TESTOSTERONE AND BEHAVIOR IN FEMALE MARMOSETS

BY

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DISSERTATION

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ABSTRACT

This study examines the role of testosterone in mediating behavior in common marmosets (*Callithrix jacchus*) and across the primate order. Testosterone has been primarily studied in males. Male behaviors related to mating success (competition and finding mates) are positively associated with testosterone levels in many bird and mammalian species. Conversely, male testosterone is negatively associated with infant care. The relationship between mating effort and infant care may represent a trade-off with testosterone serving as one hormonal mediator of these behaviors. Because females also exhibit mating competition and must balance mating investment with caring for offspring, I explored whether testosterone varied with female behavior in similar patterns as seen in males. I used a captive colony of common marmosets, at the Southwest National Primate Research Center in San Antonio, TX. To control for social context, I examined the relationship between testosterone and behavior during staged intruder tests and without any social manipulation. I compared female and male behavior and testosterone concentrations using non-invasively collected fecal samples. Neither female nor male testosterone showed a statistically significant increase in response to intruders, but female testosterone concentrations were indistinguishable from those of males during intruder tests. To test whether investing in mating behavior represented a trade-off with infant care in captive common marmosets, I also compared general behavior patterns between sexes. Females and males engaged in similar amounts of time spent being aggressive, engaging in sexual behavior, and caring for offspring. However, male common marmosets exhibited a correlation between aggression and distance from infants, whereas females did not. This suggests that the trade-off between mating effort and infant care exists for males but not females in common marmosets. I also compared testosterone concentrations without social manipulation between females and males and found no difference

in fecal testosterone concentrations. A comparison of the ratio of male-to-female testosterone was done across primates. Mean male:female testosterone concentrations correlated with mating system across primates. However, the comparative data set was small and more data are needed to fully explore the relationship of testosterone and behavior in primates. The idea that testosterone may mediate behavior in females has implications in the evolution of mating and parental behavior.

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CHAPTER 1: INTRODUCTION

Impetus

This dissertation was undertaken with the goal of understanding the ways in which the endocrine system relates to behavior. My interest in the hormone-behavior interface resulted from noticing a gap in our knowledge of the ways in which testosterone, most often investigated in males, may be evaluated and expressed in females. When I began investigating testosterone and female behavior in 2011, our knowledge was centered on cases of female pathology and experimentally manipulated females whose ovaries had been removed and testosterone either injected or increased with the use of hormone implants (e.g. Dixson, 1993).

Meanwhile, the understanding of testosterone's relationship with aggression and mating competition, both so-called "masculine" behaviors, had expanded. The relationship of T and behavior was known to follow a typical pattern, but one in which context was of utmost importance to understanding both species- and individual-level patterns of variation in the T-behavior relationship (Hau, 2007; Nunes, Fite, Patera, & French, 2001; Pollet, Cobey, & van der Meij, 2013; Wingfield, Hegner, Dufty, & Ball, 1990).

We know that in a broad sense, T is positively associated with male mating behaviors and negatively associated with infant care in birds and mammals (Hirschenhauser & Oliveira, 2006; Wingfield et al., 1990). Specifically, mating versus parenting has been treated as a trade-off, with T serving as a major endocrine mediator for this trade-off (Muehlenbein & Bribiescas, 2005). What stood out from these findings was that in the face of labels such as "male hormone," the behaviors of interest were also female behaviors. Females parent, have sex, seek out mates, and defend breeding status (Clutton-Brock & Huchard, 2013). Further, in primates such as the

callitrichids and humans, females invest considerable time and energy in both mating and parental investment, unlike what is seen in so-called "sex reversed" species (e.g. pipehorses) (Sear & Mace, 2008; Tardif et al., 2003; Yamamoto et al., 2014). Therefore, this led me to ask if there was a similar relationship with T and behavior in females as had been seen in males, and what was the relationship between female and male testosterone levels.

To answer this question I turned to a species of platyrrhine monkey, the common marmoset (*Callithrix jacchus*). Specifically, I asked 1) whether or not female marmosets were dimorphic in fecal testosterone concentrations and how this might fit into a broader pattern of sexual di- or mono-morphism in primates, 2) is there any difference in the relationship between behavior and fecal testosterone in females and males exposed to the same experimentally induced mating challenge, and 3) is the relationship between parenting and mating behaviors truly a trade-off?

Background

Sexual Selection Theory and Behavioral Dimorphism

The classic version of sexual selection theory (SST) bases dimorphic bodies and behavior in anisogamy (Trivers, 1972, Bateman 1948). In this model, males display intense competition for access to fertile females. In contrast, females focus on obtaining resources to support the energetic costs of reproduction. Among mammals, the energetic investment of reproduction further differs between females and males due to the female-only costs of pregnancy and subsequent lactation (Lee, 1996).

There are many species that follow the dimorphic model of female parental investment and male mating competition, although it is widely recognized that most species have some

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behavioral overlap between sexes. One example is the chimpanzee (*Pan troglodytes,* Stumpf 2011). While females are the primary caretakers of offspring and males compete for access to mates, female chimpanzees do experience intrasexual competition (Kahlenberg, Emery Thompson, & Wrangham, 2008). Also, male chimpanzees have been shown to preferentially interact with their likely offspring, even though this means staying with infants' mothers who are known to travel more slowly and may therefore represent foraging costs to the males (Murray, Stanton, Lonsdorf, Wroblewski, & Pusey, 2016). Another example of this behavior pattern is seen in macaques and baboons, where males generally compete for access to females and females provide parental care (Thierry, 2011). However, males have also been noted to spend time with infants in this taxon, including carrying infants, and there are also cases of female intrasexual competition (Huchard & Cowlishaw, 2011; Small, 1990). Generally, while behavioral sex differences are common, the completeness of this type of dimorphism varies.

Behavioral dimorphism may vary by mating and parenting systems. While many mammals follow the general "males compete, females parent" model, this pattern does not describe species that form socially monogamous partnerships, polyandrous groups, or those in which males heavily invest in offspring. In contrast, the previous examples of chimpanzees and papionins both follow polygynandrous mating systems, which do fit the traditional model. To address how SST predictions change with mating system and infant care, Kokko and Jennions (2008) investigate the conditions that would lead to deviation from males prioritizing mating over parenting. They argue that males should invest in offspring when the operational sex ratio is male-biased, thereby reducing the likelihood of finding a new mate. In their model, deserting offspring under these conditions would not lead to an increase in reproductive success, and may lead to a decrease if the existing offspring do not survive (Kokko & Jennions, 2008). Clutton-

Brock and Huchard (2013) also describes female competition, better documenting the varied circumstances in which females must and choose to compete. Female mating competition is well documented in some primate species, such as in tamarins and marmosets (Digby, Ferrari, & Saltzman, 2011) The current model of SST accounts for variation in operational sex ratios and variation in parental investment (Clutton-Brock, 2017; Kokko & Jennions, 2008).

General T dimorphism

Testosterone is generally higher in males than in females (Arnold, 2004; Ketterson, Nolan Jr, & Sandell, 2005). This is not surprising as the major producer of this steroid is testicular tissue. Other sources of T are the adrenal glands in both males and females, and the ovaries in females (Abraham, 1974). In many species, T will peak in females prior to ovulation, around the same time as the estradiol peak, and then drop (Bloch, Schmidt, Su, Tobin, & Rubinow, 1998; R. D. Nadler, Graham, Collins, & Gould, 1979; R.D. Nadler, Collins, Miller, & Graham, 1983). Therefore, female T levels may be more similar to male levels depending on species and cycle stage and male reproductive strategies.

Testosterone dimorphism is clearly seen in species such as humans (Feder, 1985). However, there are examples of reduced T dimorphism in a small but growing number of species (e.g. Koren & Geffen, 2009). One major challenge to understanding patterns of endocrine dimorphism in steroids is the lack of direct female/male comparisons within the same studies of androgens (and for that matter, estrogens). Both of these challenges are further discussed in Chapter 2.

Correlation between T and SST behaviors in males and females

Male testosterone is associated with the behaviors SST predicts to differ between sexes. In a classic study, Wingfield et al. (1990) described species-level variation in male T in over 60 species of birds, focusing on the patterns of change in T secretion. They found that T increased with mating investment and decreased with parental investment. Among females, evidence shows that T plays a role in mediating behavior. For example, in aggression trials, ovariectomized mice were not aggressive, but ovariectomized females given T implants showed a dose-dependent level of aggression during staged trials (Barkley and Goldman, 1979). However, there is variation in the specifics of the T-behavior relationship between species. For instance, wild baboon females have increased T levels during periods of higher aggression (Beehner, Phillips-Conroy, & Whitten, 2005). However, Drea (2007) found that androstenedione, not T, was associated with aggression in a wild population of ring-tailed lemurs. In a review of "atypical" mammals, French et al. (2013) show that androgens, including testosterone, are important mediators of female behavior. Specifically, French et al. (2013) describe some species, such as the spotted hyena, for which aggressive behavior is better correlated with organizational effects of androgens during development as opposed to a clear "activational" effect during adulthood. In other species, including mice and marmosets, French et al. (2013) describe an activational impact with T correlating with aggression or aggressive encounters in adults. More research is necessary to better understand the social and ecological contexts surrounding the relationship of T with behavior.

Further challenges in understanding the relationship between testosterone and behavior can arise from sampling methods. Non-invasive methods to measure steroid hormones, such as fecal or urine collection, are popular because they increase the contexts in which hormones can

be studied. This is useful in wild and free-ranging populations, and in captive populations for which daily blood draws are impractical or not allowed. Fecal samples have been used to monitor hormonal changes in primate species since the 1980s (Ziegler & Wittwer, 2005). However, non-invasive sampling is a proxy measure of hormone concentrations compared to blood draws, which represent circulating levels of hormones at the moment of sampling. In contrast, urinary and fecal samples represent hormones who have been metabolized and excreted. In the case of fecal samples, testosterone has gone through the liver, been excreted into bile and then enters the intestines and passes through the gut to be excreted in fecal matter (Norman & Litwack, 1997). Fecal samples have been increasingly used since earlier reports demonstrated that increasing amounts of plasma steroids resulted in increased amounts of assayed fecal steroids, both in experimentally elevated steroids and in associations with naturally occurring shifts in steroid concentrations (Bishop & Hall, 1991, Brown et al., 1996, Cockrem & Rounce, 1994, Reslir, Wasser, & Sackett, 1987, Ziegler, Sholl, Scheffler, Haggerty, & Lasley, 1989). Whereas circulating levels are representative of what is happening in the body at the moment of sampling, fecal samples are representative of a longer time period and can be impacted by gut passage times or dietary differences (Wielebnowski & Watters, 2007). Furthermore, fecal samples will contain conjugated steroid hormone molecules, as a result of passing through the liver to be deactivated (Norman & Litwack, 1997). Assay methods therefore, include a step to de-conjugate and extract the hormone, allowing the researchers to use hormone-specific antibodies to measure the steroid of interest (Ziegler and Wittwer, 2005). Thus, fecal samples represent a portion of circulating hormone levels, after they have been metabolized by the body, rather than the exact circulating levels of the hormone during the time of interest. However, because fecal samples represent a longer time frame than blood samples, researchers do not need

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to capture the sample within the immediate time frame following the event of interest. Nor do they need to worry that the sampling method will, through interactions with the stress axis, impact the hormone values being measured. Fecal steroids have, therefore, been regularly used to address questions about steroid hormones in primates (e.g. Muehlenbein, 2006, Drea 2007, Fontani et al., 2014, Rafacz et al., 2012).

Why Marmosets?

The common marmoset (*Callithrix jacchus*) is an ideal species in which to study the relationship between testosterone and behavior in both sexes because of their deviance from the pattern of male competition and female offspring care. There is diminished behavioral dimorphism in marmosets, and females, like males, engage in mating competition (Saltzman et al., 2008). In both captivity and the wild, female common marmosets engage in high levels of intrasexual competition, including likely pheromonal suppression of ovulation in subordinates, aggressive interactions between females, and infanticide (Digby, 1995; Saltzman et al., 2008). Common marmosets are cooperative breeders and all group members, including breeding males, carry and provision offspring (Digby et al., 2011; Garber et al., 2016). The energetic costs of infant care are high in common marmosets: females give birth to litters whose weight at birth is approximately 16.5% of maternal body weight (Tardif, 1994). Furthermore, females ovulate around 20 days post-partum and will conceive, meaning both males and females invest in mating effort during the same time period that infant care is a necessity (Tardif et al., 2003).

Due to the intense female intrasexual competition and the high degree of male investment in direct infant care, this species is of interest to questions about testosterone and behavior. Coupled with having overlapping sets of offspring, and females conceiving subsequent

pregnancies prior to weaning any current offspring, common marmosets are ideal to assess predictions about competition, parental behavior, and any associated trade-offs.

How my dissertation addresses theoretical gaps in T and Behavior.

Information on T in females and associated behaviors is still lacking in comparison to the rich knowledge accrued on males. This dissertation contributes to closing this gap by describing species-variation in T dimorphism, testing the assumption of a trade-off in behaviors that may differentially influence male and female reproductive success (specifically aggressive competition and parental care), and compares the relationship between T and mating and parenting behaviors in female and male captive common marmosets.

This dissertation is organized as three separate research article-style chapters, along with this introductory chapter and a conclusion (Chapter 5). Methods are described for each chapter individually.

Chapter 2: Testosterone Dimorphism in Primates

In Chapter 2, I ask if there is a relationship between female and male testosterone concentrations. I propose a model based on sexual selection theory, predicting decreased dimorphism in testosterone when decreased behavioral dimorphism is observed. I test this prediction within common marmosets because they show greater behavioral similarity between females and males than most other primates. I place these results in context of a primate-wide analysis of testosterone dimorphism. The conclusions have implications for sexual selection theory, showing that overlap between female and male T may correlate with a species' typical parenting and mating patterns.

Chapter 3: Testosterone in a biparental primate: Testing the Challenge Hypothesis in common marmosets.

Chapter 3 looks more closely at testosterone in common marmosets. I test the predictions laid out by the Challenge Hypothesis for species with overlapping sets of offspring, thus requiring individuals to quickly shift between mating and parental effort. I again compare testosterone in both sexes, but use the context of an intruder test to further gauge similarity between sexes in both T concentrations and the response to a mating challenge. Because the Challenge Hypothesis also predicts decreased T concentrations with infant care, I assess whether individuals providing more infant care have lower testosterone. I also test if adults with infants have lower testosterone levels than adults without infants. While the lack of difference between female and male hormone differences is seen in these data the strength of the correlation between behavior, the presence of infants, and testosterone raise questions regarding the validity of a central assumption of the Challenge Hypothesis: that mating effort and parental investment represent a trade-off.

Chapter 4: Male but not female captive common marmosets show a trade-off between aggression and parental care

This chapter tests the prediction that measures of infant care will negatively correlate with measures of mating effort. I use behavioral data to first assess behavioral dimorphism in aggression, sexual behavior, and infant care between females and males. Confirming the lack of behavioral dimorphism, I also assessed the proposed trade-off in each sex. I used both distance from infants (ranging from within contact to over three body lengths away from infants) and the

rate and quality of adults' interactions with infants. I also measured sexual behaviors including solicitations, mount attempts, and copulations directed toward focal animals' breeding partners or directed at individuals in other groups the focal animals had visual access to. To assess mating competition I looked at rates of aggression. Finding the predicted correlation between aggressive behavior and infant care in males, I narrowed my focus to aggression directed outside the home group. I found that females did not display the predicted correlation between infant care and measures of mating effort but that males did. I propose that in common marmosets, females may be minimizing infant care effort to the extent possible and that increased aggression may not result in decreased infant care in this sex.

Chapter 5: Conclusion

      In this chapter, I summarize my findings and discuss how they contribute to the growing body of literature on female testosterone. Future research directions are also discussed.

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CHAPTER 2: TESTOSTERONE DIMORPHISM IN PRIMATES

Abstract

Circulating testosterone is typically higher in males than females, but the relationship between male and female testosterone levels is poorly understood. In mammals, testosterone concentrations vary across species with mating and parental efforts. Testosterone generally rises during mating periods and falls when parental care is of interest. Primate species display considerable variation in the degree of mating competition and parental investment in each sex, and are therefore of interest when exploring testosterone dimorphism. We examine sexual dimorphism in testosterone concentrations in common marmosets (*Callithrix jacchus)*, characterized by male infant care and intense female mating competition. We measured female and male fecal testosterone concentrations in a captive group of common marmosets and found no significant difference between the sexes. We also conducted an analysis of sexual dimorphism in testosterone across all non-human primates. We found that published data on female testosterone concentrations is limited compared to the data available for males. However, sexual dimorphism in testosterone values was related to the degree of departure from the sexual selection model in which males primarily compete for mates and females primarily invest in offspring. Due to the low number of species available to for comparison, further research that systematically compares testosterone between sexes is encouraged.

Introduction

Sexual dimorphism is understood to occur due to a divergence in male and female reproductive strategies (Bateman, 1948; Trivers, 1972). Among mammals, male competition for mates is commonly implicated as the driving force behind dimorphic traits, such as dimorphism in body size or canine size (Plavcan, 2001). Some hormones are also sexually dimorphic, most notably those labelled as "sex steroids" that are primarily produced by the gonads. Researchers have linked one sex steroid, testosterone (T), to variation in mating competition and parental investment (Wingfield et al. 1990). T is argued to be one mediator of a trade-off between mating effort and parental investment as it is positively associated with male-male competition and negatively associated with male infant care (Archer, 2006; Gettler, McDade, Feranil, & Kuzawa, 2011; Gray, Parkin, & Sammsvaughan, 2007; Hirschenhauser & Oliveira, 2006; Ketterson & Nolan, Jr., 1999; Saino & Møller, 1995; Wingfield, Hegner, Dufty, & Ball, 1990; Ziegler, Prudom, & Zahed, 2009).

Testosterone levels are thought to be related to the divergent reproductive strategies between sexes (French, Mustoe, Cavanaugh, & Birnie, 2013; Hau, 2007; Wallen, 2005). However, the relationship between female and male testosterone concentrations is unclear, with contradictory results emerging. For instance, Goymann and Wingfield (2014a) found no relationship between T dimorphism in maximum T concentrations and mating systems or body-size dimorphism in free-ranging populations of birds. In contrast, Ketterson et al. (2005) found a relationship between female and male maximum T levels, with reduced T dimorphism occurring with reduced plumage dimorphism. They also found that female T was higher in monogamous species than in other mating systems, but did not control for phylogeny.

Goymann and Wingfield (2014a) also found a weak relationship between female and male T, and combined with the lack of a relationship between maximum female and maximum male testosterone correlating with mating system in their study, concluded that female T may not function in a behaviorally similar way to males. However, Garamszegi (2014) re-ran Goymann and Wingfield's (2014a) data using log-transformed rather than raw values, and used a framework considering that testosterone is not acted on independently in either sex. Garamszegi (2014) demonstrates a stronger relationship between female and male T than Goymann and Wingfield (2014a). In a response, Goymann and Wingfield (2014b) concede that they likely underestimated the relationship of female and male T but still question if one sex is constrained by the other or, alternatively, if each sex is responding separately to shared ecological pressures.

Neither the Goymann and Wingfield (2014a) nor Ketterson et al. (2005) analysis explicitly looked at parental investment as previous work had shown that in birds mating system influenced species-level variation in male T more than patterns of male provisioning of offspring (Hirschenhauser, Winkler, & Oliveira, 2003). However, mammals may not follow the same pattern as birds when it comes to the relationship between dimorphic testosterone concentrations, mating systems, and infant care, and an analysis of the relationship between female and male T concentrations may still be informative in mammals.

Previous work examining T dimorphism has not accounted for variation in offspring care and has been limited to birds (Goymann & Wingfield, 2014a; Ketterson et al., 2005). Mammalian females must, unlike birds, solely invest in the costs of pregnancy and lactation and cannot shift or share this burden with males (Lee, 1996). Despite this, mammals do not universally follow a behavioral pattern in which males compete heavily for mates and females are the primary providers of infant care. Primates are a useful mammalian order in which to

examine the relationship between female and male T concentrations, due to the diversity of mating systems and infant care patterns within the taxon, leading to different levels of sexual conflict between females and males (Stumpf, Martinez-Mota, Milich, Righini, & Shattuck, 2011). Behavioral dimorphism ranges from the typical pattern of males competing heavily for mates and females providing the bulk of infant care (e.g. capuchin monkeys: Fedigan, Carnegie, & Jack, 2008) to both sexes investing heavily in infant care and also competing for reproductive opportunities (e.g. callitrichines: L. J. Digby, Ferrari, & Saltzman, 2011; Gettler, 2010; Puts, Barndt, Welling, Dawood, & Burriss, 2011). This behavioral inconsistency across species make the primates an ideal order to study questions of T dimorphism.

We propose a model, building on the Challenge Hypothesis, in which intrasexual competition and parental investment (including alloparental care in cases where paternity is not assured) should be simultaneously evaluated, accounting for cases of increased male parental care or increased female intrasexual competition. Our hypothesis predicts that we will see a greater divergence of testosterone concentrations in primate species whose females provide the bulk of direct offspring care and experience low levels of intrasexual competition. In contrast, we expect to see more similarity in female and male T values when females experience intense intrasexual competition and share the burden of offspring care with adult males.

Prediction 1:

We examine testosterone dimorphism by comparing male and female T concentrations. We examine fecal T dimorphism in captive common marmosets (*Callithrix jacchus*). Common marmosets are characterized by a mating system in which females show intense intrasexual competition for a limited number of breeding spots and males contribute to direct infant care by

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carrying and provisioning offspring as early as day one post-natally (L. J. Digby et al., 2011; Tardif et al., 2003; Yamamoto, Arruda, Alencar, de Sousa, & Araujo, 2009). Due to increased female competition and increased male infant care in this species, we predict no difference in male and female fecal T concentrations.

Prediction 2:

We also examine patterns of testosterone dimorphism across primates and hypothesize that T dimorphism is related to behavioral dimorphism. We expect to see reduced T dimorphism (male and female testosterone values will be more similar to one another) in species with reduced behavioral dimorphism (females and males both compete for mates and contribute to infant care). Specifically, we predict the least divergence in female and male T concentrations in the cooperatively breeding callitrichines (L. J. Digby et al., 2011). We expect to see the most divergence in female and male T concentrations in species forming polygynandrous mating systems with heavy male-male competition, such as seen in the papionins.

Methods:

Study Subjects

We examined female and male fecal T concentrations in a captive colony of common marmosets (*Callithrix jacchus*). Samples came from adult subjects that were older than 18 months at the breeding colony of common marmosets housed at the Southwest National Primate Research Center in San Antonio, Texas, U.S.A., a USDA approved facility (N = 24 females; 33 males). Groups were comprised of an adult (breeding) female, an adult (breeding) male, and any offspring of one or both adults. Marmosets were housed on a 12 hour light-dark cycle. Home

cage dimensions were 1.5 x 1.83 x 0.92 meters. Cages contained two nest boxes and a minimum of two food dishes (and up to six food dishes depending on family size) and two water bottles. Climbing substrates included mesh, nontoxic plastic pipes, and tree branches. Animals received varying enrichment items daily, including food enrichment, puzzle feeders, novel objects, and scent enrichment. Further details on husbandry procedures have been previously described (Layne & Power, 2003).

Hormone Assays

To individually identify fecal samples, fecal markers (green or red food coloring) were fed to focal individuals between 3:30 and 5:00 PM and plastic sheeting was placed underneath the home cage. Fecal markers were visible within 12 hours and up to 48 hours after being fed to the focal individual. Fecal samples were collected first thing the following morning between 8:00 AM and 10:00 AM.

To measure steroid hormone concentrations, we followed Nunes et al. (2000). Samples underwent an extraction step prior to hormonal assays. For each sample, feces were dried in an incubator set at 37°C, ground up using a mortar and pestle, and 5 mL of methanol solubalizer was then added to the sample. Samples were shaken vigorously for 4 hours, and then centrifuged for 20 minutes at a speed of 2,500 RPM. The supernatant was then used in hormonal assays. ELISA assay plates were coated with a T antibody ordered from Coralie Munroe (University of California, Davis) at a dilution of 1:25,000 and allowed to incubate a minimum of 18 hours. Plates were then washed 3 times using BioTek ELx50 and gently tapped to remove any excess liquid. Phosphate Buffer Saline was added across the plate. Samples, standards, and controls were added in 50uL volume followed by 50uL of a labeled T hormone conjugate in a 1:30,000

dilution. Plates were incubated for a minimum of 90 minutes at room temperature. Plates were again washed three times, and an ABTS-based chromagen was added to each well. Plates were read using BioTek ELx808 once the blank wells reached an absorbance level at 405 nm of 0.8-1.0. Samples were run in duplicate with a coefficient of variance ranging from 0.0 - 14.9%.

Literature Review

We conducted a literature review of all non-human primates using ISI Web of Science, searching through May 2017. We searched for references containing both the term "testosterone" and the genus name in the "Topic" category. We were interested in articles reporting normally occurring T concentrations in captive, free-ranging, or wild populations of primates. Of the articles that contained empirical data on systemic T values (rather than in-vitro measures of a specific tissue, or indirect measures of T, such as digit ratio), we excluded those that only reported the effects of experimentally altered prenatal androgen exposure (e.g. Herman & Wallen, 2007), looked only at immature, pregnant, or non-cycling females (e.g Prall et al., 2015), relied solely on gonadectomized individuals or those that received exogenous hormones (e.g. Phoenix & Chambers 1982), or a combination of these procedures (e.g. Kendrick & Dixson, 1985, Hagger and Bachevalier, 1991).

Prior to our exclusion criteria, there were 412 research papers reporting testosterone data in males only, compared to 62 papers reporting T data on females alone or in both sexes (a total of 13% of the published literature). Sixteen primate genera were represented in female T studies compared to 36 genera in male-only studies. Following our exclusion criteria, the remaining publications were still heavily male-biased, with only 44 articles containing female data (11% of all articles), compared with 331 articles containing data from males only (Figure 2.1). We had to

further limit our criteria to match data from females with that of males 1) of the same species and 2) that used the same type of biological sampling (e.g. feces, urine, serum, or plasma) and assay method, when a comparison to males was not available within the same article. This left only 30 research articles containing female T data that could be compared with male values, either in the same publication or in another work that used comparable methods, representing a total of 16 species from 11 genera.

In cases where data were reported as part of an experimental protocol in which T values were altered or manipulated (e.g. Appt et al., 2010), we used only control or baseline values to avoid reporting manipulated endocrine values. If control values were not available, the article was excluded. Some data were reported only using graphs; the values from these graphs were approximated using ImageJ (Schneider et al., 2012).

Statistical Analyses

Statistics were run using SPSS Statistics Version 24. In all analyses, significance was set at $P = 0.05$. Hormone data from the captive colony at SNPRC did not conform to normality, and a Mann-Whitney U test was used to compare female and male fecal T concentrations from our captive colony.

We analyzed data from the literature review in primate-wide analyses. We examined mean male and female values, along with the ranges reported for each species. To investigate variation in overlap between male and female hormones we also examined the ratio of the lowest male value to the highest female value for a given species.

We predicted greater similarity in female and male testosterone concentrations in species whose mating systems departed from the model of intense male intrasexual competition and

female-only investment in offspring, when compared to species whose behavior matched this model. The full list of species for which we had data is available in Table 2.2. We ran correlative analyses using Kendall's tau to assess predicted similarity between sexes of specific species with observed ratios of Male:Female T values.

Results

Prediction 1: Captive common marmosets will show no difference in male and female fecal T concentrations.

Adult female and male captive common marmosets (*Callithrix jacchus*) did not differ in their fecal testosterone concentrations. (Mann-Whitney U = 335.00, Z = -0.96, P = 0.324, N = 24 females; 33 males, Figure 2.2, Table 2.1).

Prediction 2: T dimorphism will be reduced in species with reduced behavioral dimorphism as predicted by mating system.

Data across primate species are available in Table 2.2. Among published data, we found the most similar values in callitrichid female and male testosterone concentrations, including the current study. We found a greater difference in T concentrations between female and male mandrills than between the callitrichids.

The ratio of Mean Male:Mean Female T values showed a statistically meaningful correlation ($\tau = 0.42$, P = 0.03, N = 15 species). The ratio of Highest Male:Highest Female T value did not correlate with mating system ($\tau = 0.32$, P = 0.44, N = 9 species). We also examined the highest female T value for each species using an ANCOVA with lowest male T value and

mating system as co-variates, in order to compare the degree of overlap between sexes in T

concentrations. The relationship with mating system was non-significant (F = 4.24, P = 0.09, N =

9 species, Figure 2.3), although the observed power of the test was low (β = 0.39). Given the

small number of available species we also qualitatively assessed the results from each taxon.

Platyrrhines:

     Callitrichids: Saguinus and Callithrix. The current study saw no statistical difference in

female or male mean T concentrations in *C. jacchus,* and the range of T concentrations

overlapped considerably. Similarly, the range of female and male T concentrations overlapped in

Saguinus oedipus, but the mean fecal T was slightly higher in males than in females (Fontani et

al., 2014). We also included data from *Callithrix kuhlii*, from a series of studies investigating T

concentrations during late pregnancy and the early post-partum period (Fite et al., 2005; Nunn et

al. 2000, 2001). The mean female and male T values were different with males showing

significantly higher T concentrations than females during this period. The callitrichids are

characterized by heavy female competition for breeding positions (L. J. Digby et al., 2011).

Females are capable of suppressing ovulation in subordinate females and will exhibit aggression

toward subordinate females should hormonal suppression fail (Digby & Saltzman, 2009;

Yamamoto et al., 2009). In all callitrichids, males provide infant care by carrying and

provisioning infants, and the presence of male helpers is vital to the survival of offspring

(Garber, 1997). This pattern of heavy female-female competition and both sexes investing in

infant care led us to predict similar T concentrations in female and males. These taxa fit our

prediction when examining range data in both sexes. *Saguinus* showed a slightly larger

difference in Mean Male:Mean Female than *Callithrix jacchus* and the difference in *Saguinus*

was statistically significant whereas it was not within the current study. Data from *C. kuhlii* show a larger mean difference in female and male T than the current study, but still smaller than the mean difference between *Alouatta* females and males.

Aotus: Owl monkeys are classified as serial, social monogamists, with a variable operational sex ratio that depends on the presence of "floating" single adults looking for a breeding partner (Fernandez-Duque & Huck, 2013). Both sexes invest heavily in infant care and male investment includes provisioning and carrying of offspring (Fernandez-Duque & Huck, 2013). Based on similar levels of intrasexual competition for mates and infant care between sexes, we predicted that *Aotus* would show similar T concentrations between females and males compared to other species except the callitrichines. We were able to assess the range of values within *Aotus,* but not means for each sex. While *Aotus* T concentrations did not display a complete overlap, as in *C. jacchus,* we did see greater similarity between *A. triverigatus* females and males than the other non-callitrichid species for which a range was available: *Brachyteles hypoxanthus*. The ranges of T between sexes only overlapped slightly in *A. triverigatus*, but these values did not include one female from Bonney et al. (1980) whose peak T value was 9.5 ng/mL plasma and was judged in the original work to be an outlier in a sample size of 4. Including this female's data point increases the overlap between Bonney et al.'s (1980) female T values and Dixson and Gardener's (1981) male T values. However, the data on males came from Dixson and Gardener (1981) who looked at both nocturnal (active period) and diurnal (inactive period) T levels, finding T concentrations were higher during the diurnal period compared to nocturnal samples. In contrast, Bonney et al. (1980) took female samples only between 10:00 AM and 1:00 PM, when the lights were on (the diurnal, inactive period). If female *Aotus* T

secretion follows the same diurnal pattern seen in males, then this methodological difference should not impact the conclusions we have drawn regarding T between the sexes, however we do not have sufficient data on females to adequately compare the range of T between the sexes. Female T concentrations are expected to be lower at night based on male patterns. However, the diurnal pattern of T secretion in *Aotus* females has not been documented, and a direct comparison between sexes within the same study would be useful.

Brachyteles: Data on ranges and means were available for *Brachyteles*. Strier et al.'s (2003) and Strier and Ziegler's (2005) data show higher male than female T concentrations. Strier et al. (2003) sampled subjects weekly, and 10 out of 21 weeks measured showed a statistical difference between sexes T concentrations. *Brachyteles* form polygynandrous groups, yet males do not harass each other when mating, and the mating system has been described as "egalitarian" (Strier, 1997). Females are the primary caregivers for offspring (Strier 1997). This taxon fits our prediction when looking at the range of T values for each sex. The ratio of Mean Male:Mean Female T falls between *Callithrix* and *Saguinus,* deviating from our prediction, but below *Alouatta* and *Cebus* which we predicted would be higher than *Brachyteles*.

Cebus: *Cebus capucinus* showed the greatest difference between mean female and male T values among primates, although data on the range of T concentrations were not available. Like *Brachyteles*, *Cebus* also forms polygynandrous groups, but unlike *Brachyteles*, there is a strong reproductive skew favoring the dominant males, and males may be evicted from their social groups by new adult males looking to join a group (Sargeant, 2014). Males will play with and tolerate infants, but females are the primary caregivers (Sargeant, 2014). Based on high levels of

male-male competition and primary offspring care by females we predicted the greatest

difference between female and male T concentrations in *Cebus* compared to the other species we

investigated for which means were available (both callitrichids, *Brachyteles,* and *Alouatta,* but

not *Aotus*), and this prediction was supported. However, because ranges were not available, it is

not possible to compare *Cebus* to any pattern of overlapping T concentrations within

platyrrhines.

Catarrhines

Pan:

     Pan paniscus: Bonobos showed the most overlap between female and male T

concentration within the catarrhines. The ratio of Mean Male:Mean Female T values was also

lower for bonobos than other catarrhines. Bonobos display a polygynandrous mating system with

low levels of aggressive competition for mating partners, and with females as the dominant sex

and primary providers of infant care (Stumpf, 2011). Due to the similarity between sexes in

intrasexual competition, we expected the greatest similarity between female and male bonobo T

concentrations within the catarrhines for whom we had data. This prediction was supported.

     Pan troglodytes: Chimpanzees females and males exhibit greater divergence in T

concentrations, than bonobos, although the degree of separation differed between studies (Table

2.2, Figure 2.3). The ratio of Mean Male:Mean Female T values in chimpanzees also was much

higher than in the closely related bonobo. There are important social differences between the two

species. Unlike bonobos, males are the dominant sex in chimpanzees and experience intense

intrasexual mating competition (Stumpf, 2011). As seen in the other catarrhines examined,

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chimpanzee males do not typically provide direct infant care; females are the primary caregivers for offspring (Hayashi & Matsuzawa, 2017). Based on mating system, we expected some divergence between female and male T concentrations in chimpanzees. This prediction was supported.

Mandrillus sphynx: Mandrills have a social system in which males compete heavily for mates and exhibit a heavy reproductive skew in favor of dominant males (Setchell, Charpentier, & Wickings, 2005). Females are also the primary caretakers of offspring (Setchell, 2016). We predicted divergence between sexes in T concentrations in mandrills. We saw the largest divergence in mandrills from any species for which we had data on the range of female and male hormone values. The ratio of Mean Male:Mean Female T values in this species was also higher than any other catarrhine, and only exceeded by *Cebus capucinus*.

Papio cynocephalus: Yellow baboons, like other catarrhines, exhibit male competition for mates and female care of offspring (Altmann & Samuels, 1992). We predicted divergence in male and female T concentrations in this species. We had both fecal and plasma data to compare. The fecal data are represented by two studies: Gesquiere et al. (2014), and Stavisky et al. (1995). Gesquiere et al. (2014) directly compared fecal T in female and male baboons. They found a higher average T concentration in the females than males (females 133.43 ng/g feces; males 89.00 ng/g feces) and concluded that fecal samples were not a reliable way to assess sex-differences in androgens in this species (Gesquiere et al., 2014). The data from Castracane et al. (1986) and Castracane and Goldzieher (1983) show a clear distinction between male and female mean plasma T with males having much higher values than females, in line with what would be

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expected based on their behavior. Therefore, Gesquiere et al.'s (2014) reservations about using feces to assess T concentrations in baboons have support. However, Stavisky et al. (1995) report a much lower fecal T concentration in free-ranging baboons than Gesquiere et al. (2014). Additional fecal data on female *P. cynocephalus* were not available to compare against these two papers. However, in a study of wild hybrid baboons (*Papio hamadryas hamadrys* x *Papio hamadryas anubis*), Beehner et al. (2005) report average individual female fecal T values ranging from 6.47 ng/g to 58.56 ng/g feces, which is similar to the *P. cynocephalus* data Stavisky et al. (1995) provide and lower than in seen in the wild population of *P. cynocephalus* sampled by Gesquiere et al. (2014). However, a comparison within the same species would better shed light on the range of fecal T concentrations in this species. Furthermore, Stavisky et al. (1995) are heavily limited by only reporting the raw values for one cycling female, especially when compared to the robust sample size used by Gesquiere et al. (2014).

Macaca: All macaque species follow a general pattern of female infant care and male dominance and competition for mates, although the degree of male competition may vary (Thierry, 2011). We found comparable and normally occurring T data for both sexes in only three species of macaques, with means reported, but not ranges in all cases. Across macaque species, male T concentrations were higher than females, which generally fits our predictions.

Macaca arctoides: The mean male T concentration was over four times higher than that of females in stump-tailed macaques, in a study publishing T values for both sexes (Rhodes et al., 1994). This ratio is lower than we saw in *M. fasicularis*, although both species have a dominance-based reproductive skew in males.

Macaca fascicularis: In the only study publishing both female and male T values for long-tailed macaques (Malaivijitnond, Hamada, Suryobroto, & Takenaka, 2007), the mean male T concentration was far greater than that of females (Table 2.2). This ratio is higher than seen in the *M. arctoides* data, although when we examine the data from the remaining individual studies, mean T ratios ranged from a 4- to 34-fold difference between male and female values. Having ranges to compare between sexes would be greatly informative in both long-tailed and stump-tailed macaques.

Macaca mulatta: Rhesus macaques do not display the same male reproductive skew as seen in long- and stump-tailed macaques. Using data from multiple studies, we saw male T concentrations were about 10 times higher than females. As with the other macaque species, data on the range of values would be informative, although the clear dimorphism between sexes fits our prediction.

Strepsirhines

Lemur catta: Male ring-tailed lemurs had higher T concentrations than females. While Drea's (2007, 2011) data show a small degree of overlap between females and males in this species, the male range of T concentrations is much larger than the female. Drea's data also show a three-fold difference between female and male mean T concentrations. This is in disagreement with Tennenhouse (2017), whose data show more similarity between female and male mean T concentrations. However, Tennenhouse (2017) used hair to measure hormone concentrations, whereas Drea (2007, 2011) used plasma. We predicted similarity between female and male T

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concentrations in ring-tailed lemurs, based on their mating system. Females are dominant in this species, and they display a polygynandrous mating system, and there is typically low reproductive skew among males (Parga et al., 2016). We therefore expected overlap between female and male T values. Drea's (2007, 2011) data did not support this prediction, but Tennenhouse's (2017) was supportive of our prediction.

Microcebus: Similar to ring-tailed lemurs, mouse lemurs are female-dominant but with a dispersed polygynandrous mating system (Atsalis, 2000; Zohdy et al., 2014). Therefore, we expected to see more similarity between female and male T concentrations in *Microcebus rufus* than in the ring-tailed lemur. Female and male mean T concentrations were similar, therefore not supporting this prediction.

Discussion

This study compares testosterone concentrations in female and male primates. We hypothesized that female and male common marmosets (*Callithrix jacchus*) would have similar T concentrations due to a mating system with behavioral overlap between the sexes in both infant care and reproductive competition. We found no sex difference in fecal T concentrations in our sample of adult, captive common marmosets. We also proposed that in species with behavioral overlap between females and males (both sexes compete for mates, both invest similarly in offspring), we would see more similar T levels between the sexes than in species with stronger behavioral dimorphism. Compared to other primates, we found the greatest similarities in female and male T concentrations in *Callithrix jacchus* and *Saguinus oedipus*. Both are cooperative breeders with a limited number of females breeding, leading to high levels

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of female intrasexual competition compared to other primate species. We saw the least overlap in *Mandrillus sphynx,* polygynandrous breeders with a heavy reproductive skew in males, and show little male investment in offspring. Mating system was significantly correlated with the ratio of Mean Male:Mean Female testosterone. We saw a non-significant relationship between the highest female and lowest male T concentrations when controlling for mating system.

Limitations

Our hypothesis that T dimorphism varies with mating system in primates was marginally supported, with strong caveats. There are limits to the current study that effect strength of the conclusions, and that point to interpretations not of a strong relationship between T dimorphism and mating system, but rather warranting continued exploration of this research area. Many studies only reported the mean T value for their population. Therefore, it is likely we are not capturing the full range of variation in hormone levels for these species. Goymann and Wingfield (2014a) made a similar argument against using a single measurement type, the maximum value, to compare female and male T values in birds. The current study examines the means, maximums, and minimums when possible to capture more variation than just the maximum value. Another valuable approach to studying T in both sexes is to examine the range of values produced by each sex rather than only the maximum or the mean. When possible, we evaluated the range of both female and male hormone concentrations, but for many species this was not possible.

We also examined the relationship of lowest male and highest female T concentrations, although due to the small number of species available the statistical power for this test was low. If significance is defined as $P = 0.10$ then a significant relationship was seen with mating system.

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More data across primates are needed to fully test whether the range of overlap between female and male T is related to mating system, but these data suggest the relationship exists. This raises the question of whether the lowest male value is biologically relevant or if it represents pathological individuals. Within the marmoset data, the male with the lowest value is unlikely to represent a pathology given that he was 2 years old and displayed a wide range of fecal T concentrations, including a peak of 2,888 ng/g dry feces when placed with a new breeding female. We also excluded any results from the literature review whose methods relied on artificially changing hormone values, including methods that would depress testosterone either through castration, androgen blockers, or as part of a stress study. Therefore, the data should represent values within the biologically relevant range.

However, the greatest challenge and limitation in addressing this research is the low availability of testosterone data for both sexes within the same species, and especially within the same study. This is further compounded by the lack of serum or plasma data available across primates. None of the plasma or serum data presented here in our dataset show comparable T concentrations between sexes. Fecal samples are often used as a non-invasive way to measure steroid hormones, due to their ability to non-invasively reflect changes in circulating hormones (Bishop & Hall, 1991, Brown et al., 1996, Cockrem & Rounce, 1994, Reslir, Wasser, & Sackett, 1987, Ziegler, Sholl, Scheffler, Haggerty, & Lasley, 1989). Fecal samples have been used to answer questions about primate endocrinology in both captive and wild settings (e.g. Beehner et al., 2005, Drea, 2007, Fontani et al., 2014, Muehlenbein 2004, Muller and Wrangham 2004). However, Gesquiere et al. (2014) concluded this method was not appropriate for sex comparisons in *Papio cynocephalus* whose females displayed statistically higher fecal T concentrations than the males in the same study. Gesquiere et al. (2014) followed a validation

technique that compares expected differences between groups (juveniles should have lower T than adults of the same sex, females should have lower T than males) with assay results. Their data contradicted these expectations, as female fecal T was statistically higher than males, and they conclude that female and male baboons metabolize testosterone differently. This conclusion is supported by plasma data in the same species (Castracane et al. 1986; Castracane and Goldzieher, 1983).

This raises the question as to the validity of any fecal data when examining testosterone. If we use the same validation criteria as Gesquiere et al. (2014) for the other species for which we had fecal data, *Lemur catta, Mandrillus sphynx, Cebus capucinus, Alouatta pigra, Brachyteles hypoxanthus,* and *Saguinus oedipus* all displayed lower female than male fecal T as would be expected. *Callithrix jacchus* and *Microcebus rufus* displayed statistically similar female and male fecal T. There are two explanations for these cases: either, like *Papio cynocephalus*, fecal testosterone cannot be compared between the sexes in these species, or these data represent genuine similarity between the sexes' circulating testosterone.

There are cases in which circulating T has been shown to be similar in females and males, although these are rare and can be context dependent. Serum T concentrations in Abert's Towhee sparrows show a context in which female serum T is equivalent or higher than male T (Davies et al., 2016a). Specifically, their sample of 9 females and 36 males showed non-significant differences in plasma T during an initial (non-stress condition) blood draw (female average is approximately 1.68 ng/mL plasma; male average is approximately 2.86 ng/mL plasma). The following blood draw (stressed condition) showed raw T values in females slightly higher than those of males (female average approximately 1.42 ng/mL; male average approximately 1.14 ng/mL), driven by a drop in male testosterone. Plasma androgen

concentrations in female bell miners (a type of honeyeater bird) are also similar to males (Poiani & Fletcher, 1994). This species displays cooperative breeding in which groups contain a single breeding female along with other non-breeding females and high levels of female intrasexual competition (Poiani & Fletcher, 1994). In a sample of 4 breeding females compared to 11 breeding males, females averaged 654 pg/mL and males averaged 571.9pg/mL (Poiani & Fletcher, 1994). The same study also measured circulating androgens in adult, non-breeding females and males, with a similar result: 8 females averaged 514.1 pg/mL and 17 males averaged 304.2 pg/mL. Davies et al. (2016b) sampled serum testosterone in a wild population of meerkats. They compared dominant and subordinate females to dominant and subordinate males. Serum T in 8 dominant females was statistically equivalent to serum T in 12 dominant and 31 subordinate males. Serum T in 33 subordinate females was significantly lower than in the dominant females. Subordinate females also had statistically lower T than subordinate, but not dominant males. Dominant and subordinate males had statistically similar T values to each other. Davies et al. (2016b) also compared fecal androgen data with their serum results from both T and androstenedione (an androgenic pre-cursor to T). Due to the similarity of patterns between serum and fecal androgens they conclude the patterns are the same, although they did not distinguish between T and androstenedione in their fecal assay. However, it is biologically possible for there to be overlapping ranges of testosterone in each sex, even if rare, and for fecal data to be usable in these cases. Therefore, dismissing the statistically equivalent data between sexes from *Callithrix* and *Microcebus* as biologically irrelevant is not warranted. However, future work should also use serum or plasma concentrations to validate these findings.

While there are logistical constraints to studying small-bodied, nocturnal primates as is common in strepsirhines, catarrhines are commonly studied in captivity and the wild and hormone information is easily obtained. Our model compares behavioral variation to hormonal variation across species, yet there were data available for only a single catarrhine that did not follow the same basic behavior pattern of female investment in offspring and male competition for mates: the bonobo. Having only a single species diverging from the general behavior patterns in catarrhines limits our statistical ability to assess behavior-hormone relationships across species, although we did see a strong correlation with our data set. In line with our model, bonobos showed greater similarity in female and male T values when compared to the closely related chimpanzees (*Pan troglodytes*) and to other catarrhines.

Testosterone is often viewed as a male hormone because the testes are the primary source of T in males and it is associated with male competitive behavior and infant care. This characterization persists, in spite of the facts that females also produce T, T conversion to estradiol influences behavior and, across mammals and other vertebrates, there is considerable variation in male-male competition, female-female competition, and the degree to which each sex invests in offspring, including sex-role reversals in which males provide the majority of infant care and females compete heavily for mates (Eens & Pinxten, 2000; Lephart, 1996; Ubuka & Tsutsui, 2014). Furthermore, T is pleiotropic and it is unlikely that selection acting on testosterone in one sex is fully independent of the other, although mechanisms to alleviate constraints likely exist (Iserbyt, Eens, & Müller, 2015; E. D. Ketterson, 2014; Mokkonen & Crespi, 2015). Female and male T has been compared in wild rock hyraxes, whose females engage in greater levels of dominance-related aggression than males, and are cooperative

breeders, sharing the burden of offspring care (Koren, Mokady, & Geffen, 2006). Female rock hyraxes exhibit fecal and hair T concentrations equivalent to or higher than males (Koren & Geffen, 2009; Koren et al., 2006). Studies have raised concerns over potentially dimorphic metabolism of steroid hormone clearance rates and patterns when substances other than blood are used during assay that would lead to spuriously results between females and males (e.g. Gesquiere et al., 2014). Therefore, while androgens are traditionally viewed as "male" hormones, given the current information on steroidogenesis and increasing reports of female T (and other androgen) values, this is no longer a tenable description of testosterone or other androgens.

Researchers are beginning to examine the relationship between testosterone and both mating competition and infant care behaviors in females (e.g. Fite et al., 2005; Melber et al., 2015). For instance, captive female Wied's marmosets (*Callithrix kuhlii*) display a positive relationship between T and both within-group sexual behavior and the amount of aggression received from a same-sex aggressor (Ross & French, 2011). In humans, single women and those in polyamorous relationships have higher T than women in committed monogamous relationships (Barrett et al., 2013; Edelstein, Chopik, & Kean, 2011). Further, females that are investing heavily in the care of young offspring have lower T than those who either do not have any offspring or whose offspring are older and less dependent than infants (Barrett et al., 2013; Gettler & Oka, 2016; Kuzawa, Gettler, Huang, & McDade, 2010).

We tested a model proposing that when mating and parenting behaviors are considered simultaneously, primate species with low behavioral dimorphism in both parental care and mating competition should also show low testosterone dimorphism. Overall, our results imply that, when viewed across species, testosterone may have a relationship with behavior in both sexes, and that testosterone dimorphism may be related to behavioral mating and parenting

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system. However, more direct comparisons of T in both sexes are needed, as seen by the low availability of these data within primates, especially for plasma or serum data which represent circulating testosterone concentrations rather than metabolites. In particular, more data on the callitrichids and *Microcebus* are of interest, given that all showed similar T concentrations between sexes, yet were ranked at the opposite ends of our behavioral continuum. Given that primate males interact with and care for infants (even when females provide the bulk of care as is seen in many species) and that female mating competition exists, future studies are needed to simultaneously examine the underlying hormonal mechanisms of these behaviors in both sexes. We urge more research with catarrhines in particular, as it would be useful to have data directly comparing T values in females and males from species such as barbary macaques (*Macaca sylvanus*), whose males sometimes provide allocare (Rincon, Maréchal, Semple, Majolo, & MacLarnon, 2017), and siamangs (*Symphalangus syndactylus*), who form socially monogamous groups and whose males heavily invest in offspring (Rafacz, Margulis, & Santymire, 2012). These species would help round out the comparative sample available for catarrhine primates and validate the correlation we observed. We encourage more research on strepsirhines, whose data are limited in part due to the logistical constraints of studying small-bodied and nocturnal primates. Having serum or plasma data from *Microcebus* is also necessary to explore why this species shows such similarity between female and male T concentrations. We also urge researchers to directly compare female and male T concentrations within the same study, and to examine the range of hormone values for each sex rather than a single variable, such as the mean or maximum observed T value. This will allow for a more robust test of our model, and is vital to understanding the evolution of female and male testosterone secretion.
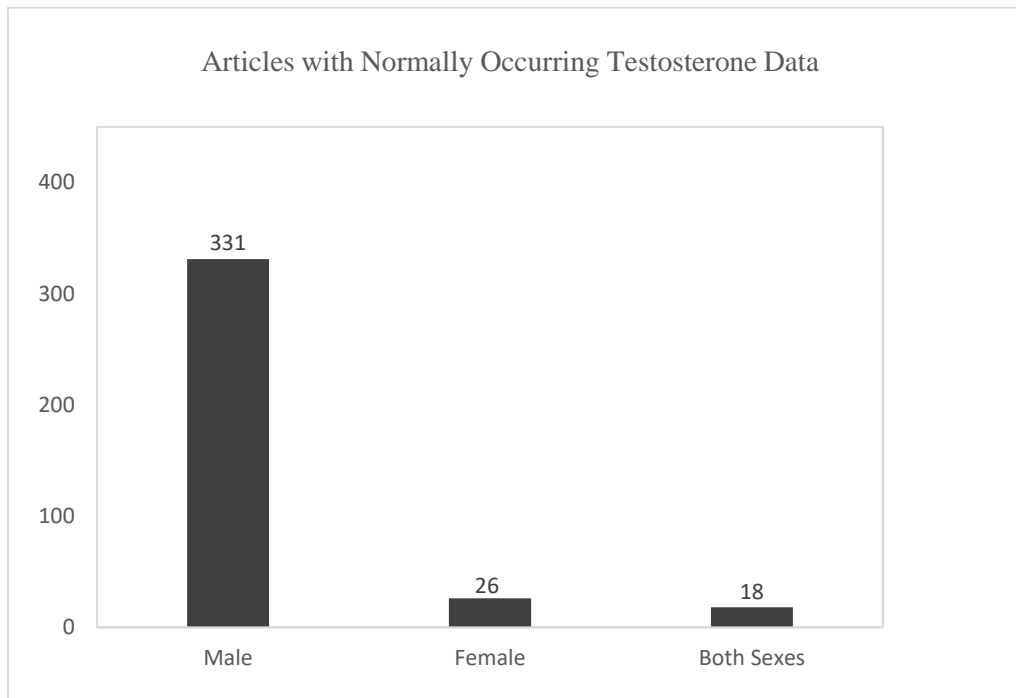
Figures and Tables

Articles with Normally Occurring Testosterone Data

Figure 2.1: Number of published research articles reporting data on normally occurring T values, by sex, in non-human primates.
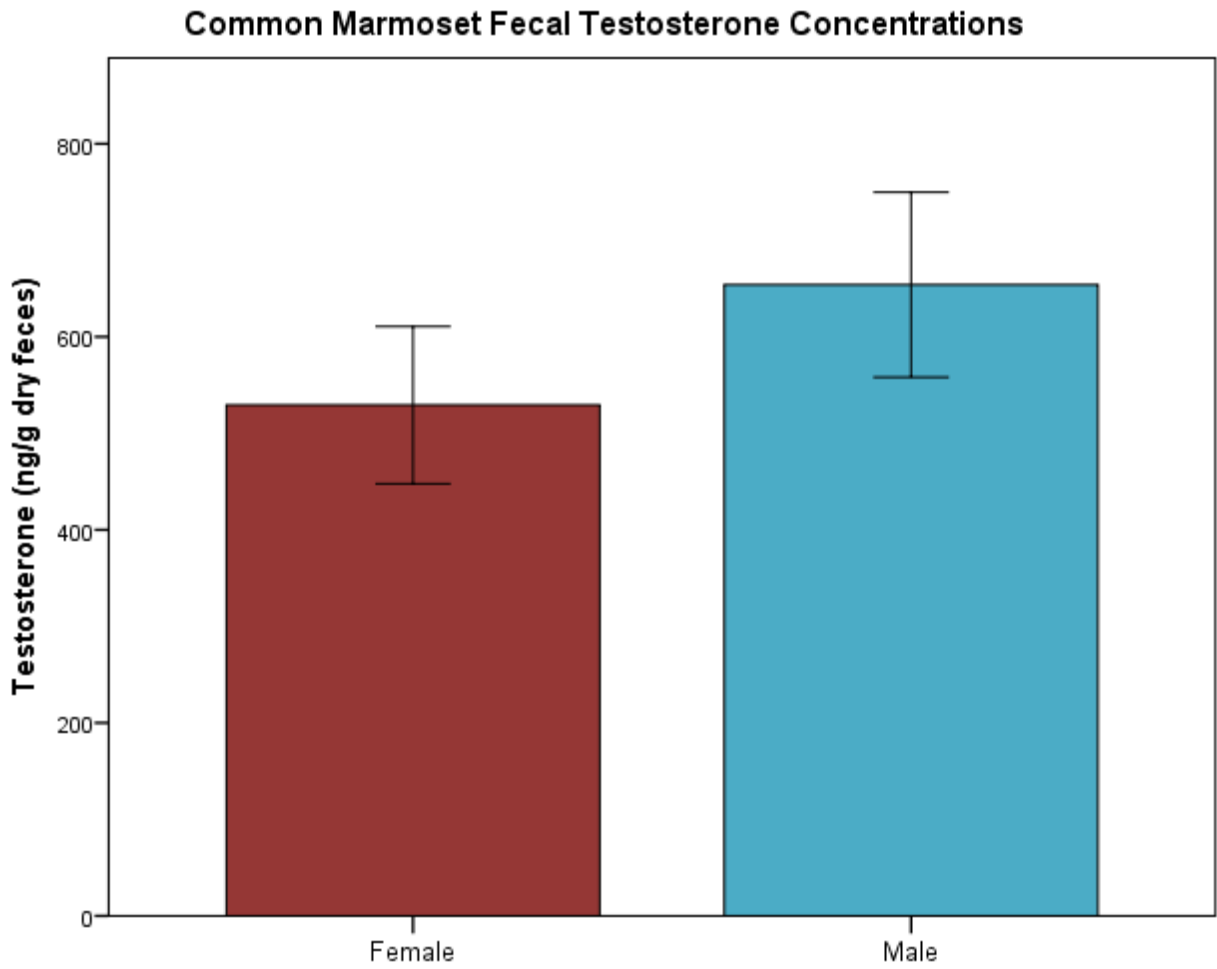
Figure 2.2: Mean fecal T concentrations from captive common marmosets. Bars represent the standard error.
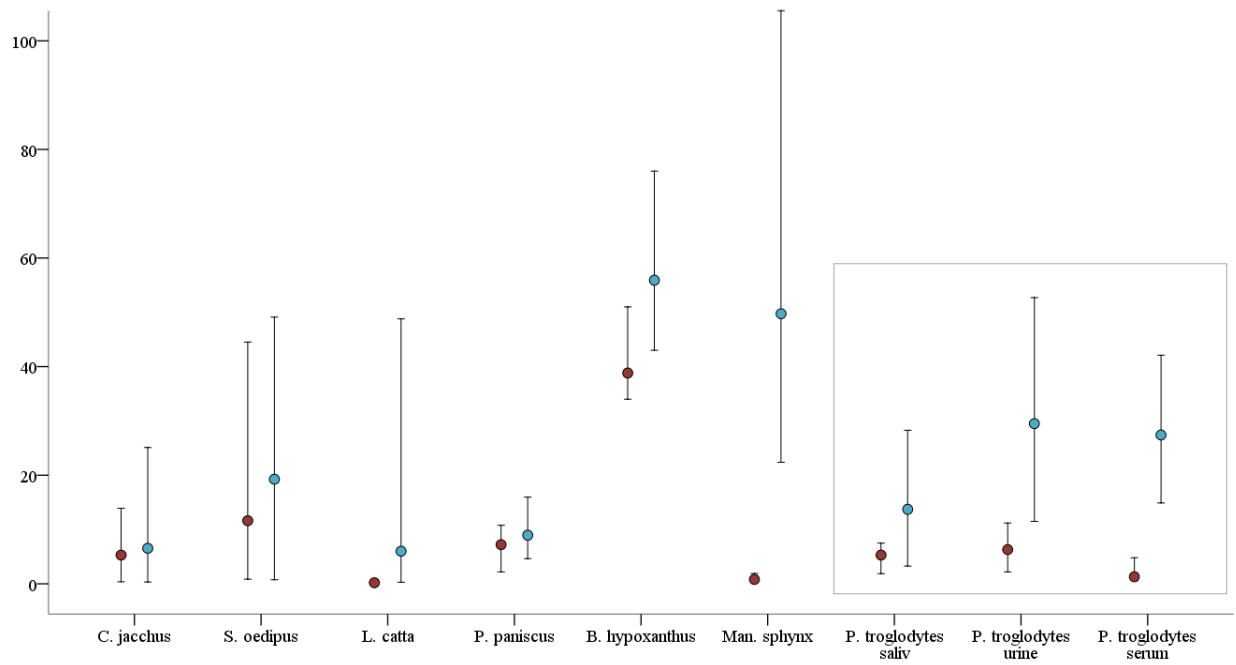
Figure 2.3: Cross species comparisons of female (dark red) and male (light blue) means and ranges of testosterone. For visual clarity, the scale of each species' data has been adjusted by changing the units of measure. Original hormone data can be found in Table 2.2. Species are ordered left to right by mating system. Missing species did not have published means or ranges available. *P. troglodytes* data comes from multiple sources with 3 sampling methods, and therefore were not combined.

Table 2.1: Female and male captive common marmoset fecal T concentrations (ng/g dry feces).

	Median	Average ± Standard Error of the Mean	95% Confidence Interval of the Mean	Sample Size
Female	403.11	529.30 ± 81.42	360.87-697.73	24
Male	534.07	654.03 ± 95.82	458.85-849.22	33

Table 2.2: Female and male T concentrations in non-human primates. When data were not available from both sexes within the same publication, male studies were matched to female studies based on sampling and assay methods.

Species	Female T values	Male T Values	Patterns of infant care	Patterns of competition	Dominant Sex	References
Callithrix jacchus	37.81 - 1,392 ng/g (range), 529.30 ng/g (mean) feces (N = 24)	34.09 - 2,510.57 ng/g (range), 654.03 ng/g (mean) feces (N = 33)	Both sexes invest in infants	Cooperative breeding mating systems are variable with monogamy, polyandry, and polygyny observed. Females compete intensely for limited breeding positions.	Context-dependent	Current study
Callithrix kuhlii *	≈ 378.68 (mean) ng/mg Creatinine (urine) (N = 6 females, 1-9 weeks post-partum)	≈ 729 (mean) ng/mg Creatinine (urine) (N = 12 males, weeks 1-8 post-partum) / 2,572 (mean) ng/mg Creatinine (urine) (N = 9 males, weeks 1-8 post-partum)			Context-dependent	Fite et al., 2005*; Nunes et al. 2000, 2001
Saguinus oedipus	≈ 8.47 - 445.11 pg/g (range), 116.24 pg/g (mean) feces (N = 7)	≈ 7.53 - 491.22 pg/g (range), 192.62 pg/g (mean) feces (N = 7)			Context-dependent	Fontani et al. 2014
Aotus trivergatus	0.35 - 4.71 ng/mL (range) plasma, means not published (N = 4)	4.7 - 24.8 ng/mL plasma (range), means not published (N = 6)	Both sexes invest in infants	Both sexes must defend socially monogamous breeding position from extra-pair individuals.	Context-dependent	Bonney et al., 1980; Dixson and Gardener, 1981
Brachyteles hypoxanthus	≈ 34 - 51 ng/g (range), 38.82 ng/g feces (mean) (N = 5)	≈ 43 - 76 ng/g (range), 54.2 - 57.6 ng/g (means) feces (N =6)	Females are primary caregivers of offspring.	Polygynandrous mating system, but males do not heavily compete for mating opportunities	Co-dominant	Strier et al., 2003; Strier & Ziegler, 2005
Alouatta pigra	ranges not published, 659.1 ng/g (mean) feces (N = 8)	ranges not published, 4,485.6 ng/g (mean) feces (N = 8)	Females are primary caregivers of offspring.	Uni- and multi-male, multi-female mating system. Group takeovers by males possible. Male infanticides reported.	Males	Rangel-Negrin et al . 2014
Cebus capucinus	ranges not published, 3.6 ng/g feces (median) (N = 10)	ranges not published, 840 ng/g feces (median) (N = 10)	Females are primary caregivers of offspring.	Polygynandrous mating with high levels of male-male competition.	Males	Weltring et al., 2012

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Table 2.2 (cont.)

Species	Female T values	Male T Values	Patterns of infant care	Patterns of competition	Dominant Sex	References
Pan troglodytes	2.2 - 11.2 ng/mg Cr (range), 6.3 ng/mg Cr (median) urine (N = 15)	11.5 - 52.7 ng/mg Cr (range), 29.5 ng/mg Cr (median) urine (N = 10)	Females are primary caregivers of offspring.	Polygynandrous	Males	Hauser et al. 2011
	0.063 - 0.475 ng/mL (range), 0.125 ng/mL (median) serum (N = 17)	1.488 - 4.213 ng/mL (range), 2.738 ng/mL (median) serum (N = 11)				
	144 - 1,090 pg/mL (range) serum, mean not published (N = 7)					Nadler et al 1985
	≈ 187.5 - 749.99 pmol/L (range), 528.64 pmol/L (mean) saliva (N = 6 data points)	≈ 326.21 - 2,827.2 pmol/L (range), 1,372.2 pmol/L (mean) saliva (N = 12 data points).				Wobber et al 2013
	ranges not published, 0.18 ng/mL (mean) saliva (N = 14)	ranges not published, 0.27 ng/mL (mean) saliva (N=13)				Wobber and Hermann 2015
		≈ 5.04 - 25.96 pg/mL (range), 14.28 pg/mL (mean) saliva (N = 4)				Kutsukake et al 2009
Pan paniscus	≈ 218-1,078.1 pmol/L (range), 721.35 pmol/L (mean) saliva (N = 6 data points)	≈ 463.61-1,597.7 pmol/L (range), 895.28 pmol/L (mean) saliva (N = 6 data points)	Females are primary caregivers of offspring.	Polygynandrous mating system, but neither sex heavily competes for mating opportunities	Females	Wobber et al 2013
	ranges not published, 0.17 ng/mL (mean) saliva (N = 11)	ranges not published, 0.22 ng/mL (mean) saliva (N = 19)				Wobber and Hermann 2015
Mandrillus sphynx	≈ 48.98 - 190.55 ng/g (range), 81.28 ng/g (mean) feces (N = 18)	≈ 2,238 - 11,220 ng/g (range), 4,974 ng/g (mean) feces (N = 14).	Females are primary caregivers of offspring.	One male units. Males compete for mates, with high reproductive skew among males.	Males	Setchell et al. 2010, 2015

Table 2.2 (cont.)

Papio cynocephalus	≈ 8.42 - 31.73 ng/g (range), 18.60 ng/g (mean) feces (N=1)	ranges not published, 89.00 ng/g (mean) feces (N = 26)	Females are primary caregivers of offspring.	One male units. Males compete for mates.	Males	Stavisky et al 1995
	ranges not published, 133.43 ng/g (mean) feces (N = 29)					Gesquiere et al 2014
	≈ ranges not published, 45.17 ng/dL (mean) plasma (N = 7 data points)	≈ 79.22-724.9 ng/dL (range), 352.61 ng/dL (mean) plasma (N = 16 individuals)				Castracane et al., 1986; Castracane et al. 1983
Macaca arctoides	ranges not published, 3.2 nmol/L (mean) serum (N = 5)	ranges not published, 14.8 nmol/L (mean) serum (N = 5)	Females are primary caregivers of offspring.		Males	Rhodes et al 1994
Macaca fascicularis	≈ ranges not published, 188 pg/mL (mean) plasma (N=31)	≈ ranges not published, 4,698 pg/mL (mean) plasma (N=17)	Females are primary caregivers of offspring.	Polygynandorus.	Males	Malaivijitnond et al. 2007
	ranges not published, 0.5, 0.54, 0.6, 0.63 ng/mL (means) serum (N= 49 females in 4 treatment groups)	≈ 1.16-10.17 ng/mL (range), 4.07 ng/ml (mean) serum (N = 17)				Appt et al. 2010; Morgan et al 2000
	ranges not published, .093 ng/mL (mean) serum (N = 86)	ranges not published, 3.24 ng/mL (mean) serum (N = 14)				Wood et al 2004; Simon et al 2004
	ranges not published, 1.13 ng/mL (means) serum (N = 10)	ranges not publised, 5.38 ng/mL (mean) serum (N = 3)				Koritnik and Marschke 1987; Tao et al. 2006
	≈ ranges not published, 0.22 ng/mL (mean) serum (N=16)	ranges not publised, 5.84 ng/mL (mean) serum (N = 8)				Kromrey et al. 2016; Lue et al. 2006
Macaca mulatta	113-754 pg/mL (range), 358.5 pg/mL (mean) plasma (N = 11)	2,000 - 12,000 pg/mL (range from non-mating to mating seasons), non-mating season: 2,280 pg/mL, mating season: 11,350 pg/mL (means) plasma (N = 7)	Females are primary caregivers of offspring.		Males	Turner et al 1989; Gordon et al 1976
	≈ ranges not published, 0.19 ng/mL (mean) serum, (N = 24 females across 4 menstrual phases)	ranges not published, 1.9 ng/mL (mean) serum (N = 6)				Dumesic et al 1997; Plant et al 1997

Table 2.2 (cont.):

Species	Female T values	Male T Values	Patterns of infant care	Patterns of competition	Dominant Sex	References
Microcebus rufus	ranges not published, 12.87 ng/g (mean) feces (N = 40)	ranges not published, 12.11 ng/g (mean) feces (N = 56)	Females are primary caregivers of offspring.	Dispersed polygyny	Female	Zohdy et al 2014
Lemur catta	0.1 - 0.7 ng ml-1 (range), 0.2 ng ml-1 (mean) plasma (N = 10); 0.05 - 0.69 ng ml-1 (range) plasma (N = 11)	0.3 - 48.8 ng ml-1 plasma (range), 6.0 ng ml-1 plasma (mean) (N = 12)	Female biased, occassional male allocare	Polygynandrous	Female	Drea 2007; Drea 2011
	≈ 40 - 143.9 ng/g (range), 52.84 ng/g, 97 ng/g (means) feces (N = 12)	≈ 112 - 724.96 ng/g (range), 230.76 ng/g, 382.7 ng/g (means) feces (N = 12)				von Engelhard et al. 2000
	≈ ranges not published, 24.29 pg/mL, 28.01 pg/mL (means) saliva (N = 12)	≈ ranges not published, 35.45 pg/mL, 76.97 pg/mL (means) saliva (N = 12)				
	≈ ranges not published, 2,289 pg/mg (mean) hair (N = 15)	≈ ranges not published, 2,274 pg/mg (mean) hair (N = 13)				Tennenhouse et al. 2017

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CHAPTER 3: TESTOSTERONE IN A BIPARENTAL PRIMATE: TESTING THE CHALLENGE HYPOTHESIS IN COMMON MARMOSETS

Abstract

Little is known about how the competing demands of mating and infant care are mediated in females. For avian and mammalian males, these behaviors are mediated by changes in testosterone, and testosterone may also mediate this trade-off in females. In marmosets, both sexes invest in mating effort while concurrently providing infant care. We examine the association between fecal testosterone concentrations and aggressive, sexual, and infant-directed behaviors in captive female and male common marmosets (*Callithrix jacchus*). We hypothesize that females and males will show similar hormonal and behavioral associations. We predict similar testosterone concentration in both sexes, and that testosterone will correlate positively with aggressive and sexual behaviors, and negatively with infant interactions. During controlled intruder trials, we collected data from marmosets housed at the Southwest National Primate Research Center. No differences were found in pre-intrusion testosterone concentrations between males and females (Mann-Whitney U = 17, P = 0.94, N = 6 males, 6 females). Intruder Test Day hormone samples did not statistically correlate with aggressive behavior toward the intruders (Spearman's r = 0.58, P = 0.10; N = 9). We also found that testosterone was lower in individuals with infants in their groups when compared to individuals in groups without offspring (Mann Whitney U = 45, Z = -2.37, P = 0.02, N = 16 with infants, 12 without offspring). Our results suggest that common marmosets exhibit reduced hormonal dimorphism and that females exhibit similar hormonal and behavioral proximate mechanisms as do males in response to intruders.

Keywords: *Callithrix jacchus*, testosterone, mating competition, sexual selection, infant care

Introduction

Mating behavior and offspring care are key components of reproductive success. Males are typically thought to be highly competitive because their reproductive success is limited by access to fertile females, whereas females' reproductive success is considered most limited by energetic resources for themselves and their offspring (Bateman, 1948; Trivers, 1972). However, in many birds and some mammals, males also provide offspring care (e.g. birds: Ketterson and Nolan Jr, 1994; hylobatids: Rafacz et al., 2012; callitrichids: Ziegler, 2000). Mating effort and parental investment may represent a behavioral trade-off (Alberts et al., 1996; Cain and Ketterson, 2013a; Dantzer et al., 2011; Emery Thompson et al., 2014; Georgiev et al., 2014, 2014; Peters, 2002; Rosvall, 2013). Across species, there are different patterns to if and when males invest in mating and offspring care. In some species, males rarely invest in offspring and are instead highly competitive (e.g. mandrills: Setchell, 2016). In others, males provide infant care, but have a mating season with little to no overlap with the birth season (e.g. night monkeys: Fernandez-Duque, Rotundo, & Ramirez-Llorens, 2002). And for some, there is either no reproductive seasonality or there may be multiple, overlapping sets of offspring (e.g. callitrichines: Digby, Ferrari, & Saltzman, 2011). The latter results in males needing to compete for mates and invest in offspring during the same season or even the same day.

The relationship of testosterone with mating effort and offspring care is described by the Challenge Hypothesis (Hirschenhauser and Oliveira, 2006; Wingfield et al., 1990). This hypothesis proposes different T secretion patterns based on a species' mating system and male patterns of offspring care. T concentrations can remain high in males if they do not provide infant care and instead compete for mating opportunities. However, high T levels may interfere

with offspring care (Hau, 2007). The Challenge Hypothesis proposes that in seasonal breeders, male T remains high during the mating season but then falls during the birth season, if males provide infant care. However, for species that lack seasonal breeding and whose males need to both compete for mates and provide offspring care in the same season or day, T concentrations should change quickly and in response to social stimuli. For example, this pattern has been observed in captive cotton-top tamarins, whose males exhibited a drop in T, 20 minutes after being exposed to the scent of their own infants (Prudom et al., 2008). Testosterone is argued to mediate the behavioral trade-off between male reproductive competition and offspring care (Hau, 2007; Ketterson and Nolan Jr, 1994).

In most mammals, females are the primary provider of infant care, yet they also experience competition related to accessing and maintaining territories, and reproductive competition associated with finding preferred mates (Buss, 1988; Cain and Ketterson, 2013; Clutton-Brock and Huchard, 2013; Puts et al., 2011; Rosvall, 2011, 2013; Scelza, 2011; Stumpf et al., 2011; Sunderani et al., 2013; Tobias et al., 2012; Vaillancourt, 2013). In primates, females may scent mark, visually advertise estrus, initiate sexual interactions, aggressively compete directly with other females, and travel long distances to find or attract mates (Bro-Jørgensen, 2002; Doran-Sheehy et al., 2009; Drea, 2011; Fairbanks, 2009; French and Inglett, 1989; Horne and Itzkowitz, 1995; Isbell, 1991; Isbell and Van Vuren, 1996; Izard and Rasmussen, 1985; Kahlenberg et al., 2008; Knott et al., 2010; Rosvall, 2011; Stumpf et al., 2009). This raises the question as to whether behavioral shifts between mating and parenting in females are associated with the shifts in T proposed by the Challenge Hypothesis for males.

Testosterone is related to female aggression and mating behaviors in mammals (e.g. Gill et al., 2007; Rosvall, 2013; Ross and French, 2011; but see Drea, 2007). For example, female

ring-tailed lemurs display increased testosterone levels along with increased aggression during the mating season (von Engelhardt et al., 2000), but Drea (2007) notes higher levels androstenedione, a steroid pre-cursor to T, than testosterone in female ring-tailed lemurs. Wild female buff-breasted wrens experimentally exposed to a same-sex intruder showed increased T concentrations compared to control females not exposed to intruders (Gill et al. 2007). Female androgen levels increased following intruder tests in cooperatively breeding cichlids (Desjardins et al. 2006). Ross and French (2011) found that female Weid's marmosets display changes in T that correlated with the intensity of received aggression during staged intruder tests. However, the relationship of T with the competing demands of infant care and sexual competition in females is not well understood. For instance, compared to controls, female dark-eyed juncos displayed increased aggression and decreased parental effort when given exogenous T (Rosvall, 2013). However, a different study of the same species found a positive correlation between experimentally manipulated T concentrations and the rate at which females provided food for offspring (Cain & Ketterson, 2013b). Furthermore, previous work has shown female T levels within the range of males in a limited number of species (e.g. rock hyrax: Koren et al., 2006; Koren and Geffen, 2009; brown mouse lemurs: Zohdy et al., 2014; meerkats: Davies et al., 2016). Yet in ring-tailed lemurs, whose females are dominant over males, female testosterone was lower than in males (Drea, 2007). Therefore, questions remain surrounding in what contexts T is related to female behavior.

This study explores the relationship of female fecal testosterone concentration to mating effort and infant care using the framework defined by the Challenge Hypothesis. While many females must balance competition and offspring care, the common marmoset (*Callithrix jacchus*) is an ideal species to explore the Challenge Hypothesis due to having overlapping sets of

offspring, intense female-female mating competition, and being cooperative breeders with both breeding and non-breeding adults, sub-adults, and juveniles all carrying and offering food to offspring (Digby et al., 2011). In both captivity and the wild, female common marmosets engage in high levels of intra-sexual competition, including suppression of ovulation in subordinates, aggressive interactions between females, and infanticide (Digby, 1995; Saltzman et al., 2008). Whereas in most mammalian species, the behavioral and energetic demands of mating generally arise once a female's prior offspring approach weaning age (Lee, 1996), in common marmosets, females and males engage in mating effort and sexual competition while simultaneously providing infant care (Tardif et al., 2003). Therefore, this species should follow the pattern outlined by the Challenge Hypothesis for males that provide infant care but either have no seasonality or have overlapping sets of offspring (Figure 3.1). We investigate if this pattern will apply to captive female common marmosets housed in family groups, and if females and males will be similar in testosterone secretion, such as seen in the rock hyrax (Koren et al., 2006; Koren and Geffen, 2009).

We conducted controlled intruder tests with six breeding pairs of common marmosets in order to test for shifts in T associated with mating competition and opportunities resulting from the presence of intruders. We tested the following predictions:

1: Male and female marmosets will have similar T concentrations.

2: T concentrations in both sexes will increase in response to intrusions and will positively correlate with aggressive and sexual behaviors during intrusions. The magnitude of T increase will positively correlate with the frequency and duration of aggressive and sexual

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behaviors.

The Challenge Hypothesis also predicts that T will negatively correlate with infant care. Therefore, we used observational data to test an additional prediction:

3: Individuals will have lower T levels when dependent infants are present in their home group versus when infants are not present. T will negatively correlate with the frequency of infant interactions, and with infant proximity.

Methods

Study Subjects

Marmosets were housed on a 12 hour light-dark cycle at the Southwest National Primate Research Center in San Antonio, Texas, U.S.A., a USDA approved facility. Groups were comprised of an adult (breeding) female, an adult (breeding) male, and any offspring of one or both breeding adults. Marmosets were kept in home cages whose dimensions were 1.5 x 1.83 x 0.92 meters. Cages contained two nestboxes, and a minimum of two food dishes and two water bottles. Climbing substrates included mesh, nontoxic plastic pipes, and tree branches. Animals received varying enrichment items daily, including puzzle feeders, novel objects, scent enrichment, and food enrichment. Multiple breeding groups were kept within the same room. As such, animals could hear and smell one another, and see each other at a distance of 1.5 meters from across a walkway. However, adjacent groups were separated by solid metal cage walls and not visible to one another. Further details on husbandry procedures have been previously described (Layne and Power, 2003).

This research consisted of two parts.

Study 1: Intruder Tests

Intruder tests occurred during August 2011 – December 2012. We utilized six focal breeding groups (Table 3.1) and two intruder pairs of common marmosets for these tests. Focal groups and intruder pairs included adult males and non-pregnant females, so that intrusions represented a mating challenge to the focal group's breeding adults. Pregnancy status was confirmed as part of an unrelated study using ultrasounds (for methods, see Tardif et al., 1998). Intruder groups were pairs currently without offspring. Home cages are mobile and we designed an experimental protocol that would cause the least stress and prevent injury to the subjects. As a result, all individuals remained in their home cages in full contact with their groups, including breeding partners. While individuals could reach out from their cage toward an intruder (and vice versa), cages were not close enough to allow physical contact, to prevent injury. The focal animal's mate was always present, simulating what would be more likely to occur in wild groups.

Focal groups were exposed to an intrusion from a breeding pair at the same time each day for two consecutive days using the same intruder group for the consecutive tests. A target animal, either the focal group's breeding male or female, was randomly chosen for the first test day. The other breeding individual was the target animal the following day. Target animals were videotaped for the entire intrusion.

The test began when intruders, in their home cages, were moved within proximity to the focal group (4 inches at the closest point) and lasted for 20 minutes. Cages were arranged to allow for handheld videotaping of the target animal. The target animal was videotaped using a Sony Handycam (models DCR-SX45 and HDR-PJ200) throughout the test. At the end of 20

minutes, the intruder group was removed from sight of the focal group. Videotaping of the target animal continued for 10 minutes post-intrusion.

Videos were later scored using continuous behavioral data collection. The same ethogram was utilized for video scoring as for general behavior data collection (Appendix A). All agonistic interactions were recorded, including threat gestures, such as tuft flicks, frown faces, genital displays, and contact aggression between the target animal and its home group. All sexual behaviors, including solicitations (such as tongue-flicking displays), mounting, and copulation were recorded. Frequencies and durations of all behaviors exhibited by the focal animal were recorded both during the intrusion and during the 10 minute post-intrusion period. The location of the focal animal during all bouts of inactivity lasting 2 or more seconds also was recorded: The front wall (closest to intruders), the middle of the home cage, or the back wall (furthest from intruders).

To assess the hormonal correlates of a social intrusion, fecal samples of the target animal were collected the morning before the first intruder test (pre-intrusion sample), the morning after the first and second intruder tests (experimental condition samples), and again two mornings after the second intruder test (post-test sample).

Study 2: Relationship between T and offspring care

Study 2 was an observational study that included 16 breeding groups of marmosets to examine the relationship between fecal T concentrations and infant interactions. These data include all groups for which infants were present and for which hormonal data were available for the focal animal. Infants are defined as individuals younger than three months old, when weaning occurs (Tardif et al., 2003). Non-experimental behavior scoring involved observing focal animals for 20 minutes during weeks in which no experimental procedures were scheduled for the target

animal's group. Data were collected using focal sampling (Altmann, 1974) with the focal animal's distance to infants recorded every two minutes (Appendix B). In addition, all social interactions directed at the infants, including play, affiliative, and agonistic behaviors were recorded *ad libitum*. Fecal samples were collected twice during each week that behavioral data were collected.

Hormone Assays

Fecal markers (green or red food coloring) were fed to target individuals between 3:30 and 5:00 PM and plastic sheeting was placed underneath the home cage. Fecal markers were used to identify the correct animal's feces and were visible within 12 hours and up to 48 hours after being fed to the target individual. Fecal samples were collected first thing the following morning between 8:00 AM and 10:00 AM.

To measure steroid hormone concentrations, we followed a protocol described by Nunes et al. (2000). Samples underwent an extraction step prior to hormonal assays. For each sample, feces were dried in an incubator set at 37°C, ground up using a mortar and pestle, and 5 mL of methanol solubalizer was then added to the sample. Samples were shaken vigorously for 4 hours, and then centrifuged for 20 minutes at a speed of 2,500 RPM. The supernatant was then used in hormonal assays. ELISA assay plates were coated with a T antibody ordered from Coralie Munroe (University of California, Davis), at a dilution of 1:25,000 and allowed to incubate a minimum of 18 hours. Plates were then washed 3 times using BioTek ELx50 and gently tapped to remove any excess liquid. Phosphate Buffer Saline was then added across the plate. Samples, standards, and controls were added in 50uL volume followed by 50uL of a labeled T hormone conjugate in a 1:30,000 dilution. Plates were then incubated for a minimum of 90 minutes at

room temperature. Plates were again washed 3 times, and an ABTS-based chromagen was added to each well. Plates were read using BioTek ELx808 once the blank wells reached an absorbance level at 405 nm of 0.8-1.0. Samples were run in duplicate with a coefficient of variance that ranged from 0.0 - 14.9%.

Statistical Analyses

Statistics were run using SPSS Statistics Version 23. In all analyses, significance was set at $P = 0.05$. Averages are reported as mean ± standard deviation. Non-parametric tests were used to compare hormone concentrations between males and females. Spearman's correlations were used to analyze the relationship between T and behavior. To test the relationship between adult T concentrations and the presence of offspring, we included matched pairs of adults who experienced both "infant present" and "infant absent" conditions during the study period, and subsequently compared these individuals to any adults who were not housed with offspring during the study period.

We ran an *a priori* power analysis for Study 1 using G*Power 3 (Faul et al., 2007) with data presented by Castro and Sousa (2005) which demonstrated differences in male marmoset fecal androgen concentrations based on age. The *a priori* power analysis showed a minimum of six individuals of each sex needed to demonstrate a statistically significant difference ($d = 2.44$, critical $t = 2.23$, power $= 0.97$, two-tailed). Six breeding pairs were included in our intruder test study, which meets the sample size required by our power analysis, but due to difficulties obtaining fecal samples over subsequent testing days, some analyses varied in sample size.

Ethical Note

This study conformed to the ethical guidelines of the Southwest National Primate Reseach

Center regarding the use of nonhuman primates in a research study and all applicable laws of the United States of America.

Results: Study 1

Prediction 1: Male and female marmosets will have similar T concentrations.

We expected similar testosterone concentrations in males and females. One male in our study was an outlier, and consistently displayed hormone values above 1.5 times the interquartile range for the sample. However, nothing about his demographics, behavior, or health explained his high hormone levels. Therefore, we included him in our analyses but additionally report values with him removed.

Comparisons of female and male T for each day of the intruder study and the average maximum T concentration for each sex can be found in Table 3.2. Without the outlying male, average female and male T concentrations and ranges were similar at all time points. When the outlier male is included, male values are higher than females and their range of values is greater, but the difference is not statistically significant (Figure 3.2). Maximum T values also did not differ between males and females (Table 3.2).

Prediction 2: T concentrations in both sexes will increase in response to intrusions.

Figure 3.3 shows that both females and males followed the same general pattern of T secretion over the testing period (pre-intrusion, post-intruder test day 1, post-intruder test day 2, outlier male included). We predicted that T concentrations in both sexes would increase in response to intrusions from another breeding pair. We used Wilcoxon signed rank tests to compare pre-intrusion T concentrations to maximum T following intrusions. Both sexes showed

non-significant changes in T in response to intruders (Females: Z = -0.73, P = 0.47, N = 4; Males: Z = -1.60, P = 0.11, N = 3, outlier male included). Since hormone concentrations were not statistically different between females and males, we also looked at this relationship by combining data from both sexes. The combined data show a difference between pre-intrusion T concentrations and maximum T concentrations, but this difference did not reach statistical significance (Wilcoxon signed rank test: Z = -1.90, P = 0.06, N = 7, outlier male included).

Prediction 2: T concentrations will positively correlate with aggressive and sexual behaviors during intrusions.

We predicted that target animals with higher T would exhibit higher rates and durations of aggressive and sexual behaviors directed at intruders than those with lower T. We compared pre-intrusion fecal T concentrations with these behaviors during the intruder test. Aggressive behavior during intruder tests did not correlate with pre-intrusion T concentrations in males or females. Pre-intrusion T concentrations correlated with female, but not male, rate of sexual behavior during the intrusion (females: Spearman's r = -0.94, P = 0.005, N = 6; males: Spearman's r = -0.31, P = 0.55, N = 6, outlier male included). The female correlation was in the opposite direction as predicted; females with increased T concentrations engaged in less sexual behavior. Given the similarity in female and male T levels we also ran this correlation with the samples combined. When the data for females and males are combined, pre-intrusion T concentrations do not correlate with sexual behavior (Spearman's r = -0.42, P = 0.17, N = 12, outlier male included).

We also examined whether Test Day T concentrations correlated with aggressive and

sexual behaviors. We saw no correlation between aggression and Test Day T concentrations in males (Spearman's r = 0.40, P = 0.60, N = 4, outlier male included) or females (Spearman's r = -0.10, P = 0.87, N=5). When we combined the female and male samples, Test day T concentrations positively correlated with the percent time spent engaging in aggression directed toward the intruder group, but did not reach statistical significance (Spearman's r = 0.58, P = 0.10; N = 9, outlier male included).

Test day T concentrations also did not correlate with rate of sexual behavior in either sex (females: Spearman's r = 0.36, P = 0.55, N = 5; males: Spearman's r = 0.78, P = 0.23, N = 4, outlier male included). Combined Test Day T concentrations show positive but non-significant correlation with rate of sexual behavior (Spearman's r = 0.60, P = 0.09, N = 9, outlier male included). Increasing sample sizes would help to further test these correlations.

Results: Study 2

Prediction 3: Individuals will have lower T levels when dependent infants are present in their home group versus when infants are not present.

We predicted that individuals would have lower T concentrations when infants were present in groups compared to when no infants were present. Matched samples show no difference between females or males with infants (defined as unweaned offspring under 3 months old) and those without infants (N = 10, Figure 3.4). We compared adults with infants, adults without infants (weaned offspring), and adults without any offspring present (Figure 3.5, Table 3.3). As predicted, adult females with infants had lower T concentrations than females in groups without any offspring (N = 6), although this was not statistically significant. Female marmosets ovulate shortly after giving birth, however, and may be pregnant. Restricting the

Infants-Present data and No Offspring data to females known not to be pregnant also showed a non-significant difference (Infants present: 290.55 ± 230.61; No offspring present: 640.98 ± 357.36, U = 7, Z = -1.15, P = 0.25, N = 5 in each group).

Similarly, males with infants did not differ from males without infants (N = 6). Males with infants had lower T concentrations than males in groups without any offspring (N = 6), but this was not statistically significant. When combining the data from both sexes, we see a significant difference between individuals with infants compared to those in groups without any offspring (infants present: average = 451.15 ± 309.47, N = 16; no offspring: average = 744.74 ± 483.24, N = 12; U = 51, Z = -2.09, P = 0.04).

Prediction 3: T will negatively correlate with the frequency of infant interactions, and with infant proximity.

We also predicted that interactions with infants would negatively correlate with T concentrations. No significant correlation was seen between T and amount of time spent in contact (carrying or huddling) with infants (Females: Spearman's r = -0.44, P = 0.23, N = 9; Males: Spearman's r = 0.19, P = 0.70, N = 7). Furthermore, no significant correlation was observed between T concentrations and rate of infant interactions (Females: Spearman's r = -0.29, P = 0.44, N = 9; Males: Spearman's r = -0.13, P = 0.78, N = 7).

Discussion

Hormonal Similarity in Response to Intruders

This study tests a series of hypotheses regarding the relationship between T and aggression, sexual behavior, and offspring care in captive common marmosets. We predicted that

female and male marmosets would display similar fecal T concentrations and show a similar response to intruders as males. Changes in female T concentrations in response to experimental intrusions were comparable to those of males in our sample. We saw no difference between female and male fecal T concentrations, throughout the experiment.

We also predicted that females and males would both experience an increase in T concentrations in response to intruders. Maximum T concentrations were higher than pre-intrusion concentrations, but the difference was not significant for either females or males. When we consider the threshold for significance to be $P \leq 0.10$, there was an increase in T in response to intruders when both male and female samples were combined. This points to the possible importance of an increased sample size for further investigation of this relationship.

Testosterone and Behavior during Intruder Tests

We predicted a positive correlation between T and sexual behaviors during the intruder tests. This prediction was not supported. We also predicted that T would positively correlate with aggressive behavior during the intruder tests. We observed a non-significant positive relationship between test-day T concentrations and the percent time the target animal spent in aggressive behavior directed at the intruder group, in our combined sample of females and males. A larger sample and confirmation in each sex separately are necessary to confirm or refute this relationship.

We designed an experimental protocol that would limit stress and prevent injury to the subjects. As a result, all individuals remained in their home cages during intruder tests with their groups intact, including breeding partners. Therefore, behavior during intrusions may have differed than had we separated the target animal from its home group. However, completely

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isolating a breeding adult from its home group for an intruder test may also mask behavior

typical of an inter-group intrusion, as naturally occurring intrusions involve multiple group

members responding to intruders (Lazaro-Perea, 2001). Previous experimental studies have used

a single intruder and reliably documented aggression directed at same sex intruders (e.g.

Anzenberger, 1985; Harrison and Tardif, 1989; Ross et al., 2004; Ross and French, 2011), and

that T concentrations increase when comparing pre-intruder to post-intruder samples the day

after the intruder test (Ross et al., 2004; Ross and French, 2011). Furthermore, the current study

provided focal animals with both a reproductive challenger and a potential mate. While there

were low rates of aggression directed between the breeding partners, which would have indicated

mate guarding, it is still possible that the presence of the breeding partner constrains an

individual's response. Specifically, a member of a breeding pair may be less likely to engage in

sexual solicitations with an intruder in the presence of their breeding partner, as has been

reported in captive common marmosets (Anzenberger, 1985; Gerber et al., 2002). We found no

relationship between sexual behavior and T, and sexual behavior was minimal altogether.

However, keeping focal animals with their home groups during the intrusion is more similar to a

natural intrusion than isolating an animal from its group members.

Testosterone and Infant Care

Last, we predicted that T concentrations would be negatively correlated with infant care

measures. We compared T concentrations in adults with infants present and those without any

offspring in their home groups. Our prediction was supported when combining hormone data

from all adults. When separated by sex, the difference in T concentrations for adults without

offspring present and those with infants was non-significant. We did not see a correlation

between T and infant proximity or rate of infant interactions. This contrasts with the results of Nunes et al. (2001) which show higher urinary T concentrations in males that carry infants less frequently, and with Ziegler et al. (2009) which demonstrate that male marmosets respond to the odor of infants with decreased T concentrations. One difference between our data and those of Nunes et al. (2001) is that we used proximity to infants as opposed to carrying, which is more specific. In our case, proximity to infants could occur by either the infant or the adult moving closer to the other.

Limitations

Our study is limited by sample size. We included six breeding pairs in the intruder tests which is the sample size required by our power analysis. However, due to difficulties obtaining fecal samples over subsequent testing days (e.g. individual changes in fecal consistency making collection impossible), some analyses had sample sizes of 3 or 4. However, we were able to compare female and male T concentrations on days in which the minimum sample was met. These data also agree with a larger dataset which found no difference between female and male fecal T concentrations (Melber et al., in prep). Despite our limited sample size, this study contributes to a growing body of literature documenting reduced endocrine dimorphism in species including another cooperatively breeding mammal, the rock hyrax (Koren et al., 2006), the polygynandrous brown mouse lemur (Zohdy et al., 2014), and Milne-Edwards' sifaka (Tecot et al., 2010), a strepsirrhine with a variable mating system. However, larger sample sizes are necessary to validate the correlations we observed between behavior and T concentrations in this study. These correlations were only significant when combining samples from both sexes. The lack of a strong correlation between T and behavior may be influenced by the social context we

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used for the intruder tests. Anzenberger (1985) showed that the presence of breeding partners influenced behavior during intruder tests. If the presence of a breeding partner constrained behavior, this may have masked the expected correlations between sexual behavior and aggression with T. Future tests should examine potential differences in T's response to intruders in both the mate-present and mate-absent social conditions.

Marmoset females ovulate shortly after giving birth and can conceive (Tardif et al. 2003). This means that when comparing T concentrations between females with unweaned versus weaned offspring, females may have been pregnant, thus confounding the comparison which showed non-significant results. Running the same analysis excluding any female that may have been pregnant still showed non-significant differences between females with unweaned infants and females without any offspring in their groups.

Non-invasive measurements of steroid hormones are critical to understanding hormone systems that may interact with the stress axis. Fecal samples are commonly used due to their ease of collection and data showing they reflect changes in circulating hormones (Bishop & Hall, 1991, Brown et al., 1996, Cockrem & Rounce, 1994, Reslir, Wasser, & Sackett, 1987, Ziegler, Sholl, Scheffler, Haggerty, & Lasley, 1989). As such, fecal samples are commonly used in primate research into testosterone (e.g. Beehner et al., 2005, Drea, 2007, Fontani et al., 2014, Muehlenbein 2004, Muller and Wrangham 2004). The lack of sexual dimorphism in fecal T concentrations in this study and other mammals should be interpreted with caution as the significance and validity of this comparison is debated. Males and females may metabolize hormones differently such that the same fecal steroid concentration in males and females may actually represent different circulating levels of the steroid in question (e.g. Gesquiere et al., 2014; Goymann and Wingfield, 2014). Gesquiere et al., 2014 compared fecal T concentrations

across ages and sexes in baboons. They found that adults of each sex had higher steroid concentrations than juveniles, but that males did not have statistically higher fecal T concentrations than females. They concluded that fecal T concentrations are a useful measurement of within sex hormone comparisons, but may not be valid for between sex investigations in *Papio cynocephalus*. Their conclusion is supported by earlier work showing males have higher plasma T than females in this species (Castracane et al., 1986; Castracane and Goldzieher, 1983), although other studies of female baboon fecal samples show lower values than Gesquiere et al.'s (2014) data (*Papio cynocephalus*: Stavisky 1995, *Papio hamadryas*: Beehner et al., 2005). Regardless, a direct comparison of fecal to circulating testosterone in *P. cynocephalus* is warranted. However, there are other cases in which females and males have shown similar testosterone levels. For instance, female bell miners (a cooperatively breeding honey eater bird) have plasma androgens that are similar to males (Poiani and Fletcher, 1994). Davies et al. (2016) also document similar serum T in female and male meerkats and subsequently compare fecal androgen data from the same population with their serum results for both T and androstenedione. While Davies et al. (2016) did not distinguish between T and androstenedione in their fecal assay, they show similar fecal excretion patterns to circulating androgens. Therefore, while fecal data showing similarity between sexes should be compared with serum or plasma data, similarity in itself does not mean the data are invalid.

Furthermore, the mechanism by which common marmosets produce androgens is sexually dimorphic (Pattison et al. 2007, 2009). Common marmoset males lack the zona reticularis, the part of the adrenal gland that produces androgens. However, female marmosets have a zona reticularis that produces androgens and increases production when ovarian function is compromised (Pattison et al. 2007). While a sexually dimorphic origin of T does not mean that

T metabolism also differs between sexes, future work should compare concentrations of serum T between female and male marmosets in order to address concerns of sexually dimorphic steroid metabolism. Regardless of the origin and metabolic pathways of T, a change in fecal T concentrations in response to social manipulation in either sex is still indicative that T responds to certain stimuli. Therefore, while the results of this paper are limited by sample size, evidence supports a lack of physiological dimorphism within *Callithrix jacchus*. The question remains whether our results present a genuine lack of dimorphism in female and male T concentrations, or whether females experience a similar pattern of hormone secretion as males, but at a smaller magnitude.

Evidence shows that female androgens function in a behaviorally meaningful capacity in other species, including humans. For instance, female androgens increased in response to intruder tests in cooperatively breeding cichlids (Desjardins et al. 2006). Female ring-tailed lemur experience increased androgen levels along with increased aggression during the mating season (Drea 2007; von Engelhardt et al. 2000). Baboon female T concentrations vary seasonally and with dominance rank (Beehner et al., 2005). In women, T concentrations correlate with the outcome of aggressive encounters (Denson et al., 2013).

However, the relationship between androgens and behavior in females may vary, including within the same population. For instance, fecal T concentrations correlate with aggressive behavior in dominant but not submissive female baboons (Beehner et al., 2005). In addition, female tree-swallows with experimentally elevated T concentrations are more aggressive and have lower hatching success than control females (Rosvall, 2013). Yet female house sparrows exposed to an intruder show decreased T concentrations compared to females

sampled without experiencing a territorial intrusion (Elekonich and Wingfield, 2000). Other hormones may influence T's association with aggression and infant care and may explain some discrepancy in results. For instance, women with high cortisol levels showed an association between T and aggressive behavior, whereas women with lower cortisol levels did not (Denson et al., 2013). Prolactin shows a positive association with infant care, and has a negative relationship with T (reviewed by Ziegler et al., 2000). Additionally, evidence suggests oxytocin and vasopressin influences infant care in common marmosets with higher concentrations of these hormones associated with greater investment in infant care (Storey and Ziegler, 2016, Taylor and French, 2015). High levels of testosterone may negatively influence reproductive function in females (Abbott et al., 2017). Therefore, it is possible that T responsiveness to the social environment may be reduced in common marmosets and that prolactin, oxytocin, vasopressin, or cortisol also play a role in mediating aggression and infant care in common marmosets. The influence of these hormones along with the relationship of aggressive competition, infant care and testosterone in females needs further research.

In marmosets, both sexes engage in intra-sexual competition and contribute to infant caregiving. Further, marmosets do not temporally separate mating and parental investment due to the fact that females resume ovarian cycling and mating behavior within two weeks post-partum (Digby et al., 2011). Therefore, females are pregnant at the same time they are lactating and providing infant care. Our results suggest that while T may respond to the presence of intruders, parental investment may be less sensitive to T concentrations than suggested by the Challenge Hypothesis. A disassociation between T and infant care has been suggested for species in which high amounts of infant care is vital to offspring survival, and therefore reproductive success (Lynn, 2008; Rosvall, 2013). Proximate mechanisms to explain elevated T concentrations

without a behavioral response have included reductions in androgen receptors, and reductions in the concentration of enzymes such as aromatase in the brain during periods when infant care is a priority (Lynn, 2008). While our study population is captive, and represents different conditions than seen in the wild, multiple studies of wild callitrichines show an association between the number of allocare providers and offspring survival rates (Garber, 1997; Koenig, 1995). We observed a non-significant increase in T concentrations in response to intruders but the associations we observed between infant care and T did not clearly follow the predictions of the Challenge Hypothesis. Therefore, we suggest that the reproductive pressures on male and female marmosets may have led to a decrease in sensitivity to changes in T.
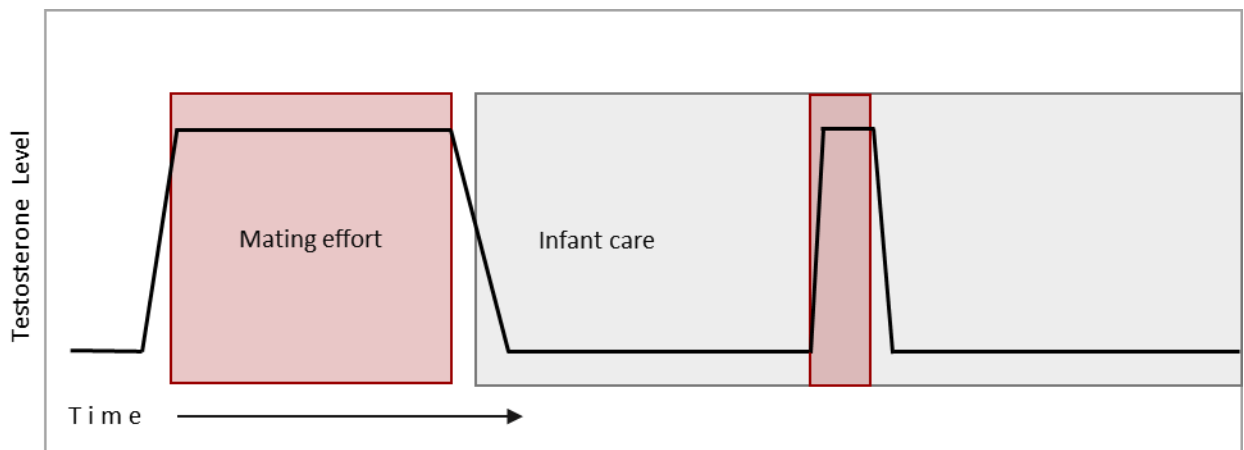
Figure 3.1: Theoretical testosterone secretion for biparental species with overlapping sets of offspring, showing changes in T with mating effort (red) and infant care (grey), Based off the model presented by Wingfield et al., 1990.
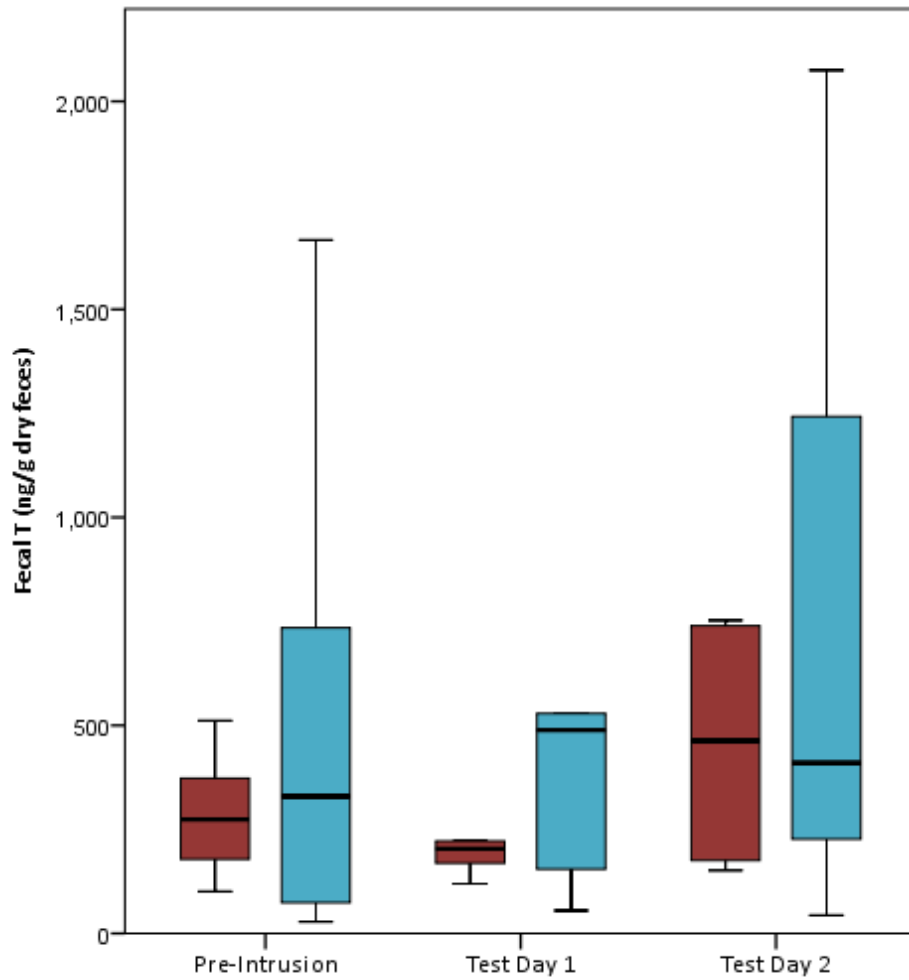
Figure 3.2: Testosterone levels for females (dark red) and males (light blue). No significant difference was observed between sexes.
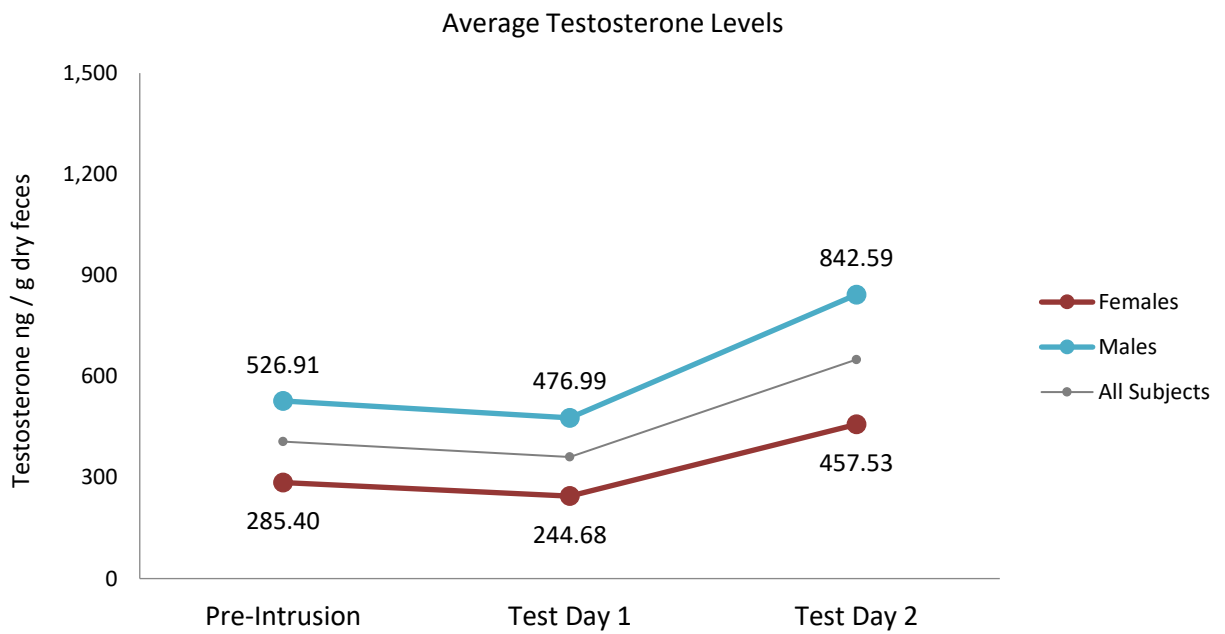
Figure 3.3: The pattern of average fecal T concentrations for females (dark red) and males (light blue) throughout intruder study. Data include the outlier male.
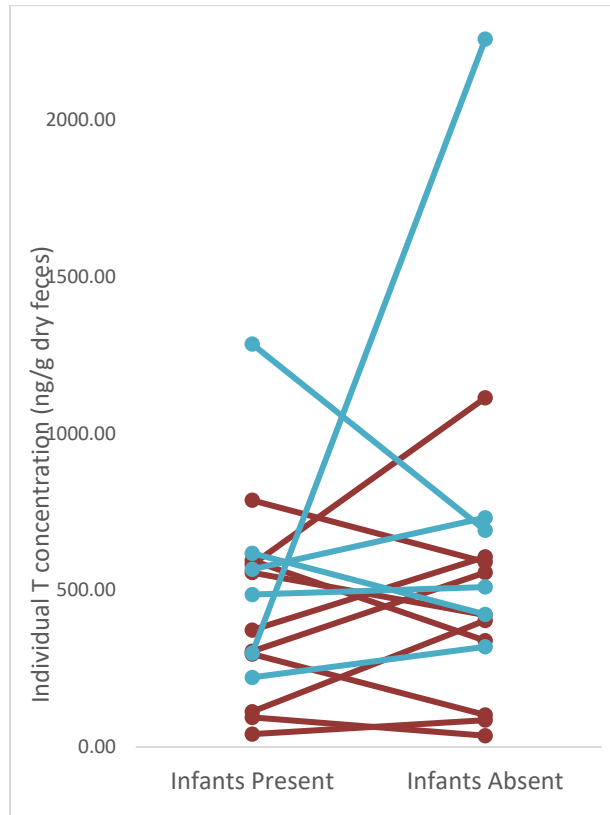
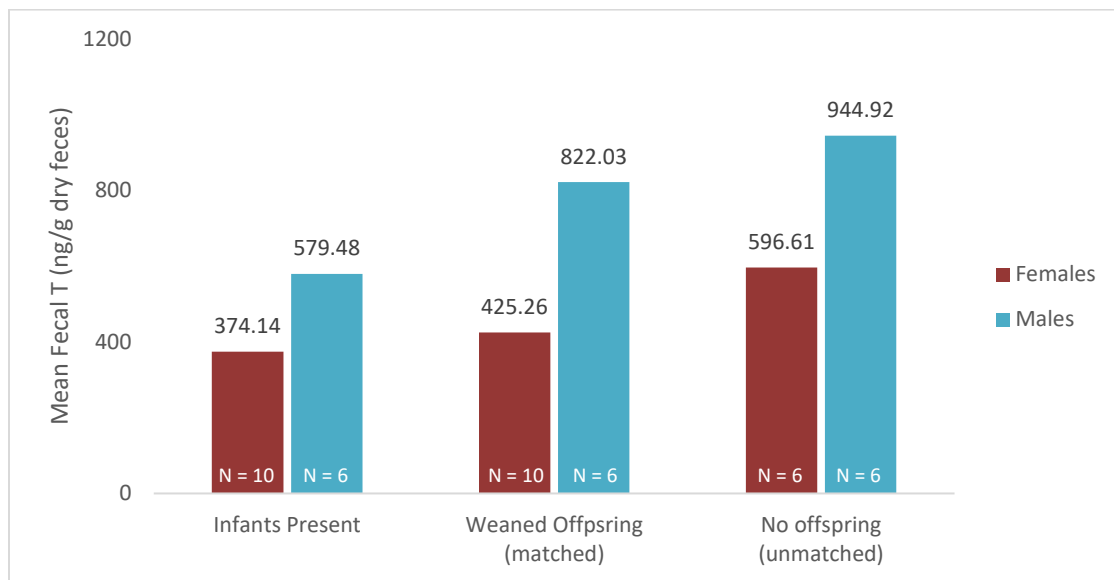**Figure 3.4:** Female (dark red) and male (light blue) T concentrations with and without unweaned infants present

Figure 3.5: Female (dark red) and male (light blue) T concentration across infant categories.

Table 3.1: Group Demographies for Intruder Tests

Group ID	Age of Female	Age of Male	Offspring present
1	4 years	6 years	1
2	4 years	4 years	6
3	4 years	8 years	4
4	4 years	5 years	5
5	2 years	2 years	0
6	2 years	8 years	2

Table 3.2: Testosterone Concentrations Before and After Intrusions. Mean ± standard deviations for female and male fecal T concentrations. Sample sizes are in parentheses. Statistics were run with the outlying male included.

	Pre-Intrusion		Test 1		Test 2		Maximum T Value
	Average	Range	Average	Range	Average	Range	Average
Females	285.40 ± 145.0 (6)	410.57	244.68 ± 154.6 (6)	431.90	457.53 ± 326.3 (4)	601.11	460.7 ± 323.0 (4)
Males (excludes outlier)	298.98 ± 292.45 (5)	706.83	343.16 ± 220.99 (5)	473.72	226.55 ± 259.11 (2)	366.44	323.72 ± 238.96 (2)
Males (with outlier)	526.91 ± 616.6 (6)	1638.80	476.99 ± 382.8 (6)	1091.11	842.6 ± 1,082.59 (3)	2031.35	907.4 ± 1,024.9 (3)
Mann Whitney U	17		14		6		5
P-Value	0.94		0.59		1		0.86

Table 3.3: Average adult T concentrations (ng/g dry feces) with and without infants.

	Unweaned Infants	Weaned Offspring	No Offspring Present
Females	374.14 ± 250.24 (N = 10)	425.26 ± 321.50 (N = 10) P = 0.58	596.61 ± 243.16 (N = 6) P = 0.15
Males	579.48 ± 378.11 (N = 6)	822.03 ± 720.62 (N = 6) P = 0.75	944.92 ± 607.00 (N = 6) P = 0.09

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CHAPTER 4: MALE BUT NOT FEMALE CAPTIVE COMMON MARMOSETS SHOW A TRADE-OFF BETWEEN AGGRESSION AND PARENTAL CARE

Abstract

Female and male behavior often differs from each other to maximize the reproductive success of each sex. While male reproduction may be limited by access to fertile females, females are often limited by access to energetic resources to support themselves and any dependent offspring. This is common in mammals, whose females must energetically support both a pregnancy and subsequent lactation costs, thus providing the majority of direct offspring care. However, some species do not display a clear separation between female and male behavior, with both sexes competing for mates and investing in offspring. Mate competition may be incompatible with parental investment. Yet, there are species such as the common marmoset (*Callithrix jacchus*) in which both sexes must compete for mates during the same time that both sexes also provide direct offspring care, offering an unusual opportunity to examine trade-offs between mating and infant care in both sexes. We examine the difference between female and male behavior in the cooperatively breeding common marmoset, *Callithrix jacchus.* We also test the hypothesis that offspring care is incompatible with mating behaviors, including aggressive competition and sexual behaviors. We find that while both sexes spend similar amounts of time in mating-related and parental investment behaviors, female sexual and aggressive behaviors do not correlate with parental investment, whereas male aggression does.

Significance statement

Mating competition and parental care may represent a behavioral trade-off. Many species avoid a direct competition between these two behavioral categories through behavioral dimorphism or seasonal breeding. However, the callitrichids are not seasonal breeders and both sexes care for

offspring. We measure the degree of behavioral dimorphism in mating behaviors and parental care in common marmosets, and test whether there is a trade-off between parental investment and aggressive or sexual behaviors. We did not see any behavioral dimorphism in time spent in mating or parental care. However, male marmosets showed a negative correlation between aggression and parental care and females did not. Our results suggest different proximate mechanisms controlling this trade-off in females and males.

Introduction

Sexual selection theory predicts sex-based behavioral differences originating from reproductive challenges specific to each sex. As originally proposed, males compete for limited access to mates, and females compete for limited access to resources necessary to support themselves and their offspring (Trivers, 1972). Many species display behavioral dimorphism with females investing in the costs of pregnancy and lactation and as the primary offspring caregivers (Lee, 1996), whereas males compete intensely for mates (e.g. hamadryas baboons; Pines et al., 2015). However, female mammals also invest in mating effort, including sexual competition (reviewed by Clutton-Brock, 2009). Female sexual competition can take the form of territory defense (e.g. ground squirrels; Luna and Baird, 2004), competition for resources (e.g. gorillas; Wright and Robbins, 2014), and competition for mates (e.g. female topi antelope; Bro-Jørgensen, 2002). Further, males of some mammalian species also directly contribute to offspring care by provisioning, carrying, or playing with offspring (Kleiman and Malcolm, 1981; Stockley and Hobson, 2016).

The degree of behavioral dimorphism is expected to vary between species. For instance, species in which sex roles are reversed exhibit behavioral dimorphism with males caring for offspring and females competing for access to mates (e.g. Pipefish: Jones et al., 2000). There are also species in which we see behavioral overlap between the sexes, with both females and males competing for mates and directly investing in offspring (e.g. callitrichids; Digby et al., 2011; owl monkeys; Fernandez-Duque and Huck, 2013). The latter is interesting in that selective pressures on one or both sexes may have led to a reduction in behavioral dimorphism.

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Mating effort and parental investment are both necessary for reproductive success in many vertebrates. However, mating behaviors may be incompatible with infant care, representing a behavioral trade-off (Magrath and Komdeur, 2003; Rosvall, 2013; Symons et al., 2011). In species where males regularly provide offspring care, evidence suggests that mating behaviors, including mate guarding and intrasexual competition, take time away from other behaviors, such as foraging and provisioning offspring (Alberts et al., 1996; Emery Thompson et al., 2014; Georgiev et al., 2014; Peters, 2002). However, there is conflicting evidence to support this trade-off in females. For example, more aggressive female dark-eyed juncos spent less time brooding, but more time feeding nestlings than less aggressive females (Cain and Ketterson, 2013). Some experimental evidence suggests that aggression related to mating success is not compatible with parental investment. For instance, hormonally manipulated individuals decrease their parental investment in favor of aggressive behavior (De Ridder et al., 2000; Hegner and Wingfield, 1987; Oring et al., 1989; Saino and Møller, 1995). In samples where other caregivers were unable to increase their investment sufficiently, the offspring of hormonally altered individuals had a lower survival rate than offspring of control individuals (Hegner and Wingfield, 1987; Rosvall, 2013; Saino and Møller, 1995; but see De Ridder et al., 2000).

Many species are able to avoid a direct conflict between mating and infant care as the birth season is temporally distinct from the mating season (e.g. ring-tailed lemurs; Koyama et al., 2001). Yet, some species must cope with infants requiring high energetic investment for survival simultaneously with breeding opportunities and mating challenges (e.g. tamarins and marmosets; Digby et al., 2011).

The callitrichids, including common marmosets (*Callithrix jacchus*), display a temporal overlap in the period in which offspring are unweaned (thus requiring a large energetic investment) and subsequent breeding (Digby et al., 2011; Tardif et al., 2003). Therefore, this species offers an important opportunity in which to study the relationship between mating effort and infant care. While common marmosets may form socially monogamous pairs, variability is found in their mating system (Yamamoto et al., 2009; Garber et al., 2016). After giving birth, common marmoset females typically ovulate within 20 days, resulting in mating effort and pregnancy during a period in which the current litter is still highly dependent (Tardif et al. 2003). The energetic cost of litters is high and infant survival rates are higher in groups with more allocare providers (Bales et al., 2000; Garber, 1997). Males invest in offspring by carrying offspring and food sharing (Digby et al., 2011). Further, both sexes experience intrasexual competition. In both captive and wild common marmosets, dominant, breeding females reproductively suppress subordinate females by inhibiting ovulation (Abbott et al., 1997). Dominant females also direct physical aggression toward subordinates, and in some cases may commit infanticide against subordinates' infants should suppression fail (Digby, 1995; Digby and Saltzman, 2009; Saltzman et al., 2008). Further, dominant females are intolerant of same-sex intruders, which may represent a threat to their territorial, and therefore, breeding status (Lazaro-Perea, 2001). If subordinate females breed, dominant females face competition for allocare givers, decreased infant survival, and increased inter-birth intervals (Yamamoto et al., 2009).

This study examines common marmosets to test whether aggressive and sexual behaviors are compatible with offspring care behaviors, and whether female and male behavior is dimorphic in captive groups of common marmosets. Because females use different competitive

mechanisms than males to address intragroup breeding competition, but are still subject to reproductive competition during intergroup encounters, we also investigate similarities between females and males in behavioral response to intruders.

We test the following predictions:

1. Females and males exhibit similar activity budgets, showing similar rates of aggression, sexual behavior, and offspring care.

2. Rates of aggression and sexual behavior are negatively correlated with infant care.

3. Females and males respond similarly to an intruder pair in level of engagement, and rates and percent time spent in aggression and soliciting intruders.

Methods

Study Subjects: Marmosets were housed at the Southwest National Primate Research Center in San Antonio, Texas, U.S.A., a USDA approved facility. Groups were comprised of an adult (breeding) female, an adult (breeding) male, and any offspring of one or both breeding adults. Marmosets were housed on a 12 hour light-dark cycle in home cages whose dimensions were 1.5 x 1.83 x 0.92 meters. Cages contained two nestboxes, and a minimum of two food dishes and two water bottles. Climbing substrates included mesh, nontoxic plastic pipes, and tree branches. Animals received varying enrichment items daily, including puzzle feeders, novel objects, scent enrichment, and food enrichment. Multiple breeding groups were kept within the

same room. As such, animals could hear and smell one another, and see each other at a distance of 1.5 meters from across a walkway. However, adjacent groups were separated by solid metal cage walls and not visible to one another. Further details on husbandry procedures have been previously described (Layne and Power, 2003).

General Behavior:

   25 breeding groups (49 individuals) were observed. Focal animals were observed in their home groups without any manipulation, on a randomized schedule between the hours of 8:00 AM and 5:00 PM. General activity budgets and proximity to infants (Appendixes A and B) were recorded using instantaneous focal sampling with 2 minute intervals. Infants were defined as any individuals under 3 months old. Behavioral data sessions lasted 20 minutes with a 3 minute habituation period prior to the start of the behavior session to allow the group to habituate to the presence of a researcher. Aggressive behaviors, sexual behaviors, and infant interactions were recorded *ad-libitum* for the focal animal during the session. Aggressive behaviors were defined as contact fighting, chasing, hitting, biting, and threatening another individual. Sexual behaviors were defined as soliciting, mounting, and copulation. Infant care behaviors included picking up, carrying, and feeding infants. Aggressive and affiliative interactions with infants were also recorded.

Intruder Tests:

   Six breeding pairs (12 individuals) were exposed to an intrusion from a separate breeding pair on two consecutive days at the same time each day. A target individual was chosen randomly between the focal group's breeding male and female for the first test day. The other

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breeding individual was the target animal the following day. Target individuals were kept within their home cages and with their groups for the duration of the intruder tests. Each test day, the intruder group was brought within close proximity of focal group's home cage (3 inches at the closest point). The test began when the intruders were brought within proximity of the focal group and lasted for 20 minutes. The target animal was videotaped using a hand-held video camera throughout the test. At the end of 20 minutes, the intruder group was removed from the sight of the focal group. Videotaping of the target animal continued for 10 minutes post-intrusion. Videos were later scored using continuous behavioral data collection. The same ethogram was utilized for video scoring as for general behavior data collection (Appendix A). Frequencies and durations of all behaviors exhibited by the focal animal were recorded both during the intrusion and the 10 minute post-intrusion period.

Statistics:

All statistics were run using SPSS Statistics versions 23 and 24. Behavioral data did not conform to normality, and two-tailed, nonparametric tests were used with P-values set at 0.05 to compare female and male behavior.

Results

Hypothesis 1: Females and males exhibit similar activity budgets, and similar rates of aggression, sexual behavior, and offspring care.

We found that females and males displayed similar activity budgets. The sexes did not differ in percent observations spent in aggressive, sexual, or infant care behaviors (Table 4.1,

Figure 4.1). Average proximity to infants also did not differ between females and males (Z = -1.25, P = 0.22, N = 10 females, 10 males).

Hypothesis 2: Rates of aggression and sexual behavior are negatively correlated with infant care.

Neither female aggressive or female sexual behavior showed a relationship with infant care (Table 4.2). Male sexual behavior also did not show a relationship with infant care. Male aggressive behavior was negatively correlated with the rate of infant interactions and the rate of affiliative infant interactions, but not with rate of aggressive infant interactions. Male aggressive behavior was positively correlated with distance from infants. This indicates that males with lower rates of aggression were in closer proximity to infants than males with higher rates of aggression.

Since aggression directed at a focal animal's home group does not represent mating competition in the captive environment, we further broke down aggressive behaviors to test if aggression directed at non-group members correlated with infant care. Male extra-group aggression negatively correlated with overall rate of infant interactions (Spearman's rho = -0.74, P = 0.006, N =12), but no significant correlations were seen when infant interactions were broken down by quality (affiliative: Spearman's rho = -0.50, P = 0.10, N = 12, neutral: Spearman's rho = -0.47, P = 0.13, N = 12, aggressive: Spearman's rho = -0.18, P = 0.58, N = 12). Male extra-group aggression also correlated with male distance from infants (Spearman's rho = 0.78, P = 0.008, N = 10, Figure 4.2).

Since males but not females showed a correlation between aggression and measures of infant care, we compared variance across sexes in rates of aggression, infant interactions, and infant distance scores. Levene's test showed no difference between female and male rates of aggression or infant interactions (aggression: W = 0.26, P = 0.61, infant interactions: W = 0.06, P = 0.81). However, females showed less variance than males in infant distance scores (female σ = 0.66, male σ = 1.00, W = 4.63, P = 0.05).

Hypothesis 3: Females and males respond similarly to an intruder pair in level of engagement, and rates and percent time spent in aggression and soliciting intruders.

Both sexes spent significantly more time on the front wall (in proximity to intruders) than on the back wall (away from intruders) during the intruder test (Wilcoxon Signed Rank tests: females: Z = -2.20, P = 0.03; males: Z = -2.20, P = 0.03). Both sexes spent more time on the front wall during the intrusion than post intrusion, when the intruder pair had been removed (Wilcoxon Signed Rank tests: females: Z = -2.20, P = 0.03; males: Z = -1.99, P = 0.05). We found no difference between females and males in the percent time spent in proximity to intruders (Mann-Whitney: female mean = 56 ± 18%; male mean = 63 ± 10%; U = 9.00, P = 0.18) or away from intruders (female mean = 7 ± 7%; male mean = 2 ± 3%; U = 12.00, P = 0.39) during the intruder test.

There was no significant difference in rate of scent marking per minute between females and males (female mean: 0.06 ± 0.07; male mean: 0.31 ± 0.67; U = 16.5, Z = -0.26, P = 0.82). Females exhibited a higher rate than males of self-directed scratching per minute during the intrusion (female mean: 0.11 ± .8; male mean: 0.01 ± 0.02; U = 3.50; Z = -2.48; P = 0.02).

We compared frequencies and durations of aggressive behaviors directed toward the

focal animal's home group with those directed at intruders. Neither sex randomly distributed

aggressive behavior, focusing instead on directing most aggressive behaviors toward the intruder

group (Wilcoxon Signed Rank test: Females: $Z = -2.20$, $P = 0.03$; Males: $Z = -2.20$, $P = 0.03$).

Males were aggressive towards intruders significantly more often than females (Mann Whitney:

$U = 6.00$, $Z = -1.92$, $P = 0.07$; Male average 2.98/min. ± 2.17; female average 1.29/min. ± 0.84).

Males spent significantly more time in extra-group aggression than did females (males: 0.08%,

females: 0.02%, $U = 4.00$, $Z = -2.24$, $P = 0.03$).

There was no significant difference between females and males in rates of sexual

behavior, either directed at the focal animal's breeding partner ($U = 17.50$, $Z = -0.12$, $P = 0.94$) or

toward the intruder group ($U = 13.00$, $Z = -0.90$, $P = 0.49$). Percent times spent sexually

soliciting, mounting, accepting mounts, or copulating during intrusions was low: On average,

0.07% total duration was spent soliciting the intruder group, and no time was spent engaging in

sexual behaviors between established breeding pairs during intruder tests.

Discussion and Conclusions

We explored behavioral differences between females and males in captive common

marmosets housed in family groups. We predicted that females and males also behave similarly

in the presence of an intruding pair. This hypothesis was partially supported. Both females and

males spent similar amounts of time in proximity to the intruders. We observed no difference in

sexual behavior between females and males during the intrusion of a breeding pair. However,

females also showed a greater amount of self-directed scratching than males, a behavior

indicative of stress (Norscia and Palagi, 2011). Females also spent less time aggressing toward

intruders than males. This result contradicts the findings of Harrison and Tardif (1989) who

found that males and females showed similar amounts of aggression during intruder tests. Like the current study, focal groups were kept in their home cages, but restricted to one half as intruders were given access to the other half of the home cage. Further, we used an intruding pair whereas Harrison and Tardif (1989) used a single intruder. Therefore, the contradictory results may be due to the different methods.

As predicted, we found no difference between female and male time spent aggressing, in sexual behavior, or in infant care during the non-experimental focal animal observations. Under these circumstances, we observed no sexual dimorphism in investment toward parental investment or mating in captive common marmosets.

We hypothesized that offspring care would negatively correlate with aggressive and sexual behaviors during non-experimental observations. We saw no correlation between sexual behavior and offspring care in either sex. Females also showed no correlation between offspring care and aggression. However, male aggression (e.g. genital displays, slit stares, tuft flicks) correlated negatively with infant interactions and positively with distance from infants. Variance in rate of infant interactions was similar between sexes. However, females showed less variance than males in distance from infants.

Limitations

While our prediction that females and males would behave similarly in the context of an intruder test was partially supported, our methods differed slightly from other intruder paradigms because we used breeding pairs as intruders. Previous experimental studies have used a single intruder and reliably documented aggression directed at same sex intruders (e.g. Anzenberger, 1985; Harrison & Tardif, 1989; C. Ross, French, & Patera, 2004; C. N. Ross & French, 2011).

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The current study provided focal animals with both a reproductive challenger and a potential mate. While there were low rates of aggression directed between the breeding partners, which would have indicated mate guarding, it is still possible that the presence of the breeding partner constrains an individual's response. Specifically, a member of a breeding pair may be less likely to engage in sexual solicitations with an intruder in the presence of their breeding partner, as has been reported in captive common marmosets (Anzenberger, 1985; Gerber, Schnell, & Anzenberger, 2002). However, keeping focal animals with their home groups during the intrusion is more similar to a natural intrusion than isolating an animal from its group members.

It should also be noted that while captive marmosets are most often kept in breeding pairs, wild marmosets display a broader range of mating systems (Garber et al., 2016, but see Yamamoto et al., 2009). Therefore, sexual behavior may differ between captive and wild groups. However, the typical captive group composition of a single breeding individual of each sex still represents one wild condition, despite not representing the full range of variation seen in common marmoset mating systems.

Our results suggest that although we saw no difference in general activity budgets of females and males, the underlying mechanisms influencing behavior may differ between sexes. In males, the androgen testosterone is proposed to mediate investment in mating effort versus parenting (Goymann et al., 2007; Wingfield et al., 1990). Previous research by Melber et al. (in prep) provides evidence that captive female marmosets have similar fecal T concentrations as males. Previous studies also show that androgens correlate with aggression in some mammalian females, although there is variation in which androgen (e.g. testosterone versus its precursor androstenedione) correlates with aggressive female behavior (reviewed by French et al., 2013).

Male investment in offspring is rare among mammals (Kleiman and Malcolm, 1981). However, callitrichids have high levels of non-maternal, and particularly male, care of offspring compared to other primates. Ross and Maclarnon (2000) report that common marmoset offspring receive 47.6% of their care from caregivers other than the mother (including fathers and siblings). This is in contrast to other, non-callitrichid primates, such as the genus *Macaca* who, at most, showed 13% of infant care came from non-maternal care givers (Ross and MacLarnon, 2000). One hypothesis that may address increased levels of male care suggests that infant care is a courtship strategy in male callitrichids (Ferrari, 1992; Garber, 1997). However, Tardif and Bales (1997) compared male carrying behavior with mating frequency in captive common marmosets and did not find support for this hypothesis. Our results also do not support this hypothesis as no measure of offspring care correlated with sexual behavior.

As an alternative to the male courtship strategy hypothesis, both female and male infant care may be explained by the relationship between energy availability and litter size in common marmosets. In species producing litters, such as common marmosets, Stockley and Hobson (2016) argue that mammalian females take advantage of male offspring care to increase the number of offspring they can produce, and that increased litter size is a consequence of males provisioning offspring rather than the cause. If so, this suggests that the motivation for providing offspring care may differ between females and males. While common marmosets most commonly produce twins, in captivity triplets are common and quadruplets are possible when females have access to increased energetic resources (Tardif and Ross, 2009). Thus, a positive energy balance could result in more fertilizable eggs. Since female common marmosets ovulate within 20 days of giving birth and must then support a pregnancy and the costs of lactation (Tardif et al., 2003), females should immediately minimize energy spent on parental investment

of the current litter. Males, in contrast, should increase energy spent investing in the current litter in order to maximize the size of the future litter. In our sample, variance in rates of infant interactions were similar for females and males, but males showed greater variance in distance from infants than females. One interpretation of this difference is that females may have less flexibility than males in energetically investing in offspring. Female common marmosets must take infants in order to breastfeed but they also allow males to care for neonates immediately after birth (Ingram, 1977). Therefore, in common marmosets, it is possible that females, unlike males, have reduced their energetic investment in offspring to the extent possible, given they also need to breastfeed. It is also possible that reproductive opportunities associated with a positive energy balance in females led to an overlap in female and male behavior in which we see decreased female investment and increased male investment in offspring.

Ethical Statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in this study were in accordance with the

ethical standards of the Texas Biomedical Research Center and Southwest National Primate
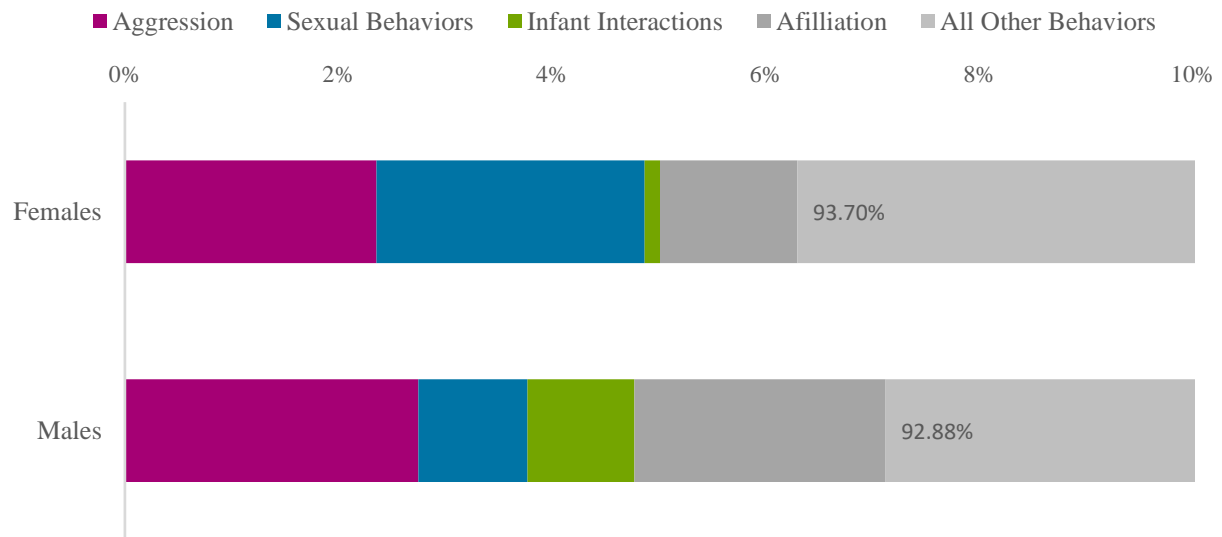
Research Center.

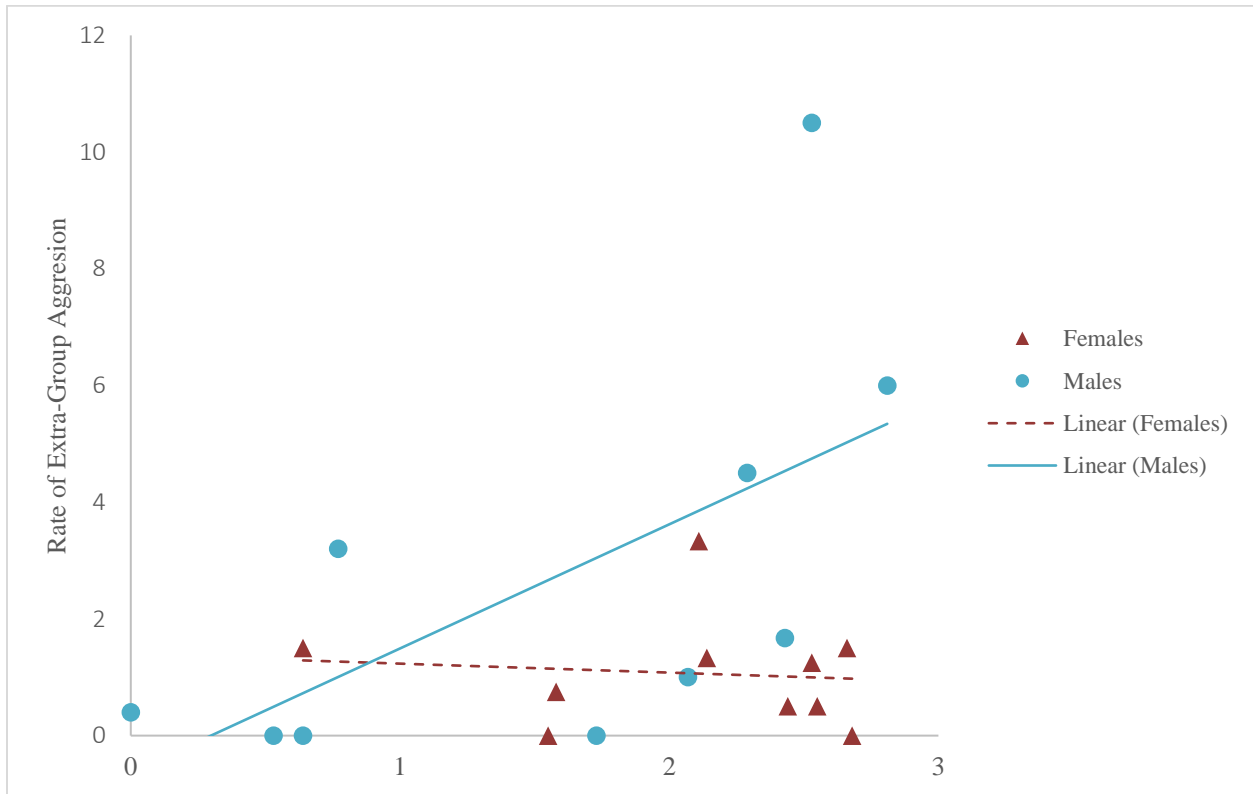**Figure 4.1:** Activity Budgets for female and male marmosets

Figure 4.2: Average Infant Distance Scores and aggression directed at non-group members. Females are in red triangles (dashed line), males in blue circles (solid line).

Table 4.1: Comparison of female (N = 25) and male (N = 24) instantaneous data.

Behavior	Female mean ± standard deviation	Male mean ± standard deviation	Mann-Whitney U	Z	P-Value
Contact Aggression	0.0% ± 0.00%	0.0% ± 0.00%	287.5	0.00	1.00
Non-Contact Aggression	2.36% ± 3.32%	2.75% ± 4.36%	286.5	-0.02	0.98
All Aggression	2.36% ± 3.32%	2.75% ± 4.36%	286.5	-0.02	0.98
Copulate	0.24% ± 0.96%	0.12% ± 0.62%	274	-0.66	0.51
Mount/Allow Mount	1.93% ± 5.95%	0.73% ± 1.53%	285	-0.08	0.94
Sexual Solicit	0.34% ± 1.06%	0.16% ± 0.63%	273	-0.56	0.57
All Sexual Behavior	2.51% ± 5.99%	1.02% ± 2.18%	256	-0.86	0.39
Retrieve Infant	0.00% ± 0.00%	0.80% ± 4.00%	276	-0.96	0.34
Resist Infant Steal	0.00% ± 0.00%	0.00% ± 0.00%	287.5	0.00	1.00
Attempt Infant Steal	0.00% ± 0.00%	0.00% ± 0.00%	287.5	0.00	1.00
Allow Infant Steal	0.00% ± 0.00%	0.08% ± 0.38%	276	-0.96	0.34
Remove Infant	0.10% ± 0.49%	0.00% ± 0.00%	275	-1.04	0.30
Harass Infant	0.04% ± 0.19%	0.13% ± 0.63%	287	-0.03	0.98
All Positive Infant Behaviors	0.00% ± 0.00%	0.80% ± 4.00%	276	-0.96	0.34

Table 4.2: Correlations between aggressive and sexual behaviors with infant care. Values given are Spearman's correlation coefficient. Sample sizes are 15 females and 15 males.

	Total Infant Interactions		Positive Infant Interactions		Negative Infant Interactions		Distance from Infants	
	Spearman's Rho	*P-value*	Spearman's Rho	*P-value*	Spearman's Rho	*P-value*	Spearman's Rho	*P-value*
Female Aggression	0.17	*0.65*	0.05	*0.9*	0.56	*0.1*	0.45	*0.19*
Female Sexual Behavior	-0.42	*0.26*	-0.17	*0.66*	0.26	*0.48*	0.11	*0.76*
Male Aggression	-0.65	***0.03***	-0.72	***0.01***	0.14	*0.66*	0.83	***0.003***
Male Sexual Behavior	0.04	*0.91*	0.19	*0.57*	-0.41	*0.18*	-0.52	*0.12*

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CHAPTER 5: CONCLUSION

Overview of Concepts and Hypotheses

      This dissertation was framed by the observation that females and males can exhibit similar behaviors, such as intrasexual competition (Clutton-Brock, 2009), and that testosterone, investigated more often in males than females, plays a role in mediating behaviors such as aggression. This led me to question the relationship between female T and the same behaviors. I sought to understand the ways that sexual dimorphism is exhibited in both testosterone and the testosterone-behavior relationship.

      Previous work has examined the relationship between female and male testosterone. Testosterone is pleiotropic, evidence exists female and male T levels are correlated, and other authors have seen a correlation in T concentrations between full siblings (Goymann & Wingfield, 2014; Ketterson, Nolan Jr, & Sandell, 2005). Goymann and Wingfield (2014) also found a correlation between female and male maximum T concentrations across 51 bird species. At the time I started this dissertation, variation in the degree of dimorphism was appearing in the literature and a limited number of studies showed no dimorphism between sexes in T values (rock hyrax: Koren & Geffen, 2009; Koren, Mokady, & Geffen, 2006; mouse lemurs: Zohdy et al., 2014).

      Cross-species comparisons of female and male T had been limited to birds and had conflicting results: Ketterson et al. (2005) saw a correlation between mating system and the relationship of maximum female and male T concentrations in a study of 44 bird species. However, Goymann and Wingfield (2014) did not find the same correlation. Goymann and Wingfield (2014) had more stringent inclusion criteria, only focusing on free-ranging studies of birds with plasma samples measured by RIA, and also controlled for phylogeny, unlike

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Ketterson et al (2005). They conclude that testosterone does not function in females as it does in males with regard to behavior or ecology. Both studies looked at mating system, but not parental investment, as previous work in birds showed the latter to be less influential on T concentrations (Hirschenhauser, Winkler, & Oliveira, 2003). However, because mammalian females differ from avian females in that they are unable to share the costs of gestation or lactation with males, I looked to the non-human primates to investigate female and male testosterone dimorphism. Both bird studies also used the maximum male to maximum female T value, which Goymann and Wingfield (2014) note may mask variation. Therefore, I looked also at the ranges of values each sex displayed in T concentrations. Because it is not possible to compare raw values of hormones that have been assayed using different sample types, I compared the ratio of lowest male to highest female value to capture overlap in the sexes.

Patterns of testosterone secretion in males have often been explained by the Challenge Hypothesis, which views testosterone as related to a trade-off between infant care and mating efforts (Wingfield, Hegner, Dufty, & Ball, 1990). Primate studies have shown support for this hypothesis, and a more recent reevaluation of the Challenge Hypothesis confirmed the relationship between parenting, mating behavior, and testosterone secretion in birds and mammals (Hirschenhauser & Oliveira, 2006). Work examining whether this relationship extends to females has shown conflicting results. Multiple studies have shown a relationship between parental status and T in women (Gettler & Oka, 2016; Kuzawa, Gettler, Huang, & McDade, 2010). Women involved in monogamous relationships have lower T than those either single or in polyamorous relationships (Barrett et al., 2013; Edelstein, Chopik, & Kean, 2011). Aggression is correlated with female T in some contexts, but not others. For instance, female buff-breasted wrens displayed increased T concentrations following intruder tests during seasons in which

territory protection was important but not during seasons where parental care was a priority, despite displaying aggressive behavior in both contexts (Gill, Alfson, & Hau, 2007). If the Challenge Hypothesis is applicable to females, then I expected to see a correlation between testosterone and both mating effort (including mating-related aggression), and infant care behaviors. Common marmosets were an ideal species to assess this, since they exhibit a pattern of mating and infant care that, if the Challenge Hypothesis were applicable, should result in measurable changes in T following specific events, such as being exposed to intruders or providing infant care.

One assumption of the Challenge Hypothesis and related work is that testosterone is involved in and may mediate trade-offs such as between parenting and mating effort (Hau, 2007). Support for the mating-parenting trade-off is seen in some, but not all, contexts. For instance, Cain and Ketterson (2013) compared testosterone, aggressiveness and territoriality, and parental investment in female dark-eyed juncos by conducting intruder tests. They found that more females with a larger T response to intrusions were less broody, as expected, but spent more time provisioning offspring than females with a smaller increase in T. The results from Chapter 3 showed a relationship between T and these behaviors, but not as strongly as expected based on the predictions of the Challenge Hypothesis. Therefore, I felt testing whether or not a behavioral trade-off was present in common marmosets was warranted.

Summary and Synthesis of Results

In Chapter 2, I examined testosterone dimorphism in both captive common marmosets and a primate-wide analysis of female and male testosterone values. I found no difference between female and male fecal T concentrations in common marmosets. When placing these

results in the larger primate-wide analysis, a pattern did emerge. I expanded from the approach of Goymann and Wingfield (2014) or Ketterson et al. (2005), which both focused on maximum T values for each sex in each species. Similar to the results in birds, I did not find a relationship between Maximum Male:Maximum Female T concentrations when looking across primates by mating and parenting system. However, when I shifted to looking at the overlap between sexes a positive correlation was seen: species with more overlap between female and male T concentrations also had more overlap between sexes when it came to parental investment and mating competition, but since much of the available data came from fecal samples, future research focusing on serum or plasma T comparison are needed to confirm or invalidate this correlation.

In Chapter 3, I tested the Challenge Hypothesis in female common marmosets and compared the response of female T to that of males. This study used 6 marmoset groups to assess response to intruders, and 16 groups were available to test response to infants. Female and male T concentrations were not distinguishable under any context. Correlations between behavior and T values were observed, but often not statistically significant. Sample size limited the intruder study, but we did expect to see a stronger correlation between behavior and testosterone than what was observed. This led me to question the validity of the trade-off between mating effort and parental investment.

In Chapter 4, I tested whether adult marmosets displayed a behavioral correlation between infant care and both aggression and sexual behavior. Sexual behavior was not correlated with infant care in either sex. However, male common marmosets showed the predicted negative correlation between aggressive behavior and infant care. When I isolated aggression to aggressive acts directed outside of the home-group, this correlation held. Therefore, I concluded

this supported the trade-off between infant care and mating competition in males. What was surprising was that despite also showing the amount of time spent in infant care, aggressive behavior, or sexual behavior was the same for males and females, that females did not show the same evidence of a behavioral trade-off as did males. Fite et al. (2005) showed that in *Callithrix kuhlii*, females reduced infant care investment when made possible by the presence of allocare givers. I interpreted the current findings in light of this study, and hypothesize that the lack of trade-off in females may result in females having already reduced their investment as much as possible. Typically, captive common marmosets group members all help carry infants, however mothers must take them to nurse. Therefore, reducing investment may not be possible in this context without compromising infant survival.

Future Directions

When examining the Challenge Hypothesis, callitrichids are of particular interest. Our results were limited by sample size for hormone data during the intruder experiment, but do suggest a correlation between aggression and testosterone, and fit within the existing literature in other callitrichids (Ross & French, 2011). Furthermore, Harrison and Tardif (1989) found that social context impacted behavior during intruder tests in common marmosets. Since our results suggested a response in T to the social event of an intruder test, a larger study comparing T changes following intrusions across varied social contexts would lend further clarity to the relationship between behavior and testosterone in both sexes.

Clearly, more research is needed to better understand the relationship between infant care and mating effort, and in particular aggression in common marmosets. We did not find evidence for this trade-off in female marmosets, but did in males. One area that needs further exploration

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is whether or not this relates to group size. Garber (1997) found in the cooperatively breeding saddle-back tamarins that the number of helpers was positively associated with infant survival in the wild. Yet, mothers must still lactate and there is a limit to the amount of energetic reduction they can accomplish without risking infant death. I hypothesize that energy balance may explain the difference in trade-offs seen between sexes. This could be tested by using a comparative framework with *Callimico goeldii,* another callitrichine, but who typically gives birth only to a single offspring (Porter 2001) or by studying groups in which one twin is removed, thereby reducing the energetic burden of infant care. Further, in our captive colony, infants could safely be left on the walls of their cages until other group members retrieved them. Therefore, it may be that females in smaller groups with fewer helpers to choose from may be more likely to show this trade-off, if it exists. Alternately, the captive environment may mask the trade-off, if females can leave infants hanging on mesh substrates without worrying they will be left behind. However, data examining both captive and wild marmoset groups showed that breeding females showed the least variance in infant carrying compared to other group members (Yamamoto, Box, Albuquerque, & de Fátima Arruda, 1996). Further data from the wild and with varied group sizes should be used to validate our conclusions on trade-offs.

One of the main opportunities to expand this research is to fill in the comparative data set for female and male testosterone values. I was able to find only 16 species in which to compare females and males, and often had to turn to data from other publications rather than the more ideal scenario in which the same authors have run the analyses in the same lab. As a comparison, Goymann and Wingfield (2014) were able to compare 51 species of birds, even while limiting their dataset to free-range populations in studies whose authors used a specific sampling and

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assay technique. Primate studies are far behind those of birds, yet have a lot to offer this area due to the diversity in both mating and parental investment patterns exhibited by primates.

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Behavior	Description
Contact Aggression	Focal animal attacks (tumble fights) with another animal.
General Agonism	Includes threat gestures and overt aggression directed toward another group member.
Copulate	Male has mounted female and thrusting is observed.
Mount/Allow Mount	Male grips back of female with hands and possibly feet.
Sexual Solicit	Includes tongue flicking, lip smacking, staring at partner with slit eyes and flat tufts.
Social Play	Focal animal is involved in a play-bout with at least one other group member. May include tumbling, chasing, wrestling, etc. No pilo-erection is seen.
Infant retrieve	Focal animal retrieves infant; picks up or allows infant to climb onto adult.
Submissive	Focal animal exhibits a submissive stance or gesture directed at another group member
Groom	Focal animal is grooming another group member: parting fur and possibly removing debris
General Affiliation	Includes behaviors such as hand, clap, hug, groom directed toward another group member.

Resist infant steal	Tries to prevent another individual from removing infant off focal.
Infant remove	Focal removes infant from its body.
Infant Harass	Focal inappropriately handles infant.
Allow infant steal	Focal animal allows another individual to remove infant from the focal animal
Attempt Infant Steal	Focal animal attempts to remove infant from another individual who was not trying to remove the infant.
Allow food steal	Another group member is allowed to take food from focal. Food was not overtly offered.
Attempt food steal	Individual tries to steal food from other group member.
Receive Agonism	Individual receives threat gesture or aggression from another group member.
Receive Groom	Focal animal is groomed by another group member
Receive Affiliation	Focal individual receives affiliative gestures from another group member.
Feed/Forage	Individual is searching through, manipulating, or ingesting food items.
Resist food steal	Tries to prevent group member from taking food item(s) from focal
Food share	Food items are overtly offered to other group member.
Food steal/Attempt food steal	Focal animal attempts or succeeds to take food items from other individuals.

Food beg	Individual is begging for food from another group member.
Scent Mark	Individual is rubbing scent glands on home cage features or other individual.
Locomote	Includes vertical and horizontal movement. Excludes chasing, playing.
Self/Solitary Play	Focal individual is playing alone, with or without an object
Self Groom	Individual is grooming itself.
Solitary cry	Infant is not being carried by a group member. Vocalizing ("crying") to be retrieved.
Self-Directed Behavior	Focal animal is scratching self or other self-directed behaviors that does not include self-grooming
Inactive	Animal is not moving or engaged in any other behavior on the ethogram. Eyes may be open or closed.
Other	Individual is engaged in a behavior not on the ethogram. Provide description.
Attention human	Focal animal is directing attention or behavior toward or interacting with researcher/staff member.
Out of View	Individual or their behavior is obscured from view.

APPENDIX B: INFANT DISTANCE SCORES

Score	Definition
0	In contact with infant. Includes carrying.
1	Within one adult body length of infant.
2	Between one and three adult body lengths of infant.
3	Over 3 body lengths from infant