The Hormonal Correlates of Male Chimpanzee Social Behavior

by

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DEDICATION

To my mother

I will love you forever, I will love you for always…

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ABSTRACT

Male chimpanzees are well known for their aggressive behavior. In this dissertation I investigate the hormonal correlates of three types of male chimpanzee aggression: within-group male-male aggression, between-group territorial aggression, and between species predatory aggression. Specifically, I examined how testosterone and cortisol, two steroid hormones, mediate these types of aggression.

Within groups, male chimpanzees compete with each other to obtain matings with females. While engaged in this form of reproductive aggression, males displayed increased testosterone levels, but only when competing for specific females. Rates of male aggression were elevated when they competed for parous, estrous females. In contrast, levels of aggression were relatively low in the presence of nulliparous females, who represented less attractive mating partners. Consequently, male testosterone concentrations were higher in the presence of parous, estrous females compared to their baseline levels. The presence of nulliparous, estrous females had no effect on male testosterone concentrations. These results are consistent with the Challenge Hypothesis, which proposes that testosterone correlates with aggression only when the latter enhances fitness.

In a novel test of the Challenge Hypothesis, I found that male chimpanzee testosterone concentrations increased during and shortly after territorial boundary patrols but not while they hunted. In addition, male chimpanzees displayed an

anticipatory rise in testosterone before they engaged in territorial behavior. Further analyses revealed that male testosterone levels were significantly lower after hunting, perhaps due to the tolerance associated with meat sharing. Males that shared and received meat at hunts exhibited decrements in testosterone, while males who failed to obtain meat at hunts showed no change.

In a third study, I examined all three types of aggression as they relate to stress. Male chimpanzees displayed acute stress responses and relatively high cortisol levels when they engaged in within-group reproductive aggression, between-group territorial aggression, and between species predatory aggression. As seen with testosterone, male chimpanzees showed an intriguing rise in cortisol before they began to participate in territorial and hunting behaviors. Thus, wild chimpanzees appear to be able to anticipate conflict situations. The cues that they use to anticipate these events, however, remain unknown and require further study.

CHAPTER 1

Introduction

Studies of the physiological mechanisms underlying primate behavior are a major focus of current research (Bercovitch and Ziegler 2002; Cheney and Seyfarth 2009; Anestis 2010). Considerable theoretical and empirical work has been devoted to investigating the relationships between hormones and behavior. These studies have enhanced our understanding of the evolution of complex behaviors (Wingfield et al. 1990; Ricklefs and Wikelski 2002; McGlothlin and Ketterson 2008). This dissertation contributes to this literature through an investigation of the hormonal correlates of male chimpanzee behavior. Specifically, I examine how two steroid hormones, testosterone and cortisol vary as a function of three types of aggression: within-group male-male aggression for estrous females; between-group territorial aggression; and between species predatory aggression.

Hormones are conserved evolutionarily, as similar structures are found in vertebrates, invertebrates, and even plants (Mechoulam et al. 1984; Adkins-Regan 2005). Similarly, the actions of hormones are maintained across taxa that have long diverged on separate evolutionary paths. This long evolutionary sets the stage for scientists to use changes in these relationships to track evolutionary developments. The close evolutionary history of humans and chimpanzees suggests we will share many similar physiological underpinnings of complex behaviors and any differences that exist will inform us of recent changes in human evolution. This will help us understand how behaviors evolved and continue to influence the human condition.

Chimpanzee Natural History

Chimpanzees live in communities of 20 to over 160 individuals and occupy relatively large territories that vary between 5–30 km^2 depending on habitat type and quality (review in Muller and Mitani 2005). Individuals within communities fission and fuse forming temporary parties that change in size and composition; parties include between 4-10 individuals on average (Boesch 1996; Matsumoto-Oda 1999; Mitani et al. 2002; Newton-Fisher 2002).

Male chimpanzees are philopatric. Males are quite gregarious and form strong social bonds with each other in the process (Nishida 1968; Goodall 1986; Goldberg and Wrangham 1997**;** Mitani et al. 2000; Mitani 2009). Male kin live together throughout their lives and cooperate via coalitions, meat sharing, and territorial boundary patrols (Langergraber et al. 2007). Male chimpanzees, however, also compete with each other within and between communities (Mitani 2009). Within communities, males compete for rank and form linear dominance hierarchies (Bygott 1979; Muller 2002). Between communities, male chimpanzees compete via their group territorial behavior (Watts and Mitani 2001; Wilson and Wrangham 2003; Williams et al. 2004).

Unlike males, female chimpanzees transfer between communities when they reach adolescence (Pusey et al. 1997; Williams et al. 2002; Nishida et al. 2003). Following dispersal, females spend 2-3 years in a period of adolescent sterility

(Goodall 1986; Nishida 1990; Boesch and Boesch-Achermann 2000). Once fertile, females mate during discrete estrous periods where they develop sexual swellings that last about 12 – 13 days (Furuichi and Hashimoto 2002). Females give birth only once every 5 - 6 years (Goodall 1986; Boesch and Boesch-Achermann 2000; Nishida et al. 2003; Sugiyama 2004). This long inter-birth interval leads to a highly skewed operational sex ratio within chimpanzee communities (Emlen and Oring 1977). As a consequence, males compete intensely to obtain reproductive opportunities with estrous females. This competition occurs over parous, estrous females, who have already reproduced successfully. Males do not typically compete as intensely for younger, nulliparous females when they are in estrus (Muller and Wrangham 2004a; Muller et al. 2006).

In addition to the aggression surrounding reproductively active females, male chimpanzees display high levels of aggression during interactions with members of other communities and while hunting prey (Muller and Mitani 2005). Male chimpanzees engage in boundary patrols to defend their territories (Goodall et al. 1979; Watts and Mitani 2001; Williams et al. 2004). Encounters between individuals of different communities can be extremely aggressive and occasionally result in lethal attacks (Wrangham 1999; Wilson et al. 2004; Mitani et al. 2010). Acts of lethal aggression have led to some chimpanzees expanding their territories into those of their neighbors (Mitani et al. 2010). Lethal intergroup aggression is quite rare in mammals and has been documented only in chimpanzees, humans, spider monkeys, and some social carnivores (Wilson and Wrangham 2003; hyenas:

Henschel and Skinner 1991; wolves: Mech 1994; spider monkeys: Aureli et al. 2006; lions: Mosser and Packer 2009).

Predation is another type of aggression displayed by chimpanzees. Chimpanzees frequently hunt other vertebrates (Goodall 1986; Boesch and Boesch 1989; Nishida 1990; Mitani and Watts 1999; Gilby et al. 2006). Following successful hunts, male chimpanzees frequently share meat with other conspecifics. Several hypotheses have been formulated to explain meat sharing by chimpanzees. Some researchers have suggested that male chimpanzees share meat with females to increase their mating success (Stanford et al. 1994a; Gomes and Boesch 2009). Others argue that sharing represents a form of tolerated theft (Blurton Jones 1984), as individuals are forced to relinquish meat to others who harass them (Gilby 2006). Additional research suggests that males use meat as a political tool to develop and maintain social bonds with others (Nishida et al. 1992; Mitani and Watts 2001). At some sites, theft occurs with high-ranking males stealing meat from lower-ranking individuals (Nishida et al. 1992). Nevertheless, there is a remarkable respect for ownership, whereby low ranking individuals are able to maintain possession of carcasses in the absence of harassment by higher-ranking chimpanzees (de Waal 2010). In addition, there is a considerable amount of voluntary sharing between related and unrelated individuals (Langergraber et al. 2007), some of which is reciprocated between dyads at the group level (Mitani 2006).

Beginning with the earliest accounts of chimpanzee behavior, male chimpanzees have been known for their aggressive behavior (Bygott 1979; Goodall 1986). In this dissertation, I investigate the relationships between hormones and the different forms of aggression discussed above in the Ngogo chimpanzee community in Kibale National Park, Uganda.

Steroid Hormone Physiology

Recent advances in the analysis of hormones collected non-invasively from animals in the wild has increased our understanding of the relationships between hormones and behavior (Bercovitch and Ziegler 2002; Anestis 2010). Hormones, especially the steroid hormones, have links to aggression (dominance rank, territoriality, hunting) and affiliative behaviors (parental care, grooming, social support) in many wild species (Adkins-Regan 2005; Anestis 2010).

To successfully navigate their social worlds, male chimpanzees must be physiologically prepared. The sympathetic nervous system produces steroid hormones from remote glands in the body, including the gonads and the adrenal cortex (Becker et al., 1992). Steroid hormones are derived from the same chemical base, cholesterol and are therefore lipophilic. They have relatively fast-acting effects on peripheral tissues and the central nervous system (Falkenstein et al. 2000). Both centers of activity are controlled by signals regulated by the brain. Adrenocorticotrophic hormone (ACTH) regulates adrenal activity, while luteinizing hormone (LH) activates the gonads (Baum 2002; Sapolsky 2002). In this dissertation, I focus on the activity of these two centers by assaying two steroid hormones, testosterone and cortisol.

Testosterone, the steroid hormone produced by the gonads, is secreted along the hypothalamo-hypophyseal-gonadal axis. When testosterone levels are low, gonadotropin-releasing hormone (GnRH), released by the hypothalamus, stimulates the pituitary gland to release follicle-stimulating hormone and LH. These later two hormones stimulate the gonads to synthesize testosterone. Finally increasing levels of testosterone, through a negative feedback loop act on the hypothalamus and pituitary, prevent the release of GnRH (Baum 2002). Testosterone is known to have a rapid influence on brain activity and sexual behavior (Falkenstein et al. 2000). It affects secondary sexual characteristics, such as muscle development, sexual motivation, risk taking behavior, and aggression in males (Baum 2002). Additionally, male testosterone mediates conspecific aggression. Since Berthold's (1849) classic pecking order experiments, testosterone has been considered integral for the normal expression of aggression (Soma 2006). More recent studies, however, have found that this relationship is not unidirectional, as aggression itself elevates testosterone levels (Soma 2006). Additionally, high levels of testosterone may decrease parental behavior and increase risk taking behaviors in males (Wingfield et al. 1990). Chronically elevated testosterone levels can increase metabolic rate and decrease body mass and fat stores important for survival, reduce immune function and lead to increased parasite loads (Ketterson et al. 1991; Owen-Ashley et al. 2004; Muehlenbein and Watts 2010).

Cortisol is a physiological marker of stress. It is released via activation of the hypothalamic-pituitary-adrenal axis when signals from the brain circulate through the body and stimulate the adrenal gland to produce cortisol (Selye 1956; Sapolsky 1992). Cortisol acts synergistically with arginine vasopressin, angiotensin II, and epinephrine. The stress response is an adaptive and generalized response. It evolved as a means to overcome pain, temperature fluctuations, and survival scenarios where the fight or flight is crucial (Korte et al. 2005). It allows an individual to quickly alter its physiological and behavioral profile in response to acute changes in its social and physical environments (McEwen and Wingfield 2003). This change is responsible for shunting sugar to the blood stream and exercising muscles, increasing heart rate and metabolic rate in addition to enhancing aspects of memory and learning (Sapolsky 2002). With the activation of the adaptive stress response, non-essential metabolic processes are deactivated. This consequently shuts down digestive processes, reduces immune function, and pauses somatic maintenance and growth processes (McEwen and Wingfield 2003; Sapolsky 2004; Korte et al 2005). While the acute stress response is adaptive, chronic stress leads to immune deficiency, muscle wasting, gastrointestinal dysfunction, impaired brain function, reproductive suppression, and growth reduction (Sapolsky 2002).

Steroid hormones are often associated with environmental and social challenges including dominance rank instability, food stress, and predatory aggression (Creel 2001; Rubenstein 2007; Lima et al. 2009; Gesquiere et al. 2011). The environmental conditions in Kibale National Park buffered the Ngogo chimpanzees from these variables during this study. The Kibale National Park is located in western Uganda along the eastern edge of the western Great Rift Valley, near the Rwenzori Mountains (Lwanga 1994; Struhsaker 1997). Due to its high elevation at 1.4000 m above sea level, Kibale is cooler and drier than other tropical forests. The average minimum and maximum daily temperatures during the same period were 16.7° C (SD = 0.29) and 24.7°. Mean annual rainfall is approximately 1,400 mm. The park consists of mixed old growth and colonizing forest, including montane tropical forest, grassland, woodland thicket, colonizing forest, papyrus swamp, and exotic tree plantation. Tall evergreen forest composes most (60%) of the park (Ghiglieri 1984; Lwanga 1994; Struhsaker 1997). These ecosystems are highly productive as Kibale has one of the highest densities of primates anywhere, with 11 primate species (Strusaker 1997). Overall, the environmental quality of Kibale National Park maintains an incredibly high faunal diversity, with at least 300 bird species and at least 70 species of mammals.

The Ngogo chimpanzee community is exceptionally productive compared to the Kanyawara chimpanzee community, a nearby community in Kibale National Park (Potts et al. 2011). The Ngogo chimpanzees do not experience enough food-stress to show changes in their ranging patterns even after they engage in energetically costly behaviors (Amsler 2009). The lack of food stress at Ngogo is also documented through endocrinology studies. C-peptide is a physiological marker of energy balance. When C-peptides are high, this indicates that food quality and availability are high. The Ngogo chimpanzees have higher C-peptide levels than the Kanyawara, a nearby chimpanzee community (Thompson et al. 2009). Many behavioral endocrinology studies focus on seasonality or food stress, as they have a large impact on hormone secretion (Rubenstein 2007; Gesquiere et al. 2011). During this study, at Ngogo, monthly phenology calculations and monthly steroid hormone production were not significantly correlated (Cortisol: R^2=.004, N = 28, F = 0.109, P = 0.75; Test: R^2 = 0.014, N = 28, F = 0.393, P = 0.54). Taken together, fruit production or food stress does not seem to be an important predictive factor in understanding fluctuation in urinary hormone concentrations.

The Ngogo chimpanzee community has the largest number of chimpanzees currently being studied. At the time of this study, the community was composed of over 160 chimpanzees with 27 adult males. As mentioned, these males form a linear dominance hierarchy. Dominance rank has long been connected to both testosterone and cortisol secretion (Abbott et al. 2003). However, studies produce inconsistent results. For example, low-ranking animals are victims of considerable aggression, and some studies reveal that low-ranking individuals possess chronically elevated levels of cortisol (Louch and Higginbotham 1967; Goymann et al. 2001). Additional research suggests the opposite, with high-ranking individuals showing high levels of cortisol presumably due to the stress associated with rank maintenance (Creel et al. 1996; Arnold and Dittami 1997; Cavigelli 1999; Holekamp and Smale 1998; Muller and Wrangham 2004b). However, the lack of control and predictability associated with rising or falling in rank may be a better predictor of hormone secretion (Sapolsky 1993; Abbott et al. 2003; Beehner et al. 2006; Engh et al. 2006). Though the Ngogo chimpanzees have a large number of adult males struggling for dominance, their dominance hierarchy was stable during this study and has been stable for many years (Mitani 2006). Therefore, though dominance rank was discussed, the effect of dominance instability on steroid hormone secretion could not be tested.

Though it has long been established that predators illicit hormone secretion in prey (review in Lima et al. 2009). The Ngogo chimpanzees have no natural predators in the Kibale National Park. This eliminates predatory stress as a factor of interest. However, other male chimpanzees from neighboring communities can act as predators. In fact, lethal aggression from other chimpanzees was the cause of nearly a quarter of all deaths in the Gombe chimpanzee community (Williams et al. 2008). These ecological conditions create a situation where the study of various types of aggression including territoriality may be a significant source of within individual hormone secretion.

The lack of food stress or predators, stability in the dominance hierarchy, and the exceptionally large community size, makes Ngogo the perfect site to investigate the hormonal correlates of male aggression. This dissertation uses systematic field and laboratory methods. I assayed testosterone and cortisol levels from non-invasively collected urine samples to study how they fluctuate as males operated in their natural environment. This dissertation has three main chapters: 1.) female parity, male aggression and the challenge hypothesis 2.) testosterone, tolerance and territoriality, and 3.) acute and anticipatory stress in wild chimpanzees.

Chapter 2: Female Parity, Male Aggression and the Challenge Hypothesis

In Chapter 2, I examine the relationship between within-group male-male aggression and testosterone, adopting the theoretical framework furnished by the Challenge Hypothesis (Wingfield et al. 1990). While many have assumed that there is a tight link between testosterone and aggression, early studies provided mixed results. Some studies showed that elevated testosterone was associated with increased aggression, while other studies failed to replicate this result (Wingfield et al. 2005). The Challenge Hypothesis helps to resolve these disparate results (Wingfield et al. 1990). Because chronically high testosterone levels can have deleterious health consequences, Wingfield and colleagues proposed that males limit the production of testosterone to situations where aggression is related to reproductive competition, such as mating or territory defense. In contrast, heightened testosterone levels are not associated with other forms of aggression, such as anti-predatory, irritable, and defensive aggression.

Previous research on primates supports the Challenge Hypothesis, but in most of these studies, the animals breed seasonally. In contrast, evidence from non-seasonally breeding taxa, such as chimpanzees, is generally lacking (Beehner et al. 2009). Male chimpanzees compete to mate with reproductively active females across the seasons and year. In a prior study, male aggression and testosterone levels were shown to increase in the presence of parous, estrous females (Muller and Wrangham 2004a). Similar increases were not displayed by these same males when nulliparas were present. Because males mated parous and nulliparous females equally often, increases in male testosterone appeared to be associated with the aggression used to acquire mates, rather than mating itself. In Chapter 2, I replicated this study with a larger sample of males and estrous females who differed in their parity, while controlling for potential confounds, such as male party size and the influence of the alpha male.

Chapter 3: Territoriality, Tolerance, and Testosterone

In Chapter 3, I developed a novel test of the Challenge Hypothesis by investigating the relationship between hunting and territorial boundary patrols and and testosterone. Boundary patrols occur when male chimpanzees gather together and move silently and in single file to the edges of their territory (Wrangham 1999; Wilson and Wrangham 2003). Patrollers occasionally make deep incursions into the territories of their neighbors. Boundary patrols can sometimes lead to hostile inter-community interactions, and in rare instances, result in lethal coalitionary attacks (Mitani et al. 2010). At Ngogo, patrols last about two hours on average and occur once every nine days (Mitani and Watts 2005). The frequent occurrence of patrols at Ngogo gave me a unique opportunity to test the Challenge Hypothesis through an examination of male testosterone levels as they engaged in a form of reproductive aggression between groups.

I also tested the Challenge Hypothesis in another aggressive context that has no apparent and immediate link to male reproduction, namely hunting. Male chimpanzees frequently hunt other animals. Their preferred prey is the red colobus monkey, *Procolobus spp*. (Boesch and Boesch 1989; Stanford et al. 1994b; Mitani and Watts 1999; Gilby et al. 2006). Chimpanzees hunt when they encounter prey opportunistically, but they also actively search for red colobus monkeys during "hunting patrols" (Boesch and Boesch 1989; Mitani and Watts 1999). As in territorial boundary patrols, male chimpanzees gather in groups and walk for up to six hours in search of prey (Mitani 2009). Because hunting is a foraging behavior that does not have an immediate influence on the reproductive performance of male chimpanzees, I predicted that it will not have an effect on testosterone secretion.

The Challenge Hypothesis also proposes that males will display relatively low testosterone levels as they begin to engage in parental care, as such periods are characterized by less frequent reproductive aggression (Wingfield et al. 1990). At Ngogo, meat sharing between males is similarly characterized by low levels of contest aggression and increased tolerance. Meat sharing among the Ngogo males occurs between maternal kin and unrelated individuals who form long-term friendships (Mitani and Watts 2001; Langergraber et al. 2007). Given these circumstances, I investigated the influence of meat sharing on male testosterone levels.

Chapter 4: Acute and Anticipatory Stress of Social Challenges

In chapter 4, I investigate whether these different types of aggression, male-male aggression over estrous females, territorial and predatory aggression, are stressful. Stress is a generalized physiological response to environmental challenges (Sapolsky 2002). As noted above, cortisol secretion is adaptive over the short-term because it mobilizes energy to overcome environmental threats while limiting non-essential metabolic processes (Sapolsky 2004). The acute, short-term stress response has been shown to increase cortisol levels when aggression increases. In contrast, chronic, long-term stress is associated with lower levels of aggression. Male chimpanzees are ideal subjects for studying the physiology of the short-term stress response as it relates to aggressive behaviors, such as those displayed by male chimpanzees. I investigated whether aggression elicited an acute stress response in males, as measured by increased cortisol secretion.

Male chimpanzees display aggression as they compete for estrous females, especially those who have given birth (Muller et al. 2006). The increased energetic demands created by this has been suggested to lead higher cortisol levels in male chimpanzees (Muller and Wrangham 2004b), and I accordingly tested this hypothesis.

Territorial boundary patrols and hunting are two major aspects of chimpanzee behavior that result in high levels of aggression and place high energetic demands on males (Mitani and Watts 2001; Mitani and Watts 2005). Although prior research has addressed the fitness benefits accrued by patrolling chimpanzees (Goodall et al. 1979; Mitani and Watts 2005; Mitani et al. 2002; Watts and Mitani 2001; Watts et al. 2006; Williams et al. 2004; Wilson and Wrangham 2003), their costs have been largely unexplored. During territorial patrols, males move more and feed less than they do during matched control periods (Amsler 2009). As a consequence, I predicted that males will experience relatively high levels of cortisol while conducting territorial patrols. Male chimpanzees at Ngogo occasionally spend up to five hours of a 12-hour day moving around their territory actively searching for prey (Mitani 2009). Additionally, male chimpanzees expend considerable energy during hunts as they pursue and attack prey. These considerations led me to predict that hunting will be associated with increased cortisol levels. Finally, urinary hormone analysis makes it possible to gain insight into the physiology of chimpanzees before they engage in these energetically and psychologically demanding behaviors.

Hunting and territorial boundary patrols involve a specific suite of behaviors, including long distance travel and vigilant and silent searching. Two unanswered questions concern how chimpanzees organize these events and how they communicate their intentions to do so to others. Some behavioral evidence suggests that the chimpanzees themselves are aware that such activities are about to take place. For example, females participate in hunting patrols but they drop out if present and fail to join males on territorial boundary patrols (Mitani and Watts 2001; Watts and Mitani 2001). In captivity, chimpanzees are capable of mounting an anticipatory hormone response before participating in experiments that place them in competitive situations (Wobber et al. 2010). In addition, chimpanzees and bonobos in the laboratory display elevated hormone levels in anticipation of social conflict (Hohmann et al. 2008; Wobber et al. 2010). These considerations led me to test the hypothesis that male chimpanzees in the wild will exhibit anticipatory increases in cortisol before engaging in any aggressive events associated with patrolling behavior.

In Chapter 5, I summarize the findings of this dissertation by discussing the importance of behavioral endocrinology and how this relatively new field of study is changing our understanding of aggression. I conclude by making recommendations for future research.

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CHAPTER 2

Female Parity, Male Aggression, and the Challenge Hypothesis

ABSTRACT

The Challenge Hypothesis proposes that testosterone mediates aggression during periods of heightened conflict between males, especially episodes that have important fitness consequences. Considerable evidence from seasonally breeding species provides support for this hypothesis, but few data exist in animals that mate year-round. We tested predictions generated by the Challenge Hypothesis in chimpanzees, a non-seasonally breeding primate, through a study of individuals living in an exceptionally large community at Ngogo, Kibale National Park, Uganda. Results indicated that high-ranking males were more aggressive than lower ranking males, but that the former actually had lower baseline testosterone levels than did the latter. Instead of rank influencing testosterone production, additional analyses revealed an important role for reproductive competition. Male chimpanzees displayed more aggression when they were in the same party as parous estrous females than when reproductively active females were unavailable. Male chimpanzees competed more intensely for mating opportunities with parous females than with nulliparas, and as a consequence, males displayed more aggression around the former than the latter. When males accompanied parous estrous females, urinary testosterone concentrations were significantly higher than baseline concentrations. In contrast, urinary testosterone concentrations did not exceed baseline when males associated with nulliparous estrous females. These differences in testosterone levels could not be attributed to mating per se because males copulated equally often with parous and nulliparous females. Furthermore, variation in testosterone concentrations were not due to males gathering together in large parties, as their levels in these situations did not exceed baseline. Taken together, these findings, derived from a relatively large sample of males and estrous females, replicate those from a prior study and furnish additional support for the Challenge Hypothesis. Our results suggest that the Challenge Hypothesis is likely to be broadly applicable to chimpanzees and increase our understanding of the physiological costs to males who compete for estrous females.

INTRODUCTION

Aggression has been a traditional focus of ethological study (Lorenz, 1966), with considerable research devoted to explain its causation, development, function, and evolution (Archer 1988; Nelson 2005). Studies regarding the hormonal correlates of aggression have featured prominently in discussions of the causal mechanisms underlying the aggressive behavior of vertebrates. While testosterone plays a primary role in reproduction, it has long been known to facilitate aggression, as revealed by early studies that showed castration limited the manifestation of aggression in males of several species, including humans (Baum 2002; Soma 2006).

Continued research, however, has complicated this picture. While differences between the sexes and between adult and juvenile males furnish additional support for a putative relationship between testosterone and aggression (Nelson 2011), in studies where only intact adult males are considered, a connection between testosterone and aggression does not always exist (Hirschenhauser and Oliveira 2006).

The Challenge Hypothesis helps resolve these conflicting findings by proposing that testosterone facilitates aggression only in specific social and reproductive contexts (Wingfield et al. 1990). Because chronically elevated testosterone levels can have deleterious effects on health, its production is limited to aggression that has particularly important fitness consequences. Aggression associated with competition for mates is a prime example, especially in many seasonally breeding primate species. For example, breeding season testosterone levels of male rhesus monkeys (*Macaca mulatta*) vary positively as a function of how frequently they display aggressive behavior (Higley et al. 1996). These same rhesus males exhibit relatively high levels of aggression while competing for estrous females and display correspondingly high levels of testosterone compared with male muriquis (*Brachyteles hypoxanthus*) who compete less intensely for mating opportunities (Strier et al. 1999). Additional observations reveal that males in several species of strepsirrhines and haplorrhines predictably increase their production of testosterone during the mating season and female conception cycles (strepsirrhines: Cavigelli and Pereira 2000; Fichtel et al. 2007; Gould and Ziegler 2007; Ostner et al.

2008; haplorrhines: Lynch et al. 2002; Bales et al. 2006; Girard-Buttoz et al. 2009; Ostner et al. 2011).

The Challenge Hypothesis was originally developed to explain the relationship between male aggression and testosterone secretion in seasonally breeding birds, which display pronounced temporal variation in their aggressive behavior (Wingfield et al. 1987). More evidence is needed to assess its applicability in non-seasonally breeding taxa that lack large seasonal changes in behavior (Archer 2006; Beehner et al. 2009). In this regard, chimpanzees represent a relevant species to test the Challenge Hypothesis as they mate throughout the year and are non-seasonal breeders. Despite the absence of breeding seasons, however, they still provide an opportunity to compare male testosterone concentrations in situations involving intense male reproductive aggression and other contexts. This opportunity exists because male competition varies over time and as a function of the individual identity of females (Muller and Wrangham 2004; Muller et al. 2006). Individual females mate during discrete estrous periods where they develop sexual swellings that last about 12 – 13 days (Furuichi and Hashimoto 2002). Moreover, females give birth only once every 5 - 6 years (Goodall 1986; Boesch and Boesch-Achermann 2000; Nishida et al. 2003; Sugiyama 2004), leading to an operational sex ratio that is skewed heavily toward males (Emlen and Oring 1977). As a consequence, males compete vigorously to obtain mating and reproductive opportunities with estrous females who are available only rarely (Boesch et al. 2006; Inoue et al. 2008; Wroblewski et al. 2009; Newton-Fisher et al. 2010). Aggression associated with reproductive competition can be extremely intense, especially for older, parous females, who have already reproduced successfully (Muller et al. 2006). Males compete less intensely for young nulliparous females, who represent less attractive mating partners (*ibid*.).

One previous study has taken advantage of the temporal variation in mating behavior by individual females and differences in male mating preferences to test the Challenge Hypothesis in chimpanzees (Muller and Wrangham 2004a). There it was found that male aggression and testosterone levels increased in the presence of parous estrous females. In contrast, aggression and testosterone were not elevated when males were around nulliparous estrous females and when estrous females were absent. Because mating frequencies were the same with both parous and nulliparous females, increases in male testosterone appeared to be related to aggression associated with mate acquisition, rather than the act of mating itself. Finally, a positive relationship between male dominance rank and testosterone concentrations existed, with high-ranking males possessing higher levels than lower ranking individuals during a period of rank stability. While these results support the Challenge Hypothesis, it is unclear whether they can be applied broadly to the behavior of chimpanzees because a relatively small number of females and males were observed. Because variation is a characteristic feature of chimpanzee behavior (Wrangham et al. 1996; Boesch et al. 2002), additional data from other chimpanzee communities, including larger samples of males and estrous females, are clearly needed to evaluate the generality of these findings.

One possibility not investigated by Muller and Wrangham (2004) in their previous study was that high male testosterone levels were a byproduct of large male group size. Large groups of males gather predictably around estrous females (Mitani et al. 2002), and in these, elevated rates of male aggression typically occur (Muller 2002). As a consequence, party size is a likely confound that requires examination and possible control. The presence of alpha males represents another potential confound that warrants investigation because these males are responsible for a disproportionate number of charging displays observed each day (Muller 2002). As a result, male testosterone concentrations might increase simply due to the heightened conflict that surrounds alpha males.

Chimpanzees at Ngogo, Kibale National Park, Uganda, live in an extremely large community containing over 150 individuals and many adult males and females. The unusually large size of this community creates ample opportunities to observe male-male aggression and matings between males and females that differ in their reproductive states and parity. In this study we extend the findings of previous work by examining whether associations exist between male aggression and testosterone during interactions with females who differ in parity. Our observations of male chimpanzees, as they compete for matings with multiple parous estrous females over several cycles, provide empirically sufficient samples to test the Challenge Hypothesis in this non-seasonally breeding primate. Specifically, we test the prediction that the presence of parous estrous females leads to heightened rates of male-male aggression, which elevates male chimpanzee testosterone levels above their baseline concentrations. In contrast, we do not expect a similar rise in urinary testosterone levels in males when they associate with nulliparous females.

Although males mate nulliparas, these females do not generate high levels of male competition.

METHODS

Study site and subjects

We observed chimpanzees at Ngogo, Kibale National Park, Uganda. The 30 km^2 study area lies at an altitude of about 1,400 meters above sea level and consists primarily of mature, evergreen tropical forest (Struhsaker 1997). The Ngogo chimpanzees have been under continuous observation since 1995 (Mitani 2009). As a result, they are well habituated to human presence and can be followed easily and observed closely. The Ngogo chimpanzee community is the largest described in the wild thus far and consisted of approximately 150 individuals at the time of study, including 27 adult males and 19 cycling females. The latter comprised eight parous females and 11 nulliparas.

Behavioral observations

MES conducted behavioral observations over 14 months during three field seasons, May - July 2006, May - November 2007, and February - May 2008. She observed 27 adult males for 1,378 hours, recording their identities and numbers each day and whether they followed estrous females. For purposes of the following analyses, we defined large groups of males to form on days that the party included more than half of all of the adult males in the community, i.e. ≥ 14 males. Although chimpanzees live in fission-fusion communities, whose members split apart and come together throughout the day, at Ngogo, large parties of males are fairly cohesive as they predictably form during periods of high food availability and whenever several females come into estrus simultaneously (Mitani et al. 2002).

Male aggression was scored by recording charging displays directed at specific individuals. These displays occur frequently and are particularly conspicuous and easy to observe and record. In prior research, they have been used to assay male aggression as they correlate positively with male dominance rank, with high ranking males engaging in this behavior often (Muller 2002). During charges, males become piloerect, shake and break trees and foliage, pull branches in their wake, and run quickly in a straight line through the forest on the ground. Charges typically elicit screams from recipients at whom charges are directed and nearby bystanders; they occasionally result in displaying individuals physically attacking others. We combined observations of charges with pant grunts to construct a dominance rank matrix. Pant grunts are vocal signals of submission given by low ranking chimpanzees to high-ranking chimpanzees (Bygott 1979; Hayaki et al. 1989). We ordered individuals from top to bottom so as to minimize the number of reversals in the matrix. Only 12 reversals occurred in 872 interactions, producing a linearity index of 98.6%.

Female chimpanzees display sexual swellings when they are in estrus. We considered only those females that exhibited full maximal swellings to be in estrus (Wallis 1992). While observing males during one-hour focal sampling sessions, MES recorded male copulations with females *ad libitum*. Copulations were recorded when males mounted estrous females followed by intromission and pelvic thrusting.

Hormone analyses

Urine collection

Testosterone was assayed using urine collected non-invasively from male chimpanzee subjects. We collected over 3000 samples from 26 adult males, who were included in the following analyses (X = 108 samples / male, SD = 42). Samples were collected from males while they were following estrous females, including those who were parous (X = 7.5 samples / male, SD = 3.5) and nulliparous (X = 6.8 samples / male, SD = 4.3). As noted above, these samples were obtained only when females possessed full, maximal swellings. The eight parous females were followed during 12 cycles (X = 1.5 cycles / female; SD = 0.8, range 1 - 3), while the 11 nulliparas were observed during 20 cycles (X = 1.8 cycles / female; SD = 0.8, range 1 - 3).

We collected 103 samples from 25 males on 25 days when more than half of all of the males were present in the daily party (X = 4.13 samples / male, SD = 1.9). For these, samples were not collected on days males hunted, patrolled the boundary of their territory, and followed estrous females, as these behaviors are known or hypothesized to affect testosterone production (Sobolewski et al. unpublished data). Finally, the alpha male was not observed on 20 days that we followed parous females. We collected samples from males on these days (X = 2.4 samples / male, SD =1.1) to compare with others collected from them when both the alpha male and parous estrous females were present (X = 6.0 samples / male, SD = 3.3). We also compared the former samples to their baseline levels.

Urine was collected from leaf litter on the ground with a pipette. Samples were occasionally caught in a plastic bag when males urinated overhead in trees. Samples cross-contaminated with feces or blood were discarded. Those that were retained were placed in 5 ml tubes and labeled with the male's name, date, and time. Samples were frozen within 12 hours after collection in the field and shipped to the United States on ice using certified transport equipment. All hormone analyses were conducted by MES working under the supervision of JLB at the Smithsonian Conservation Biology Institute, Front Royal, Virginia.

Testosterone analysis

We analyzed testosterone in unprocessed urine using a single antibody enzyme immunoassay (EIA) provided by Coralie Munro from the University of California, Davis (Kersey et al. 2010). Microtitre plates (96 well; Nunc-Immuno, Maxisorp) were coated with a polyclonal testosterone antiserum (R 156/157; 50 µl per well; diluted 1:7,500 in coating buffer, 0.05 M NaHCO3, pH 9.6) and allowed to set for 12–18 hours at 4^{o}C. Unabsorbed antiserum was removed with wash solution (0.149 M NaCl, 0.5% Tween 20). Testosterone standards (50 µl, range 2.3 - 600 pg/well, diluted in assay buffer, 0.1 M NaPO4, 0.149 M NaCl, 0.1% bovine serum albumin, pH 7.0) in triplicate and samples (50 µl) in duplicate were then added to the wells, followed immediately with testosterone-horseradish peroxidase (50 µl, 1:80,000 dilution in assay buffer). Following incubation at room temperature for 2 hours, plates were washed five times before 100 µl substrate buffer (0.4 mM 2, 2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) diammonium salt, 1.6 mM H2O2, 0.05 M citrate, pH 4.0) was added to each well. After incubation for 30-60 min, the absorbance was measured at 405 nm (540 reference filter) when the optical density in the total binding wells reached ~1.0. Intra-assay and inter-assay coefficients of variation (CV) for the internal controls (n = 124 assays) were below 10% and 15%, with 9.34% (mean binding, 23.6%) and 11.89% (mean binding, 69.5%) for the high and low samples, respectively, while the CV for the 50% binding point of the standard curve was 6.36%. The assay was validated for chimpanzee urine by demonstrating that serial dilutions of pooled urine samples produced displacement curves parallel to those of the testosterone standard curve and that there was significant recovery (>90%) of exogenous testosterone added to urine before analysis. We achieved high levels of recovery and accuracy of measurement and maintained strict controls for individual variation, sample quality, and assay variance.

Creatinine analysis

We indexed all urine samples for creatinine (Cr) to account for variations in water content (Taussky 1954). Creatinine is a by-product of muscle breakdown and under normal conditions is excreted at a constant rate per individual. The creatinine concentrations in urine were determined using a Jaffe reaction. Samples with creatinine concentrations below 0.01 ng/mL were considered too dilute and excluded from analysis; this involved less than 5% of all samples. Hormone concentrations were divided by creatinine concentrations and expressed as the concentration of testosterone (ng) / per mg Cr.

Statistical analyses

We determined baseline concentrations of testosterone for each male through an iterative process described by Moreira and colleagues (2001), with minor modifications. This technique has been used successfully in the past to identify baseline values from biologically relevant peaks (Moreira et al. 2001). We began by computing the mean value of all samples for each male. We then removed values that were above 2 standard deviations from the mean. We iterated this procedure until no values outside 2 standard deviations of the mean remained. We employed the resulting mean as the statistical baseline value for that male. We excluded urine samples collected before 0900 hours from analyses to control for diurnal variation in testosterone production (Muller and Wrangham 2004).

Testosterone varies considerably among individual males (Kempenaers et al. 2008). Therefore, we used each male as his own control for statistical purposes. Specifically, we employed a non-parametric Wilcoxon matched-pairs signed-ranks test to determine if male testosterone concentrations assayed on days when estrous females were present differed from their baseline levels. We also used Wilcoxon tests to investigate whether male aggression and copulation rates varied as a function of the presence and absence of estrous females. For all of these tests, we separated estrous females into two categories, those who had given birth, i.e. parous females, and those who had not, i.e. nulliparous females. In two additional Wilcoxon tests we examined whether urinary testosterone levels were elevated on days males gathered in large parties compared to their baseline levels and whether male testosterone concentrations differed on days they followed parous estrous females in the presence and absence of the alpha male. Finally, we utilized the matched-pairs design to examine whether male testosterone levels were high on days they followed parous estrous females in the absence of the alpha male. We did so by comparing these to their baseline concentrations.

RESULTS

Table 2.1 shows individual males, their dominance ranks, aggression rates, and baseline testosterone concentrations. In accord with previous studies (e.g. Muller 2002), high-ranking males at Ngogo displayed higher rates of aggression than did lower ranking males (Spearman $r = 0.70$, $p < 0.001$, $N = 26$). High-ranking males, however, did not possess high levels of testosterone. There was no relationship between male dominance rank and baseline testosterone levels (Spearman $r = 0.34$, $p > 0.05$, $N = 26$).

To test the Challenge Hypothesis, we examined the relationship between testosterone and aggression as males sought reproductive opportunities with females. Male chimpanzees compete for estrous females, as manifest by their aggressive behavior. Competition, however, varied as a function of female parity. Males displayed more aggression around parous estrous females than they did when no estrous females were present (Wilcoxon test: $Z = 4.55$, $p < 0.001$, $N = 27$

males; Figure 2.1). In contrast, male aggression was not elevated in the presence of nulliparous estrous females compared to when estrous females were absent (Wilcoxon test: $Z = 1.33$, $p > 0.15$, $N = 27$ males; Figure 2.1). As a consequence, male chimpanzees showed more aggression in the presence of parous estrous females than they did in the presence of nulliparous estrous females (Wilcoxon test: $Z = 4.13$, $p < 0.001$, $N = 27$ males; Figure 2.1). Despite these heightened rates of aggression around parous females, male chimpanzees mated these females as frequently as they did nulliparous females (Wilcoxon test: $Z = 1.37$, $p = 0.15$, $N = 27$; Figure 2.2).

The higher rates of aggression stimulated by the presence of parous females in estrus were related to male testosterone levels in predictable ways. Male testosterone was elevated above baseline levels on days when parous females were present (Wilcoxon test: $Z = 4.32$, $p < 0.01$, $N = 25$; Figure 2.3). In contrast, testosterone concentrations did not differ from baseline on days that males accompanied nulliparous females in estrus (Wilcoxon test: $Z = 0.70$, $p > 0.45$, $N = 25$; Figure 2.3). As a result, male urinary testosterone concentrations were also higher when they followed parous estrous females compared to when they accompanied nulliparous estrous females (Wilcoxon test: $Z = 3.98$, $p < 0.001$, $N = 25$; Figure 2.3).

The rise in testosterone experienced by males while following parous estrous females could not be attributed to the fact that they formed large parties on these days; male testosterone levels did not exceed baseline on days that they formed large parties in the absence of estrous females (Wilcoxon test: $Z = 1.01$, $p > 0.30$, $N = 25$). In addition, urinary testosterone concentrations of males who followed parous estrous females did not vary as a function of the presence or absence of the alpha male (Wilcoxon test: $Z = 0.03$, $p > 0.95$, $N = 23$). Male testosterone levels were also high on days that they followed parous estrous females in the absence of the alpha male; their values on these days consistently exceeded baseline (Wilcoxon test: $Z = 2.03$, $p < 0.05$, $N = 24$).

DISCUSSION

Prior research has revealed that dominance rank affects testosterone in male chimpanzees. At Kanyawara, high ranking males display relatively high testosterone levels compared with lower ranking individuals (Muller and Wrangham 2004). In contrast, there was no relationship between these two variables among males at Ngogo. Age is a likely factor that might account for the failure to document a relationship between male rank and testosterone at Ngogo. Testosterone decreases with age in human males (Bribiescas 2001), and many high-ranking male chimpanzees in our sample were old. Further research will be necessary to investigate this possibility.

Additional analyses indicate that male chimpanzees at Ngogo compete for mating opportunities for females, but that they do so primarily for parous females. Male aggression increased on days these females were present compared to days when males followed nulliparous females and on days when estrous females were absent. Testosterone is associated with the heightened aggression over more attractive parous females, as concentrations in the presence of these females were higher than baseline levels and those when males accompanied nulliparous females.

The elevation in male testosterone is directly attributable to reproductive competition for females and not associated with increased mating activity per se because males copulated with parous females as often as they did with nulliparous females. Large group size cannot explain these differences because urinary testosterone concentrations did not vary from baseline on days when males formed large parties in the absence of estrous females. In addition, the elevated testosterone levels that we documented in males as they competed for parous estrous females could not be attributed to the presence of the alpha male; male testosterone concentrations did not differ in the presence or absence of the alpha, and they continued to exceed baseline when parous estrous females were present and he was not there. Taken together, our results are consistent with the Challenge Hypothesis, which proposes that the production of testosterone will be associated with aggression directly related to reproduction.

The fact that testosterone did not increase in the presence of estrous females, who have not yet given birth, may be surprising. Nulliparas, however, are invariably recent immigrants who experience a prolonged period of infertility after moving into their new communities. As a result, these females cycle consistently, sometimes for several years, before giving birth for the first time (Goodall 1986; Boesch and Boesch-Achermann 2000; Nishida et al. 2003; Sugiyama 2004). Because many cycles do not represent legitimate reproductive opportunities, males do not compete for nulliparas as vigorously as they do for older, parous females (Muller et al. 2006).

Our findings are consistent with the only previous study that has tested the Challenge Hypothesis in wild chimpanzees. In the Kanyawara chimpanzee community, also located in the Kibale National Park, Muller and Wrangham (2004) found that males there selectively increased their rates of aggression in the presence of parous estrous females. Elevated testosterone levels were also displayed by males who accompanied parous estrous females compared with those who followed nulliparous estrous females or those who were alone in the absence of any estrous females. Because males failed to mate parous females more frequently than they did nulliparas, Muller and Wrangham (2004) concluded, like us, that the increase in testosterone could be attributed to heightened aggression around parous estrous females in general, rather than mating activity alone. One limitation of this prior study was that data were collected from a relatively small number of males (*N* = 8 - 11), nulliparous females (*N* = 2), and parous females (*N* = 3), with observations focused on a single parous estrous female during a single estrous cycle. These small samples raise the possibility that the findings of this previous study cannot be generalized to chimpanzees in other communities and as a whole. The results presented here were based on much larger samples of males (*N* = 26), parous females (*N* = 8), nulliparous females (*N* = 11), and reproductive cycles of females (parous females: *N* = 12; nulliparous: *N* = 20). Our findings support those derived from studies of other vertebrates (Wingfield 2005) and suggest that the Challenge Hypothesis is likely to be broadly applicable to chimpanzees.

This study also adds to our understanding of how social interactions influence male testosterone in a species that does not typically display pronounced seasonal variation in aggressive behavior. Most previous tests of the Challenge Hypothesis have been conducted with seasonally breeding taxa that show large fluctuations in aggressive behavior over time (reviews in Oliveira 2004; Hirschenhauser and Oliveira 2006). Few data exist regarding non-seasonally breeding species because such changes in aggression are less obvious, making it difficult to relate changes in testosterone to changes in behavior. The Challenge Hypothesis, however, proposes that social interactions that have particularly significant fitness consequences will lead to transient changes in androgen levels irrespective of the time of year, and as a consequence, it should apply to seasonally-breeding and non-seasonally breeding species alike. Because many primates mate year-round, they provide a model taxon to investigate in this regard. Obtaining additional data from these animals represents an important area for future research.

Table 1.1 Male dominance rank, aggression and testosterone levels

male	Rank	aggression (charges / hour)	baseline testosterone (ng/mgCR)
bt	1	1.04	69
ho	2	0.83	73
mi	3	0.97	113
bs	4	0.55	80
cr	5	0.58	71
mt	6	0.28	80
wb	7	0.61	104
lo	8	0.36	99
br	9	0.38	85
mo	10	0.26	94
hr	11	0.11	88
mw	12	0.13	89
bg	13	0.14	109
do	14	0.05	77
mg	15	0.18	132
ga	16	0.57	123
pk	17	0.11	95
or	18	0.24	119
ro	19	0.29	107
bf	20	0.06	77
rh	21	0.29	112
Dx	22	0.04	82
Ri	23	0.10	112
Ta	24	0.23	111
Gz	25	0.12	62
Di	26	0.13	112

Figure 2.1 Male chimpanzee aggression varies as a function of the presence and absence of estrous females. Male chimpanzee aggression varies as a function of the presence and absence of estrous females Rates of aggression, assayed by charging displays, when estrous females, either nulliparous or parous individuals, were present are compared with those when estrous females were absent. Displayed are means of individual male means ± 1 SE. N = 27 males. * $p < 0.001$ for comparisons between parous estrous females and estrous females absent and between parous estrous females and nulliparous estrous females

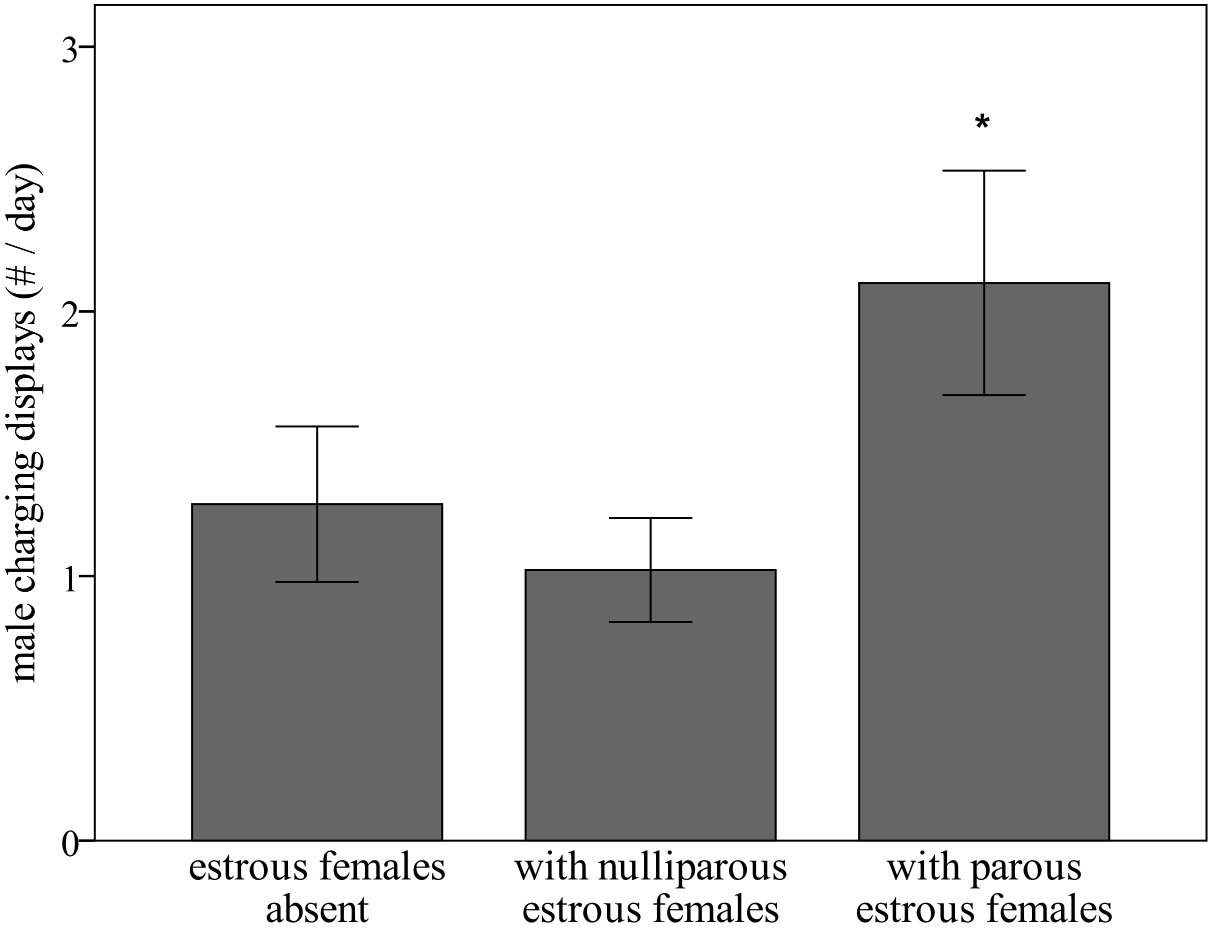


Figure 2.2 Female parity does not affect male chimpanzee copulation rates. Female parity does not affect male chimpanzee copulation rates. Male copulation rates with nulliparous and parous females are shown. Displayed are means of individual male means ± 1 SE. N = 27 males.

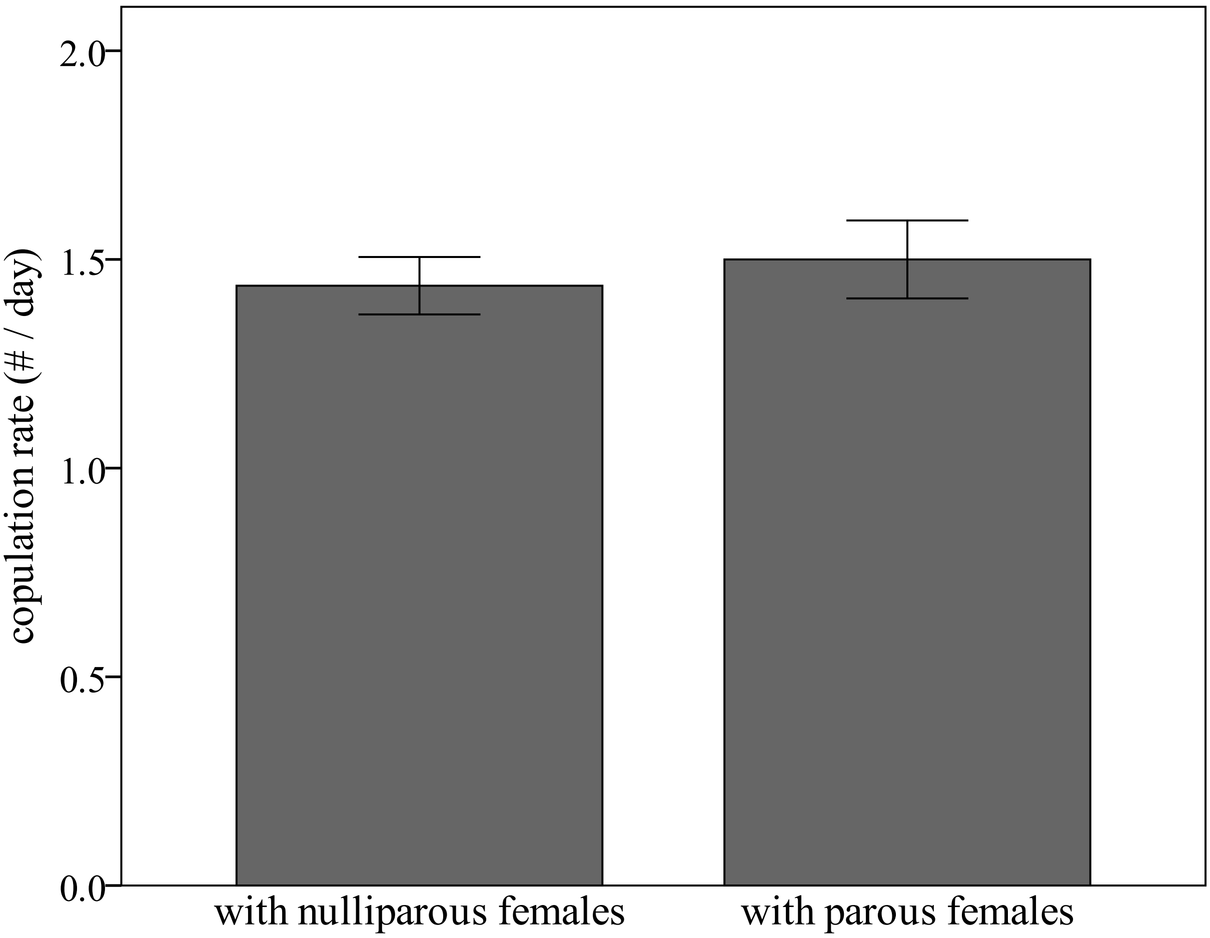
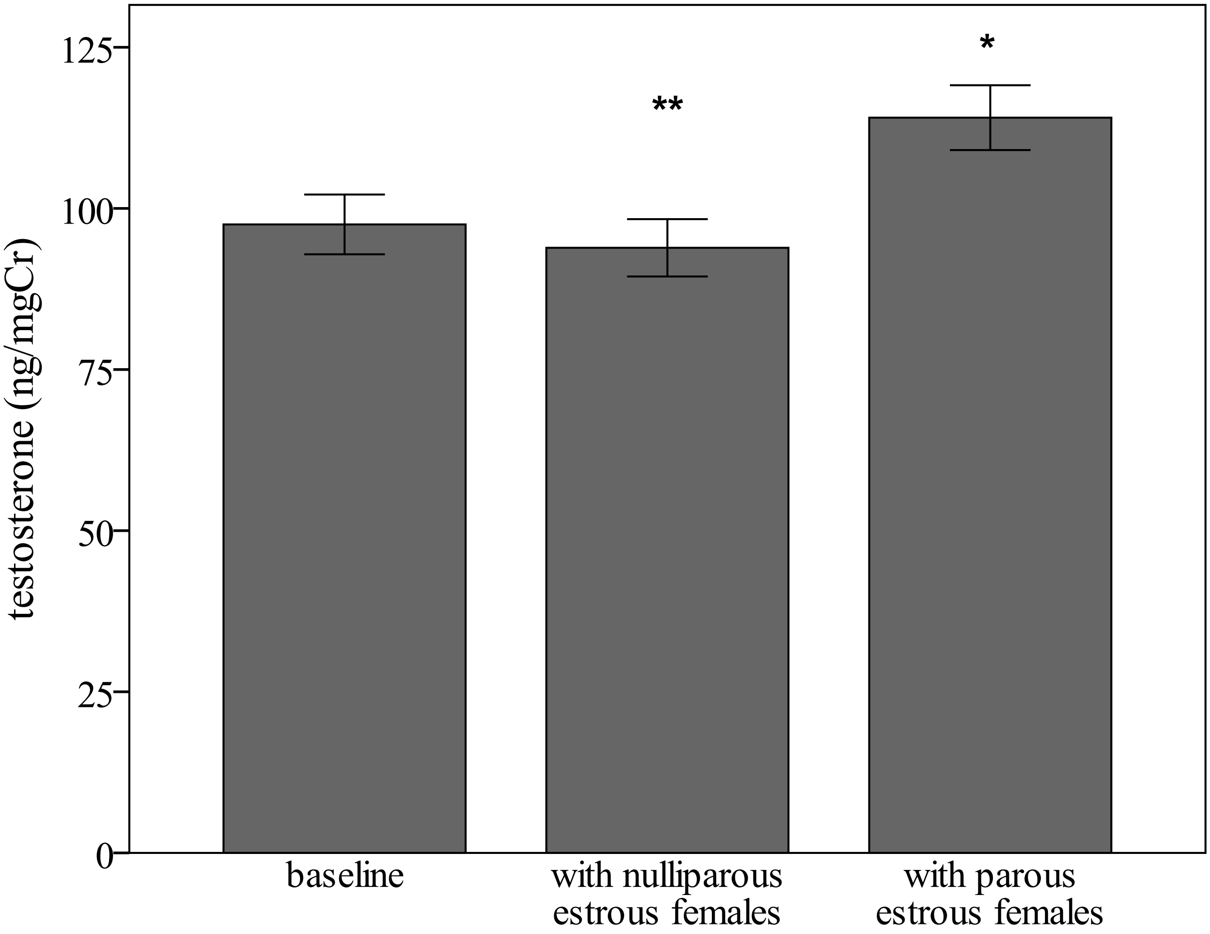


Figure 2.3 Male chimpanzee testosterone levels vary as a function of female parity. Male baseline testosterone concentrations are compared with their concentrations when they accompanied nulliparous estrous females and parous estrous females. Displayed are means of individual male means ± 1 SE. N = 25 males. * $p < 0.001$ for the comparison of male baseline concentrations and those when they followed parous estrous females. ** $p < 0.01$ for the comparison between nulliparous estrous females and parous estrous females.



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CHAPTER 3

Territoriality, Tolerance and Testosterone

ABSTRACT

Although testosterone has well known organizational and activational effects on aggression, the relationship between the two is not always clear. The Challenge Hypothesis addresses this problem by proposing that testosterone will affect aggression only in fitness-enhancing situations. One way to test the Challenge Hypothesis is to examine the relationship between testosterone and different types of aggression. Chimpanzees, *Pan troglodytes*, exhibit aggressive behaviors in several contexts and provide an opportunity for such a test. Here we show that urinary testosterone influences a form of male chimpanzee reproductive aggression, territorial boundary patrols. In contrast, testosterone does not affect predatory behavior, a form of aggression that has no immediate link to male reproduction. While these data are consistent with the Challenge Hypothesis, our results indicate that male chimpanzees experience a significant drop in urinary testosterone during hunts. Additional analyses reveal that males who share meat with others display this decrease. The reason for this decrement is unclear, but we hypothesize that the relative lack of aggression that results from voluntary sharing episodes and the tolerance engendered by such acts may be contributory factors.

INTRODUCTION

A central problem in the study of behavioral biology concerns the relationship between the steroid hormone testosterone and aggression. While it is widely acknowledged that testosterone plays an important role in regulating aggression in many species, studies of some taxa fail to show a connection between the two (Hirschenhauser and Oliveira 2006). The Challenge Hypothesis addresses these conflicting findings by proposing that testosterone affects aggression only during periods of social instability and when individuals face challenges in fitness enhancing contexts (Wingfield et al. 1990). Because chronically elevated testosterone can have deleterious health consequences, its production is limited to aggression directly related to reproduction, including territorial behavior and mate-guarding. Research on several vertebrates provides support for the Challenge Hypothesis (Wingfield 2005), but exceptions exist resulting in continued debate (Hirschenhauser and Oliveira 2006).

One potentially powerful way to test the Challenge Hypothesis is to examine the effects of testosterone on different types of aggression. Because they display aggression in several contexts, male chimpanzees provide an opportunity to conduct such a test. Within communities, male chimpanzees behave aggressively as they compete with each other to mate females (Bygott 1979; Muller 2002; Muller et al. 2006). Between communities, male chimpanzees are territorial. Territorial interactions occasionally escalate and result in lethal aggression (Goodall et al.

1979; Wilson et al. 2004; Mitani et al. 2010). Lethal aggression often occurs during boundary patrols during which male chimpanzees make deep incursions into the territories of their neighbors (Goodall et al. 1979; Mitani et al. 2010). Patrols also lead to non-lethal fights, chases, and vocal battles between members of different communities (Watts and Mitani 2001). Finally, male chimpanzees hunt vertebrate prey (Goodall 1963; Nishida et al. 1979; Boesch and Boesch 1989; Stanford et al. 1994; Mitani and Watts 1999; Gilby et al. 2006). Here aggression takes a different form, as chimpanzee hunters interact with heterospecifics in a predatory, rather than a reproductive, context. In keeping with the Challenge Hypothesis, prior research has shown that male chimpanzees display relatively high levels of testosterone when they compete for estrous females in their own community (Muller and Wrangham 2004). It remains unclear whether testosterone affects males during territorial behavior or when they hunt. The Challenge Hypothesis makes two straightforward predictions: testosterone should influence territorial behavior, but not hunting.

Chimpanzees at Ngogo, Kibale National Park, Uganda, live together in an unusually large community with over 150 individuals. Males there frequently hunt vertebrate prey successfully and share meat with others (Mitani and Watts 1999, 2001; Watts and Mitani 2002). In addition, males at Ngogo often engage in territorial boundary patrols (Mitani et al. 2010; Watts and Mitani 2001; Amsler 2010). The frequency with which the large number of males at Ngogo patrol their territory, hunt, and share meat creates an ideal situation to test the predictions outlined above regarding the relationships between these behaviors and T.

METHODS

Study Site and Subjects

We conducted fieldwork at Ngogo, Kibale National Park, Uganda. The 30 km^2 study area lies at an altitude of about 1,400 above sea level and consists primarily of mature, evergreen tropical forest. The forest is interspersed with patches of *Pennisetum purpureum* grasslands and *Phoenix reclinata* palm swamps (Struhsaker 1997). Mean annual rainfall is approximately 1,400 mm and temperature fluctuates from 16.5 to 25.0 C^0 daily. We observed members of the Ngogo chimpanzee community. The community consisted of approximately 150 individuals at the time of study, including 27 adult males who were at least 16 years old. The Ngogo chimpanzees have been under continuous observation since 1995 (Mitani 2009). As a consequence, they are well habituated to human presence and can be easily followed and observed during the events described in this paper, territorial boundary patrols and hunts.

Behavioral Observations

MES conducted fieldwork over 14 months during three field seasons, May - July 2006, May - November 2007 and February - May 2008. She collected behavioral observations of adult males and recorded the size and composition of parties daily. Large groups of males typically form around estrous females (Mitani et al. 2002), and in these, elevated rates of male aggression occur (Muller 2002). As a result, male party size is a variable that requires examination and possible control. For purposes of the following analyses, we defined large male parties as occurring on days when, during the whole or any portion of the day, more than half of the adult males were present (≥ 14 males). Although chimpanzees live in fission-fusion communities, whose members split apart and come together throughout the day, at Ngogo large parties of males are relatively stable as they predictably form during periods of high food availability (Mitani et al. 2002).

Male subjects were observed during 34 territorial boundary patrols and 36 hunts. Territorial boundary patrols are characterized by a distinctive suite of behaviors, making them readily identifiable to experienced observers (Watts and Mitani 2001; Amsler 2010; Mitani et al. 2010). During patrols, chimpanzees move en masse, typically in single file line, to the boundary of their territory. Once there, they continue to travel in single file fashion often in complete silence. Patrollers typically scan the environment; they sniff the ground and investigate signs left behind by neighboring chimpanzees, such as feces, urine, and discarded pieces of food. Patrollers can make deep incursions into the territories of their neighbors; aural or visual contact with neighbors occurs in about 30 – 40% of all patrols**,** with lethal aggression witnessed rarely (Mitani et al. 2010). Hunts were easily recognized as times chimpanzees encountered and attacked vertebrate prey. Red colobus monkeys (*Procolobus* spp.) are the favored prey of chimpanzees (Boesch and Boesch 1989; Stanford et al. 1994a; Mitani and Watts 1999; Gilby et al. 2006). Most hunts resulted in kills and the consumption of red colobus prey, as chimpanzee hunting success at Ngogo is unusually high, averaging over 80% (Watts and Mitani 2002). At

Ngogo, chimpanzees hunt opportunistically when they encounter red colobus monkeys during their normal foraging movements. Other hunts, however, occur after “hunting patrols” (Mitani and Watts 1999). Hunting patrols are characterized by a group of behaviors similar to those displayed by chimpanzees during territorial boundary patrols and closely resemble the behaviors described as ‘‘searches’’ by chimpanzees in the Tai National Park (Boesch and Boesch, 1989).

Boundary patrols and hunts occur infrequently, taking place once on average every 9 and 7 days, respectively (Watts and Mitani 2001, 2002; Amsler 2010; Mitani et al. 2010). MES therefore collected observations *ad libitum***.** She recorded the start of each patrol and hunt, and noted individuals who participated in each. Patrols and hunts were considered to have begun when chimpanzees gathered and started to display the characteristic behaviors associated with boundary and hunting patrols described above. For hunts that occurred when chimpanzees encountered prey opportunistically, start times were recorded when chimpanzees began to climb into trees and to pursue monkeys. During hunts, MES also noted the chimpanzees that made kills at hunts, and the identities of individuals with whom they shared. She recorded sharing whenever two males exchanged meat, irrespective of the behavior of the giver and taker. Collecting urine for hormone analysis (see below) at rare events such as hunts is difficult, and priority was given to obtaining these samples. As a result, specific behavioral details regarding the nature of meat sharing, i.e. whether meat was shared actively, passively, or involuntarily, were not always recorded systematically.

Hormone Analyses

Testosterone was analyzed in urine collected non-invasively from male chimpanzee subjects. All analyses were conducted by MES at the Smithsonian Conservation Biology Institute in Front Royal, Virginia, working under the supervision of JLB (Kersey et al. 2010).

Urine collection

Chimpanzees move terrestrially, which facilitated collecting their urine. Urine was typically obtained off of leaf litter on the ground with a pipette, or more rarely, caught in a plastic bag if chimpanzees urinated from a tree. Samples were analyzed only if they were obtained from individually identified males. Samples cross-contaminated with feces or blood were discarded. In the field, samples were placed in 5 ml tubes, which were immediately labeled with the male's name, date, and time. Samples were frozen at the end of the day at 0^0 C, no longer than 12 hours after collection, and were subsequently shipped to the United States on ice using medically certified transport equipment from SAF-T-PAK.

We collected 1,785 urine samples from 26 of the 27 adult male chimpanzees (X + SD = 70 + 26 samples / male, range 30 – 123). Three hundred forty six samples were obtained before and after the start of boundary patrols and hunts (patrols: X + SD = 6.7 + 2.4 samples / male, N = 25 males; hunts: X + SD = 5.8 + 3.3 samples / male, N = 26 males; Table 2). We obtained the former up to 4 hours before the start of each event. We also collected samples from each male 2 – 4 hours after the start of patrolling and hunting activity. Because there is a 2 – 4 hour time lag for hormone detection in urine (Whitten et al. 1998; Bahr et al. 2000), these samples were considered to reflect the endocrine state of males during and shortly after the two events of interest, boundary patrols and hunts. We collected multiple samples from each male both before and after patrols and hunts (before patrols: X + SD = 4.2 + 2.4 samples / male, N = 25 males; after patrols: X + SD = 2.8 + 1.5 samples / male, N = 25 males; before hunts: X + SD = 5.1 + 2.5 samples / male, N = 26 males; after hunts: X + SD = 3.8 + 1.9 samples / male, N = 24 males; Table 2).

Our initial analysis showed that males displayed a significant drop in testosterone during and shortly after hunts. To explore this finding further, we examined the potential effect of meat sharing. To do so, we subdivided males who were involved in sharing episodes between those who shared with others (X + SD = 1.8 + 0.8 samples / male, N = 12 males; Table 2) and those who were recipients (X + SD = 1.7 + 0.9 samples / male, N = 17 males; Table 2). We collected samples from several males who failed to obtain meat after they hunted unsuccessfully, and used these to determine testosterone concentrations of individuals who hunted but did not share (X + SD = 1.4 + 0.7 samples / male, N = 10 males; Table 2).

To investigate the influence of male party size on testosterone, we collected samples from males on days when they formed large parties with over half of all the community males together. This yielded 103 samples from 25 males on 25 days (X + SD = 4.1 + 1.9 samples / male). We excluded samples from days males hunted, conducted boundary patrols, and followed estrous females, as these behaviors are known or hypothesized to affect testosterone production.

Creatinine

We indexed all urine samples to their concentration of creatinine (Cr) to account for variations in water content (Taussky 1954). Creatinine is a by-product of muscle breakdown and under normal conditions is excreted at a constant rate per individual. The creatinine concentrations in urine (0.05 mL; diluted 1:20 in bovine serum albumin (BSA) –free phosphate buffer) were determined using a Jaffe reaction (Taussky 1954). Samples with creatinine concentrations below 0.01 ng/ml were considered too dilute and excluded from hormone analysis; this involved less than 5% of all samples. Hormone concentration was divided by creatinine concentration and the data expressed as the concentration of testosterone (ng) per mg Cr.

Testosterone

We analyzed urinary testosterone using a single antibody enzyme immunoassay (EIA) provided by Coralie Munro at the University of California, Davis (Kersey et al. 2010). Microtitre plates (96 well; Nunc-Immuno, Maxisorp) were coated with a polyclonal Tantiserum (R 156/157; 50 µl per well; diluted 1:7,500 in coating buffer, 0.05 M $NaHCO_3$, pH 9.6) and allowed to set for 12–18 hours at 4^o C. Unabsorbed antiserum was removed with wash solution (0.149 M NaCl, 0.5% Tween 20). Testosterone standards (50 µl, range 2.3 – 600 pg/well, diluted in assay buffer, 0.1 M $NaPO_4$, 0.149 M NaCl, 0.1% bovine serum albumin, pH 7.0) in triplicate and samples (50 µl) in duplicate were then added to the wells, followed immediately with testosterone-horseradish peroxidase (50 µl, 1:80,000 dilution in assay buffer).

Following incubation at room temperature for 2 hours, plates were washed five times before 100 µl substrate buffer [0.4 mM 2, 2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) diammonium salt, 1.6 mM H_2O_2, 0.05 M citrate, pH 4.0] was added to each well. After incubation for 30-60 min, the absorbance was measured at 405 nm (540 reference filter) when the optical density in the total binding wells reached ~1.0. We maintained strict controls for individual variation, sample quality, and assay variance. Intra-assay and inter-assay coefficients of variation (CV) for the internal controls (n = 124 assays) were below 10% and 15%, with 9.34% (mean binding, 23.6%) and 11.89% (mean binding, 69.5%) for the high and low samples, respectively, while the CV for the 50% binding point of the standard curve was 6.36%. Without extraction, we recovered biologically relevant testosterone concentrations as indicated by the levels of recovery and accuracy of measurement (Kersey et al. 2010). The assay was validated for chimpanzee urine by demonstrating that serial dilutions of pooled urine samples produced displacement curves parallel to those of the testosterone standard curve and that there was significant recovery (> 90%) of exogenous testosterone added to urine before analysis.

Statistical Analyses

We conducted a generalized linear mixed model (GLMM) analysis to examine the effects of territorial boundary patrolling and hunting on testosterone. Testosterone concentrations were not normally distributed (Kolmogorov-Smirnov test: $Z = 5.83$, $P < 0.001$), and we therefore used log transformed testosterone as the response variable in this analysis. We considered hunting status (no hunt, before hunt, after hunt) and boundary patrol status (no patrol, before patrol, after patrol) as fixed effects. Time of day was included as a covariate, as male testosterone secretion shows a characteristic decline across the day (Muller and Wrangham 2004). Because we hypothesized that male group size might influence male testosterone levels, we also treated it as a covariate. We subsequently excluded male group size, however, as it had no effect on the initial model. Testosterone concentrations typically display considerable inter-individual variation in vertebrates (Kempenaers et al. 2008). To control for this and the non-independence created by using multiple samples from the same individual, we considered individual males as a random effect. We conducted a series of post-hoc pairwise analyses using estimated marginal means of each sub-category of fixed effects. All statistical analyses were conducted using SPSS (version 19).

Our initial model revealed that male chimpanzees experience a significant drop in urinary testosterone during hunts. To investigate this finding in greater detail, we conducted another GLMM analysis in which we added meat sharing as a fixed effect. Here we considered four categories: 1) individuals who shared meat with others; 2) individuals who received meat from others; 3) individuals who did not share because they hunted unsuccessfully; and 4) individuals who did not hunt.

Our research was conducted in compliance with all legal requirements of the Republic of Uganda and adhered to the ASAB/ABS guidelines for the use of animals in research. Research was approved by the University Committee on Use and Care of Animals at the University of Michigan (Research Application 9050, July 27, 2005).

RESULTS

Table 3.1 shows the number of samples that we collected from males in different conditions and their mean urinary testosterone concentrations in each of them. Results of a GLMM analysis indicated that male chimpanzee testosterone concentrations varied across the day ($F_{1,1775} = 38.12$, $P < 0.01$). Territorial boundary patrol behavior also had a significant impact on male testosterone ($F_{2,1775} = 15.82$, $P < 0.01$; Figure 3.1). A post hoc test, controlling for time of day and male identity, revealed that male chimpanzees displayed significantly higher levels of urinary testosterone prior to conducting boundary patrols compared with those on days they did not patrol ($t = 3.66$, $P < 0.01$; Figure 3.1). Male testosterone continued to remain high during and immediately after patrols compared to levels shown on days of no patrolling activity ($t = 4.39$, $P < 0.01$; Figure 3.1). Elevated testosterone during boundary patrols could not be attributed to males forming large parties on these days because male group size did not influence testosterone (data not shown; see above).

In the initial model, hunting also had a significant impact on male chimpanzee testosterone concentrations, but in an unsuspected way ($F_{2,1775} = 4.15$, $P < 0.02$). A post-hoc test revealed that males failed to show an anticipatory response to hunting as samples collected before they hunted did not differ from those obtained on days that they did not hunt ($t = 0.39$, $P > 0.60$). In contrast, males displayed a pronounced decrease in their urinary testosterone concentrations during hunts ($t = 2.83$, $P < 0.01$).

The finding that male testosterone dropped during hunts was unexpected. An additional GLMM analysis suggested that meat sharing may have played a role as it had a significant impact on male testosterone levels ($F_{3,1775} = 6.94$, $P < 0.001$; Figure 3.2). Interestingly, when meat sharing was added to the model, the effect of hunting per se disappeared ($F_{2,1775} = 0.14$, $P > 0.80$). Post-hoc analyses indicated that both sharing and receiving meat influenced male testosterone levels. Males who hunted successfully and shared meat with others displayed significantly lower testosterone concentrations than males who did not hunt ($t = 2.80$, $P < 0.01$; Figure 3.2). Similar decrements were shown by males who received meat from others ($t = 2.33$, $P < 0.02$; Figure 3.2). The patterns displayed by males who were involved in sharing episodes differed considerably from those shown by males who hunted unsuccessfully and did not obtain meat. Males who failed to acquire meat had testosterone levels that did not differ from those that they had on days they did not hunt ($t = 1.76$, $P > 0.05$; Figure 3.2).

DISCUSSION

The preceding results provide novel tests of and support for the Challenge Hypothesis in chimpanzees. While testosterone is linked to territorial aggression in male chimpanzees, it does not appear to influence their aggression in the context of predation. Territoriality is a conspicuous aspect of male chimpanzee behavior and has significant reproductive consequences (reviews in Muller and Mitani 2005; Mitani 2009). Recently we have shown that male chimpanzees employ an extreme form of territorial behavior, lethal aggression committed largely during boundary patrols, to expand their territory at the expense of neighbors (Mitani et al. 2010). By acquiring new territory, males are able to enhance the feeding success of others in their community, and as a consequence, increase female reproduction (Williams et al. 2004). Because of these important fitness consequences, the Challenge Hypothesis predicts that testosterone will be associated with male chimpanzee territorial behavior. Our findings, however, indicate that testosterone increases before as well as during these events. This raises two important questions regarding chimpanzee physiology and behavior. First, does the increase in the production of testosterone act in a similar way to the anticipatory testosterone response observed in captive male chimpanzees prior to competitive events (Wobber et al. 2010)? Second, how do male chimpanzees know or anticipate that a patrol is imminent? Are there overt behavioral cues associated with elevated testosterone levels that might provide an observer information about an impending patrol? Additional study will be required to answer these questions.

While chimpanzee territorial aggression affects their reproduction, the reproductive consequences of hunting are less clear. Controversy exists over whether male chimpanzees hunt to obtain meat that they use to swap for matings with females (Stanford et al. 1994b; Mitani and Watts 2001; Gomes and Boesch 2009; Gilby et al. 2010). Doing so would implicate predatory aggression as a part of an evolved male chimpanzee reproductive strategy. Our results, however, do not support this hypothesis as they indicate that male urinary testosterone is low while chimpanzees hunt. This finding mirrors a study of human hunters where hunting success did not correlate with male serum testosterone concentrations (Worthman and Konner 1987).

The pronounced decrease in urinary testosterone in male chimpanzee hunters was unanticipated. One possible explanation is that success at hunts accounted for this drop, but we were unable to test this hypothesis directly. Some males obtained meat and failed to share, but we could not collect urine samples from them for analysis because these individuals typically ran away and disappeared soon after they captured prey. Despite our inability to test of this hypothesis formally, it is unlikely that successful hunting alone can account for the decrease in testosterone because males still displayed low levels when they shared but did not make kills themselves.

Two additional factors related to differences in patterns of sharing may explain the relatively low testosterone concentrations exhibited by males at hunts. Some sharing represents a form of tolerated theft (Blurton-Jones 1984), as individuals are forced to relinquish meat to others who harass them (Gilby 2006). In these cases, a decrease in sharers might reflect social failure or loss of dominance. Decrements in beggars are more difficult to explain, but one possibility is that persistent beggars might display a transient decline in testosterone as they subjugate themselves in front of others.

Voluntary sharing provides another potential explanation for the relatively low testosterone concentrations exhibited by males at hunts. Meat is sometimes shared actively between individuals, without resistance by sharers and aggression by recipients (Nishida et al. 1992; Mitani and Watts 2001). There can also be a remarkable respect for ownership, whereby carcass holders maintain possession in the absence of harassment by others (de Waal 2009). At Ngogo, there is a considerable amount of voluntary sharing between maternal half-siblings (Langergraber et al. 2007) and between unrelated individuals who use meat to develop and maintain social bonds with each other (Mitani and Watts 2001). Moreover, sharing is reciprocated at the group level, and males exchange meat for coalitionary support (Mitani and Watts 2001). These observations of how meat is shared, who shares, and why sharing occurs indicate that hunting can be characterized by tolerance and affiliation between males instead of aggression and reproductive competition. In these situations, testosterone might be down-regulated in the same way that it is in vertebrate males when they care for and affiliate with their young, another key prediction of the Challenge Hypothesis (Wingfield et al. 1990).

At present, we lack the necessary data to test these two alternative possibilities about why male chimpanzees displayed relatively low levels of testosterone at hunts. In this study, our priority was to collect urine samples for hormone analyses. Doing so at rare events, such as boundary patrols and hunts, was time consuming and demanding, and as a consequence, it was not always possible to simultaneously gather detailed observations of meat sharing. Obtaining such observations and analyzing the effects of harassment and voluntary sharing on testosterone remain important tasks for future research. These data will contribute to our understanding of the mechanisms underlying and evolution of chimpanzee behavior, and promise to help clarify the precise role that testosterone plays in animal social interactions (Eisenegger et al. 2011).

Table 3.1 Male chimpanzee testosterone concentrations (ng/mgCr) Mean testosterone values with sample sizes

male	before hunting	after hunting	no hunting	before patrolling	after patrolling	no patrolling	shared meat	received meat	unsuccessful hunt	no hunting
Bt	85 (7)	47 (6)	71 (110)	77 (4)	54 (4)	71 (115)	46 (3)	51 (3)		72 (117)
Bs	96 (8)	80 (6)	79 (92)	92 (5)	105 (1)	80 (100)	55 (1)	77 (1)	105 (1)	92 (101)
Bg	121 (7)	105 (3)	143 (88)	217 (10)	209 (3)	129 (85)	130 (2)			139 (95)
Bf	89 (1)	81 (4)	94 (30)	88 (1)	223 (3)	79 (31)	47 (1)		101 (1)	93 (33)
Br	75 (6)	77 (4)	78 (44)	105 (1)	104 (3)	90 (50)		71 (3)		92 (51)
Cr	94 (10)	58 (4)	86 (83)	88 (7)	90 (5)	78 (85)	53 (1)	50 (1)		80 (95)
Dx	96 (5)	94 (3)	87 (83)	100 (3)	175 (4)	83 (84)		89 (1)	100 (1)	88 (89)
Di	127 (3)		128 (39)	105 (3)	98 (1)	131 (38)				128 (42)
Do	86 (7)	82 (7)	87 (72)	85 (8)	77 (3)	88 (75)	72 (2)	81 (2)	79 (1)	88 (80)
Ga	127 (2)	82 (4)	128 (39)	141 (4)	164 (2)	144 (42)		43 (1)		146 (47)
Gz	85 (4)	49 (2)	87 (72)	127 (2)	100 (1)	65 (37)	42 (1)	55 (1)		70 (38)
Hr	89 (6)	82 (2)	105 (77)	94 (4)	91 (3)	111 (78)		82 (2)		110 (83)
Ho	78 (6)	54 (6)	83 (38)	127 (6)	74 (2)	73 (42)	39 (3)	37 (1)	108 (1)	82 (45)
Lo	95 (8)	83 (5)	112 (57)	109 (4)	117 (2)	108 (64)	74 (1)	75 (3)		110 (66)
Mi	139 (8)	95 (1)	125 (61)	117 (4)	116 (2)	127 (64)				127 (70)
Mg	142 (8)	101 (7)	150 (80)	218 (7)	258 (4)	136 (84)	76 (4)		136 (3)	150 (89)
Mo	109 (7)	118 (4)	109 (86)	112 (6)	206 (2)	107 (83)	101 (2)		136 (3)	108 (87)
Mt	93 (6)	70 (4)	93 (86)	150 (6)	90 (5)	88 (85)		54 (1)		93 (95)
Mw	92 (1)		94 (57)	100 (1)	107 (2)	93 (55)				94 (58)
Or	150 (4)	111 (3)	126 (50)	195 (3)	90 (2)	125 (52)	71 (2)		93 (1)	127 (54)
Pk	86 (5)	50 (2)	116 (92)	156 (7)	153 (7)	106 (85)		51 (2)		114 (97)
Rh	101 (3)	119 (6)	130 (44)	119 (3)	108 (5)	130 (45)		75 (1)		127 (52)
Ri	110 (3)	105 (2)	142 (32)	121 (2)	206 (1)	136 (34)			99 (1)	139 (36)
Ro	77 (3)	77 (1)	110 (24)			105 (28)			71 (1)	133 (29)
Ta	158 (2)	63 (1)	128 (38)	104 (1)	102 (1)	128 (39)		63 (1)		129 (40)
Wb	111 (3)	94 (3)	105 (28)	106 (4)	151 (2)	101 (28)		71 (1)		106 (32)

Figure 3.1 The effect of territorial boundary patrols on urinary testosterone concentrations. The y-axis represents the residuals of log-transformed testosterone concentrations obtained from a LMM including time of day as a covariate and male identity as a random factor. Shown are the means (+ / - 1 SEM) of individual male means for each patrol subcategory. No patrols: N = 26 males. Before patrols: N = 25 males. After patrols: N = 25 males. For comparisons between males before and after patrols vs. no patrolling: *P < 0.01.

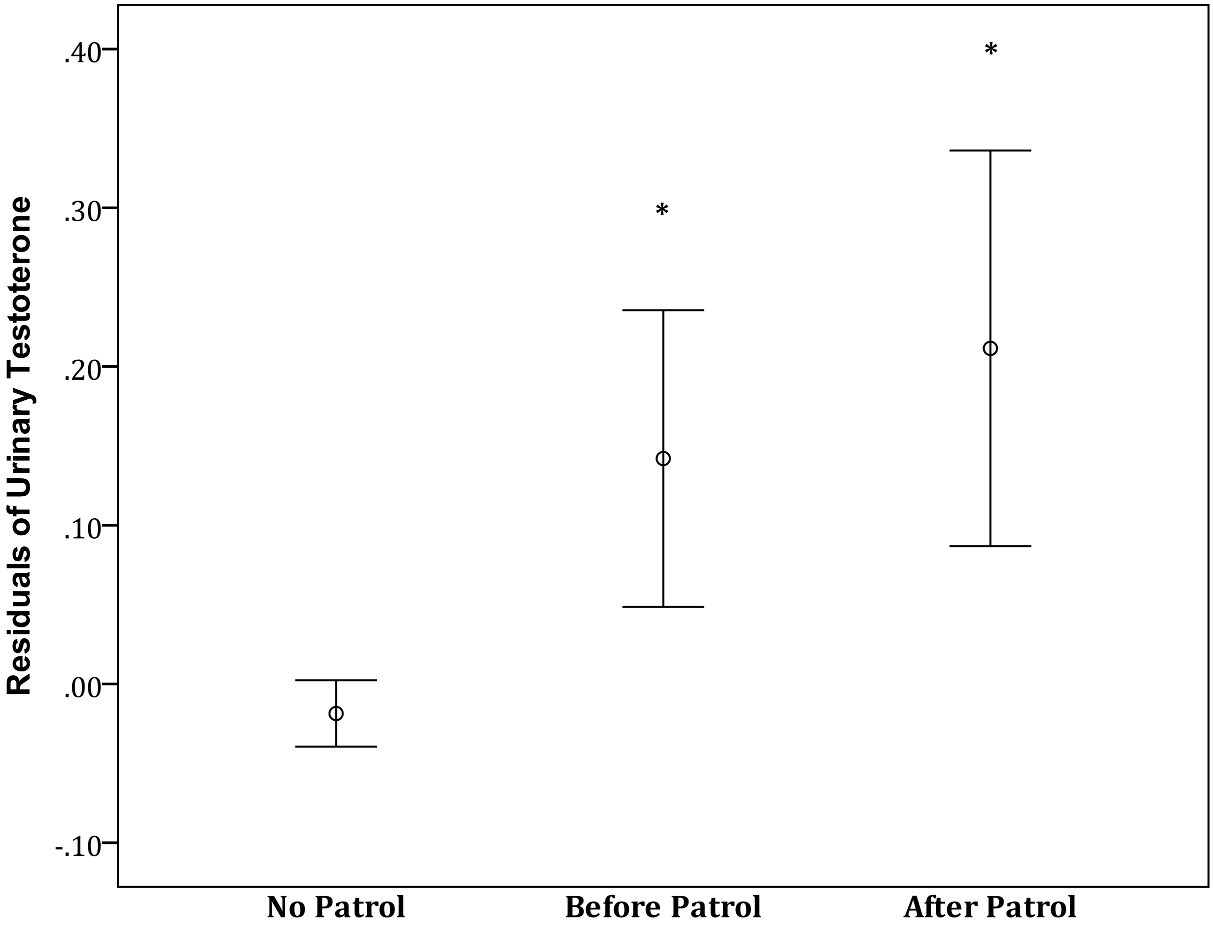
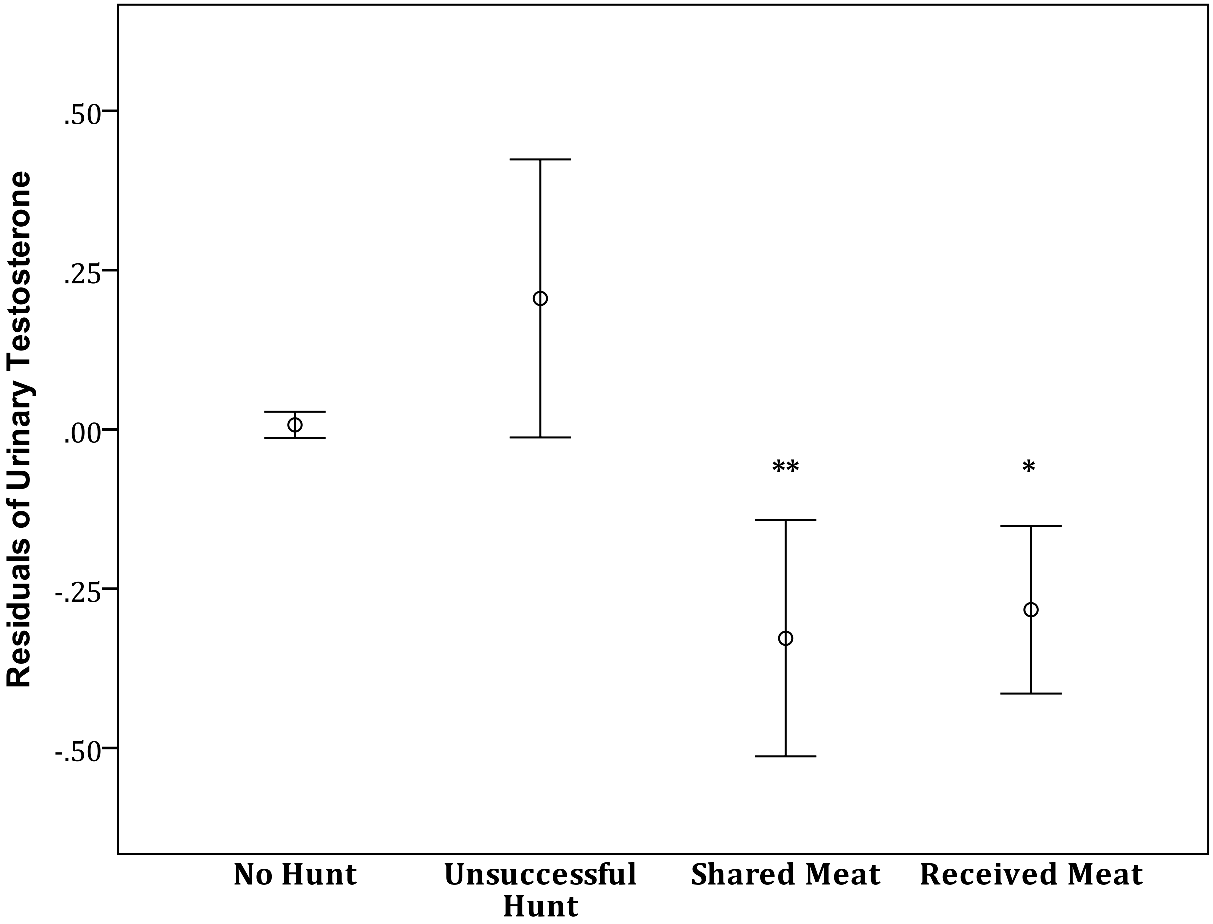


Figure 3.2 The effect of meat sharing on urinary testosterone concentrations. The y-axis represents the residuals of log-transformed testosterone concentrations obtained from a LMM including time of day as a covariate and male identity as a random factor. Shown are the means (+ / - 1 SEM) of individual male means in four sharing categories. No hunt and no sharing: N = 26 males. Unsuccessful hunt and no sharing: N = 10 males. Shared meat: N = 12 males. Received meat: N = 17 males. For comparisons between males who shared and received meat vs. no hunting and no sharing: *P < 0.02, **P < 0.01



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CHAPTER 4

Acute and Anticipatory Stress

ABSTRACT

Stress is notoriously difficult to define because what is stressful varies across species. Nevertheless, males in group living species commonly display associations between cortisol, a stress hormone, and male-male competition over estrous females. In this study, we investigate the effect that male-male aggression over estrous females has on cortisol in wild chimpanzees. In addition, we ask whether two other types of aggression involving predation and territorialty influence male cortisol levels. Our results indicate that short-term, physiologically demanding events, such as competition over parous estrous females, territorial boundary patrols, and hunting, are associated with increases in adrenal activity and cortisol production in wild chimpanzees. The discrete nature of territorial boundary patrols and hunts permitted us to also investigate anticipatory stress. Results revealed that males displayed relatively high levels of cortisol before any aggression transpired. This anticipatory increase in cortisol persisted after two potential confounds, large male party size and location in the territory, were controlled. The urinary cortisol levels of males were higher when they were in smaller groups and not when they were in larger groups that typically formed during territorial boundary patrols and hunts. Males did not display elevated cortisol levels when they were near the

periphery of their territory. Despite these findings, the potential cues that explain the observed anticipatory increases in cortisol are still unclear.

INTRODUCTION

Historically, ‘stress’ has been difficult to define yet easy to recognize (McEwen and Lasley 2002; Cheney and Sefarth 2009). The stress response allows an individual to quickly alter his or her physiological and behavioral profile in response to acute changes in the social and physical environment (McEwen and Wingfield 2003; Goymann and Wingfield 2004; Korte et al. 2005). When organisms experience a real or imagined stressor, the hypothalamic-pituitary-adrenal (HPA) axis is activated (Selye 1956; Sapolsky 1992). In addition to other hormones, glucocorticoids, like cortisol, rise above baseline within minutes causing a cascade of physiological responses including increased heart rate, mobilized sugars, and increased memory (Sapolsky 1992, 2002; McEwen and Wingfield 2003). Consequently, this response shuts down digestive processes, reduces immune function, and pauses somatic maintenance and growth processes (McEwen and Wingfield 2003; Sapolsky 2004; Korte et al 2005). Despite its costs, stress is essential for the successful navigation of changing environments that involve food shortage, predation, and aggression between conspecifics (Sapolsky 2002; McEwen and Wingfield 2003). Glucocorticoids play an especially important role in the regulation of aggression (Soma 2006). Aggression between males typically heightens as they compete for reproductive opportunities with estrous females, and male cortisol levels frequently increase during such aggression in many primate species (Bergman et al. 2005; Setchell et al. 2005, 2010; Fichtel et al. 2007).

Chimpanzees represent a particularly interesting case to examine the relationship between male aggression in the context of mating and cortisol. In chimpanzees, extremely long interbirth intervals limit the number of reproductively receptive females, creating a highly skewed operational sex ratio towards males (Emlen and Oring 1977). Males compete intensely for older, parous females, who have already reproduced successfully (Muller et al. 2006). Rates of aggression increase around these females leading males to expend considerable energy in their presence and to a concomitant stimulation of adrenal activity and cortisol production (Muller and Wrangham 2004). In addition, reduced food consumption and the psychological stress associated with male competition for females may also play a role in triggering a stress response (Bergman et al. 2005; Setchell et al. 2005, 2010; Fichtel et al. 2007). These considerations suggest that male cortisol levels will be elevated in the presence of parous, estrous females.

Male chimpanzees also display aggression in two other contexts, territorial boundary patrols and hunting. Prior studies of territorial aggression and cortisol have produced inconsistent results. Research on birds and reptiles reveals that short-term exposure to stress hormones is associated with elevated territorial aggression (Matter et al. 1998; Romero et al. 1998; Watt et al. 2003; Landys et al. 2010). However other studies indicate that there is a negative or no relationship between cortisol and territorial behavior (DeNardo and Sinervo 1994; Meddle et al. 2002; Selva et al. 2011). In free-living mammals, studies of the direct costs of territoriality are generally lacking (Landys et al. 2010), making it difficult to investigate the relationship between stress and territoriality. Additionally, little is known about the stress response of predators. Instead, most studies focus on the stress experienced by prey due to predators (review in Lima 2009). Despite the lack of data, it is often assumed that predatory behavior is associated with elevated physiological stress (Kemeny 2003).

In chimpanzees, hunting and territoriality are ubiquitous behaviors (Wrangham and Bergman-Riss 1990; Wrangham 1999; Wilson et al. 2004; Mitani 2009). Hunting and territoriality involve considerable physical exertion, are psychologically demanding, and may have significant reproductive consequences (review in Mitani 2009). Boundary patrols are an integral part of chimpanzee territorial behavior. During patrols, males make deep incursions into adjacent territories to obtain information about their neighbors or to make contact with them (Watts and Mitani 2001). Patrols last about 2 hours on average and are physically demanding as male chimpanzees move significantly more and feed less during them than other times (Amsler 2010). Hunts also involve considerable physical exertion. Male chimpanzees at Ngogo can spend up to six hours of a 12-hour waking day moving around their territory in search of prey (Mitani 2009). Boundary patrols and hunts are psychologically demanding because they both involve groups of males, who work together in a coordinated way to accomplish a goal (Watts and Mitani 2001; Boesch 2002). Finally, both behaviors have significant fitness consequences. Prior research suggests that female reproduction improves when females live in communities that are dominant over others as the result of male territorial behavior (Williams et al. 2004). Additional studies indicate that meat provides a rich source of energy, protein and other nutrients for chimpanzees (Teleki 1981; Stanford 1999) and that meat sharing provides social benefits important for maintaining cooperative social relationships (Nishida et al. 1992; Mitani and Watts 2001). Taken together, these observations suggest that male chimpanzees will display heightened levels of cortisol during hunts and while conducting territorial boundary patrols.

Whether chimpanzee hunting and territorial boundary patrols are physiologically stressful remains unclear. Equally unclear are the events leading up to these behaviors. Are males able to anticipate these behaviors and do they show an anticipatory response? In humans, many studies show that knowledge of future competition can trigger the HPA axis (Lovallo et al. 1990; Filaire et al. 2001; Gaab et al. 2005; Alix-Sy et al. 2008). Even infants, as young as six months old, are capable of mounting an anticipatory stress response (Haley et al. 2011). In captive chimpanzees and bonobos, hormone levels rise in anticipation of experiments that place participants in competitive situations (Hohmann et al. 2008; Wobber et al. 2010). Behavioral observations suggest that female chimpanzees are aware that males will initiate a territorial patrol, as they stop following males and fail to participate in patrols (Watts and Mitani 2001). These findings lead us to hypothesize that male chimpanzees will mount an anticipatory cortisol response before they start to hunt and begin a territorial boundary patrol.

What cues do males use to organize and initiate a hunt or boundary patrol? Two possibilities exist. Amsler (2009) found that most territorial boundary patrols start near the periphery of the range (25/29 = 86%). Thus, proximity to the territorial border might provide a cue to initiate a patrol. Large group size may be another factor that triggers hunts and boundary patrols, as this variable has been found to be the single best predictor of both behaviors (Mitani and Watts 1999; Mitani and Watts 2005). These considerations suggest that location in the territory and large party size may stimulate the HPA axis and lead to elevated cortisol levels in male chimpanzees. These factors represent two potentially important confounds that require statistical control in analyses investigating whether male chimpanzees display an anticipatory cortisol response before hunts and boundary patrols.

The Ngogo chimpanzees patrol and hunt at higher rates compared with chimpanzees in other communities (Goodall 1986; Boesch and Boesch 1989; Stanford et al. 1994; Mitani and Watts 1999; Boesch and Boesch-Achermann 2000, Hosaka et al. 2001; Gilby et al. 2006; Mitani et al 2010;). This, plus the extremely large size of the Ngogo chimpanzee community, provides a unique opportunity to assess the relationships between stress and male reproductive aggression, hunting, and territorial behavior. In this study, we investigate the associations between cortisol and three different types of aggression, including male-male, territorial, and predatory aggression.

METHODS

Study Site, Duration and Population

We observed chimpanzees at Ngogo in the Kibale National Park, Uganda. The 30 km^2 study area lies at an altitude of about 1,400 m above sea level and consists primarily of mature, moist evergreen forest. The forest is interspersed with patches of *Pennisetum purpureum* grassland and *Phoenix reclinata* palm swamp forest (Struhsaker 1997; Lwanga 2003). Mean annual rainfall is approximately 1,400 mm and the temperature fluctuates from 16.5^0 C to 25^0 C daily. The Ngogo chimpanzees have never been provisioned and have been under continuous observation since 1995. MES conducted fieldwork over 14 months during three field seasons, May to July 2006, May to November 2007, and February to May 2008. Urine samples were collected continuously by field assistants and MES from May 2006 to May 2008. The Ngogo community is extremely large, with over 150 individuals. We observed the behavior of 27 adult males, whose ages were estimated to range from about 20 to 40.

Behavioral Observations

MES followed adult male chimpanzees for 1,378 hours and recorded their identities, male party size, and the presence of parous estrous females. She also mapped the location of males each morning to identify the core and periphery of the territory. The core area was defined as that part of the territory in which 80% of all observations occurred (cf. Amsler 2009; Figure 4.1). Areas that fell outside of the core area were defined as the periphery of the territory. Male party size was defined as the total number of males observed that day. Chimpanzees live in fission-fusion communities, whose members split apart and come together throughout the day, and at Ngogo, large parties of males are relatively stable, leading to little diurnal variation in male party size (Mitani et al. 2002).

Male subjects were observed during 31 territorial boundary patrols and 33 hunts. Territorial boundary patrols are characterized by a distinctive suite of behaviors, making them easy to identify by experienced observers (Watts and Mitani 2001; Amsler 2010; Mitani et al. 2010). During patrols, chimpanzees move en masse, often in single file line, to the boundary of their territory. Once there, they continue to travel in single file fashion often in complete silence. Patrollers typically scan the environment; they sniff the ground and investigate signs left behind by neighboring chimpanzees, such as feces, urine, and discarded pieces of food. Patrollers can make deep incursions into the territories of their neighbors; aural or visual contact with neighbors occurs in about 30 – 40% of all patrols, with lethal aggression witnessed rarely (Mitani et al. 2010).

Hunts were easily recognized as times chimpanzees encountered and attacked vertebrate prey. Red colobus monkeys (*Procolobus* spp.) are the favored prey of chimpanzees (Boesch and Boesch 1989; Stanford et al. 1994; Mitani and Watts 1999; Gilby et al. 2006). Most hunts resulted in kills and the consumption of red colobus prey, as chimpanzee hunting success at Ngogo is unusually high, averaging over 80% (Watts and Mitani 2002). At Ngogo, chimpanzees hunt opportunistically when they encounter red colobus monkeys during their normal foraging movements. Other hunts, however, occur after “hunting patrols” (Mitani and Watts 1999), which are characterized by a group of behaviors similar to those displayed by chimpanzees during territorial boundary patrols and closely resemble the behaviors described as 'searches' by chimpanzees in the Tai National Park (Boesch and Boesch 1989).

Boundary patrols and hunts occur infrequently, taking place once on average every 9 and 7 days, respectively (Watts and Mitani 2001; 2002; Amsler 2010; Mitani et al. 2010). MES therefore collected observations *ad libitum.* She recorded the start of each patrol and hunt, and noted individuals who participated in each. Patrols and hunts were considered to have begun when chimpanzees gathered and started to display the characteristic behaviors associated with boundary and hunting patrols described above. For hunts that occurred when chimpanzees encountered prey opportunistically, start times were recorded when chimpanzees began to climb into trees and to pursue monkeys.

Hormone Analysis

Urine samples and collection

We collected 1800 urine samples from the 27 adult males *ad libitum* (X = 70 samples / male, SD = 26). We analyzed 346 samples obtained before and after the start of boundary patrols and hunts (patrols: *X* ± SD = 6.7 ± 2.4 samples / male, *N* = 26 males; hunts: *X* ± SD = 5.8 ± 3.3 samples / male, *N* = 27 males. We obtained the former up to 4 hours before the start of each event. We also analyzed samples from each male 2 – 4 hours after the start of patrolling and hunting activity. Because there is a 2 – 4 hour time lag for hormone detection in urine (Whitten et al. 1998; Bahr et al. 2000), these samples were considered to reflect the endocrine state of males during and shortly after boundary patrols and hunts. We excluded samples collected between the start time and 2 hours later, as they would represent ambiguous times relative to endogenous production. Our exclusion of samples that were collected immediately after the start of an event generates a conservative measure ensuring that the hormones signature 'before' an event was not influenced by the occurrence of any characteristic aggressive behaviors. We analyzed multiple samples from each male before and after boundary patrols and hunts (before patrols: $X \pm$ SD = 4.2 + 2.4 samples / male, N = 26 males; after patrols: $X \pm$ SD = 2.8 + 1.5 samples / male, N = 25 males; before hunts: $X \pm$ SD = 5.1 + 2.5 samples / male, N = 27 males; after hunts: $X \pm$ SD = 3.8 + 1.9 samples / male, N = 25 males; Table 4.1), and a total of 201 samples from males in the presence of parous estrous females (X ± SD = 7.0 ± 3.5 samples / male). For a subset of these urine samples (n= 1231), we possessed data regarding the location of males within the territory and male party size (X ± SD = 46 +19 samples / male).

Chimpanzees move terrestrially, which facilitated collecting urine from them noninvasively. Urine was typically obtained off of leaf litter on the ground with a pipette, or more rarely, caught in a plastic bag if chimpanzees urinated from a tree. Samples were analyzed only if they were obtained from individually identified males. Samples cross-contaminated with feces or blood were discarded. Samples were placed in 5 ml tubes, which were immediately labeled with the male's name, date, and time. Samples were frozen at the end of the day at 0^0 C, no longer than 12 hours after collection, and were subsequently shipped to the United States on ice using medically certified transport equipment from SAF-T-PAK. Samples were analyzed by MES at the Reproductive Endocrinology Laboratory at the Smithsonian Conservation Biology Institute in Front Royal, Virginia working under the supervision of Janine Brown.

Creatinine

We indexed all urine samples for creatinine (Cr) to account for variations in water content using a Jaffe reaction (Taussky 1954). Samples (0.05 mL) were diluted 1:20 in bovine serum albumin (BSA)–free phosphate buffer for Cr analysis. Samples with urinary Cr concentrations below 0.01 ng/mL were excluded from analysis as being too dilute; this involved less than 5% of all samples. Hormone concentrations were divided by Cr concentrations and expressed as the concentration of cortisol (ng) per mg Cr.

Cortisol

Cortisol was assessed in unprocessed urine using a single antibody enzyme immunoassay (EIA) provided by Coralie Munro (University of California - Davis, CA, USA) (Kersey et al. 2010). Microtitre plates (96 well; Nunc-Immuno, Maxisorp) were coated with a polyclonal cortisol antiserum (R 156/157; 50 µl per well; diluted 1:8500 in coating buffer, 0.05 M $NaHCO_3$, pH 9.6) and allowed to set for 12-18 hours at 4^0 C. Unabsorbed antiserum was removed with wash solution (0.149 M NaCl, 0.5% Tween 20). Cortisol standards (50 µl, range 3.9 –1000 pg/well, diluted in assay buffer, 0.1 M $NaPO_4$, 0.149 M NaCl, 0.1% bovine serum albumin, pH 7.0) in triplicate and samples (50 µl) in duplicate were then added to the wells, followed immediately with horseradish-peroxidase-labeled cortisol competitor (50 µl, 1:20,000 dilution in assay buffer). Following 1-hour incubation at room temperature, plates were washed five times before 100 µl substrate buffer [0.4 mM 2, 2' –azino-di-(3-ethylbenzthiazoline sulfonic acid) diammonium salt 1.6mM H_2O_2, 0.03 M citrate, pH 4.0] was added to each well. After incubation for 20-40 minutes, the absorbance was measured at 405 nm (540 reference filter) when the optical density in the total binding wells reached ~1.0. Intra-assay and inter-assay coefficients of variation (CV) were 8.81% (mean binding, 26.87%) and 9.90% (mean binding, 66.71%) for the high and low pools, respectively, while the CV for the 50% binding point of the standard curve was 5.23% (n = 126 assays). The assay was validated for chimpanzee urine by demonstrating that serial dilutions of pooled urine samples produced displacement curves parallel to that of the cortisol standard curve, and that there was significant recovery (> 90%) of exogenous cortisol added to urine before analysis.

Statistical Analysis

We conducted a generalized linear mixed model (GLMM) analysis to examine the effects of territorial boundary patrols, hunting, male group size, presence of estrous females, location, and time on cortisol. Cortisol concentrations were not normally distributed (Kolmogorov-Smirnov test: $Z = 3.81$, $P < 0.001$), and we therefore used log transformed cortisol as the response variable in this analysis. We considered hunting (before hunt, after hunt, and no hunt), territorial behavior (before patrol, after patrol, and no patrol), location (periphery or core area), and presence of estrous females (parous estrous female(s) present or parous estrous female(s) absent) as fixed effects. Because we hypothesized that male group size might influence male cortisol levels, we included it as a covariate. Time of day was also included as a covariate, as male cortisol secretion shows a characteristic decline across the day (Muller and Wrangham 2004). Finally, individual responsiveness to stress varies considerably between individuals in vertebrates (Korte et al. 2005; Overi et al. 2007). To control for this and the non-independence created by using multiple samples from the same individual, we considered individual males as a random effect. We conducted a series of post-hoc pairwise analyses using estimated marginal means of each sub-category of fixed effects. All statistical analyses were conducted using SPSS (version 20; Figure 8).

RESULTS

Table 4.1 shows the number of samples collected from males in different conditions and the mean urinary cortisol concentrations of individuals in various conditions. Results of a GLMM analysis indicated that male chimpanzee cortisol concentrations varied significantly across the day ($F_{1,1208}$ = 24.49, t = 4.95, P < 0.01, Table 4.2); however, there was no effect of location in the territory on urinary cortisol concentrations ($F_{1,1207}$ = 2.45, t = -1.57, P > 0.10, Table 4.2). Male group size had a significant effect on urinary cortisol concentrations, but contrary to expectation, male chimpanzees in small parties had higher cortisol levels than males in larger parties ($F_{1,1207}$ = 8.18, t = 2.86, P < 0.01, Figure 4.2).

Male chimpanzees displayed elevated cortisol concentrations when they were in the presence of parous, estrous females ($F_{1,1209}$ = 68.72, t = 8.29, P > 0.01, Figure 4.3). Hunting also had a significant impact on male cortisol concentrations ($F_{2,1207}$ = 10.20, *P* < 0.01, Figure 4.4); post-hoc analysis indicated that male cortisol was elevated before a hunt began and during and immediately after hunts compared with days no hunting occurred (before hunts: $F_{2,1207}$ = 10.20, t = 2.46, P < 0.05; during hunts: $F_{2,1207}$ = 10.20, t = 3.97, P < 0.01; Figure 4.4). Finally, territorial boundary patrol behavior also had a significant impact on male cortisol concentrations ($F_{2,1207}$ = 35.23, P < 0.01; Figure 4.5), with males displaying significantly higher levels of urinary cortisol prior to conducting boundary patrols compared with days they did not patrol ($F_{2,1207}$ = 35.23, t = 4.80, P < 0.01; Figure 4.5). Male cortisol continued to remain high during and immediately after patrols compared to levels shown on days of no patrolling activity ($F_{2,1207}$ = 35.23, t = 7.23, P < 0.01; Figure 4.5).

DISCUSSION

Results of our analyses reveal that parous estrous females had a significant influence on the urinary cortisol concentrations of male chimpanzees, suggesting that male competition for mating opportunities is a source of stress. These findings are consistent with studies in other species that show similar increases in the cortisol levels of males as they compete for females (Bergman et al. 2005; Setchell et al. 2005, 2010; Fichtel et al. 2007). Additional analyses indicate that males displayed relatively high cortisol concentrations while hunting and conducting territorial boundary patrols. These data suggest that heightened adrenal activity and cortisol production facilitate short-term, physiologically demanding events in wild chimpanzees.

Interestingly, male chimpanzees showed anticipatory increases in urinary cortisol concentrations before starting to hunt or engaging in a territorial boundary patrol. Similar increases have been documented in captive chimpanzees before they participate in staged competitive experiments (Wobber et al. 2010). Whether chimpanzees show an anticipatory response to analogous situations in the wild, such as hunting and boundary patrols, has not yet been investigated. In addition, our understanding of the cues that stimulate increases in cortisol before aggressive events is unclear. Captive bonobos display relatively high salivary cortisol levels when they watch their access to food resources being restricted during a food sharing experiment. Hohmann et al. (2009) concluded that the anticipation of the competitive situation was sufficient to induce social stress. In another study, captive chimpanzees displayed anticipatory increases in their cortisol concentrations when placed with a dominant partner in a food sharing experiment (Wobber et al. 2010). In these situations, individuals presumably anticipate being placed in a situation where they are forced to share with an individual that will monopolize food in the experiment. Conversely, males showed anticipatory decreases in cortisol when placed with a partner known to share food (Wobber et al. 2010). These studies suggest that chimpanzees are capable of anticipating conflict and physiologically preparing for it.

The findings presented here are consistent with the hypothesis that chimpanzees in the wild are also capable of anticipating conflict situations. We were nonetheless, unable to identify the cues chimpanzees use to anticipate such events. We investigated two potential cues. Location in the territory and large male party size are correlated with territorial and hunting behavior (Mitani and Watts 2005; Muller et al. 2006; Amsler 2010). Our results indicated, though, that male cortisol concentrations did not vary as a function of where they were in the territory. This finding eliminates the possibility that males do not show an anticipatory increase at the start of boundary patrols simply because most patrols start at the edge of the territory.

We also predicted that large male parties would be associated with elevated cortisol levels. Results of our analyses, however, indicated the opposite. Males displayed lower cortisol levels when they associated with a large number of males compared to when they moved with a smaller number of individuals. Why males show relatively low cortisol concentrations while associating with many other males is unclear. One possibility is related to lethal territorial aggression. Male chimpanzees are known to launch lethal coalitionary attacks on their neighbors. They do so only in situations where they have overwhelming numerical superiority over their opponents. In other words, large groups attack small groups. When male chimpanzees are on a boundary patrol and isolate a single male chimpanzee, the latter will frequently be attacked with lethal force. This “imbalance of power” hypothesis is consistent with our current knowledge of chimpanzee behavior and explains cases of lethal intercommunity aggression at Ngogo (Manson and

Wrangham 1991; Watts et al. 2006). These observations suggest that male chimpanzees may gain safety in numbers, which helps to explain why they display relatively low cortisol levels in larger groups. This finding also eliminates large male group size as a potential explanatory variable for the anticipatory response in cortisol secretion before territorial boundary patrols and hunts. Future studies are needed to identify the cues chimpanzees use to anticipate these events.

In sum, male-male aggression over parous females in estrus is a social stressor for wild chimpanzees. In addition, hunts and territorial boundary patrols are short-term stressors. Finally, male chimpanzees anticipate engaging in these behaviors but the cues associated with this anticipation are still unknown.

Table 4.1. Male chimpanzee cortisol concentrations (ng/mgCr). Mean cortisol values of each male are shown with sample sizes in parentheses.

male	before hunting	after hunting	no hunting	before patrol	after patrol	no patrol	parous present	no estrus
Bt	116 (7)	106 (6)	109(110)	132(4)	109(4)	109(115)	160(9)	105(114)
Bs	190 (8)	213 (6)	203 (92)	224(5)	212(1)	201(100)	246(12)	196 (94)
Bg	237 (7)	239 (3)	223 (88)	249(10)	312(3)	219 (85)	282(10)	218 (88)
Bf	415 (1)	283 (4)	320 (30)	254 (1)	525(3)	300 (31)	350 (4)	314 (31)
Br	301 (6)	244 (4)	303 (44)	376 (1)	161(3)	305 (50)	374 (7)	287 (47)
Cr	257(10)	148 (4)	220 (83)	291 (7)	314(5)	210 (85)	208 (8)	222(105)
Dx	192 (5)	248 (3)	210 (83)	339 (3)	271(4)	203 (84)	311(11)	196 (80)
Di	225 (3)		214 (39)	155 (3)	188(1)	220 (38)	339 (3)	205 (39)
Do	317 (7)	340 (7)	281 (72)	266 (8)	270(3)	292 (75)	320 (6)	289 (80)
Ga	162 (2)	137 (4)	298 (39)	232 (4)	206(2)	287 (42)	400 13)	234 (35)
Gz	129 (4)	179 (2)	180 (72)	267 (2)	488(1)	161 (37)	158 (3)	176 (37)
Hr	185 (6)	159 (2)	208 (77)	353 (4)	164(3)	199 (78)	307 (7)	195 (78)
Ho	306 (6)	279 (6)	211 (38)	248 (6)	235(2)	228 (42)	273 (6)	225 (44)
Lo	262 (8)	256 (5)	237 (57)	202 (4)	495(2)	236 (64)	371 (7)	227 (63)
Mi	257 (8)	205 (1)	251 (61)	165 (4)	430(2)	250 (64)	280 (7)	247 (63)
Mg	291 (8)	249 (7)	248 (80)	312 (7)	309(4)	244 (84)	292 (9)	248 (86)
Mo	243 (7)	247 (4)	269 (86)	199 (6)	308(2)	270 (83)	349(13)	252 (78)
Mt	301 (6)	233 (4)	237 (86)	301 (6)	516(5)	221 (85)	264 (6)	240 (90)
Mw	128 (1)		322 (57)	272 (1)	612(2)	309 (55)	429 (7)	304 (51)
Or	187 (4)	159 (3)	231 (50)	250 (3)	272(2)	220 (52)	348 (8)	203 (49)
Pk	183 (5)	202 (2)	260 (92)	350 (7)	378(7)	236 (85)	280(16)	250 (83)
Pi	338 (1)	323 (4)	232 (65)	425 (2)		233 (68)	326(10)	224 (60)
Rh	478 (3)	207 (6)	249 (44)	295 (3)	240(5)	256 (45)	280 (6)	254 (47)
Ri	326 (3)	504 (2)	299 (32)	177 (2)	201(1)	324 (34)	265 (3)	317 (34)
Ro	277 (3)	114 (1)	184 (24)			191 (28)	270 (4)	178 (25)
Ta	213 (2)	263 (1)	204 (38)	75 (1)	329 1)	206 (39)	254 (4)	201 (37)
Wb	218 (3)	253 (3)	189 (28)	264 (4)	379(2)	174 (28)	300 (2)	190 (32)

Table 4.2. Results of a Generalized Linear Mixed Model examining the effects of several variables on male chimpanzee cortisol levels.

Source	Numerator df	Denominator df	F	Significance
Intercept	1	596.40	3142.63	.000
Time	1	1207.21	24.50	.000
Group	1	1208.09	8.181	.004
Parous	1	1209.60	68.72	.000
Patrol	2	1207.01	2.449	.000
Location	1	1208.06	2.449	.118
Hunt	2	1207.46	10.204	.000

Figure 4.1 Map of the Ngogo Chimpanzee Territory. This map shows the core and periphery of the Ngogo chimpanzee territory. Paths of patrols are displayed in green. The black dots indicate that only a few patrols began in the core area.

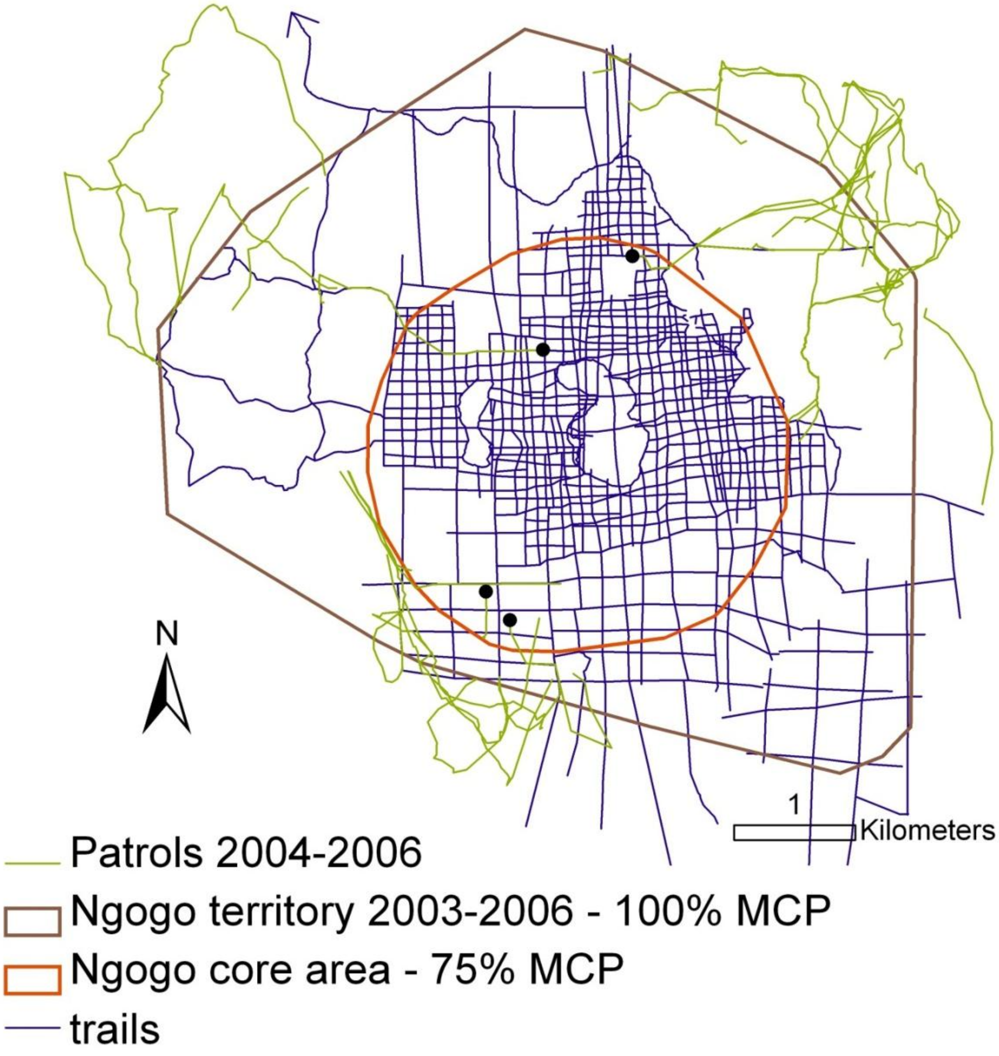


Figure 4.2 The effect of male party size on urinary cortisol concentrations. The y-axis represents the residuals of log-transformed cortisol concentrations obtained from a GLMM including time of day as a covariate, male identity as a random factor and hunting, boundary patrolling, and location in the territory as fixed effects. Each bar represents the means (+ / - 1 SEM) for parties that varied in male group size.

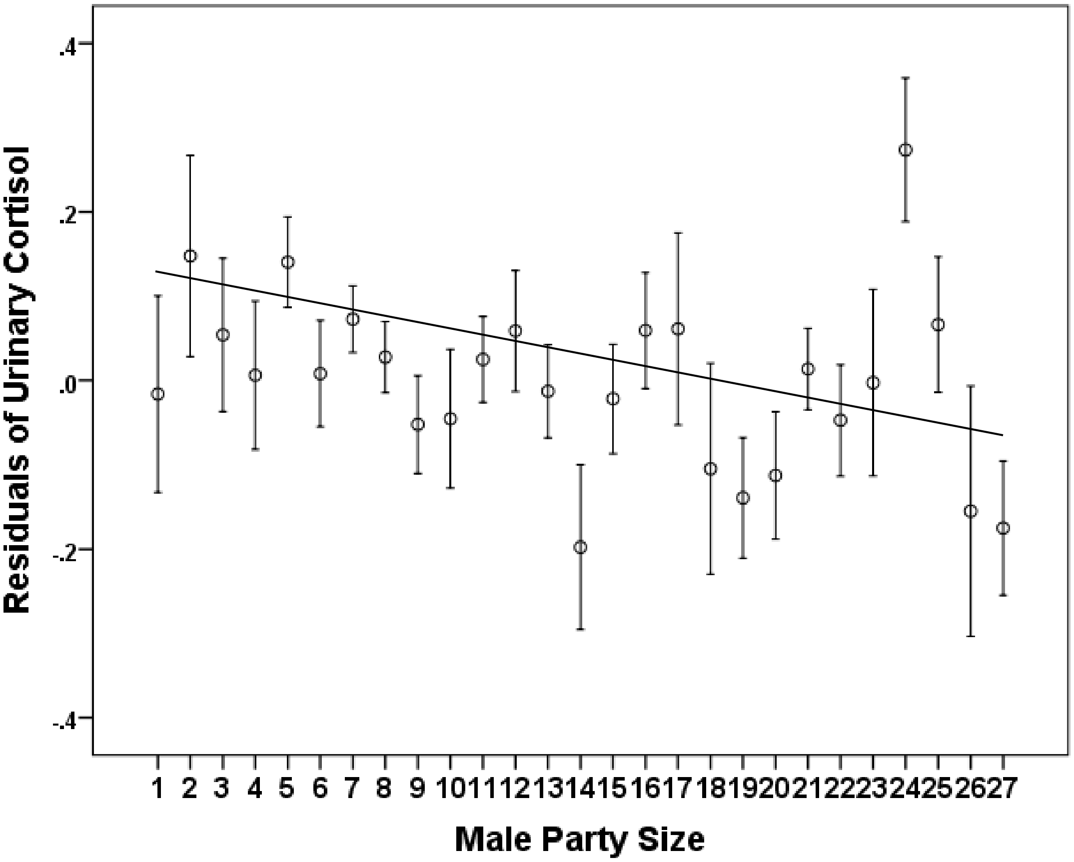


Figure 4.3. The effect of the presence of females in estrus on urinary cortisol concentrations. The y-axis represents the residuals of log-transformed cortisol concentrations obtained from a LMM including time of day as a covariate, male identity as a random factor and hunting, boundary patrolling, and location in the territory as fixed effects. Shown are the means (+ / - 1 SEM) of individual male means. Absent: N = 27 males. Present: N = 27 males. *P < 0.001

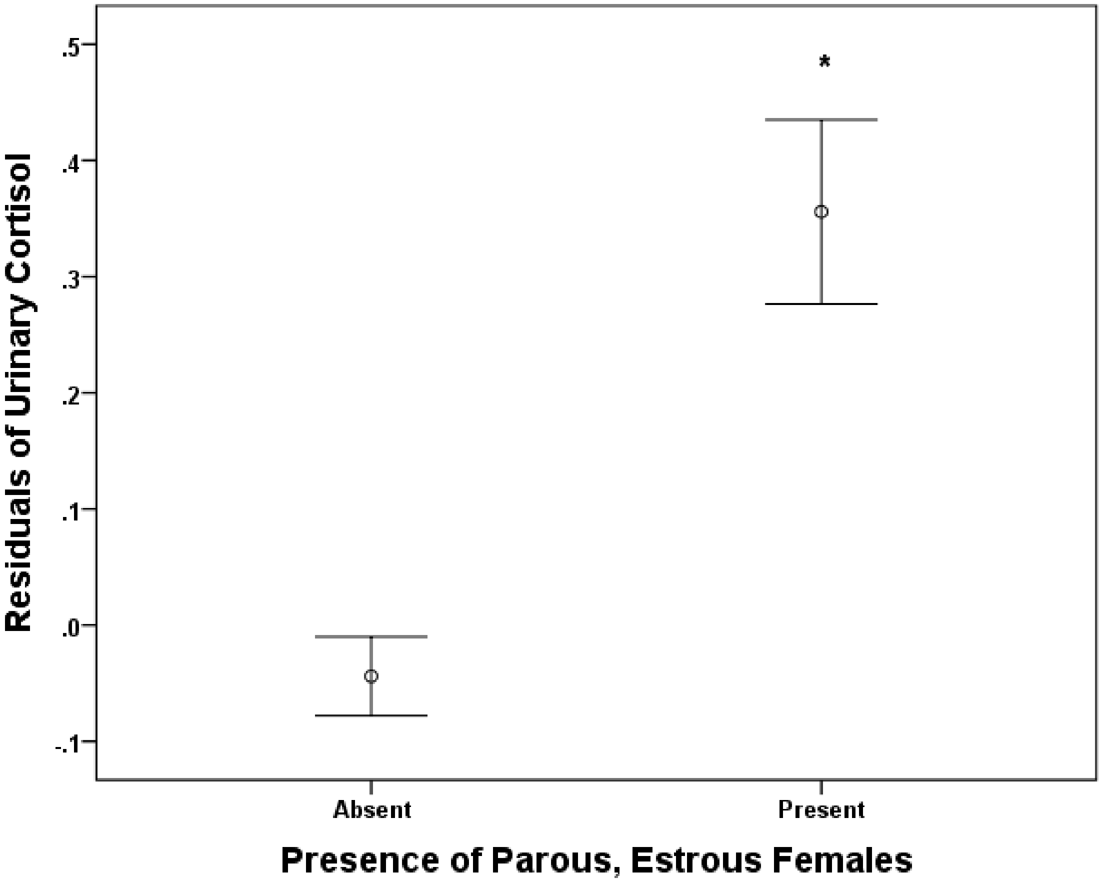


Figure 4.4. The effect of hunting on urinary cortisol concentrations. The y-axis represents the residuals of log-transformed cortisol concentrations obtained from a LMM including time of day as a covariate, male identity as a random factor and boundary patrolling, the presence of estrous females, and location in the territory as fixed effects. Shown are the means (+ / - 1 SEM) of individual male means. No hunt: N = 27 males. Before hunt: N = 27 males. After hunt: N = 25 males. For comparisons between before hunt and after hunt with no hunt: *P < 0.05, **P < 0.01

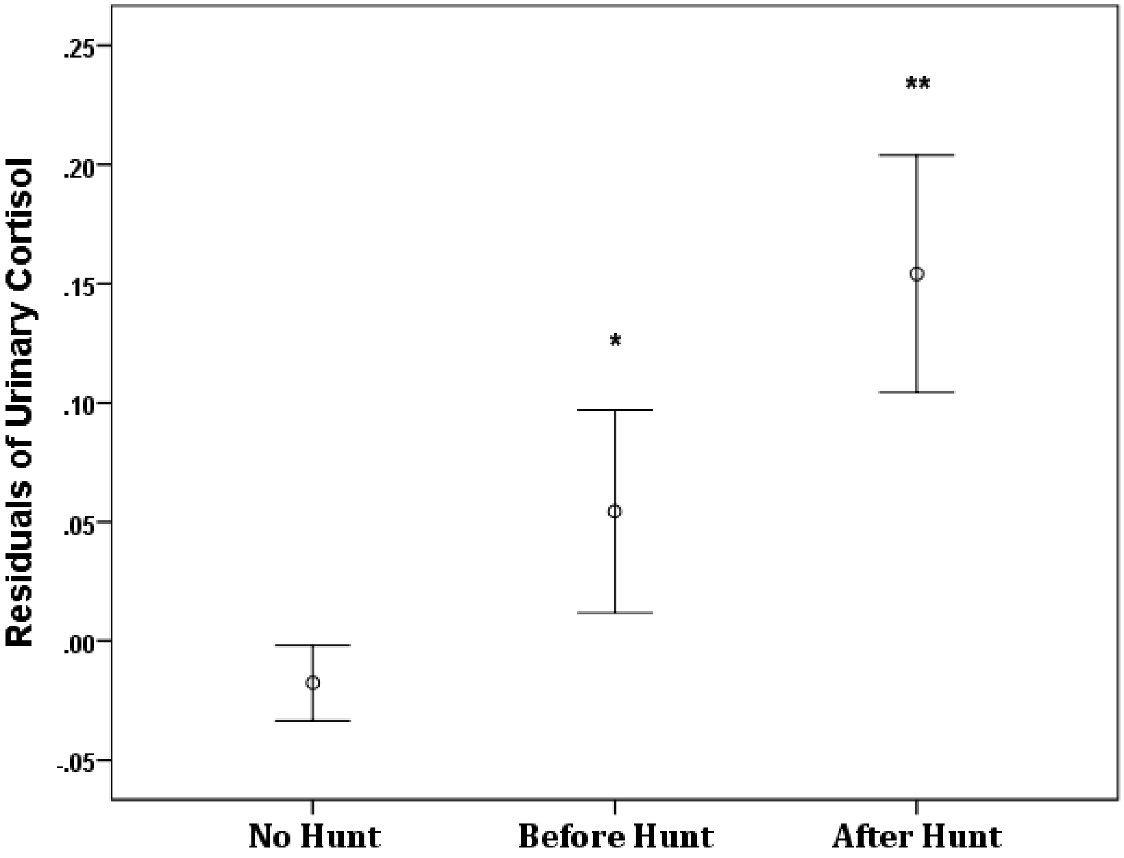
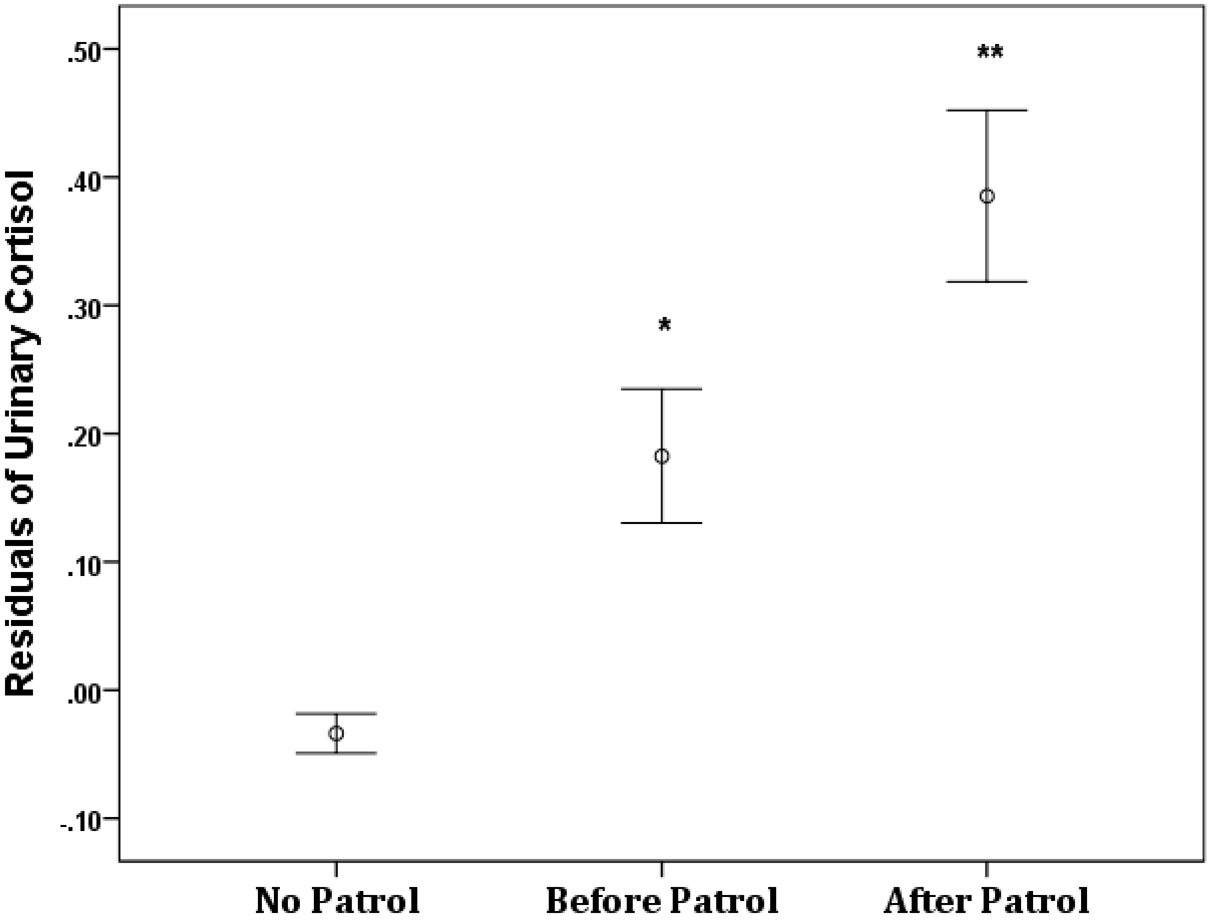


Figure 4.5. The effect of territorial boundary patrols on urinary cortisol concentrations. The y-axis represents the residuals of log-transformed cortisol concentrations obtained from a LMM including time of day as a covariate, male identity as a random factor and hunting, the presence of estrous females, and location in the territory as fixed effects. Shown are the means (+ / - 1 SEM) of individual male means. No patrol: N = 27 males. Before patrol: N = 26 males. After patrol: N = 25 males. For comparisons between before patrol and after patrol with no patrol: *P < 0.001, **P < 0.001



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Chapter 5

Conclusions

SUMMARY

This dissertation provides novel data about the physiology of our closest living relatives, chimpanzees. I combined field observations with laboratory analyses to investigate the hormonal mechanisms underlying aggression in wild chimpanzees. I focused on three different types of aggression, male-male aggression during competition for estrous females, territorial aggression, and predatory aggression, and documented the action of two steroid hormones, testosterone and cortisol, in these aggressive contexts.

In Chapter 2, I began by showing that there was no relationship between male dominance rank and baseline testosterone levels. I then proceeded to examine the influence that females have on testosterone to test the Challenge Hypothesis. This hypothesis was formulated to explain the inconsistent relationships that have been previously documented between testosterone and aggression. Because the production of testosterone has costs as well as benefits, Wingfield and colleagues (1990) suggested that increases in testosterone will be associated with aggression that has significant fitness consequences. In contrast, similar increases will not be observed in other kinds of aggression, such as anti-predatory, irritable, or defensive aggression. The Challenge Hypothesis has received broad support, especially in studies of birds and other seasonally breeding species, but more studies of non-seasonally breeding species are needed (Beehner et al. 2009).

Chimpanzees breed non-seasonally, and males compete for females when they come into estrus. I found that males did not increase their rates of aggression in the presence of nulliparous females. Instead, they elevated their rates of aggression when they competed for parous, estrous females. Despite these differences, males did not copulate more with parous females than they did with nulliparas. Because male-male reproductive aggression varied as a function of female parity, I was able to examine whether testosterone secretion increased beyond what was needed for sexual function when parous, estrous females were present. As predicted, male chimpanzee testosterone concentrations were higher when they competed for parous, estrous females compared with their levels in the presence of nulliparas. Importantly, these results still held after controlling two potential confounds, the presence of the alpha male and male party size. These findings with added controls validate and extend prior observations made in another chimpanzee community (Muller and Wrangham 2004). Taken together, they furnish strong support for the Challenge Hypothesis in chimpanzees, a non-seasonally breeding taxa.

In chapter 3, I provided a novel test of the Challenge Hypothesis by investigating two different types of male chimpanzee aggression. First, I examined the effect of between-group territorial aggression on male testosterone levels. Such aggression has important reproductive consequences, and in accordance with the Challenge Hypothesis, it had a predictable effect on male chimpanzee testosterone concentrations. The discrete nature of territorial boundary patrols permitted me to analyze samples collected a few hours before males engaged in territorial behavior. Interestingly, male testosterone levels increased before any characteristic patrol behaviors occurred, suggesting that males anticipated these activities before performing them. Questions still exist regarding how males decide to patrol and communicate their intentions to others before they do so.

In a second test of the Challenge Hypothesis, I examined male chimpanzee predatory aggression. At first blush, chimpanzee predation does not have any obvious reproductive consequences. As a result, male chimpanzees should not show any characteristic spikes in their testosterone levels while hunting according to the Challenge Hypothesis. As predicted, I found that hunting behavior was not associated with elevated testosterone levels. Somewhat surprisingly, however, I showed that male testosterone levels actually decreased during and shortly after hunting. The observed decrease is yet to be explained. I hypothesize that it may be due to the tolerance engendered during hunts due to meat sharing.

In chapter 4, I focused on the stress associated with the three types of male chimpanzee aggression. I investigated the relationships between cortisol secretion and male aggression over estrous females, territorial behavior, and predatory aggression. I found that the presence of parous, estrous females was associated with elevated male stress, as measured by their urinary cortisol concentrations. Additional results revealed that male cortisol levels increased during territorial boundary patrols and while hunting. The kinds of stress experienced by male chimpanzees in all three of these contexts are forms of acute stress and are likely to be beneficial (Haller et al. 1998). Interestingly, and in support of my findings in Chapter 3, male chimpanzees exhibit anticipatory stress before engaging in territorial boundary patrols and before hunting. Cortisol levels increased before males began to show any of the characteristic behaviors associated with boundary patrolling and hunting. I was unable to identify the cues associated with such anticipation, as large male party size and location in the territory did not affect male cortisol levels. How male chimpanzees physiologically prepare for these behaviors are still unknown.

Future Questions – Anticipation, Tolerance, and Between Individual Variation

Several fascinating questions emerge from this research and require further study. For example, the increases in cortisol and testosterone displayed by males before they begin to patrol the boundaries of their territory and hunt suggest that they anticipate these behaviors. How do male chimpanzees know that they will conduct a territorial boundary patrol and hunt before they do so? And how do they communicate their intentions to others? Another intriguing, and as yet unexplained, result documented in this thesis concerned the decrease in male testosterone during meat sharing events. Is it due to the tolerance engendered during such events? Or does it result from subjugation experienced by males who are forced to share meat with others? Observations of male chimpanzees from other study sites promise to provide insights into this issue.

Finally, the results presented in this thesis controlled for between individual variation in hormone levels. In each analysis, I used each male as his own control or employed appropriate statistical procedures to minimize the effects of between individual variation. Nonetheless, there is considerable variation between the baseline levels of males. This variation is due to a variety of sources, including physiological differences, personality, dominance rank, and coping strategies. Examining the sources of between individual variation in testosterone and cortisol remain an important area for future research.

Concluding Thoughts: Interdisciplinary Study and Biological Determinism

The fields of ethology and endocrinology gain by using interdisciplinary and evolutionary perspectives to better understand hormone-behavior relationships. Nearly 50 years ago, Niko Tinbergen outlined the four kinds of questions one can ask about the behavior of animals, namely causation, ontogeny, function, and evolution (Tinbergen 1963). To understand social behavior, these questions must be considered together under an interdisciplinary umbrella because they inform each other, inspire new hypotheses, and constrain others (Adkins-Regan 2005).

The importance of using hormones to inform our understanding of function of behavior is exemplified by the Challenge Hypothesis. Considering the costs and benefits of hormone secretion on the body changed the way ethologists identify aggression. This integration begins with an animal's natural history, which builds a picture of how physiology connects and constrains complex behavioral adaptations, such as aggression. Wingfield's 1990 paper on the Challenge Hypothesis has been cited 810 times (according to ISI Web of Science database). In the future, behavioral and endocrinological studies should formulate hypotheses that consider both the proximate mechanisms of behavior as well as their functional costs and benefits. In this way, Tinbergen's four questions can be wedded together in ways unforeseen even by him.

Another important consideration for behavioral endocrinologists is the causal nature of the hormone-behavior relationship. This relationship is nuanced. The word hormone means 'to urge on' or 'to arouse'. As hormone levels rise, the way organisms interact with stimuli in their environment changes, and therefore, the probability of a behavior occurring changes. Additionally, this relationship is not unidirectional. Hormones can urge on behavior, and behavior can stimulate hormone secretion. These two facts make untangling these relationships complicated. Endocrinological studies can contribute to eradicating persistent notions of biological determinism by highlighting these relationships and focusing on the development, environmental influences, and probabilistic nature of these hormone-behavior relationships. By highlighting how physiology and behavior are intertwined and interact, behavioral endocrinological studies reveal the fallacy of treating nature and nurture as a dichotomy.

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