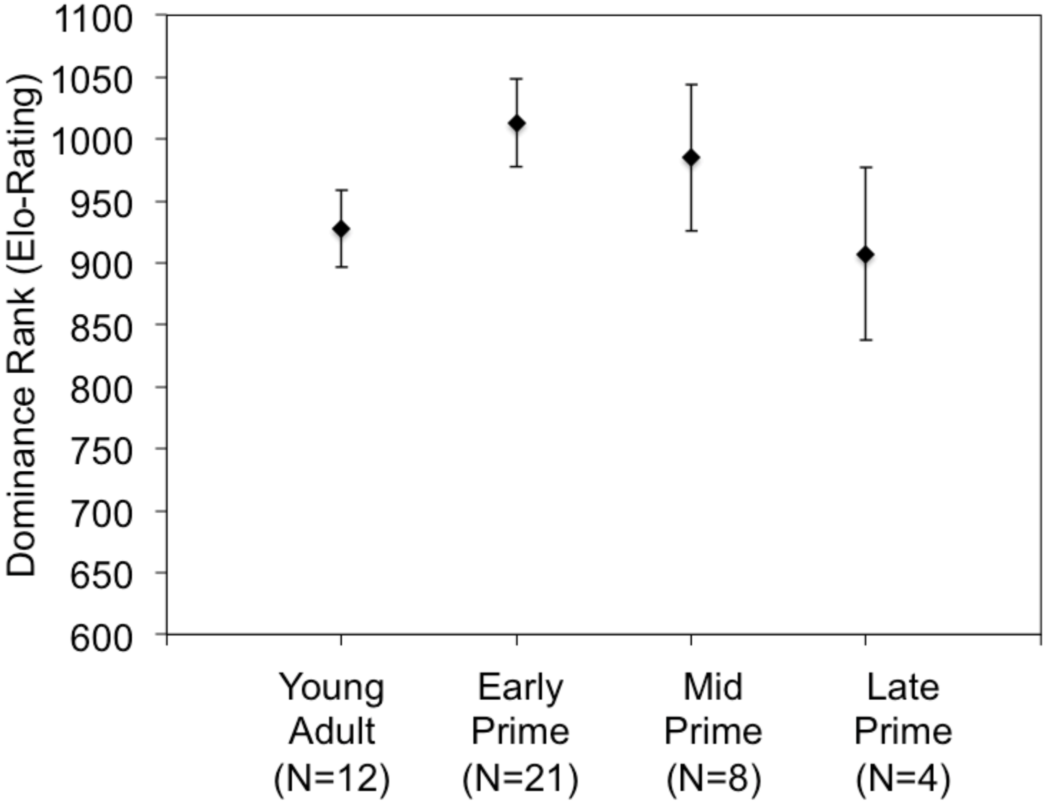

**Figure 3.4.** Elo-ratings for each bachelor male age class. Diamonds indicate mean Elo-ratings (+/- S.E.) for different age classes. A general inverse U-shaped trend suggests males rise in rank within their bachelor group, then fall after achieving high dominance in their early prime. However there was no significant association between age class and Elo-rating among bachelors (P = 0.338).

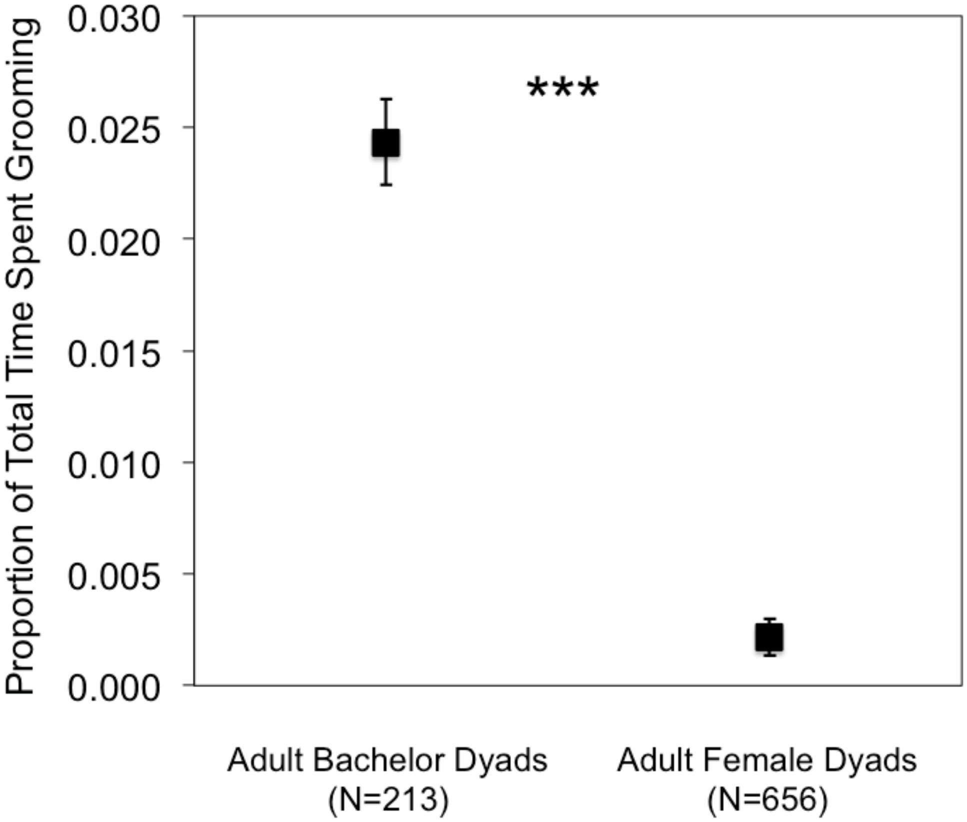

**Figure 3.5.** Comparison of grooming between adult bachelors and adult females. Squares indicate mean proportion of grooming time ($G_{ab}$) for all adult bachelor and female coresident dyads (+/- S.E.). Bachelors spend significantly more time grooming than do unit females (*** P < 0.001) Data on female grooming from Tinsley Johnson et al. (accepted).

# CHAPTER FOUR

# SOCIAL BONDS OF BACHELOR GELADAS LIVING IN ALL-MALE GROUPS

## ABSTRACT

Strong affiliative relationships, often called “social bonds,” are hypothesized to convey fitness benefits in many gregarious species. These bonds are often shared between close kin, such as siblings or parents and offspring. Bonds may also form between those of similar dominance rank or age. Given that male primates tend to be less affiliative, little is known of what factors influence bond formation, strength, and quality among primate males. In this study, we investigate the demographic, behavioral, and genetic influences on social bond formation in bachelor geladas (*Theropithecus gelada*) living in the Simien Mountains National Park, Ethiopia. Geladas are a highly gregarious primate where males disperse from their natal units and join all-male bachelor groups prior to becoming reproductively active. We use 18 months of behavioral data along with noninvasive genetic sampling to test our hypotheses. Overall, our results suggest that relatedness and age structure mediate social bond formation and grooming equality in bachelor geladas. Similar to female geladas, we discovered that grooming rather than proximity was a better indicator of bond strength. Moreover, bachelors that formed strong bonds with each other groomed each other more equally. Dominance rank did not influence bond formation or grooming equality. We suggest that social bonds among gelada males may convey fitness benefits and likely develop early in life.

## INTRODUCTION

Sexual selection favors male traits that improve competitive ability rather than cooperation. Males that are stronger, faster, and/or possess well-developed weaponry (e.g. tusks, antlers, or canines) are better able to exclude conspecific males from mating opportunities (West-Eberhard 1983; Clutton-Brock et al. 1982; Andersson 1994). Behavior is also shaped by sexual selection (Alexander 1974; Clutton-Brock & Parker 1992; Clutton-Brock 2007). As fertilizations cannot be shared, males tend to be intolerant of each other and rarely engage in affiliative behavior. This likely limits the formation of preferential male affiliative relationships (hereafter, *social bonds*) across taxa (Van Hooff & Van Schaik 1994; Hill & Van Hooff 1994). Despite this, male bonds have been observed in both male philopatric and female philopatric primate species (Mitani 2009; Silk 1994; Schülke et al. 2010). Here, we examine the factors that influence social bond formation and quality among bachelor geladas (*Theropithecus gelada*)—an Old World monkey where males disperse at puberty to join all-male bachelor groups (Pappano et al., in prep) (Dunbar & Dunbar 1975; Kawai 1979).

Within their social group, animals may form preferential associations with some conspecifics, and remain indifferent or even hostile to others (Hinde & Berman 1983; Silk 2007). Relationships that tend to be strongly affiliative are often referred to as social bonds or “friendships” (Seyfarth & Cheney 2012; Silk 2002; Dunbar & Schultz 2010), and are found in many gregarious species including: yellow baboons (*Papio cynocephalus*) (Silk et al. 2003; Silk et al. 2006; Silk et al. 2006; Silk et al. 2009), chacma baboons (*Papio ursinus*) (Silk et al. 2010; Silk et al. 2010), bonnet macaques (*Macaca radiata*) (Silk 1994), Assamese macaques (*Macaca assamensis*) (Schülke et al. 2010), chimpanzees (*Pan troglodytes*) (Mitani 2009), horses (*Equus caballus*) (Cameron et al. 2009), African elephants (*Loxodonta africana*) (Moss et al. 2011), spotted hyenas (*Crocuta crocuta*) (Smith et al. 2010), and bottlenose dolphins (*Tursiops* spp.) (Connor et al. 2000). Strong social bonds confer fitness benefits (e.g. longevity and fecundity) in both female (Silk et al. 2003; Silk et al. 2009; Silk et al. 2010) and male primates (Schülke et al. 2010), as well as female horses (Cameron et al. 2009). Given this pattern, bonding is hypothesized to be adaptive for many gregarious, long-lived species such as primates. When observed, females tend to form preferential bonds with close relatives due to shared indirect benefits, and with females of similar age or rank (Silk et al. 2006; Silk et al. 2006). Few studies have examined what factors influence bond formation among male primates (but see (Mitani 2009; Silk 1994). It is likely that when present, preferential social bonds among males mirror female bonds in that they are stratified according to kinship, age, and/or rank.

Inclusive fitness theory predicts that individuals should bias affiliative behavior towards kin rather than non-kin (Hamilton 1964; Hamilton 1964; West Eberhard 1975). Therefore, kinship may influence the development of male social bonds. The patterns of sex-biased dispersal and philopatry set the stage for the development of social bonds for both males and females (Pusey & Packer 1987; Silk 2007). Because females tend to the philopatric sex in mammals (Silk 2007), the majority of research has focused on the causes and consequences of female social bonding. However, in many species male tolerance is extremely high with instances of cooperation and coordination among both kin (Packer et al. 1991; Pope 1990), non-kin (Snyder-Mackler et al. 2012; Noë 1990), or both (Krützen et al. 2003; Langergraber et al. 2007; Chiyo et al. 2011; Widdig et al. 2000). For primate species where males are the dispersing sex, social bonds are less expected because males likely disperse alone and are unable to recognize kin in the absence of social overlap with related individuals (Silk 2002). Despite this, both bonnet (Silk 1994) (*Macaca radiata*) and Assamese macaque (*M. assamensis*) (Schülke et al. 2010) males exhibit some degree of social bonding. Therefore, it is possible that kinship influences social bond formation among male primates in female philopatric species.

In order for kinship to influence bond formation among male dispersing primates, age class similarity also must play a role. Males of similar age that are born the same social group may disperse together. These males would be related through their maternal line (via female philopatry) and the paternal line if they were sired by the same male (Altmann 1979). Although the existence of paternal male cohorts is possible, it is unlikely that young males would emigrate to the same non-natal group upon dispersal. However, in species where males spend time maturing in all-male bachelor groups (e.g. snub-nosed monkeys (*Rhinopithecus* spp.) (Grueter & van Schaik 2010), proboscis monkeys (*Nasalis larvatus*) (Murai 2004), Hanuman langur (*Semnopithecus entellus*), red uakari (*Cacajao calvus ucayalii*) (Bowler & Bodmer 2009), and geladas (*Theropithecus gelada*) (Dunbar & Dunbar 1975), males may find themselves among members of their paternal cohort and may develop social bonds within such groups.

Once developed, social bonds may vary in their quality and persistence. Grooming reciprocity is a widely accepted measure of bond quality in primates (Dunbar 2010). Individuals reciprocate grooming in exchange for grooming, coalitionary support, mating opportunities, food, or tolerance (Watts 2002; Port et al. 2009; Fruteau et al. 2009; Henzi & Barrett 1999; Barrett et al. 1999). Grooming reciprocity has also been linked to social bond persistence (Silk et al. 2010; Silk et al. 2010; Mitani 2009). Female baboons that shared the strongest bonds groomed each other most equally, and in turn these bonds persisted for the longest periods of time (Silk et al. 2010; Silk et al. 2010). Similar patterns are found among male chimpanzees, whose social bonds may last up to 10 years (Mitani 2009).

In this study, we examine the factors that influence bond formation and grooming equality among bachelor geladas (*Theropithecus gelada*). Geladas are a gregarious cercopithecine primate that are sister taxa to baboons (*Papio* spp.) (Jablonski 1993; Page et al. 1999). Geladas live in a modular social system where core groups of related females (called “one-male” units, or reproductive units) fission and fuse with each other over the course of a day (Kawai et al. 1983; Snyder-Mackler et al. 2012). These reproductive units contain related females, a single dominant “leader” male, and possibly subordinate “follower” males (Dunbar & Dunbar 1975; Kawai et al. 1983; Snyder-Mackler et al. 2012). Adult males that are excluded from reproductive units form all-male “bachelor” groups (Dunbar & Dunbar 1975; Kawai 1979)(Pappano et al., in prep). Like most baboons (except possibly hamadryas baboons, see (Colmenares 1992; Kummer 1968; Schreier & Swedell 2009; Swedell 2002), female geladas remain within their natal groups and form preferential bonds (le Roux et al. 2011)(Tinsley Johnson et al., accepted). Much like their baboon relatives (Silk et al. 2006; Silk et al. 2006), these bonds are shared with closely related and similarly ranked females (Tinsley Johnson et al., accepted). Within units, leader males are also very social and form bonds with dominant females (Dunbar 1983).

Geladas are ideal for studying male-male affiliative relationships for several reasons. First, gelada males are extremely gregarious at all life history stages (Dunbar & Dunbar 1975; Kawai 1979). Gelada male sociality begins early in development, as juveniles form sex-segregated peer groups (Dunbar & Dunbar 1975; Kawai 1979). These groups eventually contribute to the formation of all-male groups consisting of adult bachelors and associated subadult males (Dunbar & Dunbar 1975; Kawai 1979)(Pappano et al., in prep). Second, geladas are very tolerant of extra-unit (non-bachelor) males, even though they have limited ability to recognize these males individually (Bergman 2010). This extreme male tolerance helps facilitate the formation of large gelada herds that may function to dilute the costs of bachelor association (Pappano et al. 2012). Third, leaders may exchange mating opportunities for unit defense with their subordinate followers (Snyder-Mackler et al. 2012). Taken together, it is likely that increased tolerance for other males, coordination, and possibly cooperation among males likely play a key role in gelada evolution.

Although much is known of social relationships within mixed-sex reproductive units (Dunbar 1979; Mori 1979; Dunbar 1983; Dunbar 1983), few studies have examined the nature of social relationships within all-male groups (Dunbar & Dunbar 1975; Mori 1979). In a brief study of all-male groups, Dunbar and Dunbar (1975) suggest that prime-aged bachelors command the majority of grooming within a group yet bachelors could not be reliably ordered in a linear and transitive dominance hierarchy. In a corroborating study, Mori (1979) determined that within all-male groups, bachelors lacked strong and readily apparent dominant relationships. However, both studies noted that the majority of interactions among adult bachelors living within all-male groups are affiliative. Recent evidence from our study population also suggests bachelors exhibit low levels of aggression, high levels of affiliation, and lack strong dominance relationships (Pappano et al., in prep). Most strikingly, bachelor geladas spend more time grooming when compared to unit females (Pappano et al., in prep). Given these observations, we expect that bachelor geladas form preferential social bonds within their all-male groups.

We sought to answer two main questions concerning the nature of affiliative relationships among bachelor geladas. First, do bachelors form preferential social bonds? If so, what factors influence bond formation and strength? Because male geladas may join all-male groups with close kin (Pappano et al., in prep), we expect that the strongest bonds would exist among bachelors that are both similar in age and closely related. We predicted that age and relatedness influence bond formation among bachelor geladas. We do not expect rank to influence bond formation because previous research suggests bachelors have weak, possibly nonexistent dominance relationships (Pappano et al., in prep) (Dunbar & Dunbar 1975; Mori 1979). Second, if bachelors do form social bonds, do these bonds vary in quality? That is, do bachelors that form strong bonds also groom each other more equally as in male chimpanzees (Mitani 2009) and female baboons (Silk et al. 2010)? We predicted that bond strength would be associated with grooming equality, i.e. bachelors that form strong social bonds would also groom each other more equally. Because of the lack of strong dominance relationships among bachelors, we also predicted that rank disparity would not influence grooming equality.

## METHODS

### *Study site and subjects*

Data were collected across 18 months (Jul 2010 – Dec 2011) from a habituated population of wild geladas living in the Simien Mountains National Park, Ethiopia (13°18'N, 38°06'E, 3200-4500 m.a.s.l.) as part of the long-term University of Michigan Gelada Research Project. Demographic, behavioral, and genetic data were collected from 58 individually recognized adult bachelors living in 10 all-male groups (mean group size= 4.7 +/- 1.6, range= 2-7 bachelors) (Pappano et al., in prep). Given the male-biased dispersal pattern of geladas and the limited duration of current demographic records (< 6 years), we do not have known birthdates for most of the adult males in the population. Therefore, the ages of all males were estimated to the nearest half year using secondary sexual characteristics such as canine eruption, tooth wear, pelage coloration, and cape length (Beehner et al. 2009; Bergman et al. 2009). During this time, the mean adult bachelor age in the population was estimated to be 9.1 +/- 1.4 years (Pappano et al., in prep).

### *Behavioral data collection*

For each all-male group (AMG), we recorded the initial group size and the estimated age and identity for all adult bachelors. Because all-male groups frequently change in composition, we recorded the dates for all immigration and emigration events, as well as any known deaths for bachelors in all-male groups. Given the relative brevity of this study (18 months), we used these demographic events as a natural way to partition our behavioral data in to “stable periods” rather than choosing arbitrary periods such as months or years (Pappano et al., in prep). All behavioral indices below were calculated for each pair of bachelors (hereafter, *coresident dyad*) during each stable period (N=21).

Within their all-male group, bachelor geladas are highly affiliative and spend 3.41 +/- 1.82 minutes per focal hour grooming and have an average of 1.8 +/- 1.1 grooming partners (range 1-4) (Pappano et al., in prep). To record behavioral data, we used 15-minute focal animal sampling (N=453 hours total observation time, ~4 hours per male, range = 1-13 hours) (Altmann 1974). During each 15-minute focal, we recorded all affiliative and agonistic behaviors involving the focal animal, including all bouts of grooming. Additionally, we conducted neighbor scans at 5-minute intervals during a focal sample to obtain proximity measures. For each neighbor scan, we recorded the identity and distance (<1 m, 1-5 m, 5-10 m, 10-20 m, >20 m) of all bachelors within the same all-male group. Within each all-male group, focal samples were evenly distributed across bachelors on any given day and conducted in a semi-random order to reduce sampling bias.

### *Bachelor dominance relationships*

We used an Elo-rating system to determine if dominance rank contributes to bond formation and grooming equality in bachelor geladas (Elo 1978; Albers & de Vries 2001). The Elo-rating is a progressive ranking system and is hypothesized to simulate how rank is generated within animal social groups (Albers & de Vries 2001; Neumann et al. 2011). In brief, for each dominance interaction, a winning animal gains an adjusted value of points (k) to their Elo-rating. Additionally, the loser of the interaction has an adjusted value of points subtracted from their rating (Neumann et al. 2011). Elo-ratings are not sensitive to group size or composition (Neumann et al. 2011), thus they are ideal for bachelor geladas that live in small social groups (often < 5 individuals) that may change in composition. First, we calculated a mean Elo-rating for each bachelor during the study period. We then took the absolute value of the difference in mean Elo-rating (hereafter, *rank disparity*) coresident dyads. Thus, for each coresident dyad, low values of rank disparity indicate two animals are closely ranked (hereafter "close"), and high values indicate animals are more distantly ranked (hereafter "far apart"). Following previous research on bachelor geladas, we set each bachelor's starting Elo-rating value to 1000 and k=100 (Pappano et al., in prep).

*Composite sociality index*

We used a *composite sociality index* (CSI) to measure the strength of social bonds for all coresident dyads (Silk et al. 2006; Silk et al. 2006). This index was chosen because affiliative behaviors are often strongly correlated; for example, two individuals that frequently groom each other are also often found in close proximity (Silk et al. 2006; Silk et al. 2006). To account for this correlation, the CSI weighs each behavior evenly and standardizes each coresident dyad's affiliation by the mean of all possible coresident dyad scores within an all-male group. Thus, the CSI is as follows (Tinsley Johnson et al., accepted):

$$CSI = \frac{\left(\frac{G_{ab}}{G_{AMG}}\right) + \left(\frac{P_{ab}}{P_{AMG}}\right)}{2}$$

Where $G_{ab}$ is the proportion of grooming time (ignoring directionality) for the coresident dyad *ab* and $G_{AMG}$ is the mean proportion grooming time for an all-male group during a specific stable period. Proximity, or $P_{ab}$, is the proportion of neighbor scans for the coresident dyad *ab* where the individuals were less than 1 m apart. Similar to grooming frequency, proximity is corrected for the mean proportion for all possible coresident dyads ($P_{AMG}$) within the all-male group. These adjusted grooming and proximity scores are summed and divided by two to weigh each behavior equally for all coresident dyads. By definition, the mean CSI for each all-male group's stable period was 1. Higher values of the CSI indicate coresident dyads that have stronger than average social bonds within their all-male group, whereas lower values indicate relatively weak bonds. We standardized by all-male group means rather than the bachelor population mean for two reasons. First, considerable variation in sociality may exist between all-male groups. Second, given gelada's modular social organization, some all-male groups associated with our main study band more frequently than others. Therefore we could not sample all-male groups evenly, and standardized by each all-male group mean instead of the population mean.

As geladas exhibit a high degree of spatial overlap, grooming rather than proximity might be more closely associated with social bonding. Indeed, previous research on gelada females suggests that grooming is a better indicator of social bonds than proximity (Tinsley Johnson et al., accepted). Therefore, we also were interested in whether grooming or proximity was a better predictor of social bonds among bachelor geladas. Following Tinsley Johnson and colleagues (accepted), we calculated a separate grooming and proximity indices. The *grooming index* is:

$$grooming\ index = \frac{G_{ab}}{G_{AMG}}$$

Because grooming time includes times where individuals are also in close proximity, we calculated a *proximity index* as follows:

$$proximity\ index = \frac{P_{ab}}{P_{AMG}} - \frac{G_{ab}}{G_{AMG}}$$

*Equality of social bonds*

To measure how evenly grooming is distributed within a coresident dyad, we calculated a *grooming equality index* (GEI) for each coresident dyad (Mitani 2009; Silk et al. 2010). The GEI is as follows:

$$GEI = 1 - \left|\frac{G_{ab} - G_{ba}}{G_{ab} + G_{ba}}\right|$$

Where $G_{ab}$ is the grooming seconds per focal hour directed towards individual *b* by individual *a*, and $G_{ba}$ is the grooming seconds per focal hour directed by individual *a* towards individual *b*. This index is standardized to 1 by taking the absolute value of the grooming term. As a result the GEI ranges from 0 to 1, where 0 indicates completely one-sided grooming and 1 indicates completed balanced grooming within a coresident dyad (Mitani 2009; Silk et al. 2010). All indices were calculated for all pairs of bachelors during every stable period.

*Bachelor ages*

Each all-male group often contains males of many different age classes (Pappano et al., in prep). Given the diversity of ages within all-male groups, similarity in age might structure affiliative relationships within the group. Therefore, each coresident dyad was classified as either being in the same age class or different age classes. Because we only have estimated birthdates for bachelor geladas, we considered males to be in the same age class if their estimated birthdate was < 1.5 years apart. This time (1.5 years) represents the minimum time frame for an adult life history stage (see (Table 3.1) (Bergman et al. 2009; Beehner et al. 2009) for age classification).

### *Genetics*

To estimate bachelor relatedness, we collected fecal samples from all bachelors (1-2 samples per male) and stored them in RNA later for subsequent DNA extraction and genotyping (see (Snyder-Mackler et al. 2012) for full description of methods and extraction). In brief, we genotyped extracted DNA using polymerase chain reactions at 23 human derived MapPairs microsatellite loci (Tinsley Johnson et al., accepted), which were found to be variable in this gelada population (average number of alleles/locus = 5.91). We successfully genotyped 33 of the 56 bachelors at 90% of the loci (Pappano et al., in prep). We used Wang's pairwise relatedness estimator ($r_w$) in all analyses (Wang 2002) to estimate relatedness because it provided the best estimate of relatedness between individuals of known pedigree in our population (e.g., for paternal half-siblings $r_w$=0.25). Although Wang's pairwise relatedness estimator ($r_w$) is associated with error for individuals within unknown or shallow pedigrees, previous research has found it to be robust in our study population for known and unknown relationships (Tinsley Johnson et al., accepted).

### *Data analysis*

To determine what factors are associated with bond formation in bachelor geladas we conducted three linear mixed models (LMM) using the function "lmer" in the "lme4" package (R version 2.15.1) (Sarkar & Bates 2009; R Core Development Team 2011). Because many coresident dyads were never observed to groom during our study period (N=21) or were never found in close proximity (N=30), we also conducted two generalized linear mixed models (GLMM) to see if age class, relatedness, or rank disparity influenced whether or not individuals groomed or were found in close proximity at all (Table 4.1). Due to the division of our data into stable periods, individuals and all-male groups necessarily appear repeatedly in our dataset, with some appearing more than others. Therefore, individual identity of both members of a coresident dyad was included as a random effect in each of the models. We also included all-male group identity in each model as a random effect (Table 4.1). Additionally, because each model considered a different outcome variable coupled with different combinations of bachelors and all-male groups, our sample size for each model varied accordingly (CSI model N=91, grooming index model N=77, proximity index model N=68, grooming GLMM and proximity GLMM, N=98, Table 4.1).

Each social index (CSI, grooming, and proximity) was strongly right-skewed, therefore we log transformed these variables to approximate a normal distribution (Fig. 4.1). We then modelled each outcome variable (CSI, grooming index, and proximity index), using three separate univariate models that considered each fixed effect (age class, relatedness, and rank disparity) to two multivariate models: (1) an additive model that included all fixed effects, and (2) an interactive model that included an interaction between age class and relatedness (Table 4.1). We considered the model with the lowest Akaike Information Criterion (AIC) to be the best fit for our dataset (Akaike 1973; Mundry 2011; Garamszegi et al. 2009; Symonds & Moussalli 2011). If the difference in AIC between the lowest ranking models was less than 2, then we present both of the lowest ranking models as equally good fits for our data (Symonds & Moussalli 2011).

To determine if grooming, age class, relatedness, or rank disparity are associated with grooming equality we used a generalized linear mixed model (GLMM) with our grooming equality index as the outcome variable (N=77). Because 0 indicates completed “one-sided” grooming for a given dyad (i.e. grooming was never reciprocated), all 0 values are meaningful data and needed to be included in our analysis. The GEI was bimodal, so we conducted a cluster analysis using the “kmeans” function in R to identify the peaks (cluster 1 mean=0.76, cluster 2 mean=0.08) explaining 83.8% of the variance. Therefore, we transformed GEI into a binomial outcome variable for a GLMM. All GEI scores below 0.7 were considered to be *asymmetric grooming* (N=56), whereas all scores above 0.7 were considered to be *symmetric grooming* (N=21). We modelled the effect of rank disparity, age class, relatedness, and the grooming index on grooming equality. As with our previous models we compared univariate models (each fixed effect individually) to multivariate models (an additive model of all fixed effects and an interactive model including an interaction between age class and relatedness, Table 4.1). We considered the model with the lowest AIC to be the best best-fit model for our data (Mundry 2011; Garamszegi et al. 2009; Symonds & Moussalli 2011).

## RESULTS

### *Composite sociality index*

The skewedness of the histograms of the composite sociality, grooming, and proximity indices all indicate that most bachelors form weak bonds where as a few pairs form very strong social bonds (Fig. 4.1). The skew was most pronounced in the grooming index, intermediate for the composite sociality index, and least pronounced in the proximity index (Fig. 4.1). This suggests bachelors are most selective with their grooming partners.

In our model of the composite sociality index, both our multivariate models (additive and interactive) were better fits for our data than our univariate (age class, relatedness, or rank disparity) models (all ΔAIC > 20). However, the AIC difference between our additive and interactive models was small (ΔAIC < 1). Therefore we considered both the additive and interactive models as equally good fits for CSI. In both models, we observed similar patterns. In each model several variables exhibited weak associations with CSI in accordance with our predictions. In the additive model, relatedness (β=0.97 +/- 0.57 s.e., t=1.70, P=0.09) and age class (same age class: β=0.39 +/- 0.26 s.e., t=1.49, P=0.14) were weakly positively associated with CSI (Fig. 4.2). In the interactive model, relatedness (β=0.53 +/- 0.67 s.e., t=0.78, P=0.43), the interaction between age class and relatedness (same age class x relatedness: β=1.27 +/- 1.20, t=1.07, P=0.29), and being in the same age class (β=0.34 +/- 0.27 s.e., t=1.26, P=0.21) were all unassociated with CSI. Rank disparity was unassociated with CSI in both models (additive: β=-0.00 +/- 0.00 s.e., t=-0.09, P=0.92; interactive: β=-0.00 +/- 0.00 s.e., t=-0.12, P=0.91).

*Grooming and proximity indices*

In our binomial GLMM of whether or not a pair of bachelors groomed at all, both the multivariate models (additive and interactive) were better fits for our data than the univariate models (ΔAIC > 9). Similar to our models of CSI there was only a small (ΔAIC < 2) difference between our multivariate models. In both the additive and interactive model, relatedness alone was the only significant fixed effect associated with whether or not dyads groomed at all (additive model: β=3.20 +/- 1.56 s.e., Z=2.05, P < 0.05; interactive model: β=3.60 +/- 1.84 s.e., Z=1.95, P = 0.051; Fig. 4.3). In the interactive model, the interaction between age class and relatedness was not associated with whether or not dyads groomed at all (β=-1.39 +/- 3.26 s.e., Z=-0.427, P = 0.67). In both the additive and interactive models, age class similarity and rank disparity were unassociated with grooming (all P > 0.80).

In our model of the grooming index, the interactive model was a better model than all univariate models (all ΔAIC > 20) and the additive model (ΔAIC > 2). In the interactive model, the interaction between age class and relatedness was positively associated with the grooming index (same age class x relatedness: β=3.03 +/- 1.55 s.e., t=1.95, P=0.06; Fig. 4.4). No other fixed effects were associated with the grooming index (age class: same β=-0.83 +/- 0.30 s.e., t=0.37, P=0.71; relatedness: β=0.40 +/- 0.88 s.e., t=0.46, P=0.65; rank disparity: β=0.00 +/- 0.00 s.e., t=1.41, P=0.16).

In contrast to grooming, age class, relatedness, and rank disparity did not affect proximity. Although the additive and interactive models (ΔAIC < 1) were better fits than any univariate model (ΔAIC > 9), no fixed effect in our multivariate models were significant (proximity GLMM additive model: all P > 0.20; interactive model: all P > 0.20). Our model of the proximity index corroborates the results of our proximity GLMM. Both the additive and interactive models (ΔAIC < 1) were the best fit for our proximity index (ΔAIC > 9), however no fixed effect was strongly associated with proximity. In the additive model, age class (β=-0.26 +/- 0.28 s.e., t=-0.92, P=0.36), relatedness (β=-0.32 +/- 0.58 s.e., t=-0.55, P=0.59), and rank disparity (β=0.00 +/- 0.00 s.e., t=-0.58, P=0.56), were all unassociated with the proximity index. Similarly in the interactive model, age class (β=-0.36 +/- 0.31 s.e., t=-1.15, P=0.26), relatedness (β=-0.66 +/- 0.66 s.e., t=-1.00, P=0.32), rank disparity (β=0.00 +/- 0.00 s.e., t=-0.64, P=0.53), and the interaction between age class and relatedness (β=1.71 +/- 1.39 s.e., t=1.22, P=0.22) were all unassociated with the proximity index.

### *Equality of social bonds*

In our GLMM of grooming equality, both our multivariate models (additive and interactive) were better fits for our data than our univariate models (all ΔAIC > 35). Like our previous models, the difference between our additive and interactive models was low (ΔAIC < 2). In the additive model, only the grooming index was positively associated with grooming equality (β=2.11 +/- 0.61, Z=3.48, P < 0.001; Fig. 6). All other fixed effects were unassociated with grooming equality (all $P > 0.15$). In the interactive model, the grooming index ($\beta=1.80$ +/- 0.58, $Z=3.11$, $P < 0.01$) was positively associated with grooming equality. Additionally, the interaction between being in the same age class and relatedness was also positively associated with grooming equality ($\beta=9.36$ +/- 5.41, $Z=1.73$, $P=0.08$), although this was not significant at $\alpha=0.05$. All other fixed effects were unassociated with grooming equality in the interactive model (all $P > 0.14$).

## DISCUSSION

Overall, our analysis of bond formation and grooming equality in bachelor geladas supports many of our predictions. First, the multivariate models including age class, relatedness, rank disparity, and/or the interaction between age class and relatedness were the best fit for each social index data set. Second, both relatedness and age class influence bachelor grooming more strongly than either influence proximity (Figs. 2-5). This finding supports previous results from female geladas that suggest that proximity might not be as informative as grooming relationships in shaping social bonds (Tinsley Johnson et al., accepted). Third, rank disparity plays little to no influence in shaping the social bonds of bachelor geladas. Finally, dyads with strong grooming relationships also groomed each other more equally (Fig. 6). Grooming equality was strongly influenced by the grooming index, and to a lesser extent the interaction between age and relatedness. In sum, our results indicate that relatedness and age structure bachelor social bonds and bond quality.

### *Strength of social bonds*

Our results suggest that being in the same age class, as well as relatedness, influence bond formation among bachelor geladas. Relatedness was the only predictor of whether or not bachelors groomed at all during the study period (Fig. 4.3). Both age class similarity and relatedness influenced grooming more than proximity among bachelor geladas (Figs.4.4-4.5). In our model of the grooming index, the interaction between being in the same age class and relatedness was positively associated with grooming. In other words, bachelors that are both similar in age and closely related possessed stronger grooming relationships (Fig. 4.4).

Inclusive fitness theory predicts animals will bias their affiliative behavior towards close relatives to derive indirect benefits (Hamilton 1964; Hamilton 1964). As geladas are female philopatric (le Roux et al. 2011), males are not expected to form long lasting bonds (Van Hooff & Van Schaik 1994; Pusey & Packer 1987). In our bachelor population, several coresident dyads (N=8) can be considered to be close kin ($r_w > 0.25$, e.g. paternal half siblings), and a significant number of additional dyads (N=24) were distant kin ($0.25 > r_w > 0$). Although, male geladas are unlikely to recognize paternal kin outside of the context of a common rearing environment, it is possible that form bonds as juveniles within their unit (or peer group) due to their close proximity in age (Widdig 2007; Altmann 1979). Similar kin bias among dispersing males is common in species that exhibit joint dispersal of litter-born siblings, e.g. lions (Packer & Pusey 1982), and we have observed dispersing sibling males establish an all-male group in our population (Barale, pers. com.). Alternatively, males may have been born in the same band, but in different units (i.e. distant kin). Under this scenario, males likely cultivate bonds with distant kin within their sex-segregated juvenile peer groups (Dunbar & Dunbar 1975; Kawai 1979). In both cases, both close and distant kin would certainly be in the same juvenile peer group. As these groups contribute the formation of all-male “bachelor” groups, it is likely that bonds between males may form very early in gelada development (< 5 years of age).

In contrast, rank disparity plays little to no role in bachelor social bonds. Previous research has been inconclusive regarding dominance relationships among bachelor geladas (Dunbar & Dunbar 1975)(Pappano et al., in prep). Given male geladas feed nearly entirely on grass (> 90% of their diet) an evenly distributed, low-quality food source, it is unlikely they engage in strong feeding competition (Hunter 2001). This observation coupled with the lack of females within a bachelor group, suggests bachelors have little to compete over that would influence the formation of strong dominance relationships within their group. As a result, dominance does not structure bachelor social relationships.

Although we adopted a stricter criteria than earlier research for our proximity index (<1 m) compared (< 5 m for female geladas, Tinsley Johnson et al., accepted), we were unable to detect any relationship between age, relatedness, or rank on proximity (Fig. 4.5). For female geladas, dyads that exhibited the strongest as well as the weakest grooming relationships were equally likely to be found in close proximity (Tinsley Johnson et al., accepted). This suggests that proximity is a less useful means of discerning close relationships among geladas. However, unlike reproductive units that overlap spatially (Snyder-Mackler et al. 2012), all-male groups are rarely in proximity and seem to actively avoid each other when in the same herd (Dunbar & Dunbar 1975; Pappano et al. 2012). Additionally, bachelors are often found at the periphery of the main gelada herd (> 20 m away) (Pappano et al. 2012). Therefore, bachelors rarely find themselves near extra-unit animals and proximity may be less meaningful for bachelors. Alternatively, several coresident dyads (N=9) were only in close proximity when they engaged in grooming bouts. This suggests that many bachelors may only approach and seek proximity with others in order to groom. Thus, after controlling for grooming, proximity yields no additional information about bond strength for bachelor geladas.

### *Equality of social bonds*

Similar to male chimpanzees (Mitani 2009) and female baboons (Silk et al. 2010), bachelor geladas with strong bonds groom each other more equally (Fig. 6). Additionally, we observed a nonsignificant (P = 0.08) trend suggesting that members of the same age class that are closely related also groom each other more equally. Together, these results indicate that social bonds among bachelor geladas also differ in their “quality” in their relationship. In other words, males that shared the strongest bonds also groomed each other most equally. Assuming social relationships among bachelors are long-term and develop early in life, it is possible that grooming equality is associated with bond persistence as in male chimpanzees and female baboons (Mitani 2009; Silk et al. 2010). Among male chimpanzees and female baboons, those dyads that groomed more equally maintained longer lasting bonds than those that groomed less equally (Mitani 2009; Silk 2007). For male chimpanzees, bonds may persist up to 10 years and probably longer (Mitani 2009). Given our previous result, gelada males might as exhibit bonds that last throughout their pre-reproductive lives and possibly beyond. In other words, those relationships that are characterized by high grooming equality may have the earliest origins within their all-male group.

*Future directions*

Our findings are among the first to quantify social bonds among bachelors living in all-male groups across any mammalian taxa. Given the brevity of our current data set we are unable to shed light on perhaps the most important aspect of social bonding—the persistence and stability of the relationship. Other questions regarding the development and fitness consequences of social bonding will require additional long-term, longitudinal data. Below we list three future lines of inquiry concerning the nature of gelada male social bonds.

First, when do male bonds form among geladas? Given the relationship between age class and relatedness, we expect that bonds might form early in life possibly among half-sibling males within a reproductive unit or peers from the same all-male play group.

As we are currently unable to parse the effects of paternal versus maternal kinship on bond formation, we cannot ascertain how either contributes to bond formation. Understanding this critical window for individuals of known parentage will be essential in advancing our knowledge of male social relationships in geladas. Our project is currently investigating the development of male bonds during the juvenile period to address this question.

Second, how long do male bonds persist? It is possible that bachelor social bonds are temporary and affiliative relationships are more akin to transactions in a biological market (Noë & Hammerstein 1994; Noë & Hammerstein 1995; Barrett et al. 1999). However, anecdotal evidence from our project suggests bonds might persist into the reproductive careers of males. Male gelada reproduction is contingent upon entering a reproductive unit and becoming a dominant male (Dunbar 1984). Males that are kicked out of a unit occasionally return to their former all-male group. This has occurred in all instances (N=4) where the all-male group still exists (i.e. the other bachelors have also not entered units) and when males spend less than 4 months in a unit (Pappano et al., in prep). This suggests bachelors have the cognitive capacity to recognize previous group members and are drawn to those individuals with whom they formerly associated. Additionally, bonds may persist for males from the same all-male group that enter a unit together (N = 4 pairs of bachelors, 2006-2011). In only two of these instances did the males remain in the unit together for longer than one month. Although we suspect that one of these long-term pairs are at least half-siblings (Snyder-Mackler, unpublished data), we cannot currently determine how common this pattern is within the spectrum of gelada male reproductive strategies. Taken together, it is likely that some bonds may persist throughout a male gelada's lifespan.

Finally, does bonding have fitness consequences for male geladas? In female primates, social bond strength has been linked to both fecundity and longevity (Silk et al.

2003; Silk et al. 2009; Silk et al. 2010). Bonds may convey fitness benefits to bachelor geladas through stress reduction, or by providing reliable coalition partners during aggression with leader males (Sapolsky 1990; Ray & Sapolsky 1992; Virgin & Sapolsky 1997; Widdig et al. 2000). For example, male Assamese macaques that formed strong social bonds with each other more readily formed coalitions, achieved higher dominance rank, and subsequently sired more offspring (Schülke et al. 2010). Bonds among bachelors may facilitate coalition formation in overthrowing leader males and acquiring units in general, or through entering a unit together, bonded bachelors may cooperate as a leader-follower pair and share (albeit, unequally) reproduction (Snyder-Mackler et al. 2012).

### *Conclusions*

In sum, our results indicate bachelor geladas form social bonds within their all-male group. These bonds are structured according to age and kinship. It is likely that these bonds develop early in life, possibly during the juvenile period, and may persist beyond the bachelor stage. Moreover, bachelors with the strongest social bonds also groomed each other more equally than bachelors with weak bonds. Although several additional questions remain, our study is the first step in understanding the relationship between the development of social bonds, reproductive strategies, and fitness consequences of male geladas.

**Table 4.1. A summary of linear mixed models (LMM) and generalized linear mixed models (GLMM).** For all models the social index was considered to the outcome variable. All univariate models considered only one fixed effect each. We also conducted two multivariate models for each social index: (1) an additive model of all fixed effects; (2) an interactive model: the additive model, which also included an interaction between age class and relatedness. For our GLMM of the grooming equality index, we also included the grooming index as a fixed effect.

| Social Index | Random Effects | Fixed Effects |
|---|---|---|
| *Linear Mixed Models* | | |
| Composite Sociality Index (log-transformed) | Male Identities<br>All-Male Groups | Age Class (same <1.5 years apart; different >1.5 years apart)<br>Relatedness ($r_w$)<br>Rank Disparity (Δ Elo-Rating)<br>Age Class x Relatedness (interactive model only) |
| Grooming Index (log-transformed) | | |
| Proximity Index (log-transformed) | | |
| *Generalized Linear Mixed Models* | | |
| Grooming (Y/N, binomial) | Male Identities<br>All-Male Groups | Age Class<br>Relatedness<br>Rank Disparity<br>Age Class x Relatedness (interactive models only) |
| Proximity (<1 m) (Y/N, binomial) | | |
| Grooming Equality (>0.7, symmetric; <0.7 asymmetric) | | Grooming Index (log-transformed) |

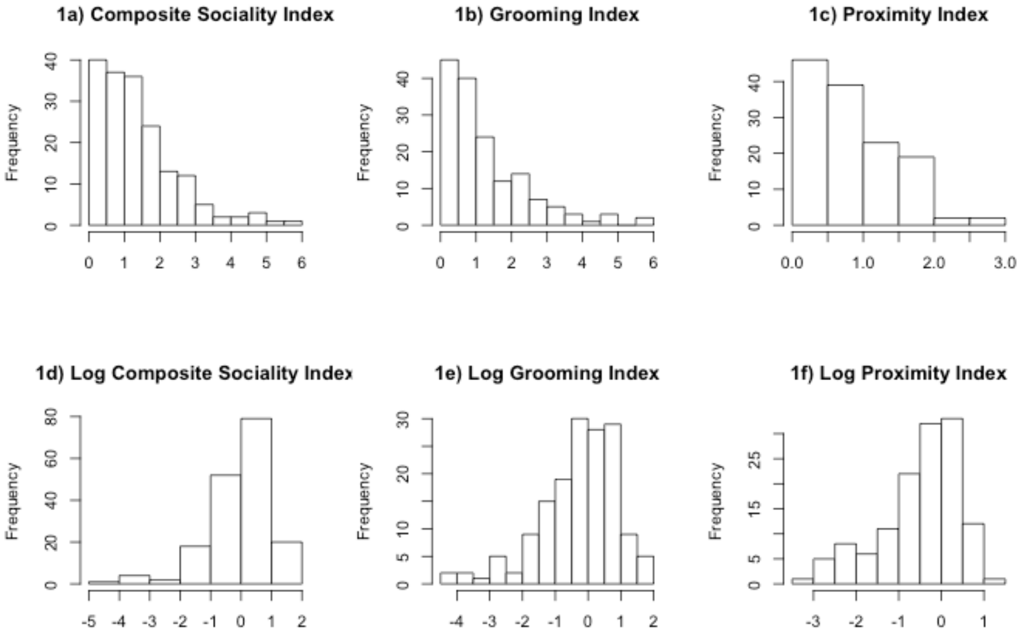

**Figure 4.1a-f.** Histograms of each social index and respective log-transformations. Each social index was right-skewed indicating many dyads had very weak indices while few dyads exhibited very strong indices. Grooming is the most skewed index suggesting strong preference for select grooming partners among bachelors. Alternatively, the proximity index is least skewed suggesting weak or no preference among bachelors for proximity after accounting for grooming (see Methods).

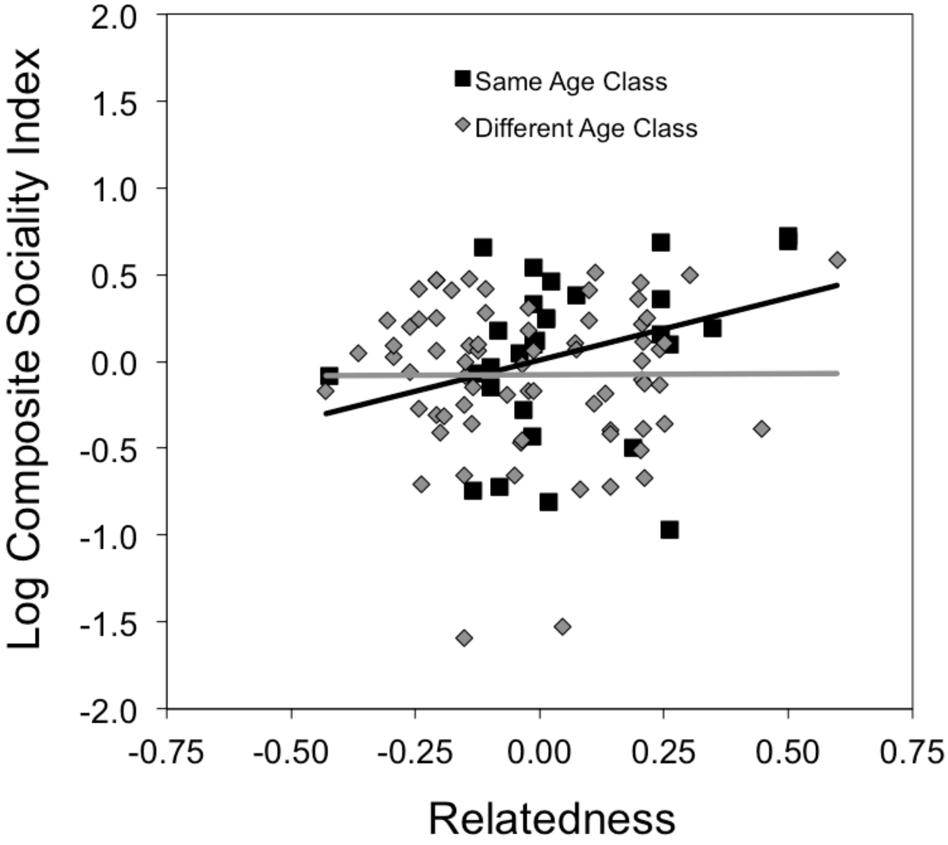

**Figure 4.2.** Scatterplot of composite sociality index (CSI). In both our multivariate models the CSI is weakly associated with relatedness (P=0.09) and age class (P=0.14).

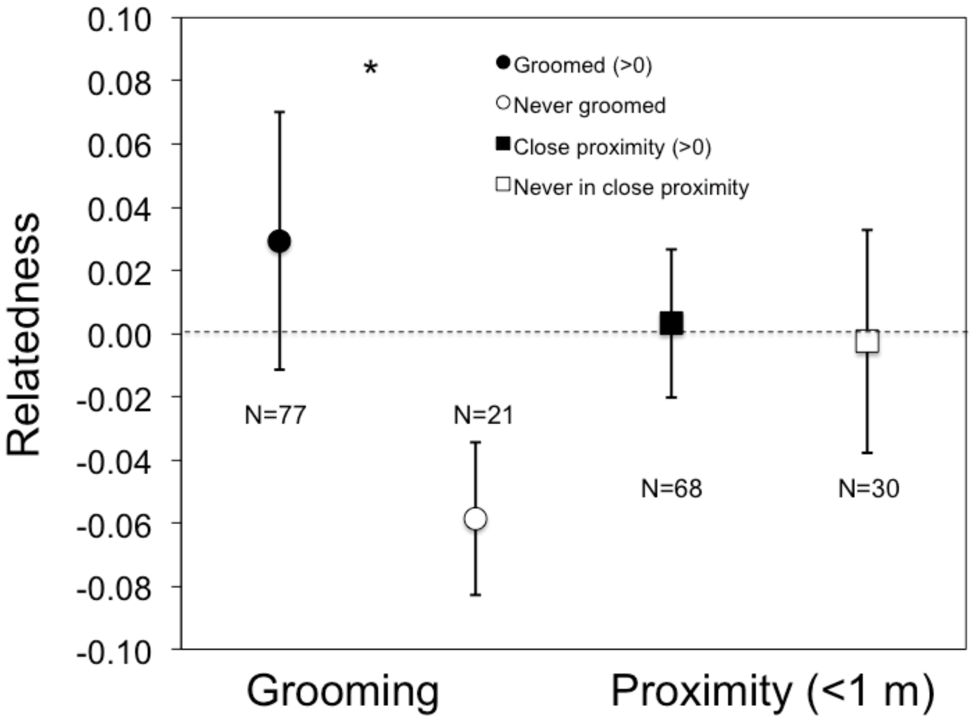

**Figure 4.3.** Relatedness, grooming, and proximity. We visualize both of our GLMM on grooming and proximity. In our grooming GLMM, relatedness was the sole predictor of whether or not a dyad was observed to groom during the study period (* $P<0.05$). None of our variables of interest (age class, relatedness, or rank disparity) where associated with whether or not a dyad was observed to be in close proximity.

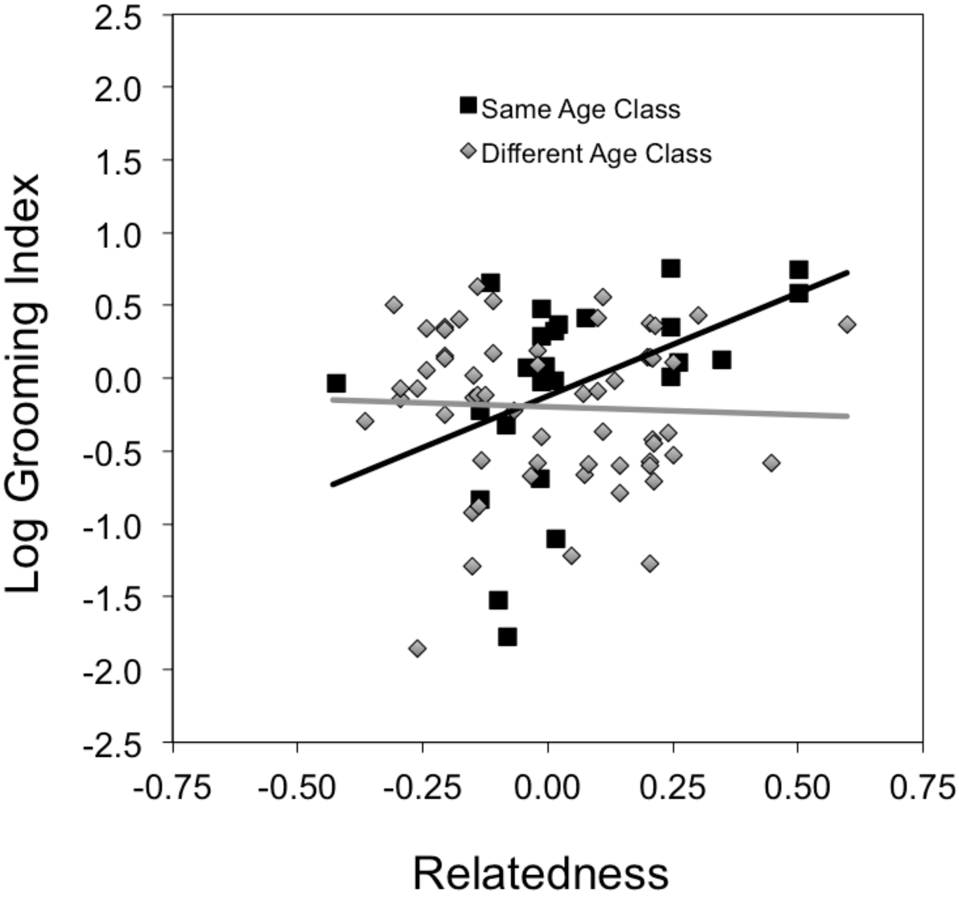

**Figure 4.4.** Scatterplot of grooming index. The grooming index is strongly associated with both age class, relatedness, and their interaction. Specifically, dyads whose members are considered to be in the same age class (<1.5 years apart estimated in age) and also closely related exhibit the strongest grooming relationships (P=0.06). Dyads whose members are far apart in age do not exhibit a relationship between relatedness and grooming.

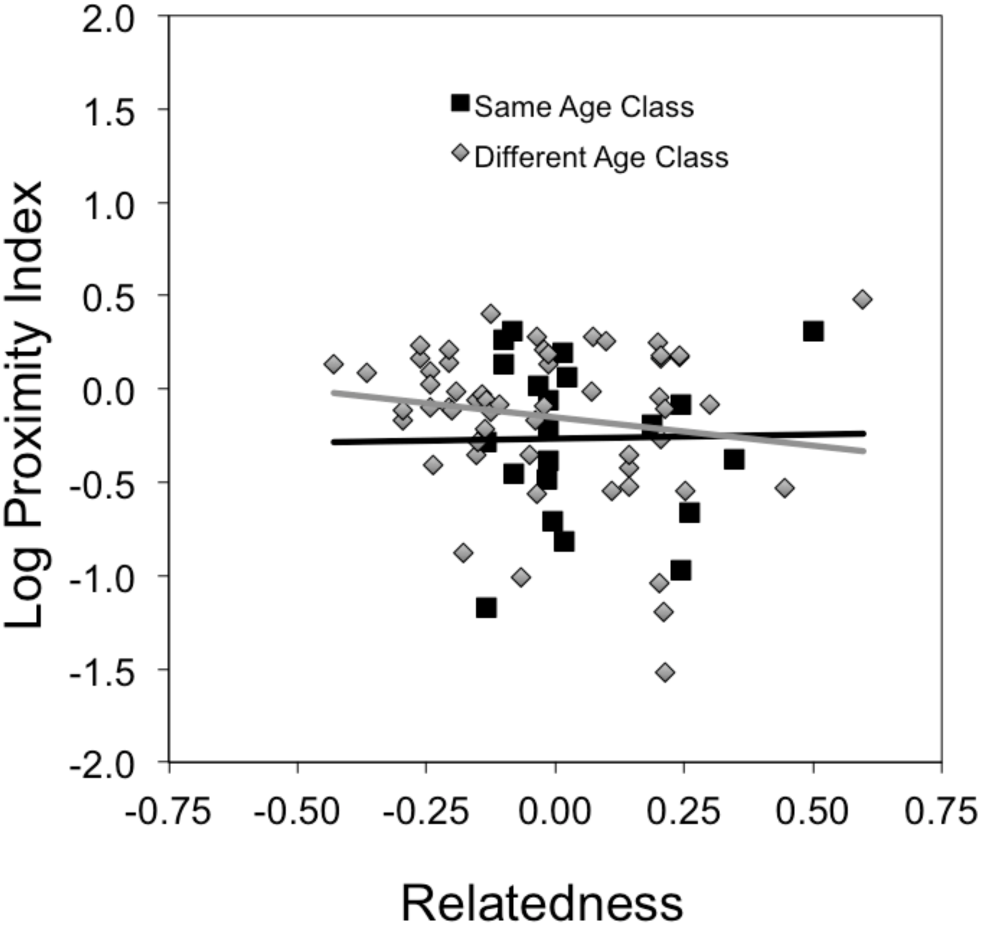

**Figure 4.5.** Scatterplot of proximity index. The proximity index is unassociated with age class or relatedness. After controlling for the amount of time dyads spent grooming, neither age nor relatedness was associated with time spent in close proximity (all P>0.20).

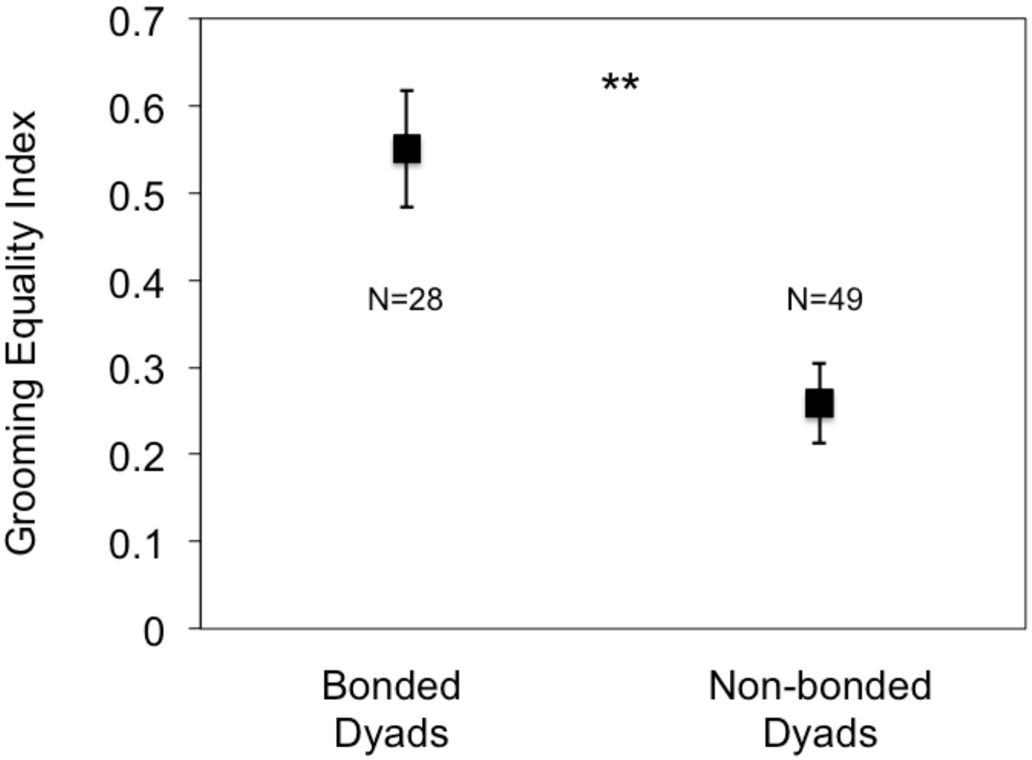

**Figure 4.6.** Grooming equality index and bond strength. We visualize our GLMM of the grooming equality index. For illustrative purposes we classified dyads based on the strength of their social bond (CSI). Dyads are considered bonded if they are each other's top or second ranking partner (** P<0.01). All other dyads are considered to be non-bonded (see Tinsley Johnson et al., accepted).

# CHAPTER FIVE

# SOCIAL ‘PREDATORS’ WITHIN A MULTILEVEL PRIMATE SOCIETY

## ABSTRACT

Group living confers a variety of benefits to individuals, particularly in predator detection and defense. Hamilton’s selfish herd hypothesis (Hamilton 1971, *Journal of Theoretical Biology*, **31,** 295–311) posits that individuals come together to reduce their own risk of predation, and numerous studies have shown that predators affect both the grouping and spacing patterns of their prey. We suggest that this hypothesis is also useful for understanding group responses to a very different threat: that posed by nonbreeding, potentially infanticidal males. In such cases, males may act as a predator-like force on the grouping patterns of breeding individuals. We hypothesized that nonbreeding males, like predators, can affect the spacing patterns of conspecifics. Specifically, we examined the effect of bachelor males on both the grouping and spacing patterns of gelada, *Theropithecus gelada*, reproductive units. First, we demonstrated that the number of bachelors was positively correlated with the number of animals in a group. Second, and more importantly, we found that bachelors exerted an acute pressure on the spacing of individuals; as bachelors approached, breeding individuals moved closer to their nearest neighbors. By approaching other breeding individuals, reproductive males and females may dilute the costs of associating with bachelor males.

## INTRODUCTION

Predator detection and defense are often invoked as benefits of group living. However, the costs and benefits of predator protection are not shared equally among group members. For example, predators generally target the nearest individual, making marginal individuals the most probable targets. Hamilton's (1971) 'selfish herd' hypothesis posits that an individual's risk of predation is positively related to its distance to conspecific neighbors (Hamilton 1971). In other words, widely spaced individuals should be targeted more often by predators (Quinn & Cresswell 2006; Wood & Ackland 2007). Hamilton (1971) suggested that by simply moving towards conspecifics, an individual reduces its risk of predation. Recent experimental (De Vos & O'Riain 2010) and naturalistic (Quinn & Cresswell 2006) studies support the central tenets of the selfish herd hypothesis in both mammalian and avian species.

Conspecifics may also pose a threat that is similar to predation. In many mammals, social units are composed of one male and several females. Unattached males mount challenges to males in mixed-sex units. Bachelors pose two direct types of threats to the fitness of breeding individuals: (1) they may replace the dominant breeding male, effectively ending his reproductive tenure; (2) they may commit infanticide, reducing the fitness of breeding males and females. The 'bachelor threat' hypothesis predicts that breeding males associate and form coalitions to avoid harassment, cuckoldry, risk of take-over (male replacement) and infanticide from unattached bachelor males (Rubenstein 1986). For example, the presence of bachelor males is the main cause of herding behavior by stallion plains zebra, *Equus burchelli* (Rubenstein & Hack 2004), and has affected the evolution of multilevel societies in some colobine primates (Grueter & van Schaik 2010). In these taxa, bachelors have a predator-like effect on the grouping behavior of individuals within core social units.

Here we examine the effect of bachelor males on the grouping and spacing patterns of a gregarious Old World monkey, the gelada, *Theropithecus gelada*. We also seek to determine whether breeding male geladas collectively defend their social group from bachelor intrusion. Gelada diet is composed nearly entirely of grasses (>90%), an abundant and evenly distributed resource that facilitates large social aggregations (Dunbar & Dunbar 1975; Kawai 1979). Geladas live in a fluid society composed of four levels: (1) the reproductive unit: 1–12 related females with their dependent offspring, one dominant ‘leader’ male, and possibly one or more subordinate ‘follower’ males that spend 100% of their time together; (2) the team: two to three units that spend at least 90% of their time together; (3) the band: a collection of 5–30 units that spend at least 50% of their time together; and (4) the community: a collection of all of the units in an area (as many as >100 units) that spend at least some time together (Dunbar & Dunbar 1975; Kawai et al. 1983; Snyder-Mackler et al. 2012). In addition, bachelor groups, comprising 2–15 mature bachelors and subadult males (Dunbar 1984), are loosely associated with reproductive units (often more than 20 m away). Reproductive units and bachelor groups fission (to form smaller groups) and fuse (to form a larger group) with each other during the day to form temporary ‘herds’ that may consist of reproductive units and bachelor groups from multiple bands (Dunbar & Dunbar 1975; Kawai et al. 1983). Leader males regularly solicit chases (‘yelping chases’) from bachelors, and previous research suggests that leader males that engage in more chases are less likely to be replaced (Dunbar & Dunbar 1975; Dunbar 1984). However, it is unknown whether leader males act collectively during these chases in a coalitionary fashion to defend the herd from bachelors.

Similar to the large aggregations of many ungulate species, it is hypothesized that the enormous size of gelada herds (>1000 individuals) is a response to predation risk (Crook 1966). However, the largest aggregations of geladas are often found at midday (Snyder-Mackler et al. 2012), and most gelada predators are crepuscular or nocturnal (e.g. spotted hyaenas, *Crocuta crocuta*, and leopards, *Pathera pardus*). Therefore, with very few predators during the daytime, we asked whether there were any factors that could affect gelada grouping and spacing throughout the day? We hypothesized that bachelors function as ‘social predators’ in gelada society because bachelors regularly harass leader males (which can result in male replacement (Dunbar 1984) as well as unit females (which can result in infanticide: (Roberts et al. 2012; Mori et al. 1997; Beehner & Bergman 2008). Specifically, we predicted that the presence and proximity of bachelors would influence the grouping and spacing patterns of breeding individuals and that leader males should collectively defend the gelada herd from encroaching bachelors. First, larger groups of geladas should form in response to increased bachelor presence, after controlling for weather variables (i.e. rainfall and temperature) that are known to influence group size (Hunter 2001). Second, the presence of bachelors should cause bands to fuse together forming larger herds throughout a given day, so that individuals may dilute their own risk of associating with bachelor groups. Third, as bachelors approach reproductive units, those units, as well as the individuals within them, should move closer together. Finally, leader males should collectively chase away bachelor males that get too close to the herd.

## METHODS

### *Study Species and Location*

Data were collected from a population of wild geladas in the Simien Mountains National Park, Ethiopia from January 2009 to April 2011 as part of the University of Michigan Gelada Research Project.  Their Afroalpine habitat includes few trees, facilitating high visibility of study animals. We collected regular observations from 229 individually recognized adults (48 unit males, 123 unit females, 58 bachelor males) across 19 units (Snyder-Mackler et al. 2012). Because of severe human encroachment, most of the natural predators of geladas, such as the spotted hyaena and leopard, are rare in the Simiens (N = 2 observed instances of predation, 2006–2011). All observers were trained for 6 weeks on data collection methods, including training with estimating known distances and monthly interobserver reliability checks for distance estimates.

### *Weather Data*

Weather data were collected using a La Crosse WS-2315U weather station (La Crosse Technology; La Crosse, WI, U.S.A.). Daily maximum and daily minimum temperatures (˚C) were recorded in addition to daily rainfall data (mm). Mean monthly rainfall was calculated as the average of rainfall from the previous 30 days.

### *Relationship between Bachelor Number and Herd Size*

Each morning (N = 381 days), observers counted all individuals as they ascended from the sleeping cliffs. Only fully mature bachelors were included in counts for bachelor groups (i.e. subadult males were excluded; for male age estimates and descriptions, see (Bergman et al. 2009; Beehner et al. 2009). Because of the high visibility conditions and spatial contiguity of the gelada herd, we counted bachelors as 'present' if they were located within 0.5 km of the herd.

First, we wanted to examine the relationship between the number of bachelors and herd size, while controlling for weather variables that are known to affect herd size. We ran five separate correlations that addressed the relationship among the number of bachelors present at a sleeping cliff, the monthly rainfall, the daily maximum temperature and the herd size: (1) number of bachelors versus herd size; (2) number of bachelors versus rainfall; (3) number of bachelors versus temperature; (4) herd size versus rainfall; and (5) herd size versus temperature. In these analyses, we did not include bachelors in our counts of herd size, and because our data were not normally distributed, we used a nonparametric Spearman's rank correlation coefficient.

Second, to establish a causal argument (i.e. does the number of bachelors cause larger herd sizes or do larger herd sizes attract more bachelors?), we then examined how bachelor number and herd size changed in response to the number of bachelors and herd size from the previous day (hereafter, termed ‘previous day’ and ‘subsequent day’). We used only pairs of consecutive days in which we observed the same band on both days (N = 71 pairs of days). We ran two separate multiple linear regressions to test the effect of bachelor number and herd size on the subsequent day’s bachelor number and herd size. In both models the number of bachelors and herd size were entered as predictor variables. The two models differed in their outcome variables. We used bachelor number on the subsequent day as the outcome variable for one model and herd size on the subsequent day as the outcome variable in the other. All predictor variables were z-transformed prior to analysis to reduce collinearity.

### *Effect of Bachelors on the Fissioning and Fusing of Herds*

Next, we examined whether the presence of bachelors influenced a herd’s probability of fissioning from or fusing with other geladas. We ran a generalized linear model using data from 113 fission and fusion events during the study period. We entered bachelor number and herd size prior to the fission or fusion event as predictor variables (in addition to rainfall and maximum daily temperature). We included the number of animals in the herd (herd size) as a predictor because there is probably an upper and lower limit to gelada aggregation size, such that larger groups are more likely to fission and smaller herds are more likely to fuse (i.e. a herd composed of only a few units is much more likely to fuse with another herd than to fission into independent units). We entered the subsequent fission or fusion event as a binomial outcome variable.

### *Effect of Bachelor Proximity on Group Spacing and Movement*

We recorded within- and between-unit distances using two independent methods: ‘neighbor scans’ and ‘unit-spread scans’. The main difference between the two spacing measurements was that for neighbor scans we only recorded the distances of three individuals to the focal animal, whereas for unit-spread scans we noted the distances of all unit members as well as the nearest extra-unit individual and bachelor to the focal unit's leader male. All distances (for both types of scans) were estimated by eye (Palombit et al. 1997), and researchers were subject to monthly interobserver reliability and accuracy checks.

### *Neighbor scans*

We conducted 15 min focal animal samples (N = 942) to record all social behavior for each individual. At 5 min intervals during focal samples, we conducted 'neighbor scans' (N = 2824) to record the identity and distance of the focal individual's nearest neighbors in three demographic categories: (1) the nearest within-unit animal, (2) the nearest extra-unit animal and (3) the distance to the nearest bachelor. We estimated distance to the nearest 0.5 m for within- and extra-unit animals and to the nearest 5 m for bachelors.

### *Unit-spread scans*

We also conducted unit 'spread scans' (N = 261), recording the distance of all unit members to the leader male (N = 1540 individual distances) as well as the distance from the leader male to the nearest member of another unit (N = 261). At the beginning of each unit-spread scan we estimated the distance from the focal unit leader male to the nearest bachelor. Bachelor distance was estimated to the nearest 5 m. Because of the large number of individuals within some units, we recorded distances from the leader male as categorical variables (<1 m, 1–5 m, 5–10 m, 10–20 m and >20 m). These scans were conducted outside of focal samples (at 20 min intervals) to ensure independence from neighbor scans.

### *Within-unit Spacing*

To determine how bachelors affect group spacing, we first examined whether the distance to bachelors affected the distance between individuals within a unit. We developed two models using the two measures of spacing: Model 1 using only neighbor scans, and Model 2 using only unit-spread scans. The outcome variable was the distance between the focal individual and the nearest within-unit individual (Model 1) or the unit's leader male (Model 2). In Models 1 and 2, unit size, group size, time of day, behavioral state (moving, feeding, resting or socializing), previous month's rainfall, maximum daily temperature and the distance to the nearest bachelors were entered as fixed effects. The individual's identity and unit identity were entered as random effects.

### *Between-unit Spacing*

In our second investigation into how bachelors affect group spacing, we examined whether the distance to bachelors was related to the distance between individuals in different units. As previously, we developed two models using our two measures of spacing: Model 3 using only neighbor scans, and Model 4 using only unit-spread scans. The outcome variable was the distance between the nearest extra-unit individual and the focal individual (Model 3) or the focal unit's leader male (Model 4). The fixed and random effects for Models 3 and 4 were the same as for Models 1 and 2. We excluded interactions between fixed effects in our models because all of our predictions were additive (i.e. what was the effect of bachelor distance on group spacing when controlling for the effect of the number of bachelors, herd size, etc.).

### *Leader Male Defense of Breeding Females*

To determine whether unit males jointly participated in herd defense against bachelors, we collected data on chases involving known unit males and bachelors during the study period. We collected all occurrences (Altmann 1974) of these chases.

However, because chase events are chaotic in nature, we may have missed some chases involving multiple males. Therefore, it is likely that we are under-reporting the number of chases that involved multiple unit males.

### *Statistical Analyses*

All statistical analyses were conducted with R statistical software (R Core Development Team 2011). We calculated all correlations using the R function ‘cor.test’. We corrected for multiple correlations using the Bonferroni correction. We ran four separate linear mixed models, fitted with a Gaussian error structure, to determine the effect of bachelors on the grouping and spacing of gelada units using the function lmer of the R package lme4 (Sarkar & Bates 2009). We found no strong collinearity among our predictors (all variance inflation factors <2; all condition numbers <4). We log-transformed our inter- and intra-unit distance measures so that they approximated a normal distribution. Following the log transformation of the outcome variables, all four of our models had approximately normally distributed residuals, which we visualized using a Q–Q plot and a histogram of residuals, and by plotting the fitted versus residual values of our models. We ran likelihood ratio tests comparing the full model (random and fixed effects) with the respective null model (only random effects) as a test of overall significance of our fixed effects. We then used 10 000 Markov-chain Monte Carlo simulations to assess significance of the full models. We present only the four full models containing all fixed effects.

### *Ethical Note*

All data collection was noninvasive. The University of Michigan University Committee on Use and Care of Animals (UCUCA number 09554) and the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC number 802996) approved all methods used in data collection.

## RESULTS

*Relationship between Bachelor Number and Herd Size*

Herd size and bachelor number were not correlated with either average monthly rainfall or maximum daily temperature (Spearman rank correlations: $-0.1 < r_S < 0.1$, Ps > 0.15). Herd size was positively correlated with the number of bachelors ($r_S = 0.37$, N = 381, $P < 0.001$; Fig. 5.1). We fitted a regression line to the correlation between number of bachelors and herd size, which revealed that for each additional bachelor, group size increased by nine individuals. Neither bachelor number nor herd size significantly affected bachelor number on the subsequent days (multiple linear regression: $F_{2,68} = 1.02$, $P = 0.37$), but herd size on the previous day was a strong predictor of herd size on the subsequent day ($F_{2,68} = 4.59$, $P<0.02$; herd size on previous day: $\beta = 40.9$, $t_{df} = 2.45$, $P < 0.02$).

*Effect of Bachelors on the Fissioning and Fusing of Herds*

Larger herds were more likely to fission into two or more smaller herds; and smaller herds were more likely to fuse with other herds ($\beta = -0.0065$, $Z = -3.43$, $P < 0.001$). The number of bachelors present did not significantly affect the probability of a herd fissioning or fusing ($\beta = 0.011$, $Z = 0.236$, $P = 0.81$). Neither of the two environmental factors, rain ($\beta = 0.075$, $Z = 1.52$, $P = 0.13$) or maximum temperature ($\beta = 0.142$, $Z = 1.40$, $P = 0.16$), significantly affected the probability of a herd fissioning or fusing.

*Within-unit Spacing (Models 1 and 2)*

Within-unit spacing was positively correlated with distance to the bachelors. The full models of within-unit spacing from two independent measures, neighbor scans and unit-spread scans, were significantly better predictors than the null models (Model 1: $\chi^2_{10}$

= 1088, P < 0.001; Model 2: $\chi^2_{10} = 413$, P < 0.001). Both models revealed the same effect; as bachelors approached units, within-unit individuals moved closer to each other and closer to their leader male. For example, if the bachelors moved from 200 m away to 100 m away from the unit, the focal individual moved 0.5 m closer to other unit animals (Table 5.1) and 2.6 m closer to the leader male (Table 5.2). Within-unit spacing was also negatively correlated with the number of bachelors present. In other words, when there were more bachelors, individuals were closer to their nearest within-unit neighbor (Table 5.1) and closer to their leader male (Table 5.2). Distance to the leader male was also affected by maximum temperature (individuals were farther from their leader on warmer days) and unit size (larger units were more spread out than smaller units).

### *Between-unit Spacing (Models 3 and 4)*

Between-unit spacing was positively correlated with the distance to the nearest bachelors as measured using both neighbor scans and unit-spread scans. The full models of between-unit spacing were significantly better predictors than the null models, which included only the random effects (Model 3: $\chi^2_9 = 327$, P < 0.001; Model 4: $\chi^2_9 = 48$, P < 0.001). Controlling for weather variables, we found that if the bachelors moved from 200 m away to 100 m away from the units, the nearest nonbachelor extra-unit individual was 1 m closer to the focal individual (Table 5.3) and 3.2 m closer to the focal unit's leader male (Table 5.4). The number of bachelors present was negatively related to between-unit spacing when measured using neighbor scans (similar to the within-unit effect mentioned above) but not when measured using unit-spread scans.

### *Leader Male Defense of Breeding Females*

We observed 72 days during which males chased bachelors from a nearby all-male group. Males from multiple units in the same herd collectively acted in chasing off the bachelors during the course of the day on 21% (15/72) of the days.

## DISCUSSION

We found several lines of evidence that bachelor males may indeed exert a predator-like effect on the gelada social system. First, we found a strong positive relationship between the number of bachelors and herd size; the more bachelors in an immediate area, the larger the herd, a finding that corroborates similar findings in plains zebra (Rubenstein & Hack 2004) and several colobine primates (e.g. *Nasalis larvatus*, *Pygathrix* spp., *Rhinopithecus* spp.) (Grueter & van Schaik 2010). However, at present, we are unable to ascertain the causality of this relationship. Analyses of consecutive days suggest that larger herds did not attract more bachelors, nor did more bachelors cause larger herds to form. Furthermore, the presence of bachelors did not influence the fission–fusion dynamics of the gelada social system. Bachelor number had no affect on fission/fusion events. Herd size alone was the only significant predicator of fissions and/or fusions. That is, larger herds were more likely to split into smaller ones and smaller herds were more likely to join with each other. Additionally, our approach (using bachelor number or herd size to predict bachelor number or herd size 1 day later) may not be fine-grained enough to uncover the causality in the relationship between number of bachelors and herd size.

If, indeed, there is no causal relationship between bachelor number and herd size (and herd size and bachelor number), this raises the question: if bachelors are neither attracted to, nor cause, the formation of larger herds, then what explains the strong correlation between bachelor number and herd size on any given day? Two alternative scenarios may be responsible for this observation.

First, reproductive units may associate with a constant proportion of bachelor groups (Dunbar & Dunbar 1975; Kawai 1979); and as multiple bands form herds, the relationship between bachelor number and herd size is merely a consequence of increasingly larger herds. This scenario is unlikely, as the association between bachelor groups and reproductive units is highly variable (10% to 100% bachelor association with certain units; Kawai et al. 1983). Preliminary results from our project suggest that bachelor groups form very loose associations with units, well below that constituting band membership in geladas (Snyder-Mackler et al. 2012), except for brief periods of 'instability' (i.e. periods of increased rates of male replacement among units) where bachelors are found with units at high association levels (le Roux & Bergman 2012). Additionally, bachelor groups themselves rarely associate with one another (D. J. Pappano & J. C. Beehner, unpublished data). When they are found in the same herd, bachelor groups are situated on opposite sides of the herd and appear to actively avoid each other.

Second, and more importantly, we found that bachelors were associated with an acute predator-like effect on the spatial relationships within and among gelada units. As bachelors were found closer to reproductive units, individuals in those units were found closer to the other members of their unit. Additionally, the units themselves were found closer together (Fig. 5.2). It is possible that both bachelors and breeding individuals are clumping in response to another unexplored variable, such as predator presence. However, we think this is unlikely because predator encounters are quite rare and bachelor groups are typically found about 35 m from the herd, a distance at which they would not reap the selfish herd benefits with respect to predation. In short, bachelor proximity was associated with the clumping of units as well as with the clumping of individuals within those units.

Despite not influencing overall grouping patterns, proximity to bachelors influences the spatial patterns of conspecifics. Although the proximity effects initially appear to be small (a few metres for every 100 m decrease in bachelors' proximity), our methods only measured distances between two individuals. Extrapolation of these results to the entire aggregation, however, indicate that all geladas within a unit were found 1–2 m closer to each other and that all geladas in the group were found 2–3 m closer together for every 100 m of bachelor proximity. This multiplicative effect of bachelors on the entire aggregation of geladas is quite substantial. Furthermore, we expect this effect to exist in other species where nonbreeding males pose a threat to breeding males and females.

We propose that the ‘selfish herd’ and ‘bachelor threat’ hypotheses can be combined into a single ‘social predator’ hypothesis. Mainly, nonbreeding mature males affect the spacing patterns of breeding individuals in a predator-like fashion. Proximity to nonbreeding males can be costly to breeding males through increased risk of cuckoldry and harassment, either of which could lead to male replacement. It is also costly to females, which are at an increased risk of infanticide (van Schaik & Kappeler 1997), harassment, and, in the case of geladas, spontaneous abortion (Roberts et al. 2012). In clumping together, breeding individuals attempt to distribute these various costs among conspecific competitors. Although both breeding males and females suffer reproductive costs from infanticide, females not currently ‘at risk’ of infanticide (i.e. those that are sexually receptive and lack dependent offspring) may not suffer any costs associated with proximity to nonbreeding males compared to their ‘at risk’ groupmates. It is possible that sexually receptive females prefer unattached males to their current mating partners, causing them to approach the bachelors. It is equally likely, however, that sexually receptive females prefer their current unit males, as geladas form strong intersex bonds within units (Dunbar 1983). Additionally, those females not ‘at risk’ of infanticide represent only a fraction of geladas, as pregnant females and females with dependent offspring represent the majority (~80%) of the females at any point in time. Furthermore, cycling and ‘at risk’ females within the same unit are all closely related and may gain inclusive fitness benefits from clumping together (N. Snyder-Mackler, J. C. Beehner, & T.

J. Bergman, unpublished data). We therefore included all adult females in our analysis, as we were interested in the effect of spacing on all adult unit individuals.

Breeding individuals may gain other benefits from coalescing, such as a dilution of encounter effect (Cresswell 1994) or possibly from a modified confusion effect (Hall et al. 1986; Neill & Cullen 1974). They may suffer some costs resulting from closer proximity to conspecifics, such as feeding competition (Majolo et al. 2008), disease transmission (Côté & Poulin 1995) and cuckoldry (Westneat & Sherman 1997). As geladas primarily feed on grass, which is evenly distributed, feeding competition is probably not a substantial cost to merging in the presence of bachelors. Moreover, a densely packed gelada unit and band may serve to ‘disguise’ larger units (since it could just as easily be several overlapping smaller units; Snyder-Mackler et al. 2012). This may benefit breeding males in larger units, since larger units are more likely to be targeted by bachelor males (Dunbar 1984; Bergman 2010). Finally, leader males occasionally simultaneously chased away bachelors from the gelada herd, and may benefit from being near preferred coalition partners during such events. However, leader males do not always join these events and might ‘free-ride’ on the efforts of males that regularly participate. We are currently investigating how relationships among leader males may influence their decision to participate in collective chases. These observations, in combination with experimental studies (Bergman 2010) suggest that breeding males (and possibly females) monitor and react to bachelors much as they would a predator. Taken together, we suggest a synthesis of social predator–prey dynamics, mate competition and spatial patterning as a new way of understanding collective movement and grouping behaviors in animal social systems.

**Table 5.1.** Mixed model describing the relationship of social and weather variables on within-unit spacing using the ‘neighbor’ scan method

| | Effect | HPD lower 95%* | HPD upper 95%† | pMCMC |
|---|---|---|---|---|
| Rainfall (mm) | -0.0013 | -0.0057 | 0.0033 | 0.5896 |
| Temperature (˚C) | 0.0050 | -0.0040 | 0.0137 | 0.2904 |
| Distance to bachelors (m) | 0.0014 | 0.0006 | 0.0022 | **0.0004** |
| Unit size | 0.0110 | -0.0008 | 0.0246 | 0.0694 |
| Group size | 0.0000 | -0.0002 | 0.0002 | 0.8858 |
| Number of bachelors | -0.0057 | -0.0113 | -0.0001 | **0.0464** |

Significant values are shown in bold.

* Highest posterior density (HPD) 95% lower bounds from the Markov-chain Monte Carlo simulation.

† Highest posterior density (HPD) 95% upper bounds from the Markov-chain Monte Carlo simulation.

**Table 5.2.** Mixed model describing the relationship of social and weather variables on within-unit spacing using the 'unit-spread' scan method

| | Effect | HPD lower 95%* | HPD upper 95%† | pMCMC |
|---|---|---|---|---|
| Rainfall (mm) | -0.0011 | -0.0088 | 0.0065 | 0.7346 |
| Temperature (˚C) | 0.0061 | -0.0004 | 0.0130 | 0.0742 |
| Distance to bachelors (m) | 0.0034 | 0.0023 | 0.0045 | **0.0001** |
| Unit size | 0.0477 | 0.0320 | 0.0643 | **0.0001** |
| Group size | 0.0002 | -0.0001 | 0.0005 | 0.2176 |
| Number of bachelors | -0.0025 | -0.0039 | -0.0009 | **0.0006** |

Significant values are shown in bold.
* Highest posterior density (HPD) 95% lower bounds from the Markov-chain Monte Carlo simulation.
† Highest posterior density (HPD) 95% upper bounds from the Markov-chain Monte Carlo simulation.

**Table 5.3.** Mixed model describing the relationship of social and weather variables on between-unit spacing using the 'neighbor' scan method

| | Effect | HPD lower 95%* | HPD upper 95%† | pMCMC |
|---|---|---|---|---|
| Rainfall (mm) | -0.0006 | -0.0046 | 0.0035 | 0.8124 |
| Temperature (˚C) | 0.0186 | 0.0102 | 0.0267 | **0.0001** |
| Distance to bachelors (m) | 0.0021 | 0.0013 | 0.0028 | **0.0001** |
| Group size | 0.0003 | 0.0001 | 0.0005 | **0.0058** |
| Number of bachelors | -0.0105 | -0.0154 | -0.0055 | **0.0001** |

Significant values are shown in bold.

* Highest posterior density (HPD) 95% lower bounds from the Markov-chain Monte Carlo simulation.

† Highest posterior density (HPD) 95% upper bounds from the Markov-chain Monte Carlo simulation.

**Table 5.4.** Mixed model describing the relationship of social and weather variables on between-unit spacing using the 'unit-spread' scan method

| | Effect | HPD lower 95%* | HPD upper 95%† | pMCMC |
|---|---|---|---|---|
| Rainfall (mm) | 0.0201 | 0.0049 | 0.0361 | **0.012** |
| Temperature (˚C) | 0.0089 | -0.0052 | 0.0217 | 0.1822 |
| Distance to bachelors (m) | 0.0033 | 0.0013 | 0.0055 | **0.002** |
| Group size | -0.0009 | -0.0016 | -0.0002 | **0.0092** |
| Number of bachelors | -0.0004 | -0.0035 | 0.0029 | 0.8134 |

Significant values are shown in bold.

* Highest posterior density (HPD) 95% lower bounds from the Markov-chain Monte Carlo simulation.

† Highest posterior density (HPD) 95% upper bounds from the Markov-chain Monte Carlo simulation.

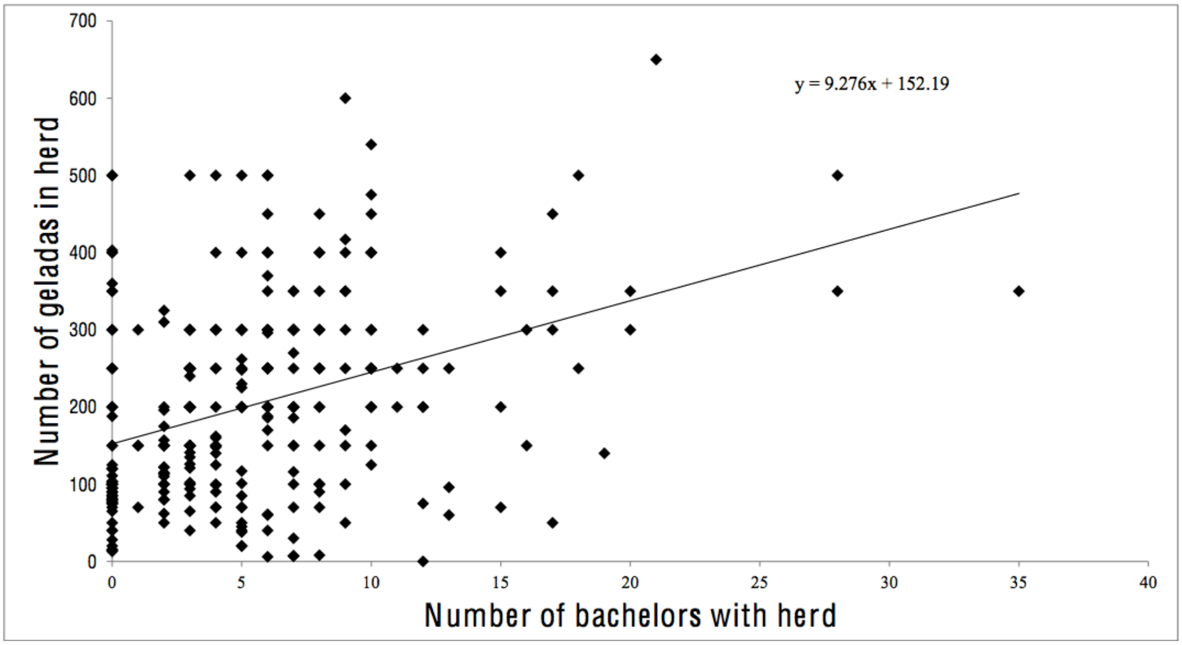

**Figure 5.1.** Linear regression showing the relationship between the number of bachelors associating with a gelada herd and the total number of geladas within a herd, excluding bachelors (Spearman rank correlation: $r_S = 0.37$, $P < 0.001$).

**Figure 2**

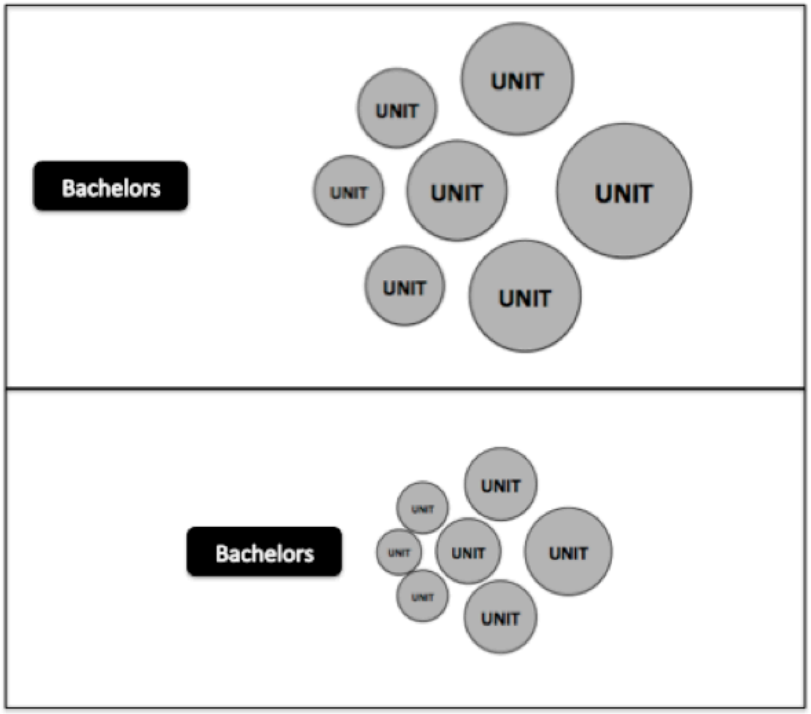

**Figure 5.2.** Heuristic schematic showing the effect of bachelor proximity (distance) on reproductive unit spread. The radius of the circles (units) represents the within-unit distance from a central individual to the most distant members of a unit. The distance between the edges of each circle represent the distance between the closest adults belonging to separate reproductive units (between-unit distance). The top panel shows the bachelors at a relatively far distance from the edge of the gelada herd, while the bottom panel shows the bachelors very close to the herd edge. Note that all units here are assumed to be of equal size; thus, the spread within and between them is only related to bachelor proximity

# CHAPTER SIX

# TESTOSTERONE AND THE REPRODUCTIVE TRAJECTORIES OF MALE GELADAS

## ABSTRACT

Among male vertebrates, the “challenge hypothesis” (Wingfield et al. 1990, *The American Naturalist*, **136**, 829-846) predicts patterns in androgen secretion to vary in accordance with mating system, male-male competition, and degree of paternal care. For non-seasonally breeding species, increases in male androgen levels are often associated with competition for rank or access to females. In order to acquire reproductive success, gelada (*Theropithecus gelada*) males must first overthrow a dominant leader male for access to a unit of females and then, as the new dominant male, fend off rival males. Here, we examine the social and seasonal factors contributing to variation in adult male gelada androgen (fecal testosterone) levels using noninvasive fecal hormone sampling (N=2730 samples and 133 males) collected over 6 years (2006-2011). Although dominant males exhibited the highest levels of testosterone overall, bachelor males exhibited significantly higher testosterone levels across a few months each year characterized by a high rate of takeover. Among bachelors, future dominant males exhibited higher testosterone levels than males that remained as bachelors. Most importantly, future dominant males exhibited elevated testosterone levels approximately 3 months prior to taking over a reproductive unit. Our results suggest that for support findings in other primate species, suggesting that testosterone predicts the reproductive trajectories of males. We discuss these findings in light of applying the challenge hypothesis to long-lived non-seasonally breeding species.

## INTRODUCTION

The role of testosterone in male vertebrate development and behavior is well understood. Testosterone promotes sexual dimorphism (Bardin & Catterall 1981), regulates spermatogenesis (Woodley 1994), causes the development of secondary sexual characteristics (Hau 2007), influences paternal care (Hunt et al. 1999; Peters et al. 2002) and mating effort (Raouf et al. 1997; Stoehr & Hill 2000). It is essential for male reproductive competition, as high testosterone levels are associated with well-developed weaponry (C et al. 2003) and sexually selected signals (Galeotti et al. 1997; Ligon et al. 1990; Madsen et al. 2007; Setchell et al. 2008). Testosterone also facilitates aggression to maintain or acquire reproductive access to females through achieving high dominance rank or status and establishing and maintaining territory (Beehner et al. 2006; Hunt et al. 1995). Despite its reproductive benefits, testosterone can impose costs on males. Elevated levels can be immunosuppressive (Mills et al. 2008; Grossman 1985) and inhibit paternal behavior (Hunt et al. 1999; Peters et al. 2002). Therefore, testosterone regulation should balance costs and benefits in accordance with socioecological pressures that change over time and with different life-history stages.

The “challenge hypothesis” is a framework for understanding the relationship between male androgen (e.g. testosterone, dihydrotestosterone, and androstenedione) levels and socioecological factors, such as mating system, degree of paternal care, male-male competition, and seasonality (Wingfield et al. 1990). Originally described for seasonally breeding avian species, the challenge hypothesis proposes that male androgen levels rise at the beginning of the mating season from a non-breeding level to a breeding baseline in order to facilitate sperm production and reproductive behavior (Wingfield et al. 1990). Androgens may also rise above this breeding baseline to a physiological maximum level (Wingfield et al. 1990; Goymann et al. 2007). Although the rise from the non-breeding baseline to the breeding baseline is periodic and strongly influenced by environmental cues (e.g. change in photoperiod), the rise above the breeding baseline is facultative and is influenced by social challenges from rival males or exposure to receptive females (Wingfield et al. 1990; Goymann et al. 2007). The central predictions of the challenge hypothesis have been generalized to many seasonally and non-seasonally breeding vertebrate species (Oliveira 2004; Hirschenhauser & Oliveira 2006) including humans (Archer 2006).

Over the past decade, advances in fecal steroid hormone monitoring have allowed researchers to examine the relationship between androgen levels and male reproductive competition in many wild taxa (Brown et al. 1994; Whitten et al. 1998). In particular, primates have garnered special attention in this area as they exhibit considerable variation in mating system and breeding seasonality coupled with long-life histories (Brockman & van Schaik 2005). For many male primates, reproductive success is often associated with dominance rank. According to a priority-of-access model, dominant males can monopolize access to receptive females therefore males should compete aggressively for rank or status (Alberts et al. 2003; Alberts et al. 2006; Altmann 1962). Given this relationship between rank and reproductive success, researchers often “test” the challenge hypothesis in primates by examining the relationship between testosterone and competition for mates, through rank acquisition (Beehner et al. 2006; Higham et al. 2013), or, direct conflict over receptive females (Muller & Wrangham 2004). Additionally, primatologists might examine the relationship between testosterone and “static” rank, i.e. dominant males are expected to exhibit the highest testosterone levels overall (Brockman et al. 1998; Mendoza et al. 1978; Setchell et al. 2008). However, given the long lifespan and small group size of many primates, many studies often only observe 1 or 2 dominant males during the study period (but see Gesquiere et al. 2011), or are limited to a single breeding season. Thus, associations between age and testosterone may confound the relationship between rank and testosterone levels, especially when prime-aged, high testosterone males are also high-ranking. Therefore, in order to "test" the challenge hypothesis in a wild primate, researchers must control for age as well as other ecological factors (e.g. temperature and rainfall) that may influence testosterone secretion (Girard-Buttoz et al. 2009; Gesquiere et al. 2005; Gesquiere et al. 2011; Muroyama et al. 2007).

In this study, we examine the seasonal and social influences on testosterone secretion in male geladas (*Theropithecus gelada*)—a primate species where dominance status mediates male reproduction (Snyder-Mackler et al. 2012). Geladas are an ideal primate species examine the factors that influence testosterone secretion for several reasons. First, gelada males may experience variation in testosterone levels due to seasonal variation in temperature or rainfall (Brockman & van Schaik 2005; Girard-Buttoz et al. 2009; Gesquiere et al. 2011). Their Afroalpine environment experiences a pronounced "wet" season where nearly 80 percent of the annual rainfall occurs between June-September (Hunter 2001; Beehner et al. 2006). Additionally, previous research has shown that geladas are "cold-stressed," demonstrating a direct link between their ecological environment and endocrine function (Beehner & McCann 2008).

Second, geladas live in extremely large social groups facilitating large amounts of cross-sectional data collection (Crook 1966; Dunbar & Dunbar 1975; Kawai 1979). Geladas live in a modular (or multilevel) society consisting of four levels: (1) the "one-male unit"- a mixed-sex core group of related females, a single dominant "leader" male, and possibly several (0-4) subordinate "follower" males; (2) the team- two or three units that spend >90% of their time together; (3) the band- a group of 5-30 units are found together >50% of the time; and (4) the community- a collection of all the units (>100) in a given area that share a common home range (Kawai et al. 1983; Snyder-Mackler et al. 2012). Temporary herds of geladas may contain over 1000 individuals from different bands (Kawai et al. 1983; Snyder-Mackler et al. 2012). Although one-male units are predominantly unimale, approximately one-third of units contain follower males (Snyder-Mackler et al. 2012). Follower males are often recently deposed leaders, but can also be young males that enter a unit as subordinates (Dunbar 1984). Dominant males account for all the reproduction within unimale units, but followers sire approximately 17% of offspring in multimale units (Snyder-Mackler et al. 2012). Although followers acquire some reproductive success, it is unlikely that "following" represents a true gelada reproductive strategy (Snyder-Mackler et al. 2012). Given the presence of follower males in some units, we call all "one-male" units simply units or reproductive units.

Third, gelada groups experience frequent bachelor incursions that result in a high rate of dominant male replacement (hereafter, takeover). Bachelor geladas live in all-male groups that loosely associate with reproductive units (Kawai et al. 1983). In order for a bachelor gelada to acquire reproductive access he must enter a reproductive unit by directly challenging and overthrowing a dominant leader male (i.e., "takeover") or submissively entering as a follower (Dunbar 1984). Challenges for reproductive access to females for geladas generally take the form of ritualized chases (also called "yelping chases," Dunbar & Dunbar 1975). Chases are hypothesized to indicate a leader male's competitive ability, as leaders who initiate chases more frequently are less likely to be overthrown (Dunbar 1984). During these chases, a leader male approaches a group of bachelors, solicits a chase, and gallops in the opposite direction (Dunbar & Dunbar 1975). Bachelors may or may not pursue the leader male. The chase ends when a leader male leaps into a tree and gives a loud display ("ee-yeow") call (Dunbar & Dunbar 1975), or with contact aggression and a challenge for reproductive access to the unit's females. When a chase escalates to fighting, the victorious bachelor will replace the leader male as the dominant animal in his reproductive unit (Dunbar & Dunbar 1975; Dunbar 1984). These attempts to overthrow males are often brief (less than a few minutes), but have been observed to last several days (Beehner and Bergman, unpublished data). If a bachelor does not directly overthrow and replace a male as leader of a given unit, he may submissively enter a unit as a follower male or remain a bachelor (Dunbar 1984).

We sought to answer three main questions concerning the relationship between male-male competition and testosterone secretion in geladas. First, do geladas exhibit seasonal patterns in male-male competition and takeovers? Previous research suggests that herd size is strongly related to bachelor presence, with the largest herds occurring during periods of increased bachelor number, however neither rainfall nor temperature predicted bachelor-herd association (Pappano et al. 2012). Despite this, we have observed that the majority of successful takeovers in our population occur at the end of the dry season (Feb-May). Given this observation, we predicted that aggression between males would be associated with increased takeovers. Moreover, we expect takeovers to follow a seasonal pattern with the majority occurring at the end of the dry season.

Second, we asked what are the sources of variation in gelada male testosterone levels over the course of a year? Prior research has shown that geladas exhibit a sharp and circumscribed rise in testosterone during early adulthood that coincides with peak potential reproductive years (Beehner et al. 2009). However, it is unknown if rainfall or temperature affect variation in gelada male testosterone values over the course of a year. In their sister taxa (baboon, *Papio* spp.), males living in the Amboseli Basin exhibited lower testosterone levels during the dry season compared to the wet season, and lower testosterone in months of high daily maximum temperature (Gesquiere et al. 2011). In the same population, dominance rank was also associated with testosterone levels with high-ranking males exhibiting the highest testosterone levels (Gesquiere et al. 2011). As geladas are closely related to baboons, we expect gelada males to also exhibit depressed testosterone during hot and dry periods of the year. We also predict age and status to influence testosterone levels. We expect that as adult gelada males increase in age their testosterone levels will decrease. After controlling for age and possible seasonal effects, we predict that leader males will exhibit higher testosterone levels than bachelors or followers across the year.

Finally, we were interested in the relationship between testosterone levels and a gelada male's reproductive trajectory. Recent evidence suggests that a peak testosterone level in geladas is associated with a life-history stage where males might expect to engage in reproductive competition, e.g. early adulthood (Beehner et al. 2009). However, it is unclear as to whether this peak in testosterone is strictly socially mediated or is simply due to age (Beehner et al. 2009). Moreover, in closely related taxa (e.g. mandrills, Wickings & Dixson 1992, and chacma baboons, Beehner et al. 2006) testosterone levels predict the reproductive trajectory of males. In other words, males with high testosterone are rising in dominance rank, whereas males with low testosterone are falling. Given these results in related species, we expect that testosterone will have a similar predictive influence on gelada male reproductive trajectories. That is, bachelors with relatively high testosterone will be those most like to become future leader males. Moreover, within specific takeovers we expect the "winning" bachelors to exhibit higher testosterone than the "losing" former leader males (Mazur et al. 1992).

## MATERIALS AND METHODS

### *Subjects and study site*

Data were collected from a population of geladas living in the Simien Mountains National Park, Ethiopia as part of the long-term University of Michigan Gelada Research Project. Over the 6-year study period (2006-2011), we collected behavioral, demographic, and hormonal data from all adult males from 21 reproductive units and 13 all-male groups (N=133 males, including leaders, followers, and bachelors). Throughout we refer to leaders and followers as “unit males” to distinguish them from “bachelor males”. This population has been under near-daily observation since 2006. All males are individually recognized and habituated to observers on foot.

The Simien Mountains National Park encompasses an area of Afroalpine habitat (150 km$^2$, 3200-4500 m.a.s.l.) including open grassland plateau and a few remnant forests. The region experiences pronounced “wet” and “dry” seasons each year (Beehner & McCann 2008; Hunter 2001). The wet and dry seasons are variable each year, but generally occur from June-September (wet season mean monthly rainfall = 310.8 +/- 30.3 mm; 2006-2011) and October-May (dry season mean monthly rainfall = 38.0 +/- 7.6 mm; 2006-2011), respectively (Beehner & McCann 2008). Temperatures in the Simien Mountains can approach freezing at night, but daily means range from 7.99˚C +/- 0.04 (mean minimum temperature, N=1843 days) to 17.66˚C +/- 0.07 (mean maximum temperature, N=1843 days). Rainfall and maximum and minimum temperatures are recorded on a daily basis using a rain gauge and digital thermometer centrally located in the gelada’s home rangeBeehner 2008.

### *Behavioral data collection*

Because extensive behavioral data collection of bachelor males did not begin until 2009, we restrict the behavioral data analysis to the latter 3 years (2009-2011). We used all-occurrence sampling to record competition between unit males and bachelors. This competition often began as a ritualized chase between two or more males (hereafter “chase”). Such chases occasionally escalated to direct physical contact (hereafter “fight”) between males. For all observed chases (N=133) and fights (N=38) we recorded the identity of participants, which males initiated the encounter (bachelors or unit males), and whether the encounter escalated from a chase (i.e., no contact aggression) to a fight (i.e. with contact aggression). We calculated monthly rates for all chases and fights (corrected for observation days).

Status categories (leader, follower, or bachelor) were determined by observations of group membership and dyadic dominance interactions. Among unit males, follower males are always subordinate to leader males, and (to our knowledge) no dominance relationships appear to exist among leader males across reproductive units (Dunbar & Dunbar 1975; Bergman et al. 2009). Therefore, we denote all dominant males “leaders” and all subordinate males “followers”. Takeovers are conspicuous and (for the most part) discrete events where a bachelor challenges and subsequently overthrows a dominant leader male. After takeovers, the former leaders have been observed to: (1) disappear (and presumably die, since we were unable to find these males during censuses of all surrounding groups), (2) remain in the reproductive unit as a subordinate follower male, or (3) return to a bachelor group. A successful takeover ensued if the former leader exhibited submissive behaviors (e.g., fear barking, cowering, displacement) towards the new leader (i.e., the former bachelor) and if the new leader copulated with females after the takeover. We recorded the date for all takeovers (N=77, 2006-2011) and the males involved.

Most males occupied only a single status category across the 6-year study (N=44 bachelors, N=10 followers, N=21 leaders). However, we observed several transitions between status categories. Sixteen previously-known bachelors became leader males, 22 leaders were deposed and became followers within their unit, and 5 males transitioned from bachelor, to leader, and finally to follower.

### *Fecal hormone collection*

We collected fecal hormone samples in a targeted fashion from unit males; fecal samples were collected for all leader (N=1376) and follower males (N=684) once per month during the entire study period (2006-2011). Bachelor fecal samples (N=676) were collected opportunistically between 2006-2009 and once per month from 2010-2011. In total, we collected 2730 fecal samples from 133 known males (~14 samples/male; range 2-91 samples/male). Field processing of fecal hormone metabolites is described elsewhere (Beehner & McCann 2008; Beehner et al. 2009; Pappano et al. 2010). But, in brief, we collected a pea-sized aliquot of homogenized fecal sample in 3 ml of a methanol:acetone solution (8:2). Samples were vortexed and later filtered and extracted using a solid-phase cartridge. All samples were washed with 2.0 ml of 0.1% sodium azide ($NaN_3$) solution (2006-2010) or 20% MeOH (2011), placed in a sterile Whirl-pak bag with a silica dessicant, and stored frozen until shipment to the Core Assay Facility at the University of Michigan for radioimmunoassay (RIA). Dry fecal weights from all samples were obtained to the nearest +/- 0.0001 g.

*Fecal hormone analysis*

Beehner et al., (2009) previously validated a testosterone antibody for use in geladas (Diagnostics Systems Laboratory (DSL); Beckman Coulter). However, the manufacturer discontinued the DSL testosterone antibody at the end of 2009, and we continued our analysis with a new testosterone antibody (Pantex testosterone direct $I^{125}$kit, Santa Monica, CA) and a different wash step (20% MeOH used for 2011 only) for the solid-phase extraction. Therefore, it was necessary first to validate this antibody both analytically and biologically for use in geladas and second to control for the new Pantex testosterone antibody and methanol wash (20%) in our analyses.

*Analytical validation*. The Pantex antiserum is known to cross-react with testosterone (100%), 5α-dihdryotestosterone (6.9%), androsterone (0.52%), and anadrol (0.15%). First, a dose-response curve using a serially-diluted adult male fecal pool was shown to be parallel to a standard curve. Second, we spiked 9 standards with a low sample (12.5

µl) and a high sample (100 µl) and found a strong linear relationship between expected and observed values for both low ($y=1.21x+0.36$, $R^2=0.996$, $P<0.0001$) and high ($y=1.63+4.65$, $R^2=0.986$, $P<0.0001$) spiked samples. The sensitivity of the Pantex antibody was 0.20 ng/ml. Third, we used a low (~20% binding) a mid (~50% binding) and a high (~80% binding) fecal pool control in all assays. The respective inter-assay coefficients of variation were 28.9% (low), 13.3% (mid), and 15.1% (high). Our intra-assay coefficient of variation was 4.0% (N=6 samples at 100 µl).

*Biological validation*. For our biological validation we adhered to the same method used in Beehner et al., (2009). We assayed males of different age classes (N=16 juveniles, 30 prime-aged, and 12 late prime males) in a single assay (to control for inter-assay variation) to examine whether prime-aged males exhibit the highest testosterone values. As predicted, results indicated an inverse-U shape between age and testosterone. Juveniles and late-prime males exhibited the lowest testosterone levels, while prime-aged males exhibited the highest (Kruskal-Wallis: $\chi^2=39.77$, df=2, $P<0.0001$).

*Controlling for different methods.* To correct for variation in methods, we included two separate fixed effects in all of our linear mixed models. These effects are “antibody” (DSL or Pantex) and “wash” ($NaN_3$ or 20% MeOH; Table 6.1).

### *Is there a seasonal pattern to male-male competition?*

First, we asked if male-male competition follows a seasonal pattern. To do this, we examined the pattern of takeovers (the presumed result of aggression) as well as the aggression itself (chases and fights). First, we examined the relationship between aggression rates and takeovers. We calculated a monthly rate for each form of aggression (chases and fights) and a total number of takeovers for each month. We then used a Pearson correlation to determine if increased rates of aggression are correlated with increased number of takeovers. We used circular statistics (Zar 1999) to statistically test whether takeovers follow a seasonal pattern each year. Each takeover date was converted into a vector and binned according to 14-day periods (N=26 per year) for our analysis. We summed the number of takeovers within each bin to calculate the angle and length for each vector. For our dataset, we were interested in two particular measures of seasonality in takeovers: the mean vector length (*r*) and the mean vector angle (*a*) (Zar 1999). The mean vector length exhibits values between 0 and 1, with 0 indicating no clustering in time and 1 indicating all events occurring on the exact same day. The mean angle can be converted into a date to describe approximately when in a given year takeovers tend to occur. We used a Rayleigh test to determine if the distribution of *r* deviated from a uniform distribution across years. All circular statistics were calculated using the R (v. 2.15.1) package "circular" (Lund & Agostinelli 2011; R Core Development Team 2011).

### *What factors influence testosterone for all males?*

It stands to reason that testosterone levels should accompany this seasonal aggression for male geladas. However, we also need to control for several factors that are known to affect male testosterone in other primates, such as weather, age, and social status (Girard-Buttoz et al. 2009; Gesquiere et al. 2005; Gesquiere et al. 2011; Muroyama et al. 2007) For rainfall, we designated each sample as a "wet" or "dry" season sample if the cumulative rainfall for the previous month was above or below the median (53.7 mm), respectively (N=1365 wet season, 1365 dry season). For temperature, we grouped samples based on whether they were collected during hot days (max temperature > median maximum temperature, 17.5 ºC, N=397), cold days (minimum temperature < median minimum temperature, 7.9 ºC, N=214), or average temperature days (all other samples, N=2119). For age, we do not have known dates of birth for adult males because gelada males disperse from their natal groups (Dunbar

1983; le Roux et al. 2011). Thus, adult male ages were estimated to the nearest half year using secondary sexual characteristics such as canine eruption, tooth wear, pelage coloration, and cape length (Bergman et al. 2009; Beehner et al. 2009). For status, we split males into their broadest status categories (leader, bachelor, follower). And, for competition, we included the number of takeovers in the population for each month (range 0-5 takeovers/month).

Using the function “lmer” in the lme4 package for R (v 2.15.1) (Sarkar & Bates 2009; R Core Development Team 2011), we ran a linear mixed model (LMM) with fecal testosterone (fT) as the outcome variable to determine the effect of weather and social factors on male testosterone (Table 6.1). We log-transformed the outcome variable to approximate a normal distribution. Because each individual male had multiple data points (N=2730, 64 leaders, 52 followers, 79 bachelors, range 2-91 samples per male), we included individual identity as a random effect. In addition to our methods-based fixed effects (antibody and wash, see above) we included rainfall, temperature, male age, status, and number of takeovers on testosterone levels (Table 6.1). We compared univariate models that considered only a single fixed effect to multivariate models that considered a combination of fixed effects and/or interactions between fixed effects (Table 6.1). We compared all models (N=23) using Akaike Information Criterion (AIC) and considered the model with the lowest AIC to be the best fit for our dataset (Akaike 1973; Garamszegi et al. 2009). If the difference in AIC was less than 2 for the lowest ranked models, we considered both models to be equally good fits for our data (Symonds & Moussalli 2011).

### *What factors influence testosterone for leader males?*

The testosterone levels of leader males may also be influenced by several additional social factors that do not apply to bachelors; for example, mating behavior, the presence of within group competitors (i.e. follower males), group size (i.e. number of females), and stability of their social environment. Therefore, we ran a separate LMM comparison (N=38 models) for leader males only. This model was similar to our first model, however we included additional fixed effects (Table 6.1). We included only leader males (N=881: 56 leader males, range 2-45 samples per male). As in our previous model, individual identity was included as a random effect. We included temperature, rainfall, age, and number of takeovers as fixed effects. We then added several fixed effects unique to leader males. Because variation in unit size might influence reproductive competition among males (i.e. through a priority-of-access), we included whether follower(s) were present in the unit as well as the number of adult females (defined by first onset of sexual swelling) as separate fixed effects. We also included whether or not a male was involved in a recent takeover (within the past 90 days). Frequently, during the first three months after a takeover, males are quickly overturned, thus this period often represents a chaotic period of "unit instability" (see le Roux et al. 2011). Finally, we included whether or not a male was observed mating during the previous 24 hours (see Beehner & McCann 2008). Similar to our first model, we compared univariate and multivariate models using AIC (Table 6.1) (Akaike 1973; Garamszegi et al. 2009; Symonds & Moussalli 2011).

### *What factors influence testosterone for bachelors?*

The testosterone levels of bachelors are also subject to at least one social factor that does not apply to unit males; for example, competition within their all-male group. As with our previous model selection procedures, we included antibody, wash, rainfall, age, and month as fixed effects. We then added (as fixed effects) all-male group size, dominance rank, and whether or not the bachelor eventually became a leader male or remained a bachelor (Table 6.1). We calculated each bachelor's dominance rank using an Elo-Rating system (Albers & de Vries 2001; Elo 1978; Neumann et al. 2011). The Elo-Rating system is a progressive ranking system and allows researchers to assign dominance ranks on the day of sample collection (see (Neumann et al. 2011) for description). Because we needed detailed behavioral data to adequately calculate an Elo-Rating for each male, we restricted this model to data collected from 2010-2011 (N=462 samples, 45 bachelors, range 2-30 samples per male). As with previous models, we compared univariate models to multivariate models (N=22) using AIC (Table 6.1). For the all models (all males, leaders, and bachelors), we checked the normality of the residuals using a Q–Q plot and a histogram of residuals, and by plotting the fitted versus residual values of our model.

### *Do testosterone profiles differ for winners vs losers?*

Finally, we were interested in taking a detailed look at the testosterone trajectories of male “winners” (bachelors that went on to become leader males) and “losers” (leaders that were defeated) during the 6 months before and after each takeover. To address this question, we included only males that were themselves involved in takeovers (N=67). Males were either classified as “winners” (N=42) or “losers” (N=25). To control for variation due to weather, age, and methodological confounds, we used as our dependent variable the residual testosterone values calculated from our previous LMM for all males (random effect: identity; fixed effects: antibody, wash, rainfall, temperature, and age). We then calculated mean residual testosterone levels for all winners and losers for the following time periods: (a) 180-91 days pre-takeover, (b) 90-16 day(s) pre-takeover, (c) 15 days before until 15 days after the takeover (day 0), (d) 16 days post-takeover until 90 days post-takeover, (e) 91-180 days post-takeover. We excluded all winners with tenure lengths of less than 12 months (since tenure lengths of less than 12 months means that these males qualify as both winners and losers simultaneously). We made a few exceptions to this to increase our sample size for pre-takeover winners. Mainly, we included data from known bachelors that took over unknown units (N=7) even though we ceased data collection on these males one month after takeover. We visualized the trajectories of winners and losers based on these categories.

## RESULTS

### *Is there a seasonal pattern to male-male competition?*

Takeovers followed a strong seasonal pattern over the study period with the majority of takeovers occurring during the end of the dry season. The distribution of takeovers differed significantly from a random distribution (Rayleigh test, Z=18.51, P<0.001) and was strongly seasonal (*r*=0.490). The majority of takeovers in each year occurred between February and May, and the mean peak date in takeovers was May 1 (Fig. 6.1). Neither chase rate (r=0.262, P=0.17), nor fight rate (r=0.260,P=0.18) were correlated with the number of takeovers in a given month.

### *What factors influence testosterone for all males?*

The multivariate interactive model was a better fit for our data set than all other models (all ΔAIC>20). After controlling for methodological variables (antibody and wash), our model yielded several significant associations. Higher testosterone was associated with warmer days (hot days: β=0.103, s.e. = 0.026, t=4.97, P<0.01) and more rainfall (wet season: β=0.185, s.e. = 0.015, t=12.28, P<0.01). Additionally, older males had lower testosterone (β=-0.066, s.e. = 0.007, t=-8.80, P<0.001). Leaders exhibited higher testosterone overall (β=0.116, s.e. = 0.033, t=3.56, P<0.01; Fig. 6.2), and all males exhibited elevated testosterone during months of increased takeovers (β=0.058, s.e. = 0.010, t=5.55, P<0.01). However, a significant interaction between number of takeovers and status suggests that unit males experience depressed testosterone during months of increased takeovers (leader x takeover month: β=-0.063, s.e. = 0.012, t=-5.10, P<0.01; follower x takeover month: β=0.038, s.e. = 0.014, t=-2.68, P<0.05; Fig. 6.3)

*What factors influence testosterone for leader males?*

The multivariate model including all fixed effects was a better fit for our data than all univariate and multivariate models (all ΔAIC>5). Similar to our previous analysis, temperature (hot days: β=0.128, s.e. = 0.037, t=3.45, P<0.01) and rainfall (wet season: β=0.208, s.e. = 0.026, t=8.08, P<0.01) were positively associated with testosterone. During periods of unit instability, leader males exhibited elevated testosterone levels (β=0.217, s.e. = 0.042, t=5.17, P<0.01). Group size (β=0.037, s.e. = 0.041, t=0.87, P=0.38), previous mating behavior were associated with an increase in testosterone (β=0.038, s.e. = 0.033, t=1.12, P=0.26), and the presence of a follower(s) (β=0.014, s.e. = 0.048, t=0.28, P=0.78) all were not associated with leader male testosterone.

*What factors influence testosterone for bachelors?*

As with our previous models, the multivariate model including all fixed effects was the best fit for our data (all ΔAIC>8). Bachelor testosterone was influenced by the same variables and in the same direction as unit males: temperature (hot days: β=0.035, s.e. = 0.060, t=5.77, P<0.01) and rainfall (wet season: β=0.094, s.e. = 0.040, t=2.36, P<0.05) were positively associated with testosterone. Neither dominance rank (β=0.001, s.e. = 0.000, t=1.16, P=0.24) nor group size β=-0.075, s.e. = 0.043, t=-1.76, P=0.08) was associated with testosterone levels in bachelors. Bachelors that became leader males at some point during the study period exhibited higher testosterone levels than males that remained bachelors (β=0.210, s.e. = 0.061, t=3.37, P<0.01; Fig. 6.4). Finally, the number of takeovers each month was positively associated with bachelor testosterone levels (0.090, s.e. = 0.016, t=5.65, P<0.01).

*Do testosterone profiles differ for winners vs losers?*

After controlling for age, temperature, and rainfall, the testosterone profiles for males that became leaders (“winners”) exhibited a very different trajectory than that of males that eventually lost their units (“losers”). Winners displayed an upward trajectory that continued well past the takeover. By contrast, losers had a temporary increase in testosterone surrounding the takeover, which then plummeted after 3 months (Fig. 6.5).

## DISCUSSION

We draw several major findings from our investigation of the social and seasonal influences on gelada male testosterone. First, despite being classified as a non-seasonal breeder (Brockman & van Schaik 2005), male-male competition and testosterone secretion in geladas exhibits a strikingly seasonal pattern (Fig. 6.1). Second, male dominance rank is associated with testosterone (Fig. 6.2), however this relationship is contingent on social stability (Fig. 6.3). Leader males exhibit the highest levels of testosterone overall, except during a few months of the year characterized by a high rate of takeovers. These months tend to occur at the end of the dry season (Fig. 6.1), when male testosterone is overall at its lowest. Most importantly, testosterone predicts the reproductive trajectories of male geladas. After controlling for age and other socioecological factors, bachelors that became leader males during our study period exhibited higher testosterone levels than their group mates that remained as bachelors. Additionally, males that “won” units during specific takeovers exhibited elevated levels of testosterone before actually becoming the dominant males within their respective units. Taken together, these results each have important implications for future studies of the challenge hypothesis in long-lived animals, such as primates.

### *Seasonal influences on male testosterone levels*

Although patterns of female endocrine secretion are well understood for non-seasonal breeders, very little research has been conducted on how seasonal factors influence male endocrine levels (but see Girard-Buttoz et al. 2009 and Gesquiere et al. 2011). Recent evidence suggests that in strongly seasonal environments, patterns of male androgen secretion may converge on patterns similar to that of seasonal breeders (Brockman & van Schaik 2005). For example, androgen levels of long-tailed macaque (*Macaca fascicularis*) males begin to increase approximately 1-2 months prior to the mating season in preparation for competition (Girard-Buttoz et al. 2009). Additionally, Gesquiere and colleagues (2011) demonstrated that male baboons exhibit depressed testosterone levels during hot and dry periods.

Although geladas and baboons shared similar rainfall and age-based patterns of testosterone secretion, the two species differ with respect to temperature-based effects on male testosterone. Few studies have directly addressed the relationship between testicular function and ambient temperature in wild animals, but experimental studies have demonstrated that high temperatures impair spermatogenesis and affect sperm quality (see (Hansen 2009) for review). Male baboons exhibit a negative relationship between temperature and testosterone (Gesquiere et al. 2011), where as gelada males exhibited a positive relationship. This is likely due to the relationship between testicular function and ambient temperature for two closely related animals living in dramatically different temperatures. Baboons in the Amboseli basin are regularly exposed to temperatures exceeding 34 (and as high as 40) degrees Celsius (Gesquiere et al. 2008). Hence the baboon's external temperatures often meet or exceed their internal temperature (Gesquiere et al. 2008). This contrasts with the near freezing temperatures of the Simien Mountains. Given that geladas are generally "cold-stressed" and share a similar physiology with baboons (Beehner & McCann 2008), it is possible that warm days promote healthy testicular function rather than inhibit it. Hence, our observed positive relationship between temperature and gelada male testosterone levels is due to similar effects of ambient temperature on testicular function in primates.

In addition to temperature, rainfall also influences gelada male testosterone levels. That is, gelada males exhibit higher testosterone levels during the wet season. This is likely due to the relationship between rainfall, food availability, and metabolic costs of androgen secretion (Pérez-Rodríguez et al. 2006). Increased rainfall is positively associated with green grass cover, and hence increased food availability for gelada males (Hunter 2001). Although we currently lack data in foraging efficiency and food quality in the Simiens, we suspect that the lack of green grass cover during the dry season influences overall condition of gelada males. In rhesus macaques, testosterone levels decreased after periods of temporary fasting (Wahab et al. 2008). Similar results have been observed in human males and other primates (Cameron et al. 1993; Cameron 1996). Taken together, it is likely that all gelada males (leaders, bachelors, and followers) suffer from negative energy balance during the dry season and thus cannot maintain high levels of testosterone.

### *Seasonality of male-male competition*

Our results suggest that male-male competition among geladas has converged on a seasonal pattern with the majority of aggression occurring at the end of the dry season (Fig. 6.1). This “takeover season” is characterized by increased successful takeovers by bachelors. We were unable to detect a significant association between rates of aggression and takeovers, however we believe this is likely due to sampling bias and possibly unobservable activities. Chases and fights among geladas are by their nature chaotic and difficult to follow. Therefore, it is difficult to get accurate data on an entire chase or fight. Additionally, these fights occur on the sleeping cliffs (at night) when units and all-male groups are forced into close proximity on the steep cliffs. Very often, we don’t observe the “takeover” fight directly, but rather find the unit the following day with a new leader male (pers. obs.). As such, our measured aggression rates likely do not reflect the true rates in the population. Despite this, a pattern of increased aggression during the late dry season is apparent in our data (Fig. 6.1). Alternatively, or perhaps additionally, the rate of fights in our population might only be weakly related to takeover success. Very often, fights end quickly suggesting a mismatch in fighting ability between the challenging bachelors and current leaders (pers. obs.). As chases are likely a low-cost way for males to evaluate each other’s fighting ability it is likely that the few chases that escalate to fights only occur between evenly matched males (Maynard Smith & Price 1973; Maynard Smith 1974). Moreover, multiple bachelors are often involved in fights with leader males (Dunbar & Dunbar 1975). This considered, leader males have very little chance at holding onto their reproductive unit once targeted by the bachelors.

Given the positive relationship between rainfall and testosterone among gelada males, this seasonal reproductive competition may be indirectly mediated by changes in male body condition. At the end of the dry season, all males are presumably in poor condition due to the lack of food in their habitat. Bachelors, who by their nature, are younger and presumably better able to buffer themselves against ecological hardship may enter this period of time in better condition than leader males. It is possible that this difference in relative body condition alone can explain the seasonal pattern in takeovers, however it is likely that bachelors rely on some information about leader male quality when assessing their potential rivals (le Roux & Bergman 2012). This information might be contained in the loud display call of leader males or the red patch of sexual skin on their chests that might indicate competitive ability, condition, or testosterone levels (Bergman et al. 2009). Given that leader male displays are hypothesized to indicate male condition (Dunbar 1984), we expect that bachelors are likely preferentially targeting males that are either in poor condition, have low testosterone, or both. Indeed, males that eventually lose their units appear to have relatively low testosterone levels at least 6 months prior to being overthrown (Fig. 6.5).

*Testosterone and predicting the reproductive fate of males*

Most strikingly, our results suggest that testosterone levels predict the reproductive trajectories of male geladas (Fig.4-5). After controlling for age, the only significant social factor associated with bachelor male testosterone was whether or not he eventually became a leader male. Moreover, in comparing “winning” to “losing” males for all takeovers, bachelors that eventually won units exhibited higher testosterone levels than leader males that eventually lost their unit in the months before the actual takeover (Fig. 6.5). This effect is in addition to our finding that all bachelors exhibit relatively elevated testosterone during periods of increased takeovers (Fig. 6.3). These results support similar findings in related species (e.g. mandrills, Wickings & Dixson 1992, and chacma baboons, Beehner et al. 2006) that suggest that current testosterone levels predict the future reproductive success of males. It is possible that this predictive element of primate male testosterone is the conserved pattern for Papionini (baboons, drills, geladas, macaques, mandrills, and mangabeys) and possibly Cercopithecines in general.

In addition to this predictive power of male testosterone, our results also broadly support central predictions of the challenge hypothesis. During the time period surrounding a takeover and in the months shortly thereafter, both “winner” and “loser” testosterone was elevated. This suggests that males are still contesting for leader status within a unit even though most takeovers result in a clear distinct dominance relationship (pers. obs.). Given the pattern of our data (Fig. 6.2, Fig. 6.5), we suspect that “winners” and “losers” differ shortly after the first six months post-takeover. Both of these findings support predictions of the challenge hypothesis in that males contesting for reproductive access should exhibit elevated levels of testosterone mediated by social challenges.

### *Conclusion*

In this study, we demonstrated that seasonal and social factors influence testosterone levels in male geladas. We used the framework of the challenge hypothesis in an attempt to parse the complex relationship between testosterone, seasonality, and male competition. We have drawn three major conclusions from our study. First, male-male competition in geladas follows a seasonal pattern with the majority of aggression and successful takeovers occurring at the end of the dry season. Second, bachelor geladas may be maximizing their chances of successful reproductive competition by targeting dominant males while their testosterone levels are depressed. And finally, testosterone levels predict the future dominance status of bachelor geladas, as bachelors with high testosterone were more likely to become future dominant males. Our study supports several key predictions of the challenge hypothesis, however we do not yet know how bachelors make decisions about what leader males to challenge in order to begin their reproductive tenure.

Table 6.1. Description of random and fixed effects in linear mixed models.

| | All Males | Leader Males | Bachelor Males |
|---|---|---|---|
| Outcome Variable | Log Testosterone | Log Testosterone | Log Testosterone |
| Random Effects (RE) | Male Identity | Male Identity | Male Identity |
| Fixed Effects (FE) | Antibody (DSL, Pantex) | Antibody | Antibody |
| | Wash (20% MeOH, $NaN_3$) | Wash | Wash |
| | Rainfall (dry, wet season) | Rainfall | Rainfall |
| | Temperature (hot, average, or cold) | Temperature | Temperature |
| | Male Age (years) | Male Age | Male Age |
| | Status (leader, bachelor, follower) | Mating (Y/N) | Dominance Rank (Elo-Rating) |
| | Number of Takeovers in Month | Number of Takeovers | Number of Takeovers |
| | | Group Size (small: 1-3, medium: 4-6, large: 7-10) | Group Size (small: 2-5, large: 6-9) |
| | | Follower (present v. absent) | Future Leader (Y/N) |
| | | Unit Stability (>90 days post-takeover = stable, <90 days post-takeover = unstable for each unit) | |
| Interactions (I) | Takeover Month x Status | N/A | N/A |
| Models | Univariate (RE + each FE) | Univariate (RE + each FE) | Univariate (RE +each FE) |
| | Multivariate (RE + all FE) | Multivariate (RE+ all FE) | Multivariate (RE +all FE) |
| | Multivariate Interactive (RE + all FE + I) | | |

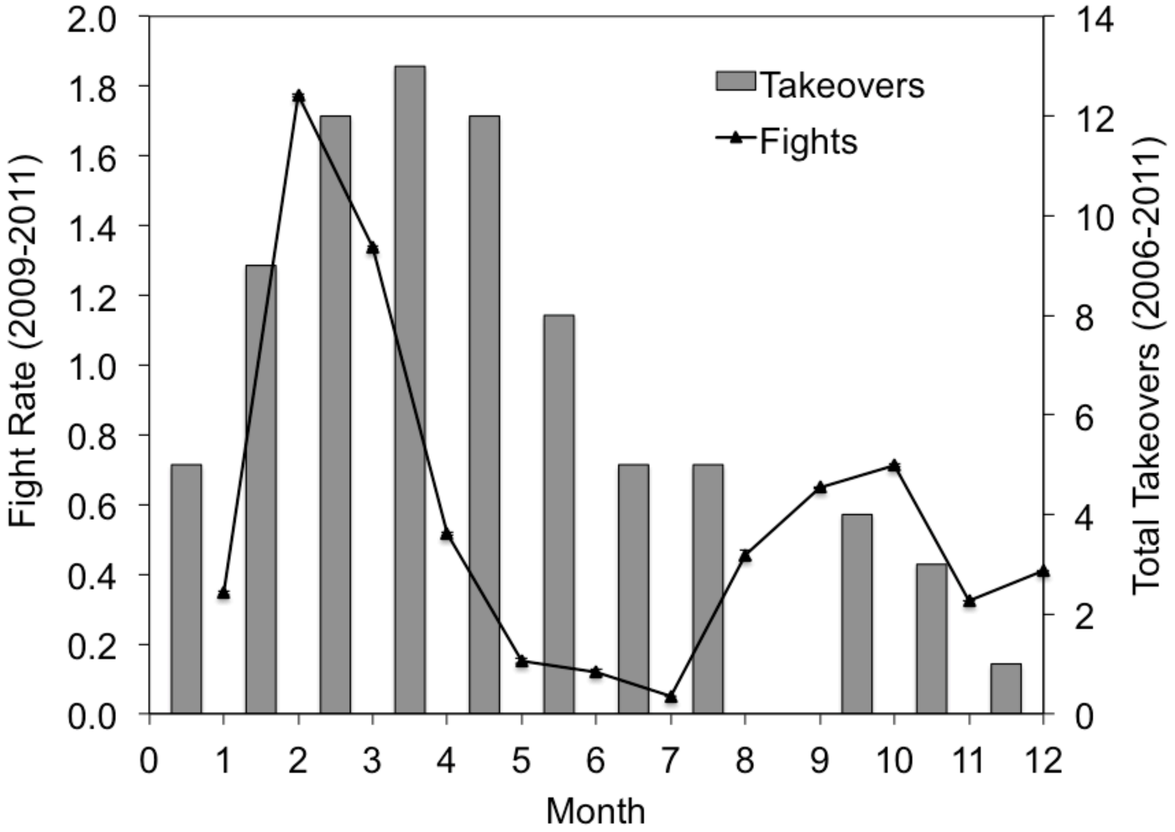

**Figure 6.1.** Seasonal distribution of takeovers and aggression. A histogram showing the distribution of successful takeovers (N=77, 2006-2011) in the study population. A second plot indicates the mean rate of aggression in the study population (mean fights per observation day per month +/- s.e.). Takeovers exhibited a seasonal pattern with the majority of takeovers occurring at the end of the dry season (Feb-May).

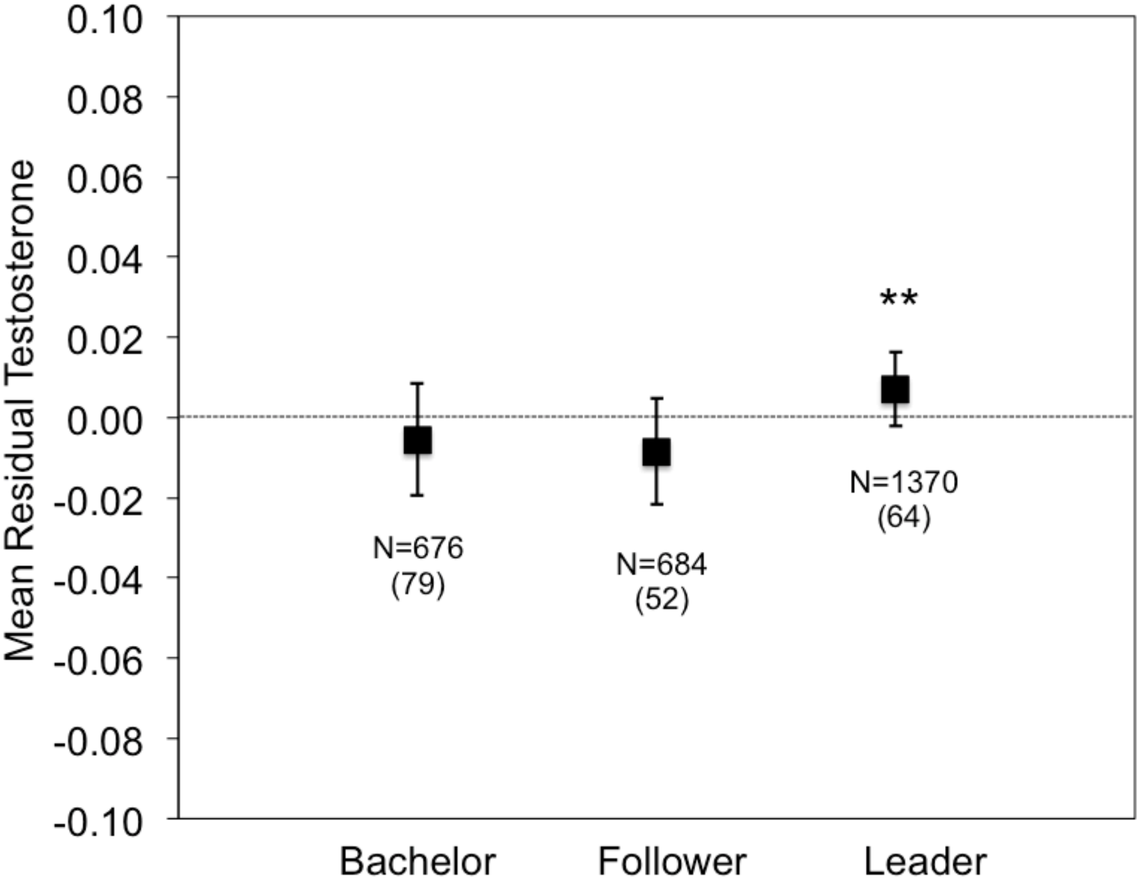

**Figure 6.2.** Status and testosterone levels. Mean residual testosterone levels (+/- s.e.) for males of each status category. Leader males exhibited the highest testosterone levels overall after controlling for age, rainfall, temperature, and methodological effects (** $P<0.01$). Sample size indicated by N = number of samples (number of males).

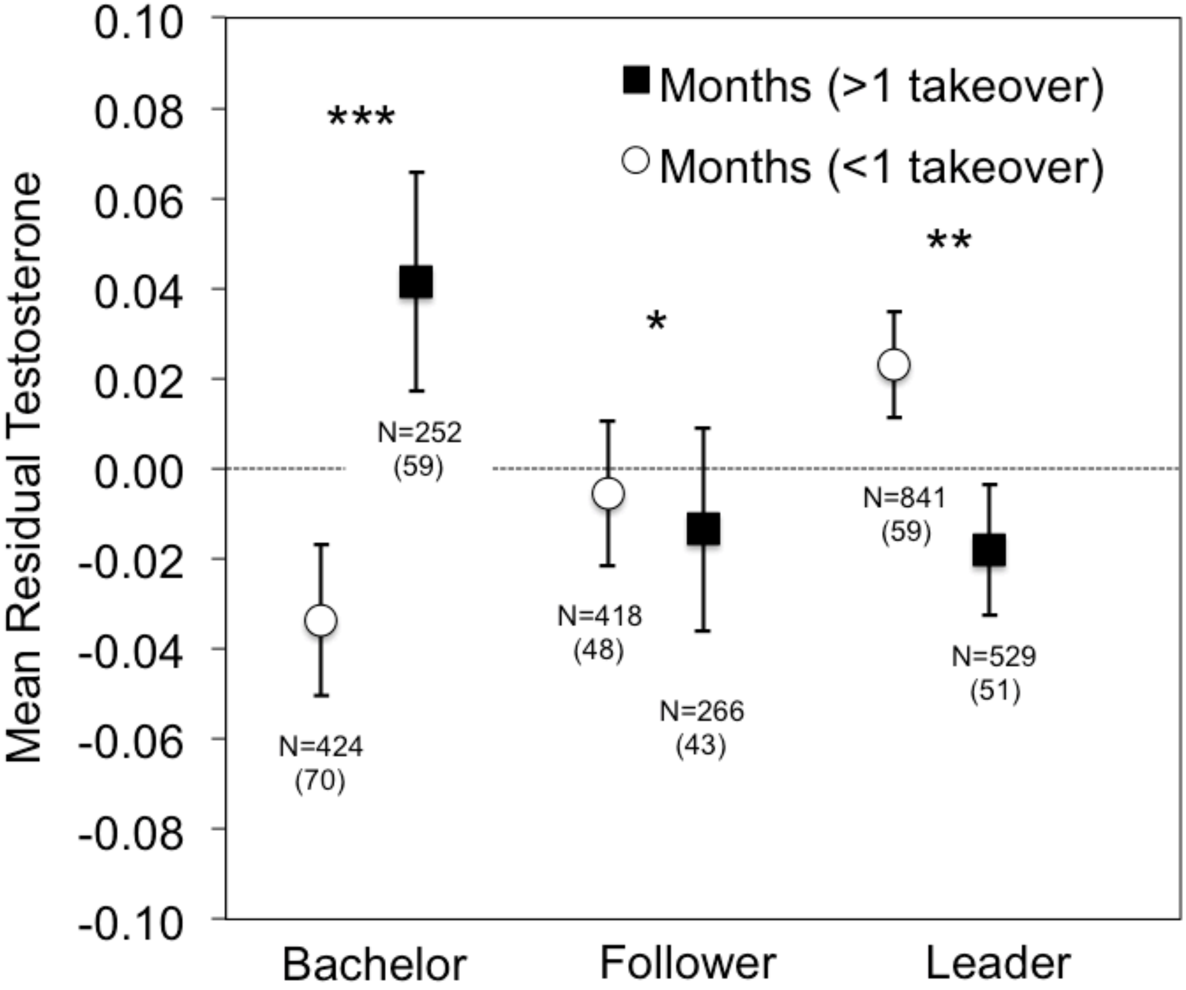

**Figure 6.3.** Testosterone levels during months of increased takeovers. Mean residual testosterone levels (+/- s.e.) for males of different status categories during takeover (>1, N=22) versus non-takeover months (<1, N=50). Bachelors exhibit elevated testosterone levels during takeover months (*** P<0.001). Unit males exhibit depressed testosterone levels during takeover months (* P<0.05, ** P<0.01). Sample size indicated by N = number of samples (number of males contributing to each period).

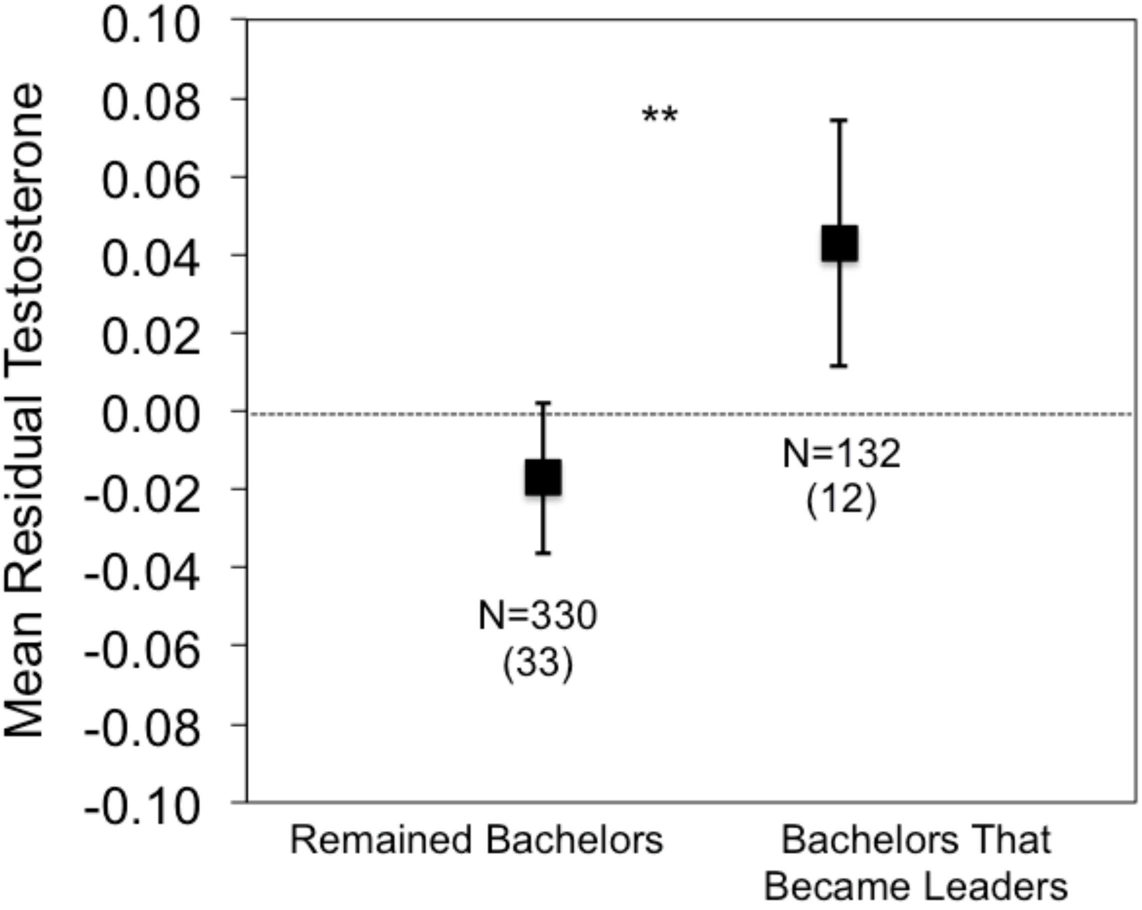

**Figure 6.4.** Testosterone levels of bachelors. Mean residual testosterone levels (+/- s.e.) for bachelors that remained bachelors during the study period compared with bachelors that became leader males during the study period. Bachelors that became leader males exhibited higher testosterone levels than bachelors that remained bachelors (** $P<0.01$). Sample size indicated by N = number of samples (number of males).

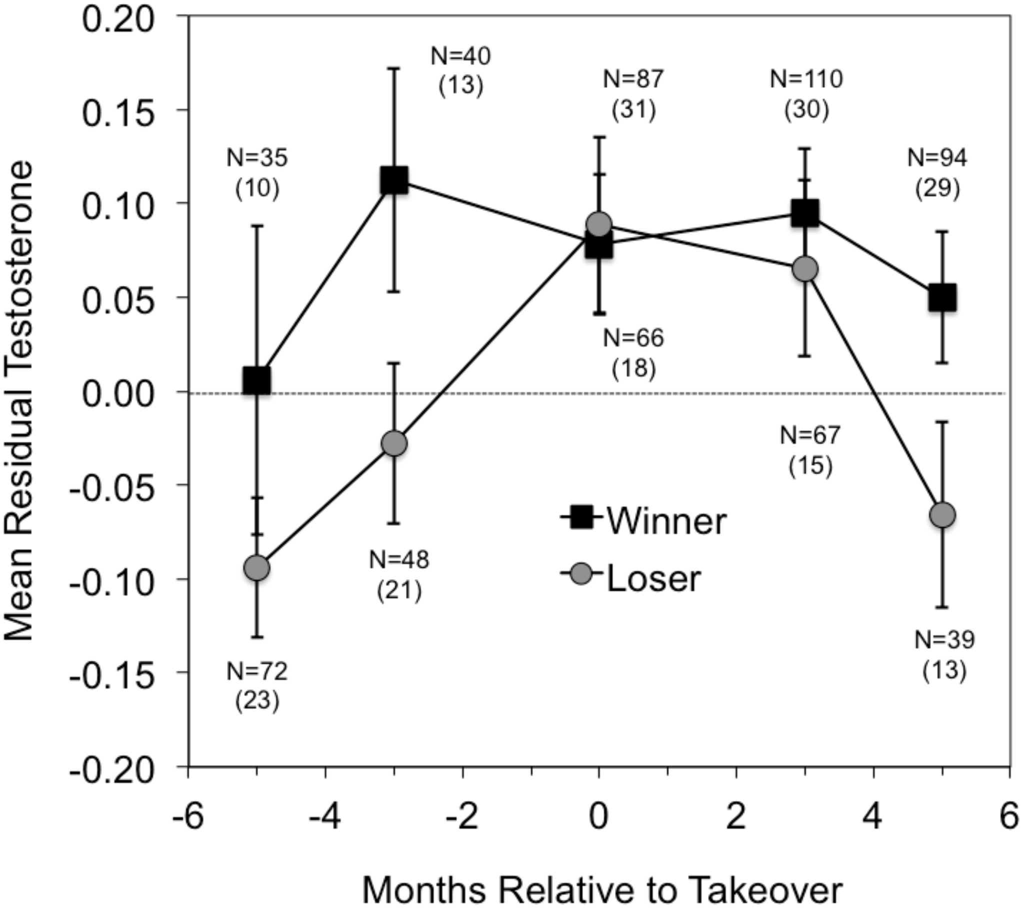

**Figure 6.5.** Testosterone levels and trajectories of "winners" and "losers". Mean residual testosterone levels (+/- s.e.) for "winners" (N=42) and "losers" (N=25) of specific takeovers. Winners are bachelors transitioning to dominant "leaders" status, where as losers are leaders transition to follower status. Both transitions occur at the takeover. Means were calculated for each category across five separate time periods: (1) 180-91 days pre-takeover, (2) 90-16 days pre-takeover, (3) 15 days pre-takeover until 15 days post-takeover, (4) 16-90 days post takeover, and (5) 91-180 days post-takeover. Winners exhibited higher testosterone that losers before the takeover occurred (90-16 days pre-takeover). Sample size indicated by N = number of samples (number of males contributing to each period).

# CHAPTER SEVEN

# CONCLUSIONS

## OVERVIEW

In this dissertation, I answered several questions regarding the reproductive trajectories of male geladas. This topic required that I first describe the formation of all-male groups and the social dynamics between bachelors living in all-male groups. The strong affiliative relationships among bachelors within these groups suggest a role for increased male-male tolerance and possibly cooperation within the gelada lineage. I then addressed the nature of reproductive competition between bachelor and leader geladas. I showed that bachelors exert a “selfish herd” effect on gelada reproductive units. Finally, I demonstrated that a bachelor’s testosterone level predicts his reproductive trajectory. I presented demographic, behavioral, genetic, and hormonal data to support these findings. Below I restate my central questions and summarize my conclusions.

### *Question 1: What are the patterns of kinship and age structure within all-male groups?*

In Chapter 3, I demonstrated that relatedness influences all-male group formation, however most bachelors within all-male groups are non-kin. All-male groups contained males of all age classes, but most bachelors were young adults and early prime males. I concluded that all-male groups likely arise from a combination of means. Young males may disperse alone and join previously established all-male groups, disperse with members of their paternal cohort and join an all-male group, or form an all-male group with members of their peer group as they mature. The presence of few close kin within all-male groups suggest that at least a few males join all-male groups with members of their paternal cohort. Additionally, members of our research project have observed such a group form with several paternal siblings and an older unrelated male. I argued that these groups likely convey fitness benefits to bachelors through stress reduction or coalition formation in agonistic contests against leader males.

*Question 2: What are the social dynamics among bachelors within all-male groups?*

In Chapters 3 and 4, I presented evidence for social bonding among bachelors within their all-male group. These bonds are characterized by relatively equal grooming relationships. Additionally, these bonds tend to form between individuals that both are closely related and in the same age cohort. This reinforces the hypothesis that all-male groups contain several pairs of males that likely disperse from the natal units or peer groups together. Moreover, I suggested that grooming rather than proximity is a better indicator of bond strength among bachelor geladas. This supports previous research on female geladas and adds to an understanding of bond development within the gelada species (Tinsley Johnson et al, submitted). I suggested that bonds between male geladas likely arise early in life, possibly within peer groups or among paternal siblings within their reproductive unit.

*Question 3: How do bachelors interact with reproductive units?*

Association with bachelor geladas may be costly to reproductive males and females. When a bachelor takeovers a reproductive unit, he not only ends the tenure of the leader male, he may also commit infanticide  (Beehner & McCann 2008; Roberts et al. 2012). Both of these outcomes have reproductive consequences for unit males and females. In a previously published manuscript, my coauthors and I demonstrated that bachelor geladas exert a “selfish-herd” effect on reproductive units  (Pappano et al. 2012)(see Chapter 5). Bachelor number was positively associated with gelada herd size, and bachelor proximity was associated with inter-individual distances. That is, as bachelors approached reproductive male and female geladas, those individuals moved closer to each other. I suggested that bachelors are analogous to "predators" in that they affect the grouping and spatial patterns of the gelada herd.

*Question 4: What are the social and ecological factors that influence testosterone secretion in male geladas?*

In Chapter 6, I examined the social and ecological factors that influence gelada male testosterone secretion and reproductive competition. As predicted, age, rainfall, temperature, and status all influenced gelada male testosterone. After controlling for age, weather, and methods-related confounds, I demonstrated that leader males possessed the highest levels of testosterone. Additionally, I demonstrated that among the bachelors, "future leaders" had higher testosterone levels than their fellow group mates. Moreover when I examined individual takeovers, "winners" (i.e. bachelors that eventually became leader males) peaked in testosterone as early as three months before taking over a reproductive unit. This contrasted with "losers" (i.e. males that eventually lost their unit). These "losers" appeared to have lower testosterone even before the takeover occurred. Finally, I observed a seasonal pattern in takeovers within our study population. Overall, this suggests that bachelors may be able to selectively target leader males with low testosterone through a variety of means including display calls or chest color variation.

## LIMITATIONS OF STUDY & FUTURE DIRECTIONS

Primates are long-lived animals. In the absence of long-term, longitudinal data it is impossible to draw specific conclusions about the relationship between behavioral strategies and reproductive success. Primatologists often use proxies (e.g. dominance rank/status) to measure reproductive success in short-term cross-sectional studies. My original intent for this project was to follow this model and examine how social and hormonal factors influenced the acquisition of leader male status. However, acquisition of dominance status in only the first step in a gelada male's reproductive career (Dunbar 1984). After becoming a leader male, a gelada must hold on to the reproductive unit and fight off rival bachelors. Although several bachelors became leader males during my study period (N=12) few of those males (N=4) took over habituated units. Therefore, I was unable to track many of these known bachelors throughout the early part of their reproductive lives. As the UMGRP collects more longitudinal data on males as they transition from bachelor to leader, I plan on examining the relationship between social bonds and reproductive success more extensively.

One of the paramount findings of my study was that bachelor geladas form preferential social bonds within their all-male group (Chapter 4). However, given the brevity of my study I cannot determine how long these bonds persist. I observed several males (N=4) return to their bachelor groups after unsuccessful attempts at entering reproductive units. Additionally, I have observed males from the same bachelor group enter units together (one as a leader and the other as a follower, N=4 pairs), however only two pairs of these males only remained in a unit together for greater than a few months (Chapter 4). This suggests that bachelor geladas, like female baboons (Silk et al. 2006; Silk et al. 2006) and male chimpanzees (Mitani 2009), form long-lasting social bonds. However, we must first know when bonds form among male geladas in order to determine how long they persist. Another researcher associated with the UMGRP is currently focusing on the development of male-male relationships during the juvenile period. I anticipate collaborating with this researcher (C. Barale) on future manuscripts concerning the ontogeny of male social bonds.

If pairs of males do not takeover units together (Chapter 4), then it is possible that social bonding is a consequence of selection on increased gregariousness within gelada males. That is, social bonds among bachelors may be indicative of broader social personality that may have fitness benefits later in life (Gosling & John 1999; Sih et al. 2004; Cote et al. 2008) . For example, male long-tailed manakins (*Chiroxiphia linearis*) that were well integrated into their social networks early in life were five times more likely to achieve high status later in life (McDonald 2007). It is possible that "social" bachelors are more likely to become leader males, or as leader males will form stronger bonds with females. Leader males that readily form bonds with key females may benefit from increased tenure, and subsequently sire more offspring (Dunbar 1983; Dunbar 1984).

## CONCLUDING REMARKS

In this dissertation, I focused on the broad themes of cooperation and competition among male geladas. This thesis includes the first quantitative study of the social dynamics of bachelor geladas (Chapters 3-4). I also used a novel approach, the "selfish herd" hypothesis (Chapter 5), to understand the spatial and grouping response of gelada units to bachelor presence and proximity. Additionally, I applied the "challenge hypothesis" to understand gelada male-male reproductive competition and testosterone secretion (Chapter 6). All chapters advance our understanding of gelada socioecology. Future research will focus on the ontogeny of gelada male bonds as well as the fitness consequences of bond formation among male geladas. Through an understanding of the causes and consequences of variation in male gelada social behavior, we will be better able to understand how primate males, including humans, survive and reproduce.

# REFERENCES

Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. *Second international symposium on information theory,* 1, 267-281.

Albers, P.C. & de Vries, H. 2001. Elo-rating as a tool in the sequential estimation of dominance strengths. *Animal behaviour,* 61, 489 - 495.

Alberts, S.C., Buchan, J.C., & Altmann, J. 2006. Sexual selection in wild baboons: From mating opportunities to paternity success. *Animal Behaviour,* 72, 1177-1196.

Alberts, S.C., Watts, H.E., & Altmann, J. 2003. Queuing and queue-jumping: Long-term patterns of reproductive skew in male savannah baboons, Papio cynocephalus. *Animal behaviour,* 65, 821 - 840.

Alexander, R.D. 1974. The evolution of social behavior. *Annual Review of Ecology and Systematics,* 5, 325-383.

Altmann, J. 1974. Observational study of behavior: Sampling methods. *Behaviour,* 49, 227-267.

Altmann, J. 1979. Age cohorts as paternal sibships. *Behavioral ecology and sociobiology,* 6, 161-164.

Altmann, J., Schoeller, D., Altmann, S.A., Muruthi, P., & Sapolsky, R.M. 1993. Body size and fatness of free-living baboons reflect food availability and activity levels. *American journal of primatology,* 30, 149-161.

Altmann, S.A. 1962. A field study of the sociobiology of rhesus monkeys, macaca mulatta. *Annals of the New York Academy of Sciences,* 102, 338-435.

Andersson M.B 1994. *Sexual selection: Monographs in behavior and ecology.*Princeton, N.J.: Princeton University Press.

Archer, J. 2006. Testosterone and human aggression: An evaluation of the challenge hypothesis. *Neuroscience and biobehavioral reviews,* 30, 319-345.

1987. *Primate societies.*(Ed. by B.B. Smuts, D.L. Cheney, R.M. Seyfarth, R.W. Wrangham, & T.T. Struhsaker) Chicago: University of Chicago Press.

Bardin, C.W. & Catterall, J.F. 1981. Testosterone: A major determinant of extragenital sexual dimorphism. *Science,* 211, 1285-1294.

Barrett, L., Henzi, S.P., Weingrill, T., Lycett, J.E., & Hill, R.A. 1999. Market forces predict grooming reciprocity in female baboons. *Proceedings of the Royal Society B: Biological Sciences,* 266, 665-670.

Barton, R.A., Byrne, R.W., & Whiten, A. 1996. Ecology, feeding competition and social structure in baboons. *Behavioral Ecology and Sociobiology,* 38, 321-329.

Bayly, K.L., Evans, C.S., & Taylor, A. 2006. Measuring social structure: A comparison of eight dominance indices. *Behavioural processes,* 73, 1-12.

Beehner, J.C. & Bergman, T.J. 2008. Infant mortality following male takeovers in wild geladas. *American journal of primatology,* 70, 1152-1159.

Beehner, J.C. & McCann, C. 2008. Seasonal and altitudinal effects on glucocorticoid metabolites in a wild primate (Theropithecus gelada). *Physiology & behavior,* 95, 508-514.

Beehner, J.C. & Whitten, P.L. 2004. Modifications of a field method for fecal steroid analysis in baboons. *Physiology & behavior,* 82, 269-277.

Beehner, J.C., Bergman, T.J., Cheney, D.L., Seyfarth, R.M., & Whitten, P.L. 2006. Testosterone predicts future dominance rank and mating activity among male chacma baboons. *Behavioral Ecology and Sociobiology,* 59, 469-479.

Beehner, J.C., Gebre, B., Bergman, T.J., & McCann, C. 2007. Population estimate for geladas (Theropithecus gelada) living in and around the simien mountains national park, ethiopia. *SINET: Ethiopian Journal of Science,* 30, 149-154.

Beehner, J.C., Gesquiere, L., Seyfarth, R.M., Cheney, D.L., Alberts, S.C., & Altmann, J. 2009. Testosterone related to age and life-history stages in male baboons and geladas. *Hormones and behavior,* 56, 472-480.

Bergman, T.J. 2010. Experimental evidence for limited vocal recognition in a wild primate: Implications for the social complexity hypothesis. *Proceedings. Biological sciences / The Royal Society,* 277, 3045-3053.

Bergman, T.J. & Beehner, J.C. 2008. A simple method for measuring colour in wild animals: Validation and use on chest patch colour in geladas (Theropithecus gelada). *Biological Journal of the Linnean Society,* 94, 231-240.

Bergman, T.J., Ho, L., & Beehner, J.C. 2009. Chest color and social status in male geladas (Theropithecus gelada). *International Journal of Primatology,* 30, 791-806.

Bowler, M. & Bodmer, R. 2009. Social behavior in fission-fusion groups of red uakari monkeys (cacajao calvus ucayalii). *American journal of primatology,* 71, 976-987.

Brockman, D.K., Whitten, P.L., Richard, A.F., & Schneider, A. 1998. Reproduction in free-ranging male propithecus verreauxi: The hormonal correlates of mating and aggression. *American journal of physical anthropology,* 105, 137-151.

Brockman D.K. & van Schaik C.P. 2005. Seasonality and reproductive function. *In: Seasonality in primates: Studies of living and extinct human and non-human primates.* (Ed. by D.K. Brockman & C.P. van Schaik) pp.269-306. New York: Cambridge University Press.

Brockman D.K. & van Schaik C.P. 2005. *Seasonality in primates: Studies of living and extinct human and non-human primates.*Cambridge University Press.

Brown, J.L., Wasser, S.K., Wildt, D.E., & Graham, L.H. 1994. Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured noninvasively in feces. *Biology of reproduction,* 51, 776-786.

Butovskaya, M.L. & Kozintsev, A.G. 1996. Gender-related factors affecting primate social behavior: Grooming, rank, age, and kinship in heterosexual and all-male groups of stumptail macaques. *American journal of physical anthropology,* 101, 39-54.

Byrne, R.W., Barnard, P.J., Davidson, I., Janik, V.M., McGrew, W.C., Miklósi, A., & Wiessner, P. 2004. Understanding culture across species. *Trends in cognitive sciences,* 8, 341-346.

C, L., Littlejohn, L., Corson, C., & Suttie, S. 2003. Effects of testosterone on pedicle formation and its transformation to antler in castrated male, freemartin and normal female red deer (cervus elaphus). *General and comparative endocrinology,* 131, 21 - 31.

Cameron, E.Z., Setsaas, T.H., & Linklater, W.L. 2009. Social bonds between unrelated females increase reproductive success in feral horses. *Proceedings of the National Academy of Sciences of the United States of America,* 106, 13850-13853.

Cameron, J.L.  1996. Regulation of reproductive hormone secretion in primates by short-term changes in nutrition. *Reviews of Reproduction,* 1, 117-126.

Cameron, J.L., Helmreich, D.L., & Schreihofer, D.A. 1993. Modulation of reproductive hormone secretion by nutritional intake: Stress signals versus metabolic signals. *Human Reproduction,* 8, 162-167.

Chiyo, P.I., Archie, E.A., Hollister-Smith, J.A., Lee, P.C., Poole, J.H., Moss, C.J., & Alberts, S.C. 2011. Association patterns of african elephants in all-male groups: The role of age and genetic relatedness. *Animal behaviour,*

Clapham, P.J., Palsbøll, P.J., Mattila, D.K., & Vasquez, O. 1992. Composition and dynamics of humpback whale competitive groups in the west indies. *Behaviour,* 122, 3-4.

Clutton-Brock, T.  2007. Sexual selection in males and females. *Science (New York, N.Y.),* 318, 1882-1885.

Clutton-Brock, T.H. & Parker, G.A. 1992. Potential reproductive rates and the operation of sexual selection. *The Quarterly Review of Biology,* 67, 437-456.

Clutton-Brock, T.H., Hodge, S.J., Spong, G., Russell, A.F., Jordan, N.R., Bennett, N.C., Sharpe, L.L., & Manser, M.B. 2006. Intrasexual competition and sexual selection in cooperative mammals. *Nature,* 444, 1065-1068.

Clutton-Brock T.H., Guinness F.E., & Albon S.D. 1982. *Red deer : Behavior and ecology of two sexes.*pp.378 Chicago: University of Chicago Press.

Colmenares, F. 1992. Clans and harems in a colony of hamadryas and hybrid baboons: Male kinship, familiarity and the formation of brother-teams. *Behaviour,* 121, 61-94.

Connor R.C., Wells R.S., Mann J., & Read A.J. 2000. The bottlenose dolphin: Social relationships in a fission-fusion society. *In: Cetacean societies: Field studies of dolphins and whales.* (Ed. by J. Mann, R.C. Connor, P.L. Tyack, & H. Whitehead) pp.91-126. Chicago: University Of Chicago Press.

Cote, J., Dreiss, A., & Clobert, J. 2008. Social personality trait and fitness. *Proceedings. Biological sciences / The Royal Society,* 275, 2851-2858.

Côté, I.M. & Poulin, R. 1995. Parasitism and group size in social animals: A meta-analysis. *Behavioral Ecology,* 6, 159-165.

Cresswell, W. 1994. Flocking is an effective anti-predation strategy in redshanks, tringa totanus. *General and comparative endocrinology,* 47, 433 - 442.

Crockford, C., Wittig, R.M., Whitten, P.L., Seyfarth, R.M., & Cheney, D.L. 2008. Social stressors and coping mechanisms in wild female baboons (Papio hamadryas ursinus). *Hormones and behavior,* 53, 254-265.

Crook, J.H. 1966. Gelada baboon herd structure and movement: A comparative report. *Symposium of the zoological society of London,* 18, 237-258.

David, H.A. 1987. Ranking from unbalanced paired-comparison data. *Biometrika,* 74, 432-436.

Dugatkin L.A 1997. *Cooperation among animals.*New York: Oxford University Press US.

Dunbar, R.I. 2010. The social role of touch in humans and primates: Behavioural function and neurobiological mechanisms. *Neuroscience and biobehavioral reviews,* 34, 260-268.

Dunbar, R.I.M. 1979. Structure of gelada baboon reproductive units I. Stability of social relationships. *Behaviour,* 69, 72-87.

Dunbar, R.I.M. 1983. Structure of gelada baboon reproductive units. II. Social relationships between reproductive females. *Animal behaviour,* 31, 556-564.

Dunbar, R.I.M. 1983. Structure of gelada baboon reproductive units. III. The male's relationship with his females. *Animal Behaviour,* 31, 565-575.

Dunbar, R.I.M. & Bose, U. 1991. Adaptation to grass-eating in gelada baboons. *Primates; journal of primatology,* 32, 1-7.

Dunbar, R.I.M. & Dunbar, P. 1974. The reproductive cycle of the gelada baboon. *Animal behaviour,* 22, 203-210.

Dunbar, R.I.M. & Schultz, S. 2010. Bondedness and sociality. *Behaviour,* 147, 775-803.

Dunbar R.I.M 1984. *Reproductive decisions : An economic analysis of gelada baboon social strategies* .Princeton, N.J. : Princeton University Press,.

Dunbar R.I.M. & Dunbar P. 1975. *Social dynamics of gelada baboons* .New York : S. Karger.

Elo A.E 1978. *The rating of chessplayers, past and present.*Batsford London.

Fashing, P.J., Nguyen, N., & Fashing, N.J. 2010. Behavior of geladas and other endemic wildlife during a desert locust outbreak at guassa, ethiopia: Ecological and conservation implications. *Primates; journal of primatology,*

*F*ischhoff, I.R., Dushoff, J., Sundaresan, S.R., Cordingley, J.E., & Rubenstein, D.I. 2009. Reproductive status influences group size and persistence of bonds in male plains zebra (equus burchelli). *Behavioral ecology and sociobiology,* 63, 1035-1043.

Fruteau, C., Voelkl, B., van Damme, E., & Noë, R. 2009. Supply and demand determine the market value of food providers in wild vervet monkeys. *Proceedings of the National Academy of Sciences of the United States of America,* 106, 12007-12012.

Galat-Luong A., Galat G., & Hagell S. 2006. The social and ecological flexibility of guinea baboons: Implications for guinea baboon social organization and male strategies. *In: Reproduction and fitness in baboons: Behavioral, ecological, and life history perspectives.* (Ed. by L. Swedell & S.R. Leigh) pp.105-121. New York: Springer US.

Galeotti, P., Saino, N., Sacchi, R., & Møller, A.P. 1997. Song correlates with social context, testosterone and body condition in male barn swallows. *Animal Behaviour,* 53, 687-700.

Gammell, M.P., de Vries, H., Jennings, D.J., Carlin, C.M., & Hayden, T.J. 2003. David's score: A more appropriate dominance ranking method than clutton-brock et al.'S index. *Animal behaviour,* 66, 601 - 605.

Garamszegi, .Z., Calhim, S., Dochtermann, N., Hegyi, G., Hurd, .L., Jorgensen, C., Kutsukake, N., Lajeunesse, .J., Pollard, .A., et al. 2009. Changing philosophies and tools for statistical inferences in behavioral ecology. *Behavioral Ecology,* 20, 1363-1375.

Gesquiere, L.R., Altmann, J., Khan, M.Z., Couret, J., Yu, J.C., Endres, C.S., Lynch, J.W., Ogola, P., Fox, E.A., et al. 2005. Coming of age: Steroid hormones of wild immature baboons (Papio cynocephalus). *American journal of primatology,* 67, 83-100.

Gesquiere, L.R., Khan, M., Shek, L., Wango, T.L., Wango, E.O., Alberts, S.C., & Altmann, J. 2008. Coping with a challenging environment: Effects of seasonal variability and reproductive status on glucocorticoid concentrations of female baboons (Papio cynocephalus). *Hormones and Behavior,* 54, 410-416.

Gesquiere, L.R., Learn, N.H., Simao, M.C.M., Onyango, P.O., Alberts, S.C., & Altmann, J. 2011. Life at the top: Rank and stress in wild male baboons. *Science (New York, N.Y.),* 333, 357-360.

Gesquiere, L.R., Onyango, P.O., Alberts, S.C., & Altmann, J. 2011. Endocrinology of year-round reproduction in a highly seasonal habitat: Environmental variability in testosterone and glucocorticoids in baboon males. *American Journal of Physical Anthropology,* 144, 169-176.

Girard-Buttoz, C., Heistermann, M., Krummel, S., & Engelhardt, A. 2009. Seasonal and social influences on fecal androgen and glucocorticoid excretion in wild male long-tailed macaques (macaca fascicularis). *Physiology & behavior,* 98, 168-175.

Glenn M.E., Matsuda R., & Bensen K.J. 2002. Unique behavior of the mona monkey (cercopithecus mona): All-Male groups and copulation calls. *In: The guenons: Diversity and adaptation in african monkeys.* (Ed. by M.E. Glenn & M. Cords) pp.133-145. New York: Kluwer Academic/Plenum Publishers.

Gosling, S.D. & John, O.P. 1999. Personality dimensions in nonhuman animals A cross-species review. *Current Directions in Psychological Science,* 8, 69-75.

Goymann, W., Landys, M.M., & Wingfield, J.C. 2007. Distinguishing seasonal androgen responses from male-male androgen responsiveness-revisiting the challenge hypothesis. *Hormones and behavior,* 51, 463-476.

Grossman, C.J. 1985. Interactions between the gonadal steroids and the immune system. *Science,* 227, 257-261.

Grueter, C.C. & van Schaik, C.P. 2010. Evolutionary determinants of modular societies in colobines. *Behavioral Ecology,* 21, 63.

Hall, S.J., Wardle, C.S., & MacLennan, D.N. 1986. Predator evasion in a fish school: Test of a model for the fountain effect. *Marine biology,* 91, 143-148.

Hamilton, W.D. 1964. The genetical evolution of social behaviour. II. *Journal of theoretical biology,* 7, 17 - 52.

Hamilton, W.D. 1964. The genetical evolution of social behaviour. I. *Journal of theoretical biology,* 7, 1 - 16.

Hamilton, W.D. 1971. Geometry for the selfish herd. *Journal of theoretical biology,* 31, 295-311.

Hansen, P.J. 2009. Effects of heat stress on mammalian reproduction. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 364, 3341-3350.

Hau, M. 2007. Regulation of male traits by testosterone: Implications for the evolution of vertebrate life histories. *BioEssays : news and reviews in molecular, cellular and developmental biology,* 29, 133-144.

Heitor, F. & Vicente, L. 2010. Dominance relationships and patterns of aggression in a bachelor group of sorraia horses (equus caballus). *Journal of Ethology,* 28, 35-44.

Henzi, S.P. & Barrett, L. 1999. The value of grooming to female primates. *Primates; journal of primatology,* 40, 47-59.

Higham, J.P., Heistermann, M., & Maestripieri, D. 2013. The endocrinology of male rhesus macaque social and reproductive status: A test of the challenge and social stress hypotheses. *Behavioral ecology and sociobiology,* 67, 19-30.

Hill, D.A. 1994. Affiliative behaviour between adult males of the genus macaca. *Behaviour,* 130, 293-308.

Hill, D.A. & Van Hooff, J.A.R.A.M. 1994. Affiliative relationships between males in groups of nonhuman primates: A summary. *Behaviour,* 130, 143-149.

Hinde R.A. & Berman C.M. 1983. *Primate social relationships : An integrated approach* .Sunderland, Mass. : Sinauer Associates,.

Hirschenhauser, K. & Oliveira, R.F. 2006. Social modulation of androgens in male vertebrates: Meta-analyses of the challenge hypothesis. *Animal behaviour,* 71, 265-277.

Hunt, K., Wingfield, J.C., Astheimer, L.B., Buttemer, W.A., & Hahn, T.P. 1995. Temporal patterns of territorial behavior and circulating testosterone in the lapland longspur and other arctic passerines. *American Zoologist,* 35, 274-284.

Hunt, K.E., Hahn, T.P., & Wingfield, J.C. 1999. Endocrine influences on parental care during a short breeding season: Testosterone and male parental care in lapland longspurs (calcarius lapponicus). *Behavioral Ecology and Sociobiology,* 45, 360-369.

Hunter C.P 2001. Liverpool (United Kingdom): University of Liverpool.

Jablonski N.G 1993. *Theropithecus : The rise and fall of a primate genus* .Cambridge [England] ; New York, NY, USA : Cambridge University Press,.

Jolly, C.J. 2001. A proper study for mankind: Analogies from the Papionin monkeys and their implications for human evolution. *American journal of physical anthropology,* Suppl 33, 177-204.

Kappeler, P.M. & van Schaik, C.P. 2002. Evolution of primate social systems. *International Journal of Primatology,* 23, 707-740.

Kappeler P.M 2000. *Primate males : Causes and consequences of variation in group composition* .pp.316 Cambridge, UK ; New York : Cambridge University Press.

Kawai, M., Dunbar, R.I.M., Ohsawa, H., & Mori, U. 1983. Social organization of gelada baboons: Social units and definitions. *Primates; journal of primatology,* 24, 13-24.

Kawai M 1979. *Ecological and sociological studies of gelada baboons* .Tokyo : Kodansha ; New York : S. Karger,.

Kawai M 1979. *Ecological and sociological studies of gelada baboons* .Tokyo : Kodansha ; New York : S. Karger,.

Keay, J.M., Singh, J., Gaunt, M.C., & Kaur, T. 2006. Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: A literature review. *Journal of Zoo and Wildlife Medicine,* 37, 234-244.

Khan, M.Z., Altmann, J., Isani, S.S., & Yu, J. 2002. A matter of time: Evaluating the storage of fecal samples for steroid analysis. *General and comparative endocrinology,* 128, 57-64.

Kitchen, D.M. & Beehner, J.C. 2007. Factors affecting individual participation in group-level aggression among non-human primates. *Behaviour,* 144, 1551-1581.

Krützen, M., Sherwin, W.B., Connor, R.C., Barré, L.M., Van de Casteele, T., Mann, J., & Brooks, R. 2003. Contrasting relatedness patterns in bottlenose dolphins (tursiops sp.) With different alliance strategies. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* 270, 497.

Kummer H 1968. *Social organization of hamadryas baboons: A field study.*Karger New York.

Langergraber, K.E., Mitani, J.C., & Vigilant, L. 2007. The limited impact of kinship on cooperation in wild chimpanzees. *Proceedings of the National Academy of Sciences of the United States of America,* 104, 7786-7790.

le Roux, A. & Bergman, T.J. 2012. Indirect rival assessment in a social primate, Theropithecus gelada. *Animal behaviour,* 83, 249-255.

le Roux, A., Beehner, J.C., & Bergman, T.J. 2011. Female philopatry and dominance patterns in wild geladas. *American journal of primatology,* 73, 422-430.

Lettevall, E., Richter, C., Jaquet, N., Slooten, E., Dawson, S., Whitehead, H., Christal, J., & Howard, P.M. 2002. Social structure and residency in aggregations of male sperm whales. *Canadian Journal of Zoology,* 80, 1189-1196.

Levréro, F., Gatti, S., Ménard, N., Petit, E., Caillaud, D., & Gautier-Hion, A. 2006. Living in nonbreeding groups: An alternative strategy for maturing gorillas. *American journal of primatology,* 68, 275-291.

Ligon, J.D., Thornhill, R., Zuk, M., & Johnson, K. 1990. Male-male competition, ornamentation and the role of testosterone in sexual selection in red jungle fowl. *Animal behaviour,* 40, 367-373.

Lund, U. & Agostinelli, C. 2011. *Circular Statistics,*

Lynch, J.W., Khan, M.Z., Altmann, J., Njahira, M.N., & Rubenstein, N. 2003. Concentrations of four fecal steroids in wild baboons: Short-term storage conditions and consequences for data interpretation. *General and comparative endocrinology,* 132, 264-271.

Madsen, V., Valkiūnas, G., Iezhova, T.A., Mercade, C., Sanchez, M., & Osorno, J.L. 2007. Testosterone levels and gular pouch coloration in courting magnificent frigatebird (fregata magnificens): Variation with age-class, visited status and blood parasite infection. *Hormones and behavior,* 51, 156-163.

Majolo, B., de Bortoli Vizioli, A., & Schino, G. 2008. Costs and benefits of group living in primates: Group size effects on behaviour and demography. *Animal behaviour,* 76, 1235-1247.

Mateo, J.M. & Cavigelli, S.A. 2005. A validation of extraction methods for noninvasive sampling of glucocorticoids in free-living ground squirrels. *Physiological and biochemical zoology : PBZ,* 78, 1069-1084.

Maynard Smith, J.  1974. The theory of games and the evolution of animal conflicts. *Journal of Theoretical Biology,* 47, 209-221.

Maynard Smith, J. & Price, G.R. 1973. The logic of animal conflict. *Nature,* 246, 15-18.

Mazur, A., Booth, A., & James, M.M.D. 1992. Testosterone and chess competition. *Social Psychology Quarterly,* 55, 70-77.

McDonald, D.B.  2007. Predicting fate from early connectivity in a social network. *Proceedings of the National Academy of Sciences of the United States of America,* 104, 10910-10914.

Mendoza, S.P., Coe, C.L., Lowe, E.L., & Levine, S. 1978. The physiological response to group formation in adult male squirrel monkeys. *Psychoneuroendocrinology,* 3, 221-229.

Mills, S.C., Hazard, L., Lancaster, L., Mappes, T., Miles, D., Oksanen, T.A., & Sinervo, B. 2008. Gonadotropin hormone modulation of testosterone, immune function, performance, and behavioral trade-offs among male morphs of the lizard uta stansburiana. *The American naturalist,* 171, 339-357.

Millspaugh, J.J. & Washburn, B.E. 2003. Within-sample variation of fecal glucocorticoid measurements. *General and comparative endocrinology,* 132, 21-26.

Millspaugh, J.J. & Washburn, B.E. 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: Considerations for application and interpretation. *General and Comparative Endocrinology,* 138, 189-199.

Mitani, J.C.  2009. Male chimpanzees form enduring and equitable social bonds. *Animal Behaviour,* 77, 633-640.

Mori, A., Iwamoto, T., & Bekele, A. 1997. A case of infanticide in a recently found gelada population in arsi, ethiopia. *Primates; journal of primatology,* 38, 79-88.

Mori U. 1979. Development of sociability and social status. *In: Ecological and sociological studies of gelada baboons.* InM. Kawai, editors. pp.125-155. New York: Karger.

Mori U. 1979. Individual relationships within a unit. *In: Ecological and sociological studies of gelada baboons.* InM. Kawai, editors. pp.94-124. New York: Karger.

Moss C.J., Croze H., & Lee P.C. 2011. *The amboseli elephants: A long-term perspective on a long-lived mammal.*University of Chicago Press.

Muller, M.N. & Wrangham, R.W. 2004. Dominance, aggression and testosterone in wild chimpanzees: A test of the "challenge hypothesis". *Animal behaviour,* 67, 113-123.

Mundry, R.  2011. Issues in information theory-based statistical inference—a commentary from a frequentist's perspective. *Behavioral ecology and sociobiology,* 65, 57-68.

Murai, T.  2004. Social behaviors of all-male proboscis monkeys when joined by females. *Ecological Research,* 19, 451-454.

Muroyama, Y., Shimizu, K., & Sugiura, H. 2007. Seasonal variation in fecal testosterone levels in free-ranging male japanese macaques. *American journal of primatology,* 69, 603-610.

Neill, S.R.J. & Cullen, J.M. 1974. Experiments on whether schooling by their prey affects the hunting behaviour of cephalopods and fish predators. *Journal of zoology,* 172, 549-569.

Neumann, C., Duboscq, J., Dubuc, C., Ginting, A., Irwan, A.M., Agil, M., Widdig, A., & Engelhardt, A. 2011. Assessing dominance hierarchies: Validation and advantages of progressive evaluation with elo-rating. *Animal Behaviour,* 82, 911-921.

Noë, R.  1990. A veto game played by baboons: A challenge to the use of the prisoner's dilemma as a paradigm for reciprocity and cooperation. *Animal behaviour,* 39, 78 - 90.

Noë, R. & Hammerstein, P. 1994. Biological markets: Supply and demand determine the effect of partner choice in cooperation, mutualism and mating. *Behavioral Ecology and Sociobiology,* 35, 1-11.

Noë, R. & Hammerstein, P. 1995. Biological markets. *Trends in ecology & evolution (Personal edition),* 10, 336-339.

Nunn C.L. & Lewis R.J. 2001. Cooperation and collective action in animal behavior. *In: Economics in nature.* (Ed. by R. Noë, J.A.R.A.M. van Hooff, & P. Hammerstein) pp.42-66. Cambridge: Cambridge University Press.

Oliveira, R.F. 2004. Social modulation of androgens in vertebrates: Mechanisms and function. *Advances in the Study of Behavior,* 34, 165-237.

Packer, C. & Pusey, A.E. 1982. Cooperation and competition within coalitions of male lions: Kin selection or game theory? *Nature,* 296, 740-742.

Packer, C. & Ruttan, L. 1988. The evolution of cooperative hunting. *American Naturalist,* 159-198.

Packer, C., Gilbert, D.A., Pusey, A.E., & O'Brien, S.J. 1991. A molecular genetic analysis of kinship and cooperation in african lions. *Nature,* 351, 562-565.

Page, S.L., Chiu, C., & Goodman, M. 1999. Molecular phylogeny of old world monkeys (cercopithecidae) as inferred from γ-globin DNA sequences. *Molecular Phylogenetics and Evolution,* 13, 348-359.

Palme, R. 2005. Measuring fecal steroids: Guidelines for practical application. *Annals of the New York Academy of Sciences,* 1046, 75-80.

Palombit, R.A., Seyfarth, R.M., & Cheney, D.L. 1997. The adaptive value of 'friendships' to female baboons: Experimental and observational evidence. *Animal behaviour,* 54, 599-614.

Pappano, D.J., Roberts, E.K., & Beehner, J.C. 2010. Testing extraction and storage parameters for a fecal hormone method. *American journal of primatology,* 72, 934-941.

Pappano, D.J., Snyder-Mackler, N., Bergman, T.J., & Beehner, J.C. 2012. Social ‘predators’ within a multilevel primate society. *Animal behaviour,*

*P*eters, A., Cockburn, A., & Cunningham, R. 2002. Testosterone treatment suppresses paternal care in superb fairy-wrens, malurus cyaneus, despite their concurrent investment in courtship. *Behavioral Ecology and Sociobiology,* 51, 538-547.

Pérez-Rodríguez, L., Blas, J., Vińuela, J., Marchant, T.A., & Bortolotti, G.R. 2006. Condition and androgen levels: Are condition-dependent and testosterone-mediated traits two sides of the same coin? *Animal behaviour,* 72, 97-103.

Pope, T.R. 1990. The reproductive consequences of male cooperation in the red howler monkey: Paternity exclusion in multi-male and single-male troops using genetic markers. *Behavioral Ecology and Sociobiology,* 27, 439-446.

Port, M., Clough, D., & Kappeler, P.M. 2009. Market effects offset the reciprocation of grooming in free-ranging redfronted lemurs, eulemur fulvus rufus. *Animal behaviour,* 77, 29-36.

Port, M., Kappeler, P.M., & Johnstone, R.A. 2011. Communal defense of territories and the evolution of sociality. *The American naturalist,* 178, 787-800.

Pörschmann, U., Trillmich, F., Mueller, B., & Wolf, J.B. 2010. Male reproductive success and its behavioural correlates in a polygynous mammal, the galápagos sea lion (zalophus wollebaeki). *Molecular ecology,* 19, 2574-2586.

Pusey A.E. & Packer C. 1987. Dispersal and philopatry. *In: 1843550.Pdf.* pp.250-266. Chicago: University of Chicago Press.

Quinn, J.L. & Cresswell, W. 2006. Testing domains of danger in the selfish herd: Sparrowhawks target widely spaced redshanks in flocks. *Proceedings. Biological sciences / The Royal Society,* 273, 2521-2526.

Rajpurohit, L.S. 1995. Temporary splitting or subgrouping in male bands of hanuman langurs, presbytis entellus around jodhpur, western india. *Mammalia,* 59, 3-8.

Rajpurohit, L.S. 1997. Sociobiology of an all male band in hanuman langurs, presbytis entellus around jodhpur. *Uttar Pradesh Journal of Zoology,* 17, 70-74.

Raouf, S.A., Parker, P.G., Ketterson, E.D., Nolan, V., & Ziegenfus, C. 1997. Testosterone affects reproductive success by influencing extra-pair fertilizations in male dark-eyed juncos (aves: Junco hyemalis). *Proceedings: Biological Sciences,* 264, 1599-1603.

Ray, J.C. & Sapolsky, R.M. 1992. Styles of male social behavior and their endocrine correlates among high-ranking wild baboons. *American journal of primatology,* 28, 231-250.

R Core Development Team. 2011. R: A language and environment for statistical computing.

Robbins, M.M. 1996. Male-male interactions in heterosexual and all-male wild mountain gorilla groups. *Ethology,* 102, 942-965.

Roberts, E.K., Lu, A., Bergman, T.J., & Beehner, J.C. 2012. A bruce effect in wild geladas. *Science (New York, N.Y.),* 335, 1222-1225.

Ross, C. 1993. Predator mobbing by an all-male band of hanuman langurs (presbytis entellus). *Primates; journal of primatology,* 34, 105-107.

Rubenstein D.I. 1986. Ecology and sociality in horses and zebras. *In: Ecological aspects of social evolution.* pp.282-302. Princeton: Princeton University Press.

Rubenstein D.I. & Hack M. 2004. Natural and sexual selection and the evolution of multi-level socieites: Insights from zebras with comparisons to primates. *In: Sexual selection in primates: New and comparative perspectives.* (Ed. by P.M. Kappeler & C.P. van Schaik) pp.266-279. Cambridge: Cambridge University Press.

Rusu, A.S. & Krackow, S. 2004. Kin-preferential cooperation, dominance-dependent reproductive skew, and competition for mates in communally nesting female house mice. *Behavioral ecology and sociobiology,* 56, 298-305.

Sachs, J.L., Mueller, U.G., Wilcox, T.P., & Bull, J.J. 2004. The evolution of cooperation. *The Quarterly Review of Biology,* 79, 135-160.

Sapolsky, R.M.  1990. Adrenocortical function, social rank, and personality among wild baboons. *Biological Psychiatry,* 28, 862-878.

Sarkar, D. & Bates, D. 2009. Lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-225.

Schreier, A.L. & Swedell, L. 2009. The fourth level of social structure in a multi-level society: Ecological and social functions of clans in hamadryas baboons. *American journal of primatology,* 71, 948-955.

Schülke, O.  2001. Differential energy budget and monopolization potential of harem holders and bachelors in hanuman langurs (semnopithecus entellus): Preliminary results. *American journal of primatology,* 55, 57-63.

Schülke, O., Bhagavatula, J., Vigilant, L., & Ostner, J. 2010. Social bonds enhance reproductive success in male macaques. *Current biology : CB,* 20, 2207-2210.

Schwarzenberger, F., Möstl, E., Palme, R., & Bamberg, E. 1996. Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. *Anim. Reprod. Sci,* 42, 515-526.

Setchell, J.M., Smith, T., Wickings, E.J., & Knapp, L.A. 2008. Social correlates of testosterone and ornamentation in male mandrills. *Hormones and behavior,* 54, 365-372.

Seyfarth, R.M. & Cheney, D.L. 2012. The evolutionary origins of friendship. *Annual review of psychology,* 63, 153-177.

Shutt, K., MacLarnon, A., Heistermann, M., & Semple, S. 2007. Grooming in barbary macaques: Better to give than to receive? *Biology letters,* 3, 231-233.

Sih, A., Bell, A.M., Johnson, J.C., & Ziemba, R.E. 2004. Behavioral syndromes: An intergrative overiew. *The Quarterly review of biology,* 79, 241-277.

Silk, J., Beehner, J., Bergman, T., Crockford, C., Engh, A., Moscovice, L., Wittig, R., Seyfarth, R., & Cheney, D. 2010. Female chacma baboons form strong, equitable, and enduring social bonds. *Behavioral ecology and sociobiology,* 64, 1733-1747.

Silk, J.B.  1994. Social relationships of male bonnet macaques: Male bonding in a matrilineal society. *Behaviour,* 130, 271-291.

Silk, J.B.  2002. Using the 'F'-word in primatology. *Behaviour,* 139, 421-446.

Silk, J.B. 2007. The adaptive value of sociality in mammalian groups. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* 362, 539-559.

Silk, J.B. 2007. Social components of fitness in primate groups. *Science (New York, N.Y.),* 317, 1347-1351.

Silk, J.B., Alberts, S.C., & Altmann, J. 2003. Social bonds of female baboons enhance infant survival. *Science (New York, N.Y.),* 302, 1231-1234.

Silk, J.B., Alberts, S.C., & Altmann, J. 2006. Social relationships among adult female baboons (Papio cynocephalus) II. Variation in the quality and stability of social bonds . *Behavioral Ecology and Sociobiology,* 61, 197-204.

Silk, J.B., Altmann, J., & Alberts, S.C. 2006. Social relationships among adult female baboons (Papio cynocephalus) I. Variation in the strength of social bonds. *Behavioral Ecology and Sociobiology,* 61, 183-195.

Silk, J.B., Beehner, J.C., Bergman, T.J., Crockford, C., Engh, A.L., Moscovice, L.R., Wittig, R.M., Seyfarth, R.M., & Cheney, D.L. 2009. The benefits of social capital: Close social bonds among female baboons enhance offspring survival. *Proceedings. Biological sciences / The Royal Society,* 276, 3099-3104.

Silk, J.B., Beehner, J.C., Bergman, T.J., Crockford, C., Engh, A.L., Moscovice, L.R., Wittig, R.M., Seyfarth, R.M., & Cheney, D.L. 2010. Strong and consistent social bonds enhance the longevity of female baboons. *Current biology : CB,* 20, 1359 - 1361.

Smith, J.E., Van Horn, R.C., Powning, K.S., Cole, A.R., Graham, K.E., Memenis, S.K., & Holekamp, K.E. 2010. Evolutionary forces favoring intragroup coalitions among spotted hyenas and other animals. *Behavioral Ecology,* 21, 284-303.

Snyder-Mackler, N., Beehner, J.C., & Bergman, T.J. 2012. Defining higher levels in the multilevel societies of geladas (Theropithecus gelada). *International journal of primatology,*

Snyder-Mackler, N., Beehner, J.C., & Bergman, T.J. 2012. Defining higher levels in the multilevel societies of geladas (Theropithecus gelada). *International journal of primatology,*

Steenbeck R., Sterck E.H., de Vries H., & Van Hoof J.A.R.A.M. 2000. Costs and benefits of the one-male, age-graded, and all-male phases in wild thomas's langur groups. *In: Primate males: Causes and consequences of variation in group composition.* InP.M. Kappeler, editors. pp.130-145. New York: Cambridge University Press.

Stoehr, A.M. & Hill, G.E. 2000. Testosterone and the allocation of reproductive effort in male house finches (carpodacus mexicanus). *Behavioral Ecology and Sociobiology,* 48, 407-411.

Stoinski, T.S., Czekala, N., Lukas, K.E., & Maple, T.L. 2002. Urinary androgen and corticoid levels in captive, male western lowland gorillas (gorilla g. Gorilla): Age- and social group-related differences. *American journal of primatology,* 56, 73-87.

Swedell, L. 2002. Affiliation among females in wild hamadryas baboons (Papio hamadryas hamadryas). *International Journal of Primatology,* 23, 1205-1226.

Symonds, M.R.E. & Moussalli, A. 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using akaike's information criterion. *Behavioral ecology and sociobiology,* 65, 13-21.

Tilson, R.L., Sweeny, K.M., Binczik, G.A., & Reindl, N.J. 1988. Buddies and bullies: Social structure of a bachelor group of przewalksi horses. *Applied Animal Behaviour Science,* 21, 169-185.

Touma, C. & Palme, R. 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: The importance of validation. *Annals of the New York Academy of Sciences,* 1046, 54-74.

Van Hooff, J.A.R.A.M. & Van Schaik, C.P. 1994. Male bonds: Afilliative relationships among nonhuman primate males. *Behaviour,* 130, 309-337.

van Schaik, C.P. & Kappeler, P.M. 1997. Infanticide risk and the evolution of male-female association in primates. *Proceedings. Biological sciences / The Royal Society,* 264, 1687-1694.

Virgin, C.E. & Sapolsky, R.M. 1997. Styles of male social behavior and their endocrine correlates among low-ranking baboons. *American journal of primatology,* 42, 25-39.

De Vos, A. & O'Riain, M.J. 2010. Sharks shape the geometry of a selfish seal herd: Experimental evidence from seal decoys. *Biology letters,* 6, 48-50.

de VRIES, H.A.N. 1998. Finding a dominance order most consistent with a linear hierarchy: A new procedure and review. *Animal behaviour,* 55, 827 - 843.

Wahab, F., Aziz, F., Irfan, S., Zaman, W.-U., & Shahab, M. 2008. Short-term fasting attenuates the response of the HPG axis to kisspeptin challenge in the adult male rhesus monkey (< i> macaca mulatta). *Life sciences,* 83, 633-637.

Wang, J. 2002. An estimator for pairwise relatedness using molecular markers. *Genetics,* 160, 1203-1215.

Wasser, S.K., Hunt, K.E., Brown, J.L., Cooper, K., Crockett, C.M., Bechert, U., Millspaugh, J.J., Larson, S., & Monfort, S.L. 2000. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. *General and Comparative Endocrinology,* 120, 260-275.

Wasser, S.K., Thomas, R., Nair, P.P., Guidry, C., Southers, J., Lucas, J., Wildt, D.E., & Monfort, S.L. 1993. Effects of dietary fibre on faecal steroid measurements in baboons (Papio cynocephalus cynocephalus). *Journal of reproduction and fertility,* 97, 569-574.

Waterman, J.M. 1997. Why do male cape ground squirrels live in groups? *Animal behaviour,* 53, 809-817.

Watts, D.P. 2002. Reciprocity and interchange in the social relationships of wild male chimpanzees. *Behaviour,* 139, 343-370.

Watts D.P. 2000. Causes and consequences of variation in male mountain gorilla life histories and group membership. *In: Primate males: Causes and consequences of variation in group composition.* InP.M. Kappeler, editors. pp.169-180. New York: Cambridge University Press.

Wenzel, J.W. & Pickering, J. 1991. Cooperative foraging, productivity, and the central limit theorem. *Proceedings of the National Academy of Sciences,* 88, 36-38.

West Eberhard, M.J. 1975. The evolution of social behavior by kin selection. *The Quarterly Review of Biology,* 50, 1-33.

West-Eberhard, M.J. 1983. Sexual selection, social competition, and speciation. *The Quarterly Review of Biology,* 58, 155-183.

Westneat, D.F. & Sherman, P.W. 1997. Density and extra-pair fertilizations in birds: A comparative analysis. *Behavioral ecology and sociobiology,* 41, 205-215.

Whitten, P.L., Brockman, D.K., & Stavisky, R.C. 1998. Recent advances in noninvasive techniques to monitor hormone-behavior interactions. *American journal of physical anthropology,* Suppl 27, 1-23.

Wickings, E.J. & Dixson, A.F. 1992. Testicular function, secondary sexual development, and social status in male mandrills (< i> mandrillus sphinx). *Physiology & behavior,* 52, 909-916.

Widdig, A. 2007. Paternal kin discrimination: The evidence and likely mechanisms. *Biological reviews of the Cambridge Philosophical Society,* 82, 319-334.

Widdig, A., Streich, W.J., & Tembrock, G. 2000. Coalition formation among male barbary macaques (macaca sylvanus). *American journal of primatology,* 50, 37-51.

Wingfield, J.C., Hegner, R.E., Dufty, A.M., Jr., J., & Ball, G.F. 1990. The "challenge hypothesis": Theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *The American Naturalist,* 136, 829-846.

Wittig, R.M., Crockford, C., Lehmann, J., Whitten, P.L., Seyfarth, R.M., & Cheney, D.L. 2008. Focused grooming networks and stress alleviation in wild female baboons. *Hormones and behavior,* 54, 170-177.

Wood, A.J. & Ackland, G.J. 2007. Evolving the selfish herd: Emergence of distinct aggregating strategies in an individual-based model. *Proceedings. Biological sciences / The Royal Society,* 274, 1637-1642.

Woodley, S.K. 1994. Plasma androgen levels, spermatogenesis, and secondary sexual characteristics in two species of plethodontid salamanders with dissociated reproductive patterns. *General and comparative endocrinology,* 96, 206 - 214.

Yamagiwa, J. 1987. Intra- and inter-group interactions of an all-male group of virunga mountain gorillas (gorilla gorilla beringei). *Primates; journal of primatology,* 28, 1-30.

Yamagiwa, J. 1992. Functional analysis of social staring behavior in an all-male group of mountain gorillas. *Primates; journal of primatology,* 33, 523-544.

Zar J.H 1999. *Biostatistical analysis.*Prentice hall Upper Saddle River, NJ.

Ziegler, T.E. & Wittwer, D.J. 2005. Fecal steroid research in the field and laboratory: Improved methods for storage, transport, processing, and analysis. *American journal of primatology,* 67, 159-174.

Zinner, D., Groeneveld, L.F., Keller, C., & Roos, C. 2009. Mitochondrial phylogeography of baboons (papio spp.): Indication for introgressive hybridization? *BMC evolutionary biology,* 9, 83.