Social interactions and androgens levels in marmosets (Callithrix jacchus) in field and laboratory studies: A preliminary investigation of the Challenge Hypothesis

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1. Introduction

Living organisms need time and energy to perform basic activities in order to survive and reproduce, including growth, development, energy storage, maintenance of vital activities, and pathogen defenses, as well as mating and reproductive efforts during the life cycle (Dammhahn and Kappeler, 2012; Gittleman and Thompson, 1988). However, both time and energy are limited resources and trade-offs are necessary to optimize decisions about how to use them (Kaplan and Gangestad, 2005). Thus, the life history theory predicts that natural selection favors individuals that better allocate energy resources to promote lifetime reproductive success (Del Giudice et al, 2015; Ellison, 2017). In this context, one of the crucial trade-offs that needs to be managed is the cost of reproduction, involving mating effort versus parental effort (Muller, 2017).

The endocrine system mediates the trade-offs for energy distribution since it exerts numerous simultaneous effects that allow a coordinated chain of action (Rodney, 2016). In relation to reproductive success, the hypothalamic-pituitary–gonadal axis plays a central role in vertebrates, and in males, testosterone affects sexual, aggressive, reproductive and metabolic functions.

According to the Challenge Hypothesis (CH) (Wingfield et al, 1990), four contexts promote changes in testosterone secretion: (i) baseline production mainly devoted to maintaining physiological and reproductive functions; (ii) territorial-related social aggression, when testosterone increases beyond the basal levels, and (iii) male-male competition for receptive females where an additional increase is observed. However, during (iv) parental care, testosterone levels generally decline (Goymann et al., 2007; Wingfield, 2017). Thus, the main trade-off proposed for androgen variation is increased testosterone in contexts of aggression and sexual interactions versus a decline in testosterone during parental care. Despite this significant trade-off, it is also important to underscore that the pattern of androgen secretion in response to sexual, parental and social contexts varies in different species.

Since Wingfield et al. postulated the CH (Wingfield et al, 1990), a large number of field and laboratory studies have shown the importance of genes, age, season, time of day and social, physical and environmental characteristics in modulating the androgen response (Kempenaers et al, 2008). For instance, monogamous birds exhibit greater androgen responsiveness to social contexts than promiscuous birds (Hirschenhauser and Oliveira, 2006). In some species of non-human primates, the increase in testosterone during the breeding season correlates positively with levels of male-male aggression (Agouta catta: Cavigelli and Pereira, 2000; Eulemur fulvus: Ostner et al., 2002), whereas in others (Cebus apella, Lynch et al., 2002) it correlates with female receptivity during this period. Moreover, some species show no change in testosterone levels during the reproductive season (Brachyteles arachnoides: Strier et al, 1999) or during periods of social instability (Saguinus mystax: Huck et al., 2005), contradicting CH predictions. Therefore, reproduction frequency (seasonal or non-seasonal), type of mating system, degree of parental investment and levels of male-male aggression are important species-specific factors that modulate androgenic reactivity by influencing both the amplitude and duration of the response (Hau et al., 2008; Hirschenhauser et al. 2003; Hirschenhauser and Oliveira 2006; Kempenaers et al, 2008). In addition to the classical seasonal, male-male and male-female responses, Goymann et al. (2007) also revised the original CH to include changes in androgen levels associated with non-social environmental cues and the physiological capacity of animals to produce and secrete androgens. Additionally, change in androgen levels due to social isolation have also been studied, since in some species of birds, these levels rise during social isolation and decrease in social contexts.
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Common marmosets (*Callithrix jacchus*) are small New World primates that live in scrub and Atlantic Forest in Northeastern Brazil (Roylans and Mittermeier, 2009) in social groups that vary from 3 to 19 individuals (Stevenson and Rylands, 1988) and form extended families where mothers, fathers and older siblings participate in infant care (Yamamoto, 1993). Moreover, non-related adults belonging to the social group, other than offspring, also contribute to caregiving. They are considered relaxed seasonal breeders (Brockman and Van Schaik, 2005) and territorial animals that defend their home-range area from neighboring groups. Social interactions within natural group members involve competition and cooperation (Yamamoto et al., 2009), but social organization for both sexes is based on hierarchy of dominance. In this species, dominant females act aggressively toward other females in the group, whereas subordinates display submissive behavior to dominant members (Abbott and Hearn, 1978). The dominant female typically exhibits hormonal levels that indicate ovarian cycles whereas subordinates might exhibit complete ovarian suppression (Abbott, 1984), but daughters may ovulate in the family group (Ziegler and Sousa, 2002) or even reproduce in free-ranging groups, likely through extra-group copulation (Albuquerque et al., 2001; Sousa et al., 2005). 

Dominance hierarchy among males has also been recorded, but aggressive displays from dominant males toward subordinates are not as common or intense as those observed among females, and hormonal inhibition of subordinates is not confirmed in wild males. Studies in captivity have also shown that reproductive males experience a decrease in androgens when living in captive family groups with pregnant females. As such, in this reproductive phase, adult sons have higher androgen levels than their fathers (Castro and Sousa, 2005). According to Abbott (1984) male subordinates or male offspring are suppressed or prevented from exhibiting sexual behavior. Moreover, free-living breeding males showed a significantly higher ratio between testicular volume and body mass compared to non-breeding marmosets, suggesting that testis size varies as a function of reproductive status in the social group (Araújo and Sousa, 2008). This increase in testis functioning in dominant individuals is probably related to reproductive efforts. Thus, reproductive success of subordinate males seems to be also low as demonstrated for subordinate females. It was recently suggested that male reproductive success might occur from inheriting the reproductive position in the natural social group as documented through long-term monitoring (Yamamoto et al., 2014). Marmoset fathers provide parental care from one week after birth and they share the time spent carrying the infants equally with the mother, who is almost always the exclusive carrier of infants (Yamamoto et al., 2008). Shortly after parturition, breeding females exhibit a new fertile period (postpartum ovulation, mostly between 10 and 18 days after infants’ birth) that might result in a new pregnancy (Dixon and Lunn, 1987). Thus, during the early post-partum period, marmoset males might be simultaneously engaged in competition for exclusive sexual access to the reproductive female as well as in parental care.

Considering the aforementioned characteristics of marmoset males and in line with CH (Goymann et al. 2007; Wingfield, 2017), a number of predictions concerning androgen level fluctuations can be made: (a) a rise in androgen levels during territorial acquisition or expansion, if these contexts involve male competition, (b) an increase in androgen levels in response to males confronting unfamiliar males in competitive contexts, such as intergroup encounters, where copulations and mate guarding frequently occur (Digby, 1999) and (c) a decrease in androgen levels during paternal care. Additionally, in a review on the applicability of this hypothesis for primates, Muller (2017) suggested that a rise in androgens primarily occurs in response to interaction with fertile females.

Thus, the applicability of the Challenge Hypothesis for marmosets seems to be of great interest, given that male-male competition as well as male-female interactions and opportunities for males to access ovulating females on the borders of the home range, are common occurrences in the wild (Araújo, 1996; Lazarro-Perea, 2001; Sousa et al., 2005). Further, during the postpartum estrus of breeding females, breeding males are involved simultaneously in mating and paternal investment, which is critical for infant survival (Yamamoto et al., 1996). Although the androgen response to reproductive contexts had already been evaluated for callithricidae species (p. ex. *Callithrix geoffroyi*, Birnie et al., 2011; *Saguinus oedipus*, Ziegler et al., 2004; *Callithrix kuhlii*, Ross et al., 2004), these previous studies do not aim to test directly the CH predictions.

Muller (2017) argues that data from a range of wild primate species support the CH. However, for those species that present direct paternal care associated to nurturance more than just infant defense against infanticide and predation, the author advised that the wild data are still scarce and captive data are sometimes contradictory. Thus, the present study, which provides androgen data of male marmosets in both contexts - i.e. living in wild groups, where they are able to express their natural reproductive strategies, and in laboratory-controlled situations that simulate territorial and mating competition, can contribute to clarify the androgens’ role in shape aggressive and reproductive interactions in this cooperative breeding primate species.

2. Methods

2.1. Field data

2.1.1. Subjects and field conditions

Two natural groups were monitored from April to September 2005. Group 1 (G1) consisted of 6 individuals: 3 males (2 adults and 1 subadult) and 3 females (1 adult breeding, 1 subadult and 1 infant). During the monitoring period, a breeding female gave birth (August) to two infants, but only one survived. In the same month, the subadult male emigrated (08/16/05) (Table 1). Group 2 (G2) was composed of 5 individuals: 4 males (2 adults, 1 subadult, and 1 juvenile) and the breeding female. During the monitoring period, the subadult male emigrated and two infants were born in July (Table 1).

Before data collection, all the animals were captured and fitted with

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Groups’ composition and additional data about the animals.</th>
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</thead>
<tbody>
<tr>
<td>Composition</td>
<td>Birth and emigration/immigration records</td>
</tr>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
</tr>
<tr>
<td>Breeding male (*)</td>
<td>Born in September 2003</td>
</tr>
<tr>
<td>Non-breeding male (*)</td>
<td>Arrived as adult on Oct. 23/2004</td>
</tr>
<tr>
<td>Breeding female (#)</td>
<td>Emigrated in August/2005</td>
</tr>
<tr>
<td>Subadult male</td>
<td>Arrived as adult on Oct. 10/2004</td>
</tr>
<tr>
<td>Subadult female</td>
<td>Born in January/2004</td>
</tr>
<tr>
<td>Infant female</td>
<td>Born in June/2004</td>
</tr>
<tr>
<td>Infant (=1) (#)</td>
<td>Born in August/2005</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
</tr>
<tr>
<td>Breeding male (*)</td>
<td>Evaluated at the first capture (+)</td>
</tr>
<tr>
<td>Non-breeding male (#)</td>
<td>Evaluated at the first capture (+)</td>
</tr>
<tr>
<td>Breeding female (#)</td>
<td>Evaluated at the first capture (+)</td>
</tr>
<tr>
<td>Subadult male</td>
<td>Evaluated at the first capture (+)</td>
</tr>
<tr>
<td>Juvenile male</td>
<td>Emigrated in July/2005</td>
</tr>
<tr>
<td>Infant (=2)</td>
<td>Born in July/2005</td>
</tr>
</tbody>
</table>

(*): Focal males; (#): Pregnant females; (*)&: One infant disappeared within the first week after birth. (+): The age stages were classified according to Stevenson and Rylands (1988), and estimate age in months used morphometric measures (Lazarro-Perea, 2001; Araújo et al., 2000).

(*) Focal males; (#) Pregnant females; (&) One infant disappeared within the first week after birth. (+) The age stages were classified according to Stevenson and Rylands (1988), and estimate age in months used morphometric measures (Lazarro-Perea, 2001; Araújo et al., 2000).
a 3-colored collar to identify the individual, its group, and age in months (estimated when necessary), since the birth data and sex of some animals were known from previous group monitoring. The animals’ age was classified before group monitoring using Stevenson and Rylands (1988) categories: infant (0–5 months old), juvenile (6–10 months old), subadult (11–15 months old), and adult (> 15 months old). For individuals whose date of birth was not available, body size, weight, and morphological physical appearance such as ear tuft coloration and dentition were also considered (Ararjo et al., 2000; Lazaro-Pereia, 2001). We also dyed their tails and parts of their body to facilitate individual identification.

The animals inhabited a peri domiciliary area of an experimental farm with mango, cashew, almond and hog plum trees. In Macaiba County, 25 km from Natal, in Northeastern Brazil (5°53’S and 35°23’W). The local climate is a transition between types As and BSh according to the Köppen-Geiger classification (Geiger, 1961), with high temperatures throughout the year and rainfall from March to August. Average annual rainfall is 1227 mm and annual temperature is stable year around (26°C) (Cestaro and Soares, 2004). Both groups (G1 and G2) home ranges were 500 m apart and occupied an area of 2025 m² and 3600 m², respectively. The total area where intergroup encounters with neighboring groups occurred was around 200 m² (9.87%) and 1200 m² (30%) of the total home range area, for G1 and G2, respectively. Thus, the G2 intergroup encounter area was around six times larger than that of G1, since a substantial portion of the G2 home range overlapped with its neighboring group.

### 2.1.2. Data collection

Both groups were monitored for approximately 5 and a half months (165 days), from April 14 to September 26, 2005. Behavioral data collection took place once a week, using the instantaneous focal technique for 5 min each hour (Altman, 1974) for approximately 12 h (light phase). All records were quantified for frequency, for a total of 9563 records, using chromometers, print sheets and a voice recorder. Agonistic behaviors expressed toward individuals from the neighboring group and each other. Agonistic behaviors were recorded (Table 2) and fecal samples were collected to measure androgen levels as previously mentioned. Agonistic behaviors were recorded (Table 2) and fecal samples were collected to measure androgen levels as previously mentioned.

### 2.2. Laboratory data

#### 2.2.1. Subjects and laboratory conditions

In this part of the study 25 male and 9 female captive-born adult marmosets were used. The animals were all living in outdoor masonry cages under natural lighting, humidity, and temperature conditions. Animals were living in two types of cages depending on the situations: isolated animals or dyads were housed in 1.0 × 2.0 × 1.0 m cages, heterosexual pairs, and newly formed groups were allocated in 2.0 × 2.0 × 1.0 m cages (see situations a, b.1 and b.2 below). The front of both types of cages consisted of a glass wall with a unidirectional visor, and a water bottle and plate of food were placed in the wire mesh door on the back wall. The cages contained a nest box in which the animals slept and rested, wood plank flooring and perching branches for environmental enrichment and to allow them to move around the cage. The animals were habituated to the presence of the researchers prior to the study. Veterinary care was provided throughout the experiment. Water was available ad libitum throughout the study and all animals were fed twice a day with seasonal fruits such as banana, papaya, melon, and mango, as well as potato and a protein potage containing milk, oats, egg, and bread. A multivitamin supplement (Glicocan) was administered twice a week. Social isolated animals had no visual contact with conspecifics but were allowed auditory and olfactory contact with conspecifics. Although this allows to the animals to be maintained under environmental conditions similar to those before the beginning of the experiment, in order to avoid significant variations in the captive setting, both types of sensory contact still have the potential to be influencing our results.

All the animals were housed at the Primatology Center of the Federal University of Rio Grande do Norte, Natal, Brazil, according to IBAMA (Brazilian Institute of Environment and Renewable Natural Resources) guidelines (Normative Instruction no. 169 of February 20, 2008), and the care standards for animals established by CONCEA – National Council for Animal Experimentation Control, Law No. 11.794, (October 8, 2008). In addition, the laboratory complies with international standards for ex situ maintenance of animals as defined by the Animal Behavior Society and the International Primatological Society. The study and experimental procedures were approved by the Animal Research Ethics Committee of the Department of Physiology, Federal University of Rio Grande do Norte (UFRN), under the protocol number 07/2014 addendum 11.20.17.

#### 2.2.2. Data collection

We collected and analyzed the data of captive marmoset males in two main contexts as described below: (a) Male-male response and (b.1. and b.2) Male-female response for purpose of comparison with field data:

a. Male-male response (competition for territory) – Eight male iso sexual dyads were formed in a new cage (similar to where the animals were living previously) to analyze male-male territorial competition. To estimate the relatedness effect between marmosets in this context 4 related (R) and 4 unrelated (UR) isosexual dyads were formed. Before dyads formation, all males were living isolated for at least 7 days. In the two conditions, isolated and in dyads, fecal sampling to measure androgen levels as well as behavioral sampling were performed daily for 7 days in the early morning (between 6 and 8 a.m. to avoid circadian influence). For more detailed information see Section 2.3.

Behaviors such as autogrooming (duration) and piloerection (frequency) and scent marking (frequency) were recorded in both phases (isolated and in dyads) and social behavior such as simultaneous piloerection (frequency), contact (duration) and allo grooms (duration) were recorded only during the dyad phase. The behavioral definition followed the ethogram compiled by Stevenson and Poole (1976), Lipp (1978) and Tardif et al. (1986). To obtain the behavioral data the focal continuous method was used during 30-minute observation sessions of focal animals (Altman, 1970) (Table 2).
Table 2

Descriptions of behaviors recorded for the focal males based on Lipp (1978), Stevenson and Poole (1976), Tardif et al. (1986).

<table>
<thead>
<tr>
<th>Behaviors</th>
<th>Description</th>
<th>Context</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autogrooming</td>
<td>Act of self-grooming</td>
<td>Laboratory – simulated territorial dispute/ dyad formation</td>
<td></td>
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<tr>
<td>Affiliation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contact</td>
<td>Resting in physical body contact with another conspecific</td>
<td>Field – to characterize breeding couple</td>
<td></td>
</tr>
<tr>
<td>Proximity</td>
<td>Remaining close &lt; 2 m (for field)</td>
<td>Laboratory – simulated territorial dispute/ dyad formation</td>
<td></td>
</tr>
<tr>
<td>Allogrooming</td>
<td>Slow combing, cleaning or licking a partner fur using hands and/or teeth</td>
<td>Field – to characterize breeding couple</td>
<td></td>
</tr>
<tr>
<td>Simultaneous piloerection</td>
<td>The synchrony between animals of erection of the pelage and walking with an arched back</td>
<td>Laboratory – simulated territorial dispute/ dyad formation</td>
<td></td>
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<tr>
<td>Agonism</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Piloerection</td>
<td>Body pelage erection and walking with an arch bristle</td>
<td>Field – intragroup and intergroup encounters</td>
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<tr>
<td></td>
<td></td>
<td>Laboratory- simulated territorial dispute/ pair formation</td>
<td></td>
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<tr>
<td>Vocalization</td>
<td>Vocalizations consisting of a mixture of the calls mostly “erh-erh”</td>
<td>Field – intragroup, and intergroup encounters</td>
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<tr>
<td>Chasing</td>
<td>Pursuing and following a conspecific during locomotion</td>
<td>Field (intragroup, and intergroup encounters)</td>
<td></td>
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<tr>
<td>Scent-marking (anogenital)</td>
<td>Sit-rubbing the anogenital region along the substrate</td>
<td>Field (intragroup, and intergroup encounters)</td>
<td></td>
</tr>
<tr>
<td>Genital display</td>
<td>Raising the tail and presenting the genital region toward a target</td>
<td>Field – intragroup, and intergroup encounters</td>
<td></td>
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<tr>
<td>Sexual</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copulations</td>
<td>Mounting the back of female, gripping her with the hands and performed rhythmical pelvic thrusts</td>
<td>Field – intragroup, and intergroup encounters</td>
<td></td>
</tr>
<tr>
<td>Attempts of copulation</td>
<td>Mounting the back of female females without thrusting pelvic</td>
<td>Field – intragroup, and intergroup encounters</td>
<td></td>
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<tr>
<td>Parental care</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrying</td>
<td>Transporting infants as any position in which time infant was clinging with at least two limbs on the carrier during in resting or during locomotion</td>
<td>Field – intragroup</td>
<td></td>
</tr>
</tbody>
</table>

b. Male-female response (with and without potential mate competition) –
To assess androgens response in heterosexual pairs, two situations were created: male-female pairing without competitors (b.1. Pair formation, n = 5; 5 males/5 females), and new group formation where related males were paired with related females creating a potential context for mate competition (b.2. New group formation, n = 2; 4 males/4 females).

b.1. Pair formation – To analyze male response to the presence of a female in the absence of male competitors, five isolated male marmosets were paired with five isolated females, forming five unrelated heterosexual pairs. Both isolated and paired phases lasted seven days and male fecal samples and behavior were collected daily, there is in both isolated and paired phases, 7 fecal samples and 7 thirty-minute behavior observation sessions using the continuous focal method, were performed.

b.2. New group formation – To analyze male response to the presence of female in the context of potential mate competition, two new social groups were formed. Each group was composed by one pair of male and female twins. Males (n = 4) were monitored across two sequential experimental phases: (i) after twins be moved from their family groups and housed in new cages, and (ii) during group formation, when each pair of male twins was paired with a dyad of female twins (n = 4 females) to form a new group in another larger cage. The first experimental phase lasted four weeks, and the second two weeks. In both phases, fecal and behavioral samplings were performed in the morning (between 8:00 a.m. and 11:00 a.m.) three times a week. Thus, when living with a twin, 12 fecal samples and 12 thirty-minute observation sessions were performed, whereas during pair formation 6 fecal samples and 6 thirty-minute sessions were conducted.

The study design of wild and laboratory-housed conditions is shown in Fig. 1.

2.3. Fecal sampling and hormonal measurements

Fecal samples from both wild and laboratory marmosets were collected and placed in plastic Eppendorf tubes, with labeled information on time, animal identification and date. In all conditions, an observer monitored the animals until defection occurred, and samples were collected immediately with a spatula to avoid sample identification errors. In the laboratory, before each feces collection, the observer cleaned the cages to facilitate identification of the fresh samples.

The samples were stored at −20 °C until they were processed following the procedures described by Sousa and Ziegler (1998) for fecal enzyme-immunoassays. For the free-ranging study, the intra- and inter-assay coefficients of variation for androgens were 2.03% and 5.66% respectively. For captivity experiments, the intra- and inter-assay coefficients of variation for androgens were 6.02% and 14.64%, respectively.

2.4. Statistical analyses

For wild marmosets, descriptive statistics were used to show the individual mean and standard deviation of fecal androgen levels, and the total values observed for agonistic behaviors toward intra and extra group males, copulations and attempted of copulation during the field monitoring period of the 4 focal males from G1 and G2. These data were plotted as graphs. Also, androgens fluctuations during the monitoring period of the 4 focal males of G1 and G2 (putative breeding and
non-breeding males) and daily androgens levels of the 4 focal males from G1 and G2 within 3 weeks period before and after birth of the infants were also plotted in graphs.

The hormonal data of laboratory animals were normalized by logarithmic transformation (log). To analyze androgens levels before and after isosexual dyad formation (a.1) the general linear model (GLM) statistical test and Fisher’s post-hoc (LSD test) were used. In GLM, log-transformed of androgen-values was used as dependent quantitative variable and relatedness (related and non-related) as independent qualitative variable. For pair formation (b.1), the log of androgens was also analyzed by GLM. Additionally, non-parametric Wilcoxon test was used to analyze behavioral changes after isosexual dyad formation (a) and pair formation (b.1), and Mann-Whitney test was used to examine the influence of relatedness (related and non-related) on behaviors in (a).

For the new group formation (b.2), log-transformed androgen concentrations in the two experimental phases, before and after group formation, were compared using the Student t-test. For this experimental protocol, behavioral data were not investigated.

The significance level considered for all tests was $p < 0.05$.

3. Results

3.1. Field conditions

3.1.1. Male-male response

During the five months of monitoring the mean individual concentration of fecal excreted androgens by breeding and non-breeding males was $48.65 \pm 11.89$ and $39.96 \pm 7.7$ ng/g for Group 1 and $87.32 \pm 17.55$ and $68.44 \pm 9.38$ ng/g for Group 2, respectively (Fig. 2). The variation in androgen concentration between breeding and non-breeding males was 16% and 22% for G1 and G2 males, respectively. Monthly total number of agonistic episodes for the 4 focal males is shown in Fig. 3. The total frequency of extra-group agonistic episodes of breeding and non-breeding males were higher in Group 2 (47 and 34, respectively) than in Group 1 (6 and 4, respectively) (Fig. 2B) exhibiting a variation in agonistic interactions within groups of 66.6% and 27.7% for breeding in relation to non-breeding males of G2 and G1 respectively. However, the total frequencies of intra-group agonistic episodes of breeding and non-breeding males were very low in both (G1: breeding male toward non-breeding male = 2; non-breeding male toward breeding male = 0; G2: breeding male toward non-breeding male = 0; non-breeding male toward breeding male = 4).

3.1.2. Male-female response

Mating behavior was recorded mainly during intergroup encounters, and the monthly frequencies of sexual behaviors are shown in Fig. 3. For breeding male of G1 only copulation with breeding females were recorded. On the other hand, breeding male of G2 copulated with both breeding and extra-group females. Non-breeding males from both groups showed successful copulation only with extra-group females, but one attempt of copulation with the breeding female was recorded in G2.

Monthly means of individual androgen concentrations of the breeding and non-breeding males from G1 and G2 during the five months of monitoring are illustrated in Fig. 3. In the Group 1 reproductive pair copulated exclusively with each other and 2 copulations and 2 attempts occur in June, and 2 copulations in July ($n = 2$) before parturition and during the period of potential post-partum estrus. Non-breeding male performed 3 attempts of copulation with extra-group females in May, when androgens levels reaching values numerically higher compared to that of breeding males. Agonistic interactions were low, as mentioned before, but were higher in the month in which breeding male reach higher values in androgens, coincident with post-partum period. In relation to G2, breeding male copulates with breeding females in three occasions, twice in June and once in August. Breeding male also copulations with extra-group females, in five occasions, being once in April, June and September and twice in August. In all month episodes of agonism toward extra-group males were recorded associated with high number of episodes of sexual, agonistic or both behaviors as recorded in June and August, in the late month associated to post-partum period (Fig. 3).

3.1.3. Trade-off between mating competition and parental care

Births occurred in both groups during the study period. Androgen levels measured in three fecal samples collected from all breeding and non-breeding male from G1 and G2 within the interval of three weeks
before and three weeks after the births showed that androgens increased 20% and 175% in breeding males, respectively, after parturition. On the other hand, in non-breeding males androgen rise in the total of three samples was 2% and 39% in G1 and G2 following the births (Fig. 4). Breeding and non-breeding males were equally involved in parental care, in stationary and locomotion situations, during this period, when it is likely that post-partum estrus occurred since the breeding females were receptive to copulation (Fig. 3). Around this period males also exhibited agonistic behavior toward extra-group males during encounters (Fig. 3).

3.2. Laboratory conditions

3.2.1. Male-male response

Of the eight male dyads formed, when comparing androgen levels before and after dyads formation, only related dyads (R) (n = 4; 8 males), increased androgen levels compared to their unrelated counterparts (GLM test: F = 19.82, p = 0.00, R: p = 0.02, UR: p = 0.37) (Fig. 5A). Moreover, after dyads formation, the frequency of simultaneous piloerection and duration of contact between males were significantly higher in related dyads than in unrelated ones (Mann-

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**Fig. 3.** Monthly androgens levels in breeding and non-breeding males of G1 and G2 and its association with sexual and agonistic episodes: \( \text{●} = \) breeding male copulation with breeding female; \( \text{●} \) = breeding male attempts of copulation with breeding female; \( \text{●} = \) breeding male copulation with extra-group female; \( \text{●} = \) non-breeding male copulation with breeding female; \( \text{●} = \) non-breeding male attempts of copulation with breeding female; \( \text{●} = \) non-breeding male copulation with extra-group female; \( \text{●} = \) breeding males episodes of agonistic behavior; \( \text{●} = \) Non-breeding males episodes of agonistic behavior. Numbers within parenthesis means frequencies of sexual and agonist behaviors.
Whitney: simultaneous piloerection $U = 5395$, $p = 0.003$; contact $U = 4852$, $p = 0.003$).

3.2.2. Male-female response

3.2.2.1. Pair formation (absence of competitors). Mean androgen values for the five socially isolated males did not show a statistically significant change after pairing with five females in the first week after pairing (GLM: $F = 2.17$, $p = 0.12$) (Fig. 5B). Moreover, no significant changes in male scent-marking (Wilcoxon test: $z = -1.658$, $p = 0.097$) and piloerection (Wilcoxon test: $z = -0.114$, $p = 0.909$) behaviors were observed after pair formation.

3.2.2.2. New group formation (potential competition). Comparison of mean fecal androgen levels before and after the new pairs formation, each one containing 1 pair of male and 1 pair of female twins, showed that androgen increase was not significantly different between these two situations (Student’s $t$-test, $df = 23$, $t = -1.54$, $p = 0.1353$) (Fig. 5C).

Long term follow-up of pairs showed that pregnancy occurred later in 2 pairs of b.1 and in the putative dominant females of b.2. The summary of the results and their adequacy in terms of the Challenge hypothesis is shown in Table 3.

4. Discussion

4.1. Field conditions

Our field data show that the physiological correlates for males during periods of increased aggression at intergroup encounters involve a rise in androgen levels in both breeding and non-breeding marmosets, in line with the Challenge Hypothesis for male-male response during a territorial dispute. In this aforementioned context, the increase in androgen levels that occur during months of higher frequency of agonistic episodes was more prominent. The common marmoset home range is between 0.5 and 5.0 ha (Schiel and Souto, 2017), which must be maintained and defended on a daily basis to ensure reproduction and survival (Araújo, 1996). As such, adaptation to aggressive intergroup behavior is crucial to preserving it. This behavioral pattern is illustrated in the present study, although marmosets inhabit a peridomiciliary area where feeding resources were readily available, suggesting a strong role for the aggressive behavior used to defend their territory and guarantee both survival and reproduction.

In the present study, it was found that male-female androgen response of marmoset males living in the wild occurs during male-male response to territorial defense, since, in addition to defending the territory, intergroup encounters play an important role in marmoset reproduction by promoting mating opportunities (Lazarо-Perea, 2001). Genetic studies show that gene flow as a result of intergroup dynamics between natural groups is frequent and haplotypes from the breeding male were identified in an adult non-breeding male from a neighboring group (Faulkes et al., 2009). In a group of nine individuals, these authors also identified five matrilines in addition to that of the breeding pair. Long-term monitoring of nine wild marmoset groups has shown
that subordinate females have scarce reproductive opportunities, given that the literature contains no records of a subordinate female in-...ships within natural groups, even for non-related individuals, show high affiliation and low aggression (Digby, 1995), similar to the low aggression exhibited in groups G1 and G2 of the present study. The durable social relationships between males, primarily genetically related individuals (Yamamoto et al. 2014), might contribute to an inclusive fitness advantage, thereby increasing male-male cooperation (van Hooff and van Schaik, 1994) and favoring collaboration as a preferential strategy for the reproductive success of marmoset males (Yamamoto et al., 2010). Thus, male reproductive success in wild groups appears to depend on the opportunity for inherit breed position into the natal group (Yamamoto et al., 2014) or, eventually, through extra-group copulation with subordinate females since it seems to be the only opportunity they have to reproduce if they remain in their natal group (Araújo, 1996; Lazaro-Perea et al., 2000). However, infanticide might occur in the latter case (Arruda et al., 2005).

For wild marmosets, a preferential affiliative and sexual (copula-...ions) relationship between breeding males and females was observed when compared to that between non-breeding males and breeding females. This strong affiliation between breeding pairs was found in studies of wild marmoset groups living at the Nísia Floresta field station, 30 km from the current study area (Albuquerque et al., 2001; Digby, 1999). In relation to sexual behavior, breeding marmoset males copulate almost exclusively with breeding females. Thus, the social dynamics between wild adult males made difficult to discriminate factors that might influence androgen levels separately. As such, we can speculate that the high androgen levels observed in breeding males result from a number of factors acting in conjunction, mainly their leadership position in territorial defense and mate monopolization, that is, androgens seem to increase as a response to both territorial defense and receptive females.

It is also important to underscore that during the study period the females in both groups were pregnant and many studies have shown greater affiliation between breeding couples in captivity during this period (Sutcliffe and Poole, 1984; Woodcock, 1982), which is also an indication of early stages of pregnancy after pairing (Silva and Sousa, 1997). Indeed, a rise in male grooming of breeding females was also associated with conception in captive pairs during the postpartum period (Dixon and Lunn, 1987).

With respect to the trade-off for androgen response between competition for receptive females and paternal care in wild marmoset males, breeding males exhibited increased androgen levels after parturition. Moreover, copulations between the breeding pair were recorded in both groups in this period, suggesting the likelihood of postpartum estrus.

According to our results, the dynamics of androgens during increased parental care (first three weeks after parturition) occur jointly with postpartum estrus, that is, a male-female challenge, where copulation and female monopolization are necessary to increase reproductive success. Thus, evidence from our findings and studies on parental behavior in marmosets suggests low paternal care sensitivity to androgens as observed for C. jacchus by Ziegler and Sosa (2016) who found no decrease in father responsiveness to infant distress calls after they had received low or high testosterone doses. Moreover, for Saguinus oedipus males, Ziegler et al. (2004) showed that the increase in androgen levels, which coincides with their mate’s postpartum ovulation, do not alter father-infant interactions. Other hormones such as prolactin (Mota et al., 2006; Ziegler et al., 2009) and oxytocin (OT) may be the main hormonal modulators for parental care in marmoset males. Recent research reinforces the role of OT in marmoset carrying behavior (Pinkenwirth et al., 2016) and that the OT system (Lambert et al., 2011) is important for fathering and caregiving experience in bipari-ental rodents. Thus, future research is needed to explore these hormonal interactions.

### 4.2. Laboratory conditions

To better understand the androgens changes predicted by male-male response and male-female response, as observed under natural conditions, we conducted three experimental protocols in the laboratory. In the first, eight marmoset males were paired with related and unrelated males as isosexual dyads, to investigate relatedness modulation in androgen response in terms of territoriality. In this context, we observed different androgen variations associated with relatedness, where only related males exhibited an increase in androgens (male-male response) when they were paired as a dyad. However, these same related dyads also showed higher levels of affiliative behavior, such as contact and simultaneous piloerection, after dyad formation. Social relationships...
between marmoset males are based on cooperation, in contrast to females, who are usually engaged in competition (Arruda et al., 2005; Yamamoto et al., 2009), likely associated with adaptive mechanisms, given that during new group formation or rearrangement into the social group in the wild, the related males might inherit the breeding position (Yamamoto et al., 2014). Thus, this experimental protocol evidenced the low male androgen reactivity to the presence of unrelated males, in the absence of mate competition. Since males are more sympatric than females in the wild, and related males form the group’s social core (Yamamoto et al., 2014), when related males join the group, a rise in androgen is expected, enabling them to act cooperatively in territorial defense and potential sexual encounters. This is reinforced by the higher levels of coordinate behavior (simultaneous piloerection) and contact between related than unrelated males in the present study.

To analyze the influence of competition on male-female response, two protocols were used: pair formation (absence of competitors) and new group formation (presence of potential competitors). However, although the rise in androgens observed after group formation was not statistically significant, we cannot conclude that this result is definitive, given the small sample size (two groups). Although the retroactive monitoring of the offspring’s birth date of each breeding pair suggests that probably none of the females were ovulating at pair or group formation, long term follow-up of pairs showed that pregnancy occurred later in 2 pairs of b.1 and in the putative dominant females of b.2. Thus, despite the absence of acute androgen increase in related males after pairing in scenarios of low competition for females (2 males x 2 females), androgen increases might occur later, associated to female reproductive condition, as demonstrated by Ziegler et al., (2004) for common marmoset males exposed to the scent secretions of ovulating females.

4.3. Integrating the data to the Challenge Hypothesis

Several authors have investigated the androgen response to social challenges and the applicability of the Challenge Hypothesis (CH) in cooperative breeding species, mainly birds. However, even for this group, some questions remain unclear. Although this study investigated a small number of animals, to the best of our knowledge, it is the first to test the predictions derived from CH in a cooperative breeding primate species. Our field data suggest an increase in androgens in response to agonistic encounters with neighboring males, and as a function of mating opportunities, where androgen reactivity of breeding males was higher than that of their non-breeding counterparts. Moreover, the field results as well as data from other close related species of callitrichid, suggest that a rise in androgens when ovulating females are accessible seems do not influence paternal investment, likely as an adaptation aimed at improving future reproductive success.

Thus, given the temporal association between intrasexual competition, mating and parental challenges for marmoset males, the observed rise in androgens along with the male-male response in situations of territorial defense, male-female response and mating competition, are all predicted by the Challenge Hypothesis, but are difficult to be evaluated separately in this species.

Additionally, as evidenced by laboratory data, relatedness between males also seems to be an important aspect that shapes male-male response in the context of territoriality as well as the relationship between androgens and sexual and agonist behaviors. Given the marmosets’ complex social dynamics, these preliminary indications need to be further investigated, through either additional field studies, applying the same laboratory approaches to a large sample size or developing new experimental designs that simulate natural contexts, which would certainly contribute to better clarify the implication of CH for common marmosets.

5. Conflict of interest

The authors declare no conflict of interest.

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