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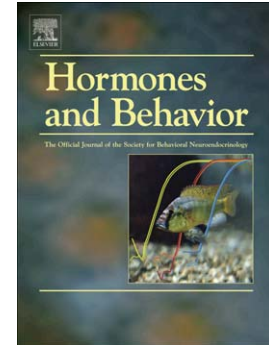
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**TITLE**

High rates of pregnancy loss by subordinates leads to high reproductive skew in wild golden lion tamarins (*Leontopithecus rosalia*)

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**ABSTRACT**

Across taxa, cooperative breeding has been associated with high reproductive skew. Cooperatively breeding golden lion tamarins (*Leontopithecus rosalia*) were long thought to have a monogynous mating system in which reproduction was limited to a single dominant female. Subordinates with few reproductive opportunities delayed dispersal and remained in the natal group to provide alloparental care to siblings, thus allowing dominant reproductive females to meet the energetic needs associated with high rates of reproduction and successful infant rearing. The goal of this study was to re-assess monogyny in wild golden lion tamarin groups based upon pregnancy diagnoses that used non-invasive enzyme immunoassay for progesterone and cortisol, combined with weekly data on individual weight gain, bi-annual physical examinations noting pregnancy and lactation status and daily behavioral observations. We established quantitative and qualitative criteria to detect and determine the timing of pregnancies that did not result in the birth of infants. Pregnancy polygyny occurred in 83% of golden lion tamarin groups studied. The loss of 64% of subordinate pregnancies compared to only 15% by dominant females limited reproductive success mainly to dominant females, thus maintaining high reproductive skew in female golden lion tamarins. Pregnancy loss by subordinate adults did not appear to result from dominant interference in subordinate hormonal mechanisms, but more likely resulted from subordinate abandonment of newborn infants to mitigate dominant aggression.

**KEYWORDS**

cooperative breeding; reproductive skew; polygyny; reproductive suppression; pregnancy loss;  
dominant control; subordination; progesterone; cortisol; *Leontopithecus rosalia*

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## INTRODUCTION

In cooperatively breeding species, reproduction is commonly limited to one or a few dominant individuals of each sex (Clutton-Brock, 2009). The young born to breeding females are reared by all group members, including non-reproductive helpers. Several mechanisms have been suggested to explain high reproductive skew in female cooperative breeders, including lower concentrations of reproductive hormones in subordinates (Creel et al., 1992; Faulkes and Bennett, 2001; Mays et al., 1991; Schoech et al., 1991), suppression of subordinate ovulation (Abbott, 1984; Faulkes and Bennett, 2001; French, 1997; Solomon et al., 2001), dominant interference in subordinate mating (Abbott, 1984), behavioral suppression of subordinate reproduction (Clutton-Brock et al., 2008; De Vleeschouwer et al., 2001; Inglett et al., 1989; Kleiman, 1979; Kutsukake and Clutton-Brock, 2006), stress-induced infertility and pregnancy loss (Gilchrist, 2006a; Pottinger, 1999; Wasser and Barash, 1983; Young et al., 2006), infanticide (Clutton-Brock et al., 1998a; Gilchrist, 2006a; Hoogland, 1985; Saltzman et al., 2009) and ecological constraints on subordinate reproduction (Clutton-Brock et al., 2001a; Creel and Creel, 1991; Emlen, 1982; Hatchwell and Komdeur, 2000; Kleiman, 1977b). If multiple females produce litters at the same time the amount of help received by the offspring of the dominant female may be reduced, resulting in lower infant growth and/or survival (Clutton-Brock et al., 2001b; Digby, 1995a; Hodge, 2009) and reduced dominant fecundity (Clutton-Brock et al., 1998b; Fite et al., 2005; Russell et al., 2003). Therefore, dominant females able to limit subordinate reproduction should have a selective advantage. Indeed, monopolization of reproduction by dominant group members by means of suppression of subordinate reproduction has been documented in several taxa of cooperative breeders including mammals (Solomon and

French, 1997), birds (Mays et al., 1991; Reyer et al., 1986; Schoech et al., 1991), fishes (Fitzpatrick et al., 2008) and invertebrates (Hamilton, 2004).

As long-term field research continues, the presence of multiple breeding females within cooperatively breeding groups originally described as monogynous or singular breeding has been observed in an increasing number of species including blue tits (*Parus caeruleus* (Kempnaers, 1994)), wolves (*Canis lupus* (Mech, 2000)), naked mole-rats (*Heterocephalus glaber* (Faulkes and Abbott, 1997)), dwarf mongooses (*Helogale parvula* (Creel and Waser, 1991)), common marmosets (*Callithrix jacchus* (Arruda et al., 2005; Digby, 1995b; Digby and Ferrari, 1994; Hubrecht, 1984)), pygmy marmosets (*Cebuella pygmaea* (Soini, 1982)), cotton-top tamarins (*Saguinus oedipus* (Savage et al., 1996)), saddle-back tamarins (*Saguinus fuscicollis* (Goldizen et al., 1996)) and moustached tamarins (*Saguinus mystax* (Garber et al., 1993; Garber et al., 1984; Ramirez, 1984; Smith et al., 2001)). Though multiple breeding females may be present, high reproductive skew remains, with subordinates having lower reproductive success than dominant females in banded mongooses (*Mungos mungo* (Gilchrist, 2006a, 2006b)), dwarf mongooses (Creel and Waser, 1997), meerkats (*Suricata suricatta* (Clutton-Brock et al., 2008; Young et al., 2006)), marmosets (Arruda et al., 2005; Digby, 1995a; Saltzman et al., 2009; Saltzman et al., 2008; Sousa et al., 2005) and tamarins (Garber, 1997; Goldizen et al., 1996). In captive and wild groups of cotton-top tamarins only one female per group gives birth regardless of the number of other pregnant females in the group (Price and McGrew, 1991; Savage et al., 1996). Non-invasive hormonal assays to determine the reproductive status of all group members can be used to reassess mating patterns in cooperatively breeding species as well as illuminate the mechanisms responsible for maintaining high reproductive skew in spite of the presence of multiple breeding females.

Marmosets and tamarins, neotropical primates in the family Callitrichidae (genera *Callithrix*, *Cebuella*, *Saguinus* and *Leontopithecus*), were long thought to have a monogynous mating system in which reproduction was limited to a single dominant female (Goldizen, 1987; Sussman and Garber, 1987). Golden lion tamarins (GLTs, *Leontopithecus rosalia*) are cooperative breeders that display a high degree of reproductive skew, with reproduction limited to one or a few dominant individuals of each sex even in groups containing as many as 13 individuals (Dietz and Baker, 1993). Dietz and Baker (1993) described the mating system in GLTs as monogyny with about a 10% incidence of polygyny. However, when examining only those groups containing more than one potentially reproductive female, 44.3% showed pregnancy polygyny (i.e. more than one female was confirmed to be pregnant within the same breeding season) and 26.2% showed rearing polygyny (more than one female reared offspring to weaning) (Baker et al., 2002; Dietz and Baker, 1993). Lion tamarin offspring typically delay dispersal and reproduction, and remain in their natal group to help with the care of infant siblings (Dietz and Baker, 1993). Cooperative care has been suggested to be instrumental in the ability of callitrichids to meet the energetic needs associated with successfully rearing the litters of twins they are capable of producing once or twice a year (Baker et al., 1993; Dietz and Baker, 1993; Kleiman, 1977a; Sussman and Garber, 1987). Therefore, lion tamarins represent a good candidate species for evaluating the occurrence of, and mechanisms underlying, singular vs. plural breeding in a cooperatively breeding mating system.

The goal of this study was to re-assess the degree of polygyny in wild GLT groups based upon pregnancy diagnoses that used non-invasive hormonal enzyme immunoassay for progesterone and cortisol combined with weekly data on individual weight gain, pregnancy and lactation status from bi-annual capture records and behavioral observations. We collected the

following data on females residing within seven groups of GLTs over three reproductive years: group demography, dominance status, reproductive status and reproductive success defined as pregnancies that resulted in the birth of live offspring. We tested two competing predictions derived from the hypothesis that reproduction is limited to a single dominant female in the majority of GLT groups. To the extent that dominant females fully ‘control’ reproduction in subordinates, we predicted that subordinate adult female GLTs would not become pregnant while residing within their natal group. In the case where dominant female control was incomplete, we predicted that pregnancies by dominant adult female GLTs would result in the birth of live offspring; whereas pregnancies by subordinate adult female GLTs would not. We also examined two alternative methods by which dominant females may control subordinate reproduction to maintain high reproductive skew. If dominant females suppress subordinate reproduction via hormonal mechanisms, we predicted that subordinates would not get pregnant, or at the least, that hormonal patterns during subordinate pregnancy would be abnormal, resulting in higher rates of subordinate pregnancy loss. Alternatively, if dominant females suppress subordinate reproduction via behavioral mechanisms, we predicted that subordinates might become pregnant, but that dominant aggression and harassment of pregnant subordinates would result in higher rates of pregnancy loss. We also predicted that the offspring of subordinate females might not survive because they would not receive infant care from group members attempting to avoid dominant aggression.

## **METHODS**

### **Study site and species**

Data were collected within the 6300 ha Poço das Antas Biological Reserve (PDA), Rio de Janeiro State, Brazil (22° 30' - 33' S, 42° 15' - 19' W) (Miller and Dietz, 2006). PDA holds the



largest remaining population of GLTs in the wild, with an estimated 350 GLTs in the secondary forests protected by the reserve (Ruiz-Miranda et al., 2008; Rylands et al., 2002).

### **Individual identification and weighing**

The animals under study at PDA are native and non-manipulated except for bi-annual live captures necessary for replacing radio collars to facilitate group location. During these routine captures, usually in May or early June and again in December or January, individuals are given identifiable markings (hair dye and tattoos), weighed and evaluated for growth and body condition including notes regarding female nipple length (reflecting parity), lactation and pregnancy (Dietz and Baker, 1993; Dietz et al., 1994). During the current study weights were also obtained weekly from August through December of each year using baited scales in the field (Bales, 2000; Bales et al., 2002; Siani, 2009).

### **Group demography**

We collected data on 7 groups of wild GLTs at PDA, each containing 2 to 13 individuals and 1 to 3 adult females. All individuals were habituated to the presence of human observers. Data were collected over three reproductive years: 2004-2005, 2005-2006 and 2006-2007. A reproductive year was defined as the 1<sup>st</sup> of March through the 28<sup>th</sup> of February in order to encompass the mating period, pregnancy and the first annual peak in infant births (October through November (Dietz et al., 1994)) as well as post-partum ovulation, mating and pregnancy that lead to the second annual peak in infant births (February (Dietz et al., 1994)). Six of these groups were observed from March 2004 through February 2007. Another group was added to the study in June of 2005, and was observed until the end of the study in February of 2007. Group sizes fluctuated, with losses typically filled by immigrants. Group composition was recorded daily including all births, deaths, emigrations and immigrations.

The ages of individuals born within study groups are known from long-term demographic data or estimated to year based upon weight, the eruption of permanent teeth and degree of tooth wear and discoloration noted at semi-annual captures (Bales et al., 2001; Dietz et al., 1994; French et al., 2003). Adults were defined as individuals older than 18 months of age (Dietz and Baker, 1993), corresponding to the average age of sexual maturation (Dietz et al., 1994; French et al., 2002; French et al., 1989; French and Stribley, 1987). Subadults were defined as individuals from 8 to 18 months of age.

Each group contained one adult female that was behaviorally dominant to other females in the group. A status of 'dominant female' was assigned to the predominant aggressor based upon archwalks, mounts and chases and winner of fights, and 'subordinate female' to the adult-aged females within each group that were targets of these agonistic displays (Bales et al., 2005; Dietz and Baker, 1993).

### **Fecal sample collection**

We collected 1176 fecal samples from individually identified female GLTs in seven free-ranging social groups from March 2004 through February 2007. Feces were collected from a total of 21 GLT females including 14 adults, 5 females that became adults and 2 females that became subadults during the study period (Table 1). We collected samples year-round during reproductive and non-reproductive months of the year. We collected fecal samples from each female once or twice per week as they left their sleeping locations or during subsequent observations. We attempted to restrict fecal collection to the morning hours to reduce diurnal variation in concentrations of fecal progesterone metabolites (French et al., 2003; Sousa and Ziegler, 1998). As a result, 28% of samples were collected before 0900h and 83% by 1200h. No more than 10 hours passed between fecal sample collection and storage in a freezer. Samples

remained frozen until analysis at the Endocrine Bioservices Laboratory, University of Nebraska at Omaha (UNO).

### **Pregnanediol-3-glucuronide (PdG) and cortisol extraction from feces**

PdG is a metabolite of progesterone excreted in feces and has been validated as a reliable indicator of circulating progesterone concentrations in lion tamarins (Bales, 2000; French et al., 2003). Cortisol is excreted directly into the feces. PdG and cortisol were extracted simultaneously from fecal samples. Fecal samples were allowed to thaw at room temperature, and large seeds, leafy material and undigested insect parts were removed. Fecal samples were dried in a drying oven at 37° C prior to weighing for hormonal extraction. Hormonal extraction was performed by briefly vortexing and then shaking 0.125 g of dried fecal matter in 2.5 ml of solubilizing extraction buffer (40% methanol (MeOH): 60% phosphate buffered saline (PBS)) for 12-16 hours on a shaker rack. Samples were then briefly vortexed to remove residue along the tube walls and centrifuged for 15 min at 2000 rpm at 6° C. The supernatant was decanted into a clean test tube and refrozen for storage until further dilution for assay.

### **PdG immunoassay**

PdG concentrations were determined through standardized enzyme immunoassay techniques (EIA) and calculations resulting from a four-parameter sigmoidal curve-fitting function as previously described (Bales, 2000; French et al., 2003; French et al., 1996). Internal quality control pools consisted of female Geoffroy's marmoset (*Callithrix geoffroyi*) urine since a more recent and extensive assay history existed for comparison. Quality control pools were run at high (1:80 dilution in 1:5 extraction buffer:PBS) and low concentrations (1:640 dilution in 1:5 extraction buffer:PBS). The intra- and inter-assay coefficients of variation for high and low concentrations of the urine quality control pool were 9.1% and 19.9% (high), and 7.4% and

26.7% (low), respectively. This EIA assay for fecal PdG was previously tested for accuracy and validated against circulating hormone levels (Bales, 2000; French et al., 2003).

Hormone concentrations that were within 10% above and below the standard curve (11,000 pg/well through 70.2 pg/well) were accepted and used at face value in calculations to arrive at ng PdG/g feces. Concentrations above (n=28) or below (n=167) the 10% cut off were assigned values of 11,000 and 70.2 pg/well, respectively. The large number of samples with very low PdG concentrations was expected due to the large number of samples from young females. This bias could not be avoided since dilution of fecal extracts by less than 1:5 or bringing more fecal extract to the EIA well would have caused interference in the assay due to the increase in methanol concentrations. As such, the risk of false positive pregnancy diagnoses based on PdG concentrations was minimal since overestimation was limited to very low PdG values, with very high PdG values conservatively underestimated.

### **Cortisol immunoassay**

Fecal cortisol concentrations were determined through standardized EIA techniques and calculations resulting from a four-parameter sigmoidal curve-fitting function as previously described (Bales, 2000; Bales et al., 2005). Internal quality control pools consisted of female Geoffroy's marmoset urine. Quality control pools were run at high (1:2,560 dilution in 1:10 extraction buffer:PBS) and low concentrations (1:20,480 dilution in 1:10 extraction buffer:PBS). The intra- and inter-assay coefficients of variation for high and low concentrations of the urine quality control pool were 3.9% and 11.4% (high), and 3.9% and 20.7% (low), respectively. This EIA assay for fecal cortisol was previously tested for accuracy and validated against circulating hormone levels (Bales, 2000; Bales et al., 2005).

Samples that were more than 10% above the highest standard were re-assayed after further dilution. A small number of samples produced results that were below the lowest standard value by 10% or more (15 cases distributed over 10 females with no female having more than 3 samples with low values). These samples were assigned the value corresponding to 10% below the lowest standard (7.02 pg/well) that was used to calculate ng cortisol/g feces.

### **Detection of ovulation, pregnancy, parturition and abortion**

We plotted PdG and cortisol concentrations over time to help visualize reproductive patterns for individual GLT females. Variation in progesterone levels corresponds to ovulation, the formation of the *corpus luteum* and placental development (Nelson, 2005; Ojeda, 1996) and has been used to trace ovarian cycles and pregnancy in GLTs (Bales, 2000; French et al., 2003; French et al., 2002; French and Stribley, 1985, 1987). We examined progesterone profiles of subordinate adult GLT females from 18 months of age until they became pregnant for the first time. Subordinate adult females were considered ovulatory if non-pregnant progesterone profiles showed cyclical elevation in PdG concentrations averaging 20 days in periodicity (French et al., 2002).

Pregnancy was diagnosed primarily by the prolonged elevation of PdG concentrations (Bales, 2000; French et al., 2003; French et al., 2002). Cortisol, a glucocorticoid responsible for mobilizing fat and protein reserves for use by the body in times of food deprivation or stress (Nelson, 2005), also shows a significant increase in concentration during the 3<sup>rd</sup> trimester of GLT pregnancy (Bales, 2000; Bales et al., 2005), so can be used as a late pregnancy diagnostic. In addition, pregnant GLT females demonstrate steady and considerable weight gain beginning in their 2<sup>nd</sup> trimester of pregnancy and continuing throughout gestation (Bales et al., 2001; Hankerson, 2008). Parturition was diagnosed by a sudden drop in PdG concentrations to baseline

levels (Bales, 2000; French et al., 2003; French et al., 2002) accompanied by a rapid and large amount of weight loss (mean=112.7 g; range 73 to 142 g; n=12 full term pregnancies for which immediate pre- and post-partum weights were available (Siani, 2009)). We defined successful pregnancies as those that were carried to full term and that resulted in observations of new infants carried and nursed during the first week after birth by the female identified as the mother. Birth dates were recorded within one or two days of parturition for most infants and within one week for all observed infants born during this study. To establish a calendar for each successful pregnancy we divided the 125-day gestation period (French et al., 2002) into trimesters. Trimesters were assigned by counting back from known parturition dates: 84-125 days (3<sup>rd</sup> trimester), 42-83 days (2<sup>nd</sup>) and 0-41 days (1<sup>st</sup>) (French et al., 2002; French and Stribley, 1985). Because hormone profiles are highly individualized, for each reproductive female we calculated average PdG concentrations during each trimester of pregnancy and during non-pregnant periods to provide “typical” values for successful pregnancies. These trimester averages were used as a guide in diagnosing suspected pregnancies for which infants were not observed and to determine the probable trimester in which pregnancies were aborted.

Pregnancy diagnoses and assignment of trimesters for pregnancies that did not result in observed infants were made by examining multiple sources of information on a case-by-case basis. We used average trimester PdG values for successful pregnancies as a reference to assign trimesters to unsuccessful pregnancies. Females that produced offspring showed mean PdG concentrations of 1500 ng PdG/g feces when they were not pregnant. Their mean PdG concentrations rose to 3500 ng PdG/g feces during the 1<sup>st</sup> trimester of pregnancy. Mean PdG concentrations above 5000 ng PdG/g feces were considered indicative of a pregnancy that had progressed into the 2<sup>nd</sup> trimester. Concentrations averaging 10,000 ng PdG/g feces or higher

were considered indicative of 3<sup>rd</sup> trimester pregnancy. Consistently high levels of PdG that suddenly dropped to baseline levels indicated pregnancy loss or parturition depending on when concentrations returned to baseline values (Bales, 2000; French et al., 2003; French et al., 2002).

Results from cortisol assays were also used as a crosscheck when diagnosing pregnancy. A large and consistent rise in cortisol reaching a mean of 15,000 ng cortisol/g feces was used to diagnose pregnancies that had proceeded into the middle to late 3<sup>rd</sup> trimester. Short rises in PdG concentrations that were not accompanied by weight gain often coincided with temporary increases in cortisol concentrations and potentially stressful events such as bi-annual captures, immigration of new individuals into social groups and increased group encounter frequencies. Extremes in nutrition and stress can affect any assay using a metabolite (PdG) rather than measuring the hormone of interest (progesterone) directly (Griffin, 1996). Thus, cortisol was also used as a crosscheck to prevent false positive early pregnancy diagnoses based upon elevated PdG concentrations resulting from stress.

We used patterns of weight gain or loss in GLT females to establish guidelines for diagnosing pregnancy from the 2<sup>nd</sup> trimester onward. Since no significant weight gain occurs during the 1<sup>st</sup> trimester of GLT pregnancy (Bales et al., 2001; Hankerson, 2008; Siani, 2009), we did not attempt to use weight to diagnose pregnancies that did not progress past the 1<sup>st</sup> trimester. Females gaining 5-10% of their non-pregnant average weight in a consistently rising pattern were considered to be in their 2<sup>nd</sup> trimester of pregnancy. Steady weight gain in excess of 10% of a female's non-pregnant body weight was used as an indicator of a 3<sup>rd</sup> trimester pregnancy. Females that gained approximately 20% of their body mass were considered to have reached full term, since neonatal weight for *Leontopithecus* is on average 20% of maternal body weight (Tardif et al., 1993). Average weight loss for 12 births during the current study for which

immediate pre- and post-partum weights were available was 18.6% of the females' post-partum weight (Siani, 2009). Rapid weight loss was considered indicative of parturition or the loss of a 2<sup>nd</sup> or 3<sup>rd</sup> trimester pregnancy. Using weight gain or loss based upon percentages of normative body weight allowed us to account for individual differences in non-pregnant body mass (Dietz et al., 1994; Siani, 2009) while remaining consistent with the average patterns of weight gain throughout pregnancy for our study population.

We used bi-annual capture records from 2004 through 2007 to provide data points during early pregnancy in late May or June when field weights were not available and pregnancy diagnoses were not often possible given hormonal concentrations that remain low during this early stage. These physical examinations included each female's weight (considered together with the weights obtained without capture as described above), nipple length (parous females having nipple lengths >3 mm in length), whether a female was lactating or not (indicative of a recent parturition whether infants were observed or not) and uterus palpation for pregnancy and trimester diagnosis at the time of capture (Dietz and Baker, 1993; Dietz et al., 1994).

*Ad libitum* field observations regarding female appearance (e.g. distended abdomen) and behavioral changes (e.g. remaining at the rear of encounters with neighboring groups and reduced daily travel lengths shortly before parturition) provided additional confirmation, though were not considered diagnostic.

### **PdG and cortisol averages by trimester**

For pregnancies without the observation of live infants and thus unknown dates of parturition we used the guidelines above, counting either forward or backward from an established trimester to obtain surrounding trimester cut off dates as well as probable conception and projected parturition dates. We then calculated average PdG and cortisol concentrations for



each trimester for all pregnancies, successful and unsuccessful, as well as for non-pregnant adult females.

## STATISTICAL ANALYSES

### **Hormonal changes associated with pregnancy**

We performed a linear mixed model analysis of variance (ANOVA) using the GLIMMIX procedure in SAS 9.2 (SAS Institute, Cary, North Carolina) to determine whether PdG and cortisol concentrations changed significantly as females became pregnant and as pregnancy proceeded from one trimester to another. To avoid hormonal variation due to differences in reproductive maturity (French et al., 2003; French et al., 2002; French et al., 1989; French and Stribley, 1987), only data for adult females were included in analyses. The data set tested consisted of 367 monthly hormonal averages from 18 adult females from seven GLT groups (n=18 females contributing 179 samples while non-pregnant, n=14 females contributing 65 samples during their 1<sup>st</sup> trimester of pregnancy, n=14 females contributing 65 samples during their 2<sup>nd</sup> trimester of pregnancy and n=14 females contributing 58 samples during their 3<sup>rd</sup> trimester of pregnancy). Samples from both successful and unsuccessful pregnancies were included. Using GLIMMIX we were able to account for correlations within the data by assigning both random effects, such as the home range occupied by each female, and repeated effects, such as individual female identity and the identity of each pregnancy. As such, our analysis addressed the problem of pseudoreplication that occurs when multiple observations are drawn from a single female or from multiple females occupying a single home range (Bales et al., 2002; Bales et al., 2001; Diggle et al., 1999). The data were log transformed to meet the assumptions of normality prior to analysis. Pairwise comparisons were performed on least squared means using a Tukey-Kramer adjustment for multiple comparisons.

### **Effect of dominance status and group size on reproductive success**

We used logistic regression analyses to test for a statistical relationship between the dominance status of the pregnant female and the likelihood that the pregnancy would result in live infants. We also included group size in the model to test whether pregnant females occupying larger groups had a greater likelihood of giving birth to live young. We performed backward selection eliminating non-significant variables (those with the largest  $p$ -value  $> 0.05$ ) one at a time. All 37 pregnancies detected during the course of the study were included in this analysis including 26 successful and 11 unsuccessful pregnancies to 14 dominant and subordinate females. We controlled for multiple pregnancies by including FEMID as a covariate in the model. The final model was accepted when every variable remaining in the model was significant, the model as a whole was significant and all assumptions for the model were met.

## **RESULTS**

### **Pregnancy polygyny in free-ranging GLTs**

All adult females over 3.9 years of age became pregnant (Table 1). A total of 37 pregnancies to 14 adult GLT females were detected. Ten females gave birth to 48 infants as the result of 26 successful pregnancies. Eleven pregnancies to seven females were unsuccessful. Of the 21 GLT females sampled, only five females did not become pregnant after reaching adulthood. Four of the five were younger than three years of age at the conclusion of the study but did show cyclical elevations in PdG concentrations indicative of ovulation. The fifth female was of unknown age and remained within a habituated study group for just three months during which time she did not become pregnant.

Six of the seven GLT groups studied contained more than one adult (post-pubertal) female, thus were potentially polygynous. Five of these six groups (83%) exhibited pregnancy

polygyny. Only four pregnancies by two subordinate adult females in two groups resulted in the birth of live young.

### **Effect of dominance status and group size on reproductive success**

Both dominance status of the pregnant female (Wald Chi-Square=7.69, df=1, p=0.0055) and the number of individuals in her group (Wald Chi-Square=4.52, df=1, p=0.0335) were significant predictors of a successful pregnancy according to a significant logistic regression model that also controlled for FEMID (Likelihood Ratio Chi-Square=15.37, df=3, p=0.0015). A pregnant female holding dominant status in her group during her pregnancy was 40.4 (95% confidence limits=3.0-552.1) times as likely to give birth to live young as a pregnant female holding a subordinate ranking. Dominant females became pregnant on 26 occasions, 22 (85%) pregnancies were successful and 4 (15%) unsuccessful. Two pregnancies to dominant females were lost in the second trimester and two were lost in the third trimester. Subordinate females became pregnant on 11 occasions, 4 (36%) pregnancies were successful and 7 (64%) unsuccessful. Infants were never observed for five subordinate pregnancies though they were carried to full-term. In addition, one subordinate female lost a pregnancy during the second trimester and another subordinate female lost a pregnancy during the third trimester.

Pregnant females residing in larger groups were more likely to have a successful pregnancy, regardless of whether they held dominant or subordinate status. For every additional group member, pregnant females increased their chances of success by 1.7 times (95% confidence limits=1.0–2.9). Given information on the dominance status of the pregnant female and the number of individuals residing within her group, we correctly classified a pregnancy as successful 88.5% of the time.

### **Hormonal changes associated with pregnancy**

Both PdG ( $F(3,342)=50.34$ ,  $p<0.0001$ ) and cortisol ( $F(3,342)=41.95$ ,  $p<0.0001$ ) concentrations were significantly affected by stage of pregnancy (Figure 1). Mean PdG concentrations increased significantly following conception (non-pregnant vs. 1<sup>st</sup> trimester;  $t(342)=5.86$ , adjusted  $p<0.0001$ ). PdG levels did not increase significantly from the 1<sup>st</sup> to the 2<sup>nd</sup> trimester ( $t(342)=0.44$ , adjusted  $p=0.9711$ ). A significant increase in PdG occurred from the 2<sup>nd</sup> trimester to the 3<sup>rd</sup> trimester of pregnancy ( $t(342)=4.85$ , adjusted  $p=0.0001$ ). Cortisol did not increase significantly following conception (non-pregnant vs. 1<sup>st</sup> trimester;  $t(342)=0.04$ , adjusted  $p=1.000$ ). However, cortisol increased significantly as females moved from the 1<sup>st</sup> to the 2<sup>nd</sup> trimester of pregnancy ( $t(342)=2.67$ , adjusted  $p=0.0395$ ). The largest increase in cortisol was from the 2<sup>nd</sup> to the 3<sup>rd</sup> trimester of pregnancy ( $t(342)=6.58$ , adjusted  $p<0.0001$ ).

## DISCUSSION

### High rates of pregnancy polygyny under stable group compositions

In the current study, all females over 3.9 years of age became pregnant regardless of whether they still resided in their natal group. Pregnancy was more common in this GLT population than previously reported (Baker and Dietz, 1993; Dietz et al. 1994; Baker et al. 2002; French et al., 2003). Subordinate females in the seven groups studied were pregnant at the same time as dominant females 11 times during three reproductive years. The 83% rate of pregnancy polygyny observed in this study represents an almost doubling of the 44.3% rate reported by Baker et al. (2002). The large increase in pregnancy polygyny reported here is likely due to the use of improved methods of pregnancy detection. The combination of non-invasive fecal hormone enzyme immunoassay for progesterone and cortisol, baited scales for obtaining weekly data on individual weight gain without capture, evaluation of reproductive status during bi-annual live captures and the observation of behavioral changes occurring during pregnancy

allowed the detection of mid- to late-term pregnancies that went unobserved in previous studies. Consistency between the trimester-based hormone profiles in this and previous studies of successful pregnancies in GLTs (Bales, 2000; Bales et al., 2005; French et al., 2003) and other callitrichids (Smith and French, 1997; Ziegler et al., 1995; Ziegler and Sousa, 2002; Ziegler et al., 2004) provides support for our pregnancy diagnoses.

Polygyny was common in large GLT groups. With each additional group member, the likelihood of a successful pregnancy increased 1.7 times, regardless of the dominance status of the female. Multiple breeding females lead to larger groups, but larger groups also provide the support necessary to care for multiple infants that arrive during the same reproductive season (in birds (Brown, 1987), coyotes (*Canis latrans* (Bekoff and Wells, 1982)), lions (*Panthera leo* (Bygott et al., 1979)), African wild dogs (*Lycaon pictus* (Malcolm and Marten, 1982)), blackbacked jackals (*Canis mesomelas* (Moehlman, 1979)), dwarf mongooses (Rood, 1990) and badgers (*Meles meles* (Kruuk, 1989))). Both the current study and earlier reports from the same research site (Baker et al., 2002) included data collected during periods when predation levels and population turnover were low. Study groups were large, stable and dominant females tended to be older, a set of circumstances that have been shown to promote polygyny in GLTs (Baker et al., 2002; Dietz, 2004; Dietz and Baker, 1993; French et al., 2003). With most or all breeding positions occupied by tenured females, eldest subordinate GLT daughters residing within large, stable groups attempted reproduction within their natal groups, which led to high rates of pregnancy polygyny.

Our report of pregnancy polygyny occurring in 83% of GLT groups may still be an underestimate. Although progesterone concentrations rise significantly during the 1<sup>st</sup> trimester of pregnancy (Figure 1), weight gain during this time is not significant (Bales et al., 2001;

Hankerson, 2008; Siani, 2009), uterine palpation fails to detect fetuses (Henry and Dietz, unpublished data) and maternal behavior does not change (Henry, personal observation). The lack of corroborating sources of evidence to diagnose early pregnancy may have resulted in non-detection of pregnancies lost during the 1<sup>st</sup> trimester.

### **Mechanisms responsible for maintaining high reproductive skew in free-ranging GLT groups**

Reproduction in subordinate adult female GLTs appears to be limited by pregnancy loss. Pregnancy polygyny followed by high rates of subordinate pregnancy loss occurs in several cooperatively breeding species including banded mongooses (Gilchrist, 2006a, 2006b), meerkats (Clutton-Brock et al., 2008; Young et al., 2006), marmosets (Arruda et al., 2005; Digby, 1995a; Saltzman et al., 2009; Saltzman et al., 2008; Sousa et al., 2005), tamarins and lion tamarins (Dietz and Baker, 1993; Garber, 1997; Goldizen et al., 1996). Banded mongooses have low preparturition reproductive skew, but high rates of subordinate pregnancy loss resulting from stress-induced abortions and infanticide tip the scales back in favor of dominant reproductive success (Gilchrist, 2006a).

Dominant female GLTs were 40 times more likely to give birth to live infants than were subordinate females. This is not surprising given the association between cooperative breeding and high reproductive skew seen across taxa (Clutton-Brock, 2009). When simultaneous breeding by multiple females dilutes the investment of helpers and/or reduces the survival or growth of offspring born to the dominant (Clutton-Brock et al., 2001b; Hodge, 2009), selection should favor dominant females able to control subordinate reproduction through hormonal and/or behavioral mechanisms.

#### **Dominant control via hormonal mechanisms**

Other studies suggest that dominant females exert control over subordinate reproduction through the reduction of reproductive hormone concentrations (Florida scrub jays (*Aphelocoma coerulescens coerulescens* (Schoech et al., 1991)), Harris' hawks (*Parabuteo unicinctus* (Mays et al., 1991)), dwarf mongooses (Creel et al., 1992) and African mole-rats (*Cryptomys* spp. and *Heterocephalus* spp. (Faulkes and Bennett, 2001))). Singular breeding in many callitrichid species has been shown to be maintained by hormonal suppression of subordinate female ovulation and the inhibition of subordinate sexual behavior (in marmosets (Abbott, 1984, 1993; Abbott and Hearn, 1978; Abbott et al., 1981; Albuquerque et al., 2001; Digby, 1999; Evans and Hodges, 1984; French, 1997; Hubrecht, 1989; Rothe, 1975; Saltzman et al., 1997; Sousa et al., 2005) and tamarins (Abbott, 1993; Epple and Katz, 1984; French, 1997; French et al., 1984)).

The genus *Leontopithecus* differs from other callitrichids in that dominant females do not appear to control subordinate reproduction through the suppression of ovulation in either captive (French, 1987; French et al., 2002; French et al., 1989; French and Stribley, 1987; Inglett, 1993; Monfort et al., 1996) or free-ranging populations (Henry, 2011). Subordinate GLT females older than 18 months of age ovulate, as demonstrated by cyclical elevations of progesterone concentrations, while residing within their natal group (Henry, 2011) and all females regardless of dominance status became pregnant by the time they were 3.9 years of age. In addition, consistency between the trimester-based hormone profiles in this study (Figure 1) and previous studies of successful GLT pregnancies (Bales, 2000; Bales et al., 2005; French et al., 2003) do not lend support to the hypothesis that pregnancies by subordinate females fail as a result of dominant interference in subordinate reproduction via hormonal mechanisms.

### **Dominant control via behavioral mechanisms**

Five of seven unsuccessful pregnancies by subordinate females were carried to full-term but infants were never observed. Aggression, eviction, stress-induced abortion and infanticide have been shown to skew post-conceptive reproductive success in favor of dominant females in a wide range of cooperatively breeding species (Arabian babblers (*Turdoides squamiceps* (Zahavi, 1990)), meerkats (Clutton-Brock et al., 2001a; Clutton-Brock et al., 1998a; Clutton-Brock et al., 2008; Kutsukake and Clutton-Brock, 2006; Young et al., 2006), dingos (*Canis familiaris dingo* (Corbett, 1988)), African wild dogs (Creel et al., 1997), dwarf mongooses (Creel and Waser, 1991, 1997; Rasa, 1994), banded mongooses (Cant et al., 2010; Gilchrist, 2006a), naked mole-rats (Faulkes and Abbott, 1997), prairie dogs (*Cynomys ludovicianus* (Hoogland, 1985)) and in Mongolian gerbils (*Meriones unguiculatus* (Saltzman et al., 2006))). In the current study, five of seven unsuccessful subordinate GLT pregnancies were carried to full term. With no hormonal evidence to indicate abnormal fetal or placental development, it seems unlikely that pregnancies lost at full term were stillborn. In common marmoset groups, where the potential hormonal and behavioral mechanisms responsible for reproductive skew have been examined in detail in captive and wild groups, infanticide appears to be responsible for limiting reproductive success in subordinate females (Saltzman, 2003; Saltzman et al., 2009). In meerkats, simultaneous litters by dominant and subordinate females dilute helping investment and reduce food intake and growth of pups, variables closely associated with survival (Clutton-Brock et al., 2001b; Hodge, 2009). Infanticide in meerkats has been interpreted as a means to reduce competition for resources among litters (Young and Clutton-Brock, 2006). In contrast, infanticide has never been observed in the long term study of GLTs at PDA (Dietz, personal communication). However, overt aggression by dominant females toward subordinate females has been suggested as a potential mechanism limiting reproduction by subordinate lion tamarin females (De



Vleeschouwer et al., 2001; French et al., 2002; Inglett et al., 1989; Kleiman, 1979). Attempts to evict subordinates upon giving birth within the natal group could lead to the abandonment of litters in order to re-enter the group as has been shown to occur in banded mongooses (Gilchrist, 2006a). In the face of dominant aggression toward themselves, their newborn infants, or toward any group member offering assistance to subordinate mothers, subordinate females may simply abandon their newborn offspring shortly after birth in favor of being allowed to remain peaceably within the group. The contradiction presented here between high rates of pregnancy polygyny and low reproductive success by subordinate females encourages a re-examination of the classical explanations for singular breeding in callitrichids.

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**TABLE LEGEND**

**Table 1.** Reproductive summary for 21 GLT females from seven free-ranging GLT groups sampled from March of 2004 through February of 2007.

**FIGURE LEGEND**

**Figure 1.** Mean ( $\pm$  standard error of the mean) fecal pregnanediol-3-glucuronide (PdG) and cortisol concentrations (ng hormone/g feces) for adult females while non-pregnant (NP, n=179 samples, from 18 females) and during each trimester of pregnancy (1<sup>st</sup> trimester, I, n=65, 14; 2<sup>nd</sup> trimester, II, n=65, 14; 3<sup>rd</sup> trimester, III, n=58, 14). Statistically significant differences in means are indicated by different letters.

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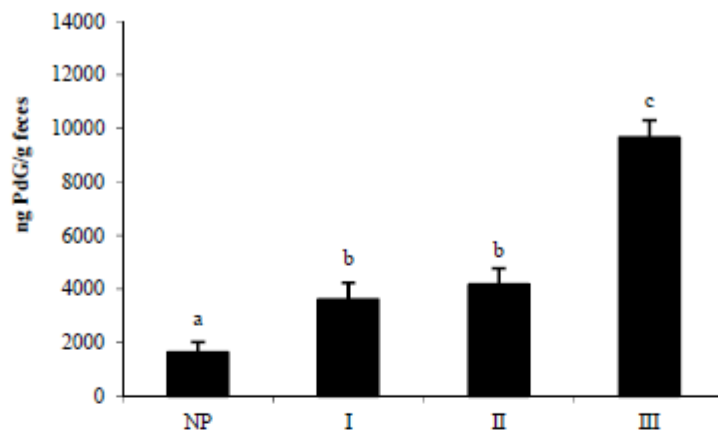
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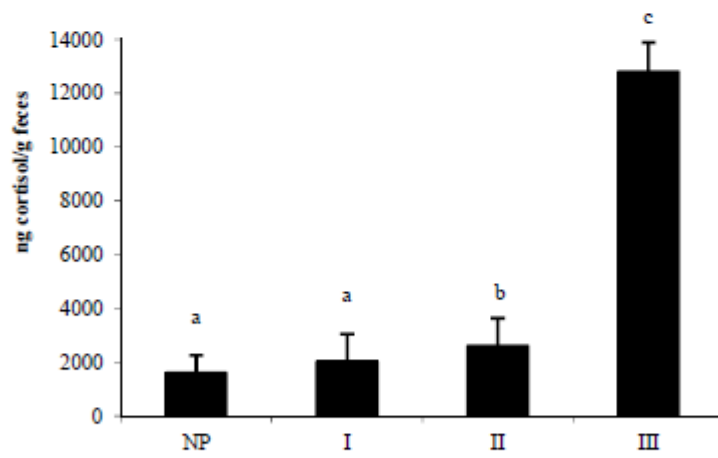
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Table 1.

Group	Group exhibits polygyny	Female ID	Dominance status	Age category (years)	Age (years) at first pregnancy if during study	Successful pregnancies	Infants born	Unsuccessful pregnancies	Fecal samples
3M5	N	782	Dominant	Adult (4.5-6.7)		0	0	1	32
AL	Y	539	Dominant then subordinate	Adult (10.6-13.3)		2	2	2	97
AL	Y	846	Subordinate then dominant	Adult (2.8-5.3)	2.9	3	6	0	78
AL	Y	1267	Subordinate	Infant to subadult (0.2-1.3)	Did not become pregnant	0	0	0	21
BO2	Y	720	Dominant	Adult (9.4-12.3)		6	11	0	125
BO2	Y	848	Subordinate	Adult (2.4-5.3)	2.5	3	6	2	136
BO2/POR	Y/N	880	Subordinate/dominant	Subadult to adult (1.4-4.1)	2.8	3	6	0	88
BO2/POR	Y/N	899	Subordinate	Subadult to adult (1.1-2.4)	Did not become pregnant	0	0	0	39
BO2/POR	Y/N	1227	Subordinate	Subadult to adult (0.7-2.9)	Did not become pregnant	0	0	0	68
BO2/POR	Y/N	1241	Subordinate	Subadult to adult (0.8-2.0)	Did not become pregnant	0	0	0	52
GF	Y	766	Dominant	Adult (5.6-6.9)		2	4	0	35
GF	Y	889	Subordinate	Adult (1.6-3.0)	2.5	0	0	1	18
GF	N	1266	Dominant	Adult (2.9-4.0)	3.4	1	1	0	32
PA	Y	869	Dominant	Adult (4.7-7.3)		4	8	0	98
PA	Y	884	Subordinate	Adult (1.7-3.3)	2.7	0	0	1	60
PA	Y	1271	Subordinate	Juvenile to subadult (0.4-1.4)	Did not become pregnant	0	0	0	32
PP3	N	750	Dominant	Adult (8.7-10.5)		1	2	3	77
PP3	N	1238	Subordinate	Juvenile to adult (0.3-1.9)	Did not become pregnant	0	0	0	56
PP3	Y	1264	Dominant	Adult (4.0-4.3)	3.9	1	2	0	12
PP3	Y	1265	Subordinate	Adult (3.3-4.2)	3.9	0	0	1	10
PP3	N	T0PP3	Dominant	Adult (Unknown)	Did not become pregnant	0	0	0	10
					$\bar{x} = 3.1 \pm 0.2$	$\Sigma = 26$	$\Sigma = 48$	$\Sigma = 11$	$\Sigma = 1176$



PdG Profile by Stage of Pregnancy



Cortisol Profile by Stage of Pregnancy

Figure 1.

### Highlights

- All female golden lion tamarins became pregnant by 3.9 yrs of age.
- Two females became pregnant in 83% of groups with multiple adult females.
- Pregnancies by dominant females were 40 times more likely to succeed.
- 5 of 7 subordinate pregnancies reached full-term but infants were never observed.
- Subordinate reproduction was limited via behavioral, not hormonal mechanisms.