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Social and Hormonal Correlates of Life History Characteristics and Mating Patterns in Female *Colobus vellerosus*

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Social and Hormonal Correlates of Life History Characteristics and Mating Patterns in
Female Colobus vellerosus

by

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A THESIS

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Abstract

This dissertation uses behavioural and hormonal data to describe female life history characteristics in *Colobus vellerosus*. It explores female behaviour patterns that may influence female reproductive success, and focuses on the effect of male group membership on female mating behaviour and investment in offspring. My team and I collected behavioural and faecal data between May 2012 and May 2013 at Boabeng-Fiema Monkey Sanctuary, in central Ghana. From June 2013 to September 2013 I extracted female reproductive hormones (progesterone and oestradiol) from the faecal samples at the Wisconsin National Primate Research Center and mapped the hormone values onto female hormone profiles. I documented ovarian cycle length (24 days), gestation length (mean=168.5 days, range=159-178 days, n = 2), age at first birth (5.87 years, range=4.66-7.08, n=8), and inter-birth interval for females whose infants survived to nutritional independence (mean=16.5 months, range=8.3-24.0 months, n=11) and for females whose infants did not survive to nutritional independence (mean=11.4 months, range=8.0-17.1 months, n=9). I investigated whether or not post-conceptive mating in female *C. vellerosus* is a by-product of fluctuating and/or elevated oestradiol and progesterone levels during pregnancy, or if it is a result of females' access to multiple males. I found that female progesterone and oestradiol levels increased throughout pregnancy and female mating patterns were associated with elevated progesterone levels. Females directed solicitations significantly differently among group types, and females directed solicitations significantly more in unstable multi-male groups than in stable multi-male groups. Females in stable and unstable multi-male groups copulated more with dominant than non-dominant males. I also investigated if female *C. vellerosus* stack

investment in their offspring by conceiving a new offspring while a previous infant is still in nipple contact. I found that eight out of 16 females stacked investment. Females in stable multi-male groups stacked investment significantly more than those in unstable multi-male groups. Females that stacked investment spent a higher proportion of time with an infant in nipple contact than did those that did not stack investment. The combination of behavioural and endocrine data used in this study contributes to a growing body of work describing primate life history variables and mating systems.

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Dedication

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List of Abbreviations

AR1	First-order autocorrelation
BFMS	Boabeng-Fiema Monkey Sanctuary
CV	Coefficient of variation
E2	Oestradiol
EIA	Enzyme immunoassay
FSH	Follicle stimulating hormone
GEE	Generalized estimating equation
GnRH	Gonadotrophin releasing hormone
IBI	Inter-birth interval
WNPRC	Wisconsin National Primate Research Centre
POP	Peri-ovulatory period
P	Progesterone
RIA	Radio-immunoassay
SUM	Stable uni-male group
SMM	Stable multi-male group
SPE	Solid phase extraction

CHAPTER 1 GENERAL INTRODUCTION AND OVERVIEW OF DISSERTATION

Research Purpose and Objectives

This dissertation aims to describe female life history characteristics in *C. vellerosus* using both behavioural and hormonal data. It also investigates female behaviour patterns that may influence female reproductive success, particularly the effect of male group membership on female mating behaviour and investment in offspring. The combination of behavioural and endocrine data used in this study contributes to a growing body of work describing primate life history variables and mating systems (Deschner *et al.* 2004, Harris and Monfort 2006, Bissonnette *et al.* 2011, Lu *et al.* 2012). My specific objectives are to:

1. Quantify female life history variables and biologically validate the results of our hormone assays by comparing the hormonal indicators of life history and reproductive events to observed events.
2. Document female post-conceptive mating patterns and explore the hormonal and social context that may influence these patterns.
3. Determine whether female *C. vellerosus* are able to stack investment in their offspring (by simultaneously conceiving and gestating with an infant still in nipple contact) and investigate the social and ecological factors that may impact this stacked investment.

Significance of Research

Life histories describe the features of an animal's life cycle and the timing of these features (Stearns 1992). The goal of describing life history characteristics is to better understand variation in these characteristics and explain how evolution may have shaped organisms to achieve reproductive success (Stearns 2000). Life history trade-offs among growth, reproduction, and survival vary within and between species, and are sensitive to ecological and demographic conditions (Stearns 1992). Describing a species' life history is important for understanding features of its morphology and behaviour, and how these features have been shaped by biological and social factors.

Detailed study of a single species can provide crucial insight into its life history and mating system, as well as the subtle effects of specific behaviors on social relationships, such as those involved in infanticide avoidance (Janson and van Schaik 2000). Primate social systems are shaped, in part, by social factors like infanticide by males (van Schaik and Janson 2000, hereafter "male infanticide") and female strategies to reduce the risk of male infanticide must then have played a role in the evolution of male-female relationships (Hrdy 2000). Although male mating strategies, such as infanticide, and female mating strategies co-evolved, only recently has research attention begun to shift from male behaviours, to document and understand the range of behaviours female use to avoid male infanticide (Bisonnette *et al.* 2011, Lu *et al.* 2012, Fedigan and Jack 2013).

Reconstructions of early hominin mating systems have often assumed that females are monandrous and males range from monogamous to polygynous (reviewed in Hrdy 2000). However, human and non-human female primates may enhance their

reproductive success by mating with multiple males at times when females are not fertile (Hrdy 2000). Indeed, recent studies of female mating tactics have shown that females mate with multiple males both within and outside the fertile period (Bissonette *et al.* 2011, Lu *et al.* 2012). In light of this perceived flexibility in female mate choice, it is important to look at female choice in relation to the social context in which a female lives, because the social context can directly affect her reproductive strategies.

Not only can social context affect female mating patterns, it can also affect female investment patterns in offspring. Female mammals invest heavily in their offspring, particularly during gestation and lactation (Boyce 1988, Altmann and Samuels 1992). Because of this substantial investment, females must balance their own needs with those of their offspring, as well as making trade-offs between investment in current and future offspring (Trivers 1972, 1974). There is a multitude of factors affecting female reproduction and offspring investment patterns (reviewed in van Noordwijk *et al.* 2013) but only a few studies have considered female social context as a factor potentially shaping female reproductive strategies (e.g. Alberts *et al.* 1992, Sapolsky 1993, Palombit *et al.* 2000, Fedigan and Jack 2013). Incorporating female social context with other ecological and social factors that may be influencing female mating and investment patterns allows us to study females in a larger framework, and explore them as flexible and opportunistic individuals (Hrdy 2000).

Study Site

This study was conducted at the Boabeng-Fiema Monkey Sanctuary (BFMS) in Central Ghana (7° 43' N and 1° 42' W), a 192 hectare forest fragment surrounded by

farmland (Fargey, 1992). BFMS is a dry semi-deciduous forest consisting of primary and secondary forest (regenerating farmland), woodland savannah, and riverine forest (Saj *et al.* 2005). Three primate species inhabit the forest: black and white colobus monkeys (*Colobus vellerosus*), Lowe's guenons (*Cercopithecus campbelli lowei*), and Demidoff's bushbabies (*Galagoides demidoff*). The black and white colobus and guenons are protected, while the bushbaby is hunted for meat (Saj *et al.* 2005, 2006, Wong and Sicotte 2006). Because the sanctuary is connected to other forest fragments by narrow, riparian forest corridors, some colobus dispersal may occur between Boabeng-Fiema and the other fragments (Wong and Sicotte 2006). Small mammals such as squirrels, bats and rodents are still found at the site, however large mammals and predators are absent (Kankam *et al.* 2010, Kankam and Sicotte 2013). Two communities, Boabeng and Fiema, border the sanctuary. The people of these communities use the sanctuary as a source of livelihood (for firewood and plant collection, hunting, and animal grazing). They protect the colobus and the guenons through traditional religious taboos (Saj *et al.* 2005, 2006). National law protects the colobus monkeys (Saj and Sicotte 2013).

BFMS is located in the Brong-Ahafo region of Ghana and is characterized by two rainy seasons (April to July, and September to October) and two dry seasons (November to March, and August) (Saj *et al.* 2005), with mean annual rainfall of 1102.1mm from 2000 to 2006 (Ghana Meteorological Agency data, Kankam and Sicotte 2013). The colobus monkeys at BFMS have been the subjects of on-going behavioural and ecological studies since 2000 under the direction of Dr. Pascale Sicotte at the University of Calgary.

Study Species

Colobus vellerosus at BFMS live in groups ranging from 9-38 individuals, including immature individuals (Wong and Sicotte 2006). Bisexual groups can be multi-male/multi-female, or uni-male/multi-female, (Teichroeb *et al.* 2003, Saj and Sicotte 2005, Saj *et al.* 2005). Dispersal is male-biased (Teichroeb *et al.* 2003) and females are facultative dispersers (Saj *et al.* 2007, Saj and Sicotte 2007, Teichroeb *et al.* 2009, Wikberg *et al.* 2012). *C. vellerosus* are folivorous and eat mature leaves (mean 34%, range 30-37%), young leaves (mean 40%, range 35-45%) (4% of leaves were unclassified; mean 79% for all leaf-types), and unripe fruit (mean 10.7%, range 9.5-12/3%), flowers and flower buds (mean 6%, range 5.7-6.3%), and seedpods (mean 4%, range 1.2-6.6%) (Saj *et al.* 2005, Saj and Sicotte 2007, Teichroeb and Sicotte 2009). Although their food trees tend to be large and evenly distributed, the density of these trees is low (Saj and Sicotte 2007) suggesting that overall food availability may be low, which may lead to between group contest competition (Isbell, 1991). Between groups, there is little difference in the quality of home ranges, monthly food availability, diet, adult female ingestion rates, and rates of travel within a food patch (Teichroeb and Sicotte 2009). However, large groups tend to have increased home-range size, day-range length, and time spent feeding, suggesting that scramble competition may be more pronounced in large groups (Teichroeb and Sicotte 2009). High rates of between-group encounters occur in important feeding areas; earlier reports from a single group suggested that females were rarely aggressive participants in these encounters (Sicotte and Macintosh 2004), but analyses in progress on several groups show a wide range of variation in female rates of aggression (Wikberg in prep). Although between-group

competition appears to be high, within-group contest competition appears low (Wikberg *et al.* 2013). Rates of within-group female-female aggression are low and female-female coalitions have only been observed during infanticide attempts (Saj *et al.* 2007). Therefore, female-female relationships have been classified as egalitarian, according to Sterck's *et al.* (1997) socio-ecological model (Saj *et al.* 2007, Wikberg *et al.* 2013).

Intergroup encounters occur in our population and resident males typically display high rates of aggression during these encounters (Sicotte and Macintosh 2004). Male incursions also occur and during these incursions group males, solitary males, and all-male bands interact with bisexual groups (Sicotte and MacIntosh 2004). Aggression directed at infants can occur during intergroup encounters and male incursions (Sicotte and MacIntosh 2004, Saj and Sicotte 2005) and infanticide has been both observed and inferred, constituting up to 38.5% of infant mortality in some years (Teichroeb and Sicotte 2008). Female dispersal occurs most frequently after male takeover involving multiple males, and has been proposed as a female counterstrategy to infanticide (Teichroeb *et al.* 2009, Sicotte *et al.* 2015). Males can provide protection against infanticide and males in uni-male groups seem to be of higher quality than males in multi-male groups (Teichroeb and Sicotte 2010) because uni-male group males display more and are able to evict other males from their group (Teichroeb *et al.* 2012).

Females mate polyandrously when they have access to multiple males (Teichroeb and Sicotte 2008). Copulations appear to be distributed relatively evenly among males of different ranks in multi-male groups (Teichroeb and Sicotte 2010). In uni-male groups, females typically mate with only the resident male, but extra-group copulations have been reported (Teichroeb *et al.* 2005). There is no mating or birth season (Teichroeb and

Sicotte 2008) and females give birth to a single infant at a time. Teichroeb and Sicotte (2008) estimated an average inter-birth interval (IBI) for *C. vellerosus* of 22 months using data from *C. polykomos* (Dasilva 1989, n=4, IBI=24 months) and *C. guereza* (Harris and Monfort 2006, n=6, IBI=22 months). When an infant dies, mean IBI is approximately 10 months (n=5) (Teichroeb and Sicotte 2008). Females show no external signs of ovulation and, although no mating synchrony between females has been observed, females who experience infanticide at the same time can become resume cycling at the same time (Teichroeb and Sicotte 2008).

Study Groups and Subjects

In two teams of three (one researcher, one research assistant, and one local assistant) we followed four groups (18 focal females) from 6am to 2pm, 6 days per week, from May 2012-May 2013 (Table 1-1; See Appendix II for detailed list of all individuals in the study groups). We also collected behavioural data from one all-male band because these males were potential mates for the females in our study groups, either during male incursions or in the event of a takeover (Teichroeb *et al.* 2005, 2011). These males were in our study area and interacted regularly with the study groups for the duration of the field season. We collected a total of 5820 contact hours and 562 focal hours (mean 30.8 hours per female, range 20.4-45.6 hours, n=18). We sampled all females opportunistically in an effort to sample each female for equal amounts of time on a weekly basis. We maintained a 30-minute time period between focal samples of the same female.

Table 1-1 List of study females. Including the group each study subject was in, her age at the start of the study period, parity (N = nulliparous; M = Multiparous), the total number of focal hours for each female, the total number of faecal samples collected, and the total number of faecal samples analysed.

Female	Group	Age (yrs.)	Parity	Total Focal Hours	Total Faecal Samples Collected	Total Faecal Samples Analysed
BE	RT	8.18	M	21.29	118	101
BL	RT	17.35	M	22.52	117	114
FV	RT	6.34	M	20.40	116	102
JI	RT	4.42	N	22.32	107	106
SU	RT	11.34	M	21.59	113	68
TR	RT	14.35	M	25.47	120	115
CT	SP	7.01	M	36.29	117	105
SA	SP	7.01	N	36.85	116	78
SE	SP	15.35	M	35.55	115	98
VE	SP	16.35	M	38.40	111	43
XE	SP	12.35	M	37.70	116	100
IS	WT	5.96	N	41.47	91	91
VM	WT	4.84	N	48.49	97	97
XY	WT	5.50	N	45.58	98	98
CR	WW	17.35	M	28.64	75	60
IT	WW	8.34	M	23.74	80	59
JN	WW	18.35	M	28.20	76	75
ML	WW	17.44	M	27.53	83	75
Total	4 Groups	-	-	562	1866	1586

We collected faecal samples in sterile collection vials each morning between 6 am and 12 pm. To avoid the potential effects of diurnal variation we did not collect samples

after 12 pm (Hodges and Heistermann 2011). We collected a total of 1866 faecal samples from 18 focal females, with a mean of 104 samples per female (range 75-120, Vayro *et al.* 2016). We extracted steroid metabolites into SPE cartridges at the field station within eight hours of collection using the techniques outlined in Strier and Ziegler (1997) and Ziegler and Wittwer (2005). In a centrifuge tube, we added 2.5 ml of ethanol and 2.5 ml of distilled water to 0.2 g of wet faecal matter. We vortexed the tube for 10 minutes, then centrifuged the tube for 10 minutes. We extracted 2 ml of the resulting supernatant using a syringe and pushed it through an SPE cartridge. The steroids adhered to the filter matrix and we pushed the remaining liquid out the bottom of the cartridge. We then rid the cartridge of any impurities by pushing 1.5 ml of distilled water through the cartridge. We capped and stored the SPE cartridges in sealed plastic bags in a dark closet between 365 and 36 days (first sample collected May 17th, 2012 and last sample collected May 5th, 2013). Extracting samples in the field allowed us to separate steroids from the actual faeces, which eliminates the need for permits to transport animal by-products in and out of Ghana and Canada. SPE cartridges are a safe storage method for multiple steroid hormones and do not provide any information about hormones such as presence or absence of hormones, or amount of a particular hormone. I processed 1586 samples at the Wisconsin National Primate Research Centre (WNPRC) at the University of Wisconsin-Madison to determine levels of oestradiol (E2) and progesterone (P) metabolites in each sample. I assayed faecal E2 metabolites using radio-immunoassay (RIA) and P metabolites using enzyme immunoassay (EIA) (Ziegler *et al.* 1987, Saltzman *et al.* 1994).

I did not process all the samples we collected because analysis is costly and I wanted to focus on periods in the females' reproductive lives that were relevant to my

study objectives. Prior to analysis, I chose which samples I would analysis by identifying key events for each female from behavioural data i.e., periods in which they were observed mating, when an infant died or disappeared, or females with older infants who may have resumed cycling (Table 1-2). I chose periods that were specific to each female and corresponded to her behaviour and observed reproductive events during the study period.

Reproductive steroid metabolites have been measured from faecal samples in animals since 1987 (et al. 1987). During a preliminary data collection and analysis period in May 2011 we confirmed that we could extract the needed hormones and validated the process of analysis for *Colobus vellerosus*. Ziegler et al. (1996) confirmed that steroid hormone levels found in faecal samples are equitable to those found in serum and can be reliably used to determine the ovulatory period within a 2-day window. As such, the steroid hormone levels found in this study are taken as representative of circulating hormone levels in the body. However, one must use caution when making comparisons between absolute hormone values found in this study and other studies. Comparisons of absolute values should only be done if the compared values are also from faecal samples, and not compared directly to levels found in serum, saliva, or urine.

Table 1-2 Justification for the faecal samples I chose to analyze, and the collection period for each female's samples.

Female	Justification for samples analyzed	Collection period for analyzed samples
VM	To determine first cycle	20 Jun-25 Feb
XY	To determine first cycle. Gave birth Nov. 19 2012. Copulated throughout collection period	20 Jun-25 Feb
IS	To determine first cycle. Gave birth Jan. 9 2013. Copulated throughout collection period	20 Jun-25 Feb
SA	Baby disappeared in Sept. 2012	1 Sep-30 Mar
SE	Had an infant, trying to capture resumption of cycling	6 Jul-30 Mar
VE	Baby disappeared Feb. 16 2013	2 Dec-31 Mar
CT	Observed copulating after Aug. 1 2012	1 Aug-21 Apr
XE	Had an infant, trying to capture resumption of cycling	5 Jul-30 Mar
TR	Had an infant, trying to capture resumption of cycling	4 Jul-22 Apr
BE	Gave birth Dec. 10 2012	1 Jul-31 Mar
BL	Observed copulating throughout study period	1 Jul-22 Apr
JI	To determine first cycle	31 Mar-22 Apr
FV	Males solicited her throughout study period	1 Jul-31 Mar
SU	Gave birth Dec. 1 2012	6 Jul-31 Dec
JN	Baby disappeared Aug 9, new baby born 1st week of March	6 Jul-13 Jan
ML	Copulated in May 2012, no baby during study period.	21 Jun-13 Jan
CR	Gave birth Aug. 21 2012	3 Aug-14 Jan
IT	Gave birth Sept. 7 2012	2 Aug-14 Jan

Research questions

My main objective is to describe female life history characteristics in female *C. vellerosus* and further investigate behavioural patterns that may influence female reproductive success, particularly in relation to male group membership. My specific research questions are:

1. What are the key life history characteristics (specifically ovulatory cycle length, ovarian hormone patterns, gestation length, inter-birth interval, and age at first birth) in female *C. vellerosus*?
2. Do the reproductive events or reproductive phases identified in the hormone profiles of female subjects correspond to events observed in each of the study females?
3. Do fluctuating hormone levels affect post-conceptive mating?
4. Does male group membership (group type, number of males, and male dominance status) affect female post-conceptive mating?
5. Do patterns of female post-conceptive mating support the infanticide avoidance hypothesis?
6. Do female *C. vellerosus* conceive a subsequent offspring when they still have a previous infant in nipple contact?
7. If so, how long is the period of overlap between conception and last nipple contact?
8. Do any characteristics of the infant in nipple contact affect female stacked investment (infant age, infant sex, proportion of time spent in nipple contact per day)?

9. Does female age affect female stacked investment?
10. Do male group membership and group stability affect female stacked investment?
11. Does food availability affect female stacked investment?

Organization of Dissertation

This dissertation is organized into five chapters. This chapter introduces the purpose and objectives of my dissertation, the significance of the research, the study site and study subjects, and my specific research questions.

Chapter Two is published in the journal *Primates* (Hormonal correlates of life history characteristics in wild female *Colobus vellerosus*, 2016) and quantifies key life history characteristics (specifically ovulatory cycle length, ovarian hormone patterns, gestation length, inter-birth interval, and age at first birth) in *C. vellerosus* using hormonal and observational data. It also biologically validates the results of my hormone assays by comparing hormone profiles, and fluctuations in oestrogen and progesterone levels, to observational data on mating and parturition events in each of the study females.

Chapters Three (The influence of reproductive hormones and male group membership on post-conceptive mating in female *Colobus vellerosus*) and Four (Factors influencing the timing and overlap of reproductive events in wild female *Colobus vellerosus*) are independent manuscripts that I will submit to peer-reviewed journals.

The goal of Chapter Three is to test whether or not post-conceptive mating in female *C. vellerosus* is a by-product of fluctuating and/or elevated oestradiol and

progesterone levels during pregnancy, or if it is a result of females' access to multiple males. I also consider whether or not patterns of female post-conceptive mating in *C. vellerosus* support the infanticide avoidance hypothesis.

Chapter Four investigates whether female *C. vellerosus* stack their offspring (i.e. conceive a subsequent offspring while the current infant is still in nipple contact) and if so, under what conditions. I consider the timing and overlap of reproductive events (cessation of nipple contact, resumption of cycling, and conception) in relation to the social conditions under which they occur, paying particular attention to male group membership and the stability of the group. I present the distribution of these events relative to one another, and explore what factors may be influencing their overlap (or lack thereof). In particular, I explore which variables may be affecting female inter-birth intervals and females' ability to invest in more than one offspring at a time.

Chapter Five is a discussion of my key results in relation to my research questions. I also consider future research directions.

Chapters Two, Three and Four are co-authored, as many people contributed to the making of this dissertation. I am lead author on all chapters because I collected the majority of the data, performed all data analyses and wrote all the papers.

CHAPTER 2 HORMONAL CORRELATES OF LIFE HISTORY

CHARACTERISTICS IN WILD FEMALE *COLOBUS VELLEROSUS*

Published in *Primates*:

Vayro JV, Fedigan LM, Ziegler TE, Crotty A, Ataman R, Clendenning R, Potvin-Rosselet E, Wikberg EC, Sicotte P. 2016. Hormonal correlates of life history characteristics in wild *Colobus vellerosus*. *Primates* 57:509-519. DOI 10.1007/s10329-016-0551-1

Introduction

Life history theory refers to the timing of key events in an organism's life that have been shaped by natural selection to maximize an individual's number of surviving offspring (Stearns 2000). Traits such as stages of infant development, speed of growth, age at sexual maturity, age at first reproduction, number of offspring, and degree of parental investment are some of the variables studied in mammalian life history (Borries *et al.* 2013). Because primates are long-lived, and variation exists both within and between females of different species (Lee and Kappeler 2003, Borries *et al.* 2013), detailed long-term study is often needed to provide good estimates of key life history variables in primates (Purvis *et al.* 2003). Understanding a species' life history traits adds not only to our understanding of the evolution of these traits, but provides the foundation for the reconstruction of life history values for extinct taxa, such as early hominins (reviewed in Borries *et al.* 2013, Hawkes *et al.* 2002). Furthermore, for those species that are under threat of extinction, life history data can contribute valuable ecological data that allow wildlife managers to assess a population's viability by providing insight into its size, distribution, and traits that affect reproduction (Ruggiero *et al.* 1994).

Detailed data collection on individually recognizable subjects studied over the long-term allows for the investigation of biological problems in long-lived species that previously could only be addressed by using rapidly developing species (Leigh 1994). Furthermore, in the last three decades, the development of non-invasive field techniques allowing for the collection of hormonal data from faecal samples has enabled researchers to more efficiently and cost-effectively collect hormonal data from wild primates (Ziegler and Wittwer 2005, Heistermann 2010). Techniques to improve the transportation, purification, and analysis of faecal steroids have made processing faecal samples in the field simpler and more reliable (Ziegler and Wittwer 2005, Heistermann 2010). These techniques also make it possible to extract multiple steroids at once, reducing the time and cost of analysis (Ziegler and Wittwer 2005, Heistermann 2010). Despite advances in non-invasive field techniques for the collection of hormonal samples, measuring steroids from faecal samples is not simple and steps must be taken to ensure the validity of the results (Buchanan and Goldsmith 2004, Ziegler and Wittwer 2005, Heistermann 2010, Murray *et al.* 2013). Hormone analyses need to be validated to ensure accuracy of the results (Buchanan and Goldsmith 2004), including assessing parallelism, accuracy, recoveries, and sensitivity (Ziegler *et al.* 1996, Buchanan and Goldsmith 2004). In addition to validation during laboratory analyses, some results also should be confirmed by retroactively comparing hormone levels and patterns with known reproductive events in the study of females' lives, a process known as biological validation (Murray *et al.* 2013).

The objectives for this paper are two-fold. We first quantify key life history characteristics (specifically ovulatory cycle length, ovarian hormone patterns, gestation

length, inter-birth interval, and age at first reproduction) in a wild colobine (*C. vellerosus*) using hormonal and observational data. We then draw comparisons between the life history characteristics of our study species and those of other colobines.

The second objective is to biologically validate the results of hormone assays by comparing hormone profiles, and fluctuations in oestrogen and progesterone levels, to observational data on mating and parturition events. This biological validation will confirm whether the reproductive events or the reproductive phases identified in the hormone profiles of the female subjects correspond to observed events in each of the study females.

Methods

This study was conducted at the Boabeng-Fiema Monkey Sanctuary (BFMS) in Central Ghana (7° 43' N and 1° 42' W), a 192 hectare forest fragment. BFMS is a dry semi-deciduous forest consisting of primary and secondary forest (regenerating farmland), woodland savannah, and riverine forest (Fargey 1991, Saj *et al.* 2005). The forest is inhabited by two species of diurnal monkeys (*Colobus vellerosus* and *Cercopithecus campbelli lowei*), which are protected through traditional religious taboos (Saj *et al.* 2005, 2006). The colobus are also protected by national law (Saj and Sicotte 2005, Saj *et al.* 2006). They are listed as 'vulnerable' in the IUCN (2015) red list.

The colobus monkeys at Boabeng-Fiema Monkey Sanctuary have been the subjects of ongoing behavioural studies since 2000 under the direction of Dr. P. Sicotte at the University of Calgary. *C. vellerosus* live in multi-male/multi-female groups, uni-male/multi-female groups, and all male bands (AMB). Grouping patterns are fluid and

shift over time. *C. vellerosus* females show no external signs of ovulation and they cycle asynchronously (Saj and Sicotte 2013). Females have no mating or birth season (Teichroeb and Sicotte 2008) and give birth to a single infant at a time. When females simultaneously experience male infanticide as a result of male takeover, there can be a clustering of females who become receptive concurrently (Teichroeb and Sicotte 2008).

We spent 5820 contact hours with four study groups from May 2012 to May 2013. All members of the study groups were individually recognizable. We collected ten-minute focal samples, scan samples and *ad libitum* data from 6am to 2pm, six days per week on 18 adult and sub-adult females in two multi-male and two uni-male groups. All females were sampled opportunistically, and our goal was to sample each female for a similar amount of time on a weekly basis. A total of 562 hours of focal observation were collected (mean 30.8 hours per female; range 20.4-45.6 hours; n=18). Fourteen females were parous and four were nulliparous at the start of the study period. We combined data from the 2012-2013 data collection period with our long-term demographic data (2004-2011) to calculate inter-birth intervals and age at first reproduction. We investigated the length of inter-birth interval for females with surviving infants and those with infants who died using a Mann-Whitney *U*-test. We set the significance level to $p < 0.05$, and the test was one-tailed.

We collected faecal samples each morning between 6am and 12pm. No samples were collected after 12pm to avoid any potential effects of diurnal variation (Hodges and Heistermann 2011). All samples were collected in a sterile collection vial and brought back to the field-station within eight hours of collection. We extracted oestradiol (E2) and progesterone (P) metabolites at the field station using the techniques outlined in Strier

and Ziegler (1997) and Ziegler and Wittwer (2005). All samples were processed at the Wisconsin National Primate Research Center (WNPRC) at the University of Wisconsin-Madison. Faecal oestradiol metabolites were assayed using radio-immunoassay (RIA) and progesterone metabolites using enzyme immunoassay (EIA) (Ziegler *et al.* 1987, Saltzman *et al.* 1994). Reproductive events from hormonal data were defined/determined using previously described parameters (see Nagle and Denari 1983, Ziegler *et al.* 2000, Carnegie *et al.* 2005, Strier *et al.* 2003).

We validated the assays using quality controls for each plate (for P) and each assay (for E2). The mean intra- and inter-assay coefficient of variation (CVs) for P were 7.03 and 25.22 for low pools, and 5.33 and 28.76 for high pools. The mean intra- and inter-assay CVs for E2 were 7.37 and 15.43 for low pools, and 4.43 and 11.75 for high pools.

Results

We collected a total of 1866 faecal samples from 18 focal females, with a mean of 104 samples per female (range 75-120). Analyses were performed on 1586 of these samples for P and E2 metabolites (mean 88 samples per female; range 43-115 per female). Over the course of the study period, the 18 focal females went through various reproductive states: six females were cycling, 11 were or became pregnant, 10 were in or entered lactational amenorrhea, two were prepubescent, and one was post-reproductive (Table 2-1).

Hormone Levels and Patterns

We mapped P and E2 levels for each female using the values obtained from the RIAs and EIAs to determine hormonal patterns for cycling, ovulation, pregnancy and lactational amenorrhea. We calculated baseline E2 and P concentrations for the two females that cycled; both were conceptive cycles. Mean baseline E2 concentration was 5.92 ng/g (n=10, 2 females, 2 cycles, range 3.12-7.89 ng/g). Mean baseline P concentrations were 161.81 ng/g (n=10, 2 females, 2 cycles, range 31.52-292.52 ng/g). During ovulatory cycles, E2 concentrations began to rise a mean of 6.5 days (n = 2 females, 2 cycles, range 4-9 days) before they peaked. P peaked a mean of 6.5 days (n = 2 cycles, range 6-7 days) before the peak in E2. Because both females conceived during the only visible cycle in their hormone profile, we could not determine the number of days that P remained elevated following the rise in E2 for a complete cycle. Peak hormone concentrations ranged from 4-48.41 ng/g for E2 (n=8, 2 females, 2 ovulations, mean 20.83 ng/g) and 70.58-2579.07 ng/g for P (n=8, 2 females, 2 ovulations, mean 1236.37 ng/g). For both females, P remained elevated after the ovulatory phase. E2 increased to a greater extent than did P, although both increased during the early stages of pregnancy (Figures 2-1 and 2-2).

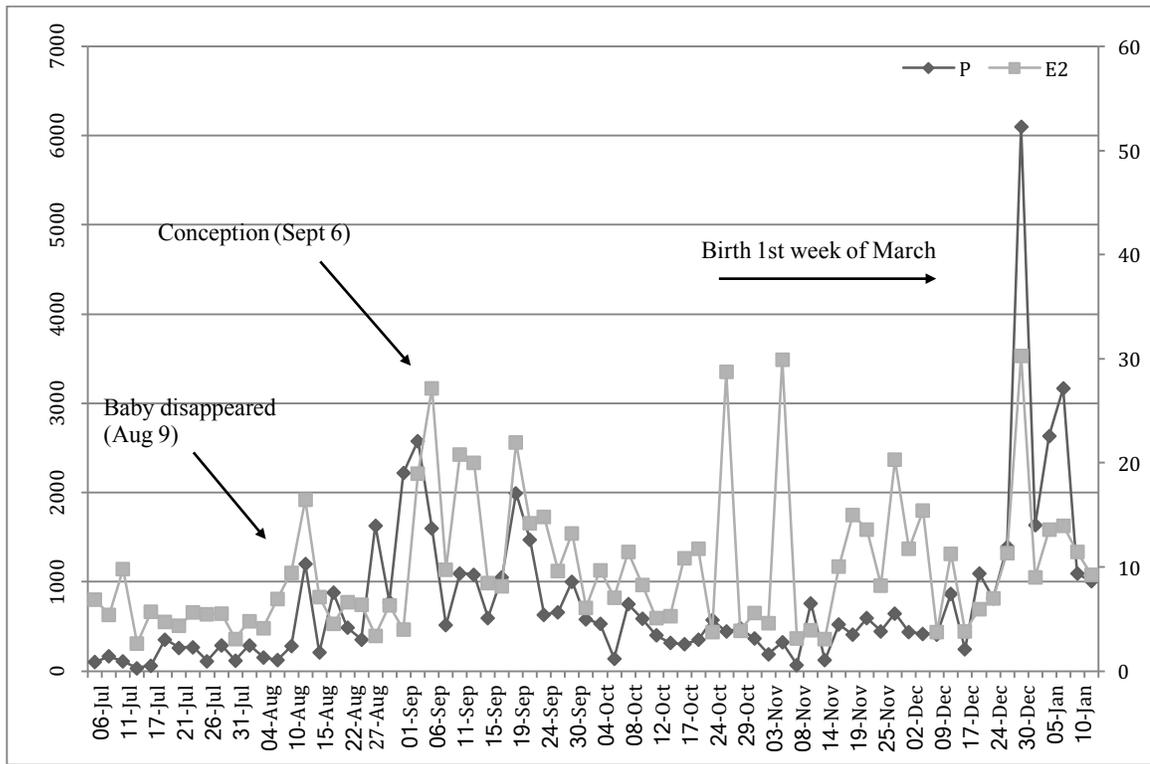


Figure 2-1 Hormone profile for female JN showing E2 and P levels throughout the study period. The date of first infant disappearance, day of conception, and duration of pregnancy are noted.

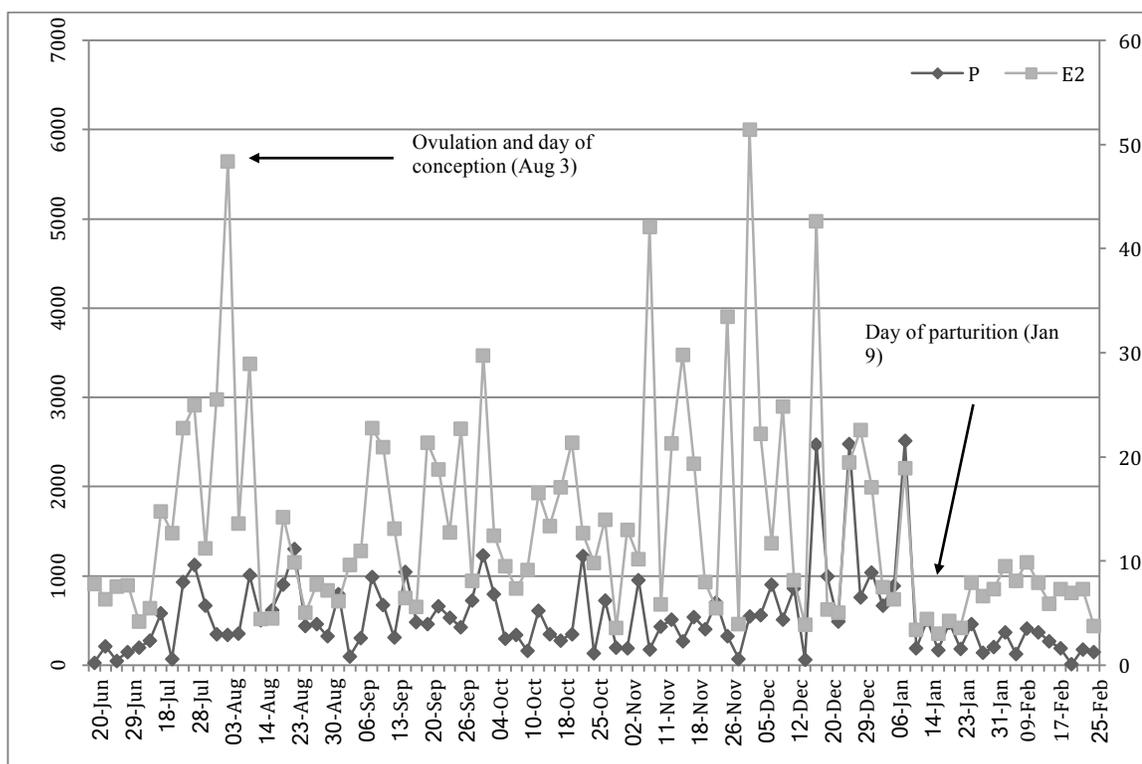


Figure 2-2 Hormone profile for female IS showing E2 and P levels throughout the study period. The day of conception, duration of pregnancy, and day of parturition are noted.

Pregnancy was characterized by a sustained rise in baseline levels of P and E2. Mean values of P throughout pregnancy were 874.15 ng/g (range 41.93-3168.15 ng/g, n=8) and for E2 were 24.07 ng/g (range 1.17-96.94 ng/g, n=8). The first four months of pregnancy were characterized by lower mean P values of 544.40 ng/g (range 41.93-6102.83 ng/g, n=6) and E2 values of 13.63 ng/g (range 3.11-96.94 ng/g, n=6) than in the final month of pregnancy. The last month of pregnancy for all females was characterized by mean values of 1149.31 ng/g for P (range 62.55-3852.78 ng/g, n=7) and 33.09 ng/g for E2 (range 3.82-154.21 ng/g, n=7). We determined pregnancy from the hormone profiles for 8 females (one from conception to parturition, one from conception but

parturition occurred after the end of the study period, and six who were pregnant before the start of the study until parturition).

Females who had dependent infants and were acyclic with low hormone (E2 and P) concentrations were deemed to be in lactational amenorrhea. For these females, the mean E2 and P concentrations were 5.04 ng/g and 280 ng/g, respectively (n=9, range 4.16-6.13 ng/g for E2, and 210.23-491.30 ng/g for P).

Life History Characteristics from Hormonal Data

Two of our 18 subjects were cycling (n=2 cycles in total). The hormone profiles for these females show day of conception, the duration of pregnancy, and day of parturition (Figures 2-1 and 2-2). Six females were pregnant prior to the start of the study, and they experienced parturition and then lactational amenorrhea during the study. Figure 2-3 shows the typical hormone patterns for a pregnant female who gave birth and remained in lactational amenorrhea for the duration of the study. Four females showed no variation in hormone levels and thus were most likely in lactational amenorrhea for the duration of the study period. The hormone profiles for two females showed a pattern beginning to conform to a typical ovulatory cycle, but they did not experience a complete ovulatory cycle, hence were probably prepubescent (Figure 2-4). One female experienced two possible ovulations that fit an atypical pattern, with 12 weeks between possible ovulations. Based on our long-term records she is at least 18 years old and therefore was probably post-reproductive (Figure 2-5). Three females fit no criteria for cycling, pregnancy or lactational amenorrhea, because their hormone profiles showed no clear patterns (Figure 2-6). Based on our demographic data, we also know that four females

conceived during the study period because they gave birth a few months (1-6 months) after the end of the study period, although their hormone profiles do not show the E2 and P levels required to identify pregnancy. Table 2-1 presents a summary of reproductive state and calculated reproductive parameters for each female.

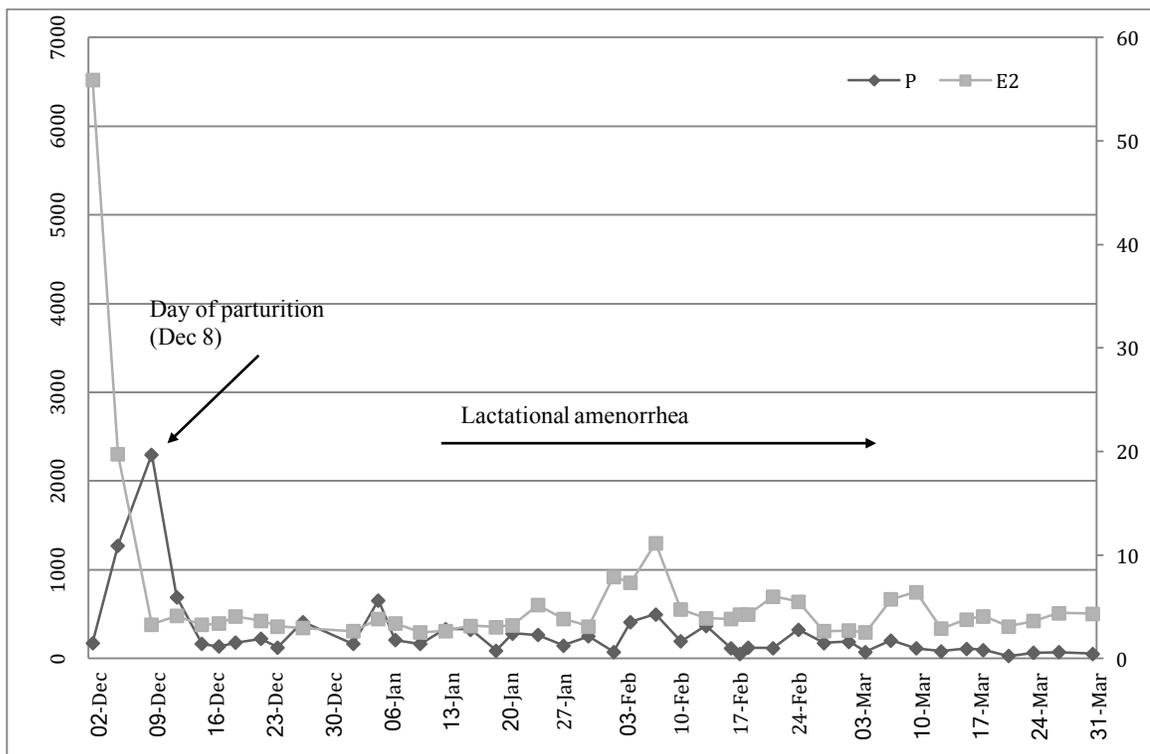


Figure 2-3 Hormone profile for female VE showing E2 and P levels throughout the study period. The day of parturition and lactational amenorrhea are noted.

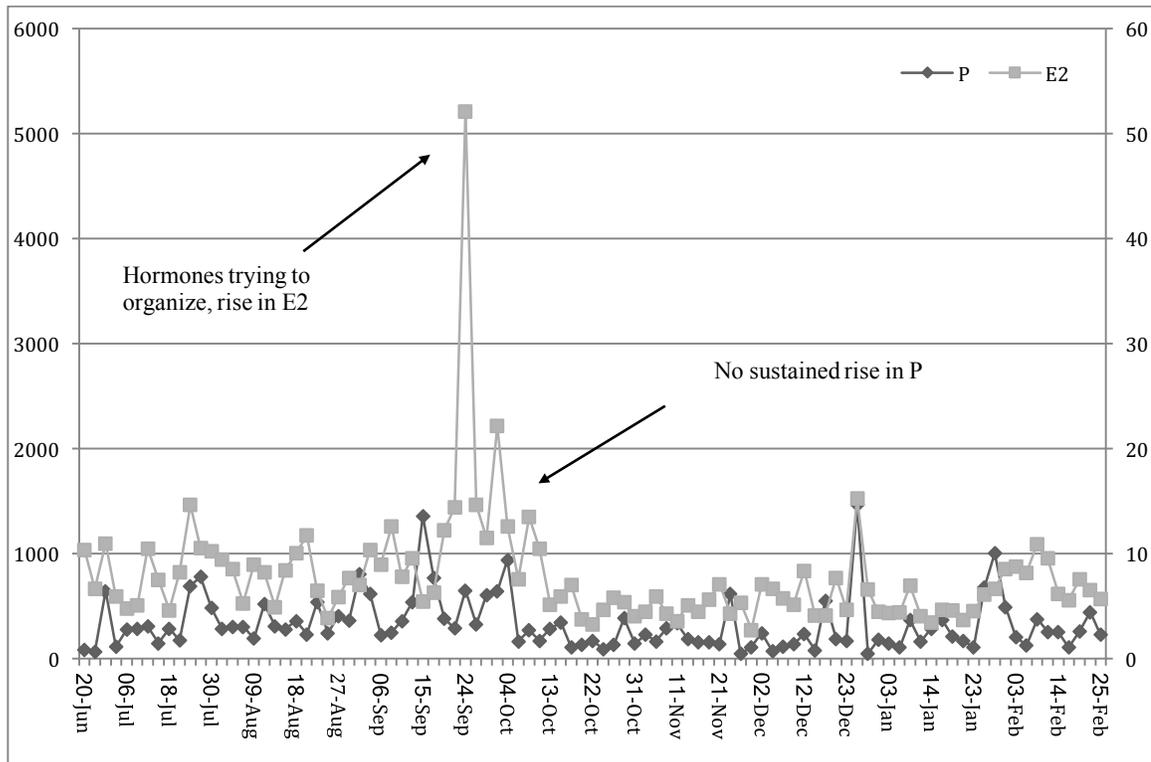


Figure 2-4 Hormone profile for female XY showing E2 and P levels throughout the study period. This profile shows a pattern beginning to conform to a typical ovulatory cycle, but the conditions for ovulation were not met, hence she was presumed prepubescent.

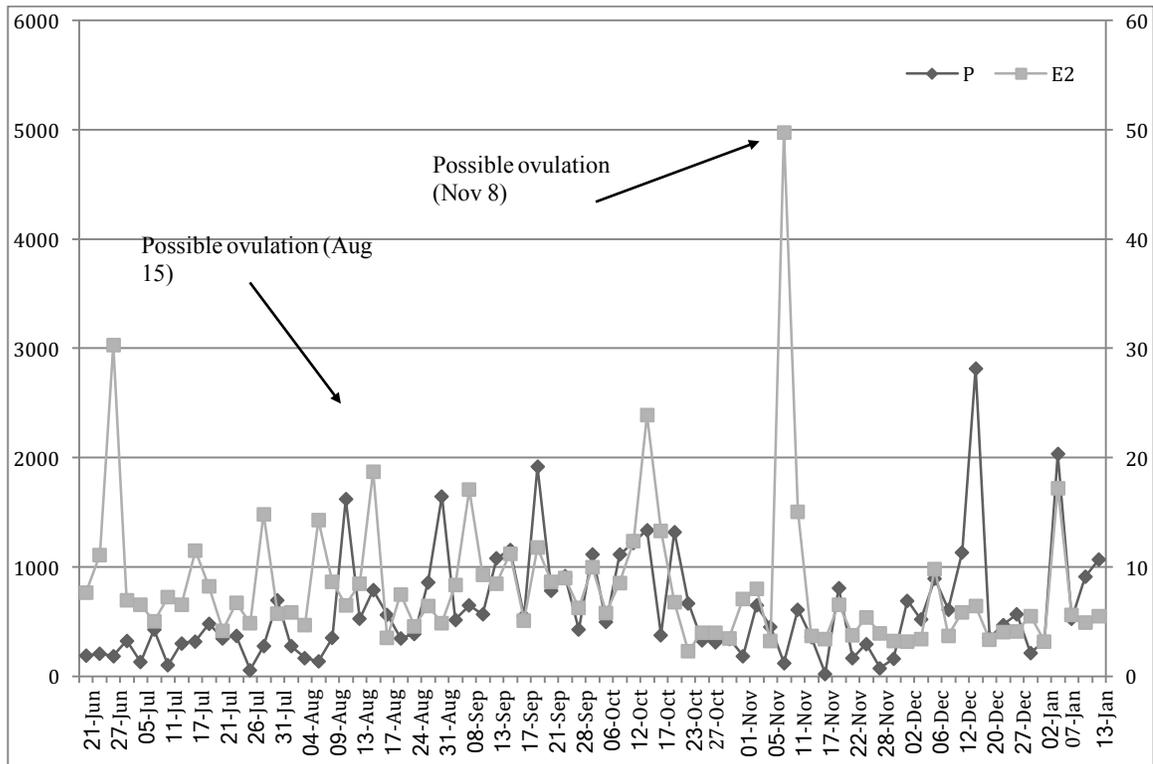


Figure 2-5 Hormone profile for female ML showing E2 and P levels throughout the study period. This is an atypical profile with 12 weeks between possible ovulations hence she was presumed post-reproductive.

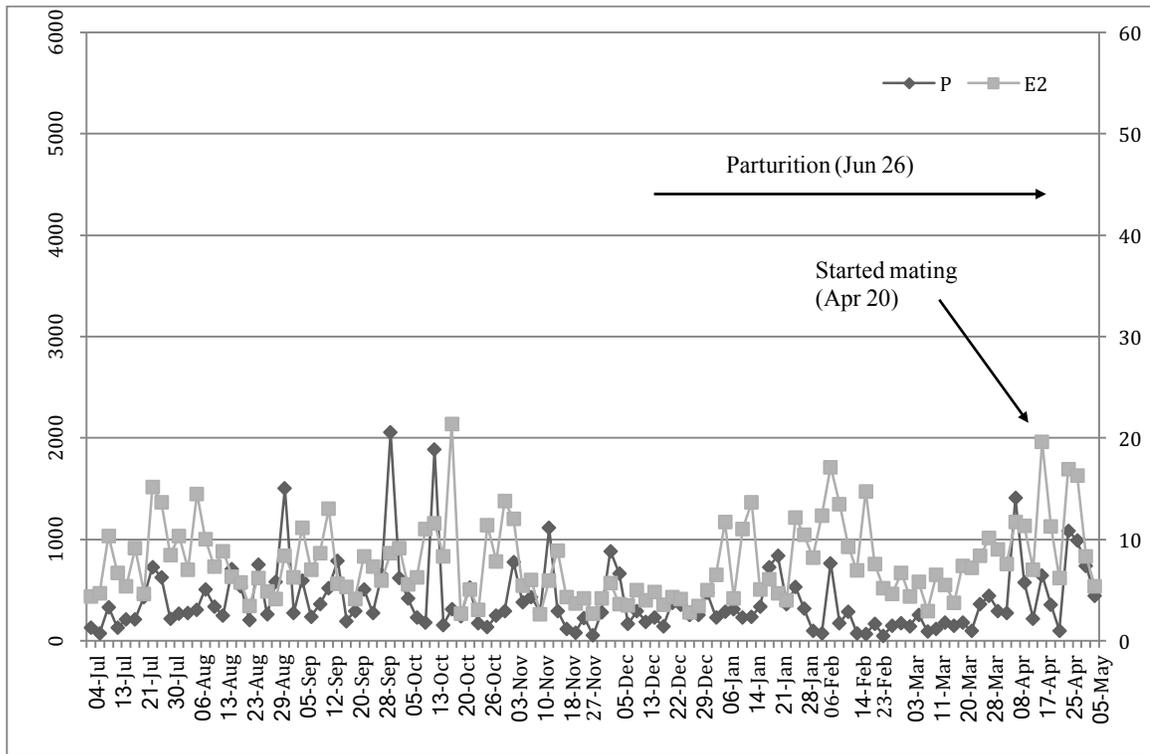


Figure 2-6 Hormone profile for female TR showing E2 and P levels throughout the study period. The profile fit no criteria for cycling, pregnancy, or lactational amenorrhea because there is no clear E2 and P pattern.

Table 2-1 Hormone steroid values for study females. Summaries of values found in this study for reproductive state, number of ovulations, and mean baseline hormone levels during lactational amenorrhea (lact. amen) and ovulation for each study female. Standard deviation is shown in brackets (S.D.). ? denotes that hormone profiles did not fit the criteria for any reproductive state, however, reproductive state was determined using observational data.

ID	Samples	Reprod. State	Mean E2 lact. amen. (S.D.)	Mean P lact. amen. (S.D.)	Presumed ovulations (n)	Mean peak ovulatory E2 (S.D.)	Mean peak ovulatory P (S.D.)
BE	101	Preg; Lact. amen.	5.31 ng/g (3.17)	184.7 ng/g (116.42)	-	-	-
BL	114	Cycling? Preg?	-	-	-	-	-
FV	102	Cycling? Preg?	-	-	-	-	-
JI	106	Prepub	-	-	-	-	-
SU	68	Preg; Lact. amen.	4.19 ng/g (1.74)	274.58 ng/g (179.51)	-	-	-
TR	115	Cycling? Preg?	-	-	-	-	-
CT	105	Lact. amen.	5.26 ng/g (6.93)	217.94 ng/g (142.32)	-	-	-
SA	78	Lact. amen.; cycling?	6.13 ng/g (3.47)	291.22 ng/g (193.87)	-	-	-
SE	98	Lact. amen.	5.99 ng/g (13.53)	226.67 ng/g (161.21)	-	-	-
VE	43	Preg; Lact. amen.	4.16 ng/g (1.71)	210.23 ng/g (155.65)	-	-	-
XE	100	Lact. amen.	4.81 ng/g (2.05)	241.02 ng/g (162.48)	-	-	-
IS	91	Cycling; Preg	-	-	1	27.54 ng/g (15.41)	678.51 ng/g (463.57)
VM	97	Prepub	-	-	-	-	-
XY	98	Preg; Lact. amen.	4.65 ng/g (1.77)	271.88 ng/g (381.64)	-	-	-
CR	60	Preg; Lact. amen.	5.43 ng/g (4.01)	491.30 ng/g (387.09)	-	-	-
IT	59	Preg; Lact. amen.	4.50 ng/g (1.87)	390.57 ng/g (302.46)	-	-	-

ID	Samples	Reprod. State	Mean E2 lact. amen. (S.D.)	Mean P lact. amen. (S.D.)	Presumed ovulations (n)	Mean peak ovulatory E2 (S.D.)	Mean peak ovulatory P (S.D.)
JN	75	Cycling; Preg	-	-	1	14.12 ng/g (10.92)	1794.23 ng/g (790.56)
ML	75	Post-reprod.?	-	-	-	-	-

Cycle length was 25 days and 23 days, respectively (mean cycle length 24 days +/- 1, n = 2 cycles. Each female cycled once). Both of these females conceived at the start of the study period during the first cycle for which we have hormonal data. It is unknown if they were cycling before the start of the study period. Gestation length was 23.4 weeks (+/- 5 days) and 21 weeks (+/- 5 days) respectively; mean gestation length was 22.2 weeks (range=21-23.4 weeks, n=2).

Life History Characteristics from Observational Data

We determined inter-birth interval for females with infants that reached nutritional independence vs. females whose infants died before reaching nutritional independence (n=15 females from this study, n=5 from 2004-2011 dataset). For females whose infants survived to nutritional independence, the mean inter-birth interval was significantly longer (17.75 months, range=8-20.75 months, n=11) than the inter-birth interval for females whose infants did not survive to nutritional independence (11.89 months, range=8-18.5 months, n=4 from this study, n=5 from 2004-2011 dataset) (U=14.5, p=.006). The shortest inter-birth interval in our sample was linked to the disappearance of an infant (approximately 2 months old) on August 9th 2012. An extra-

group male had wounded this infant during an incursion. The mother resumed cycling (and ovulated) 27 days later on September 6th 2012 and conceived during this cycle.

Eleven females were pregnant during the study period. Eight pregnancies were identified from hormone profiles and three were identified retrospectively from observed births. Of these 11 females, two showed the day of conception and full pregnancy in their hormone profile. Three became pregnant during the study period but their hormone profiles did not show the consistent rises in P and E2 required to identify ovulation, conception, or pregnancy. The hormone profiles for the six females who conceived before the start of the study period show the end of pregnancy, day of parturition, and lactational amenorrhea. Table 2-2 presents a summary of key life history characteristics associated with each female.

Table 2-2¹ Life history characteristics for study females. Values found in this study for each female including cycle length, gestation length, inter-birth interval, and age at first reproduction.

ID	Cycle length (days)	Gestation length (months)	Interbirth interval w/ infant survival (months)	Interbirth interval w/ infant death (months)	Age at first reproduction (years)
BE	-	-	8*	-	4.66 [^]
BL	-	-	14.88 [^]	-	-
FV	-	-	18.75 [^]	-	-
JI	-	-	-	-	-
SU	-	-	20 [^]	-	5.50 [^]
TR	-	-	16 [^]	-	-
CT	-	-	17 [^]	-	5.66 [^]
SA	-	-	-	14.75*	5.66 [^]
SE	-	-	20.75 [^]	-	-
VE	-	-	-	10.75 [^]	-
XE	-	-	20.25 [^]	-	-
IS	23*	5.25*	-	18.5 [^]	6.58*
VM	-	-	-	-	7.08*
XY	-	-	11.5 [^]	-	5.33*
CR	-	-	15.25 [^]	-	-
IT	-	-	16 [^]	-	6.16 [^]
JN	25*	5.85*	-	12*	-
ML	-	-	-	-	-

*denotes data from this study; [^]denotes data from long-term data

Age at first pregnancy

The mean age at first pregnancy was 5.82 years (range = 4.66-7.08, n = 3 from this study, n = 5 from 2004-2011 dataset) (Table 2-2). Four females were primiparous at the start of our study period. Of these, three conceived and two gave birth during the study period. One gave birth after data collection.

¹ Please see Appendix IV for a breakdown of the dataset and sources of data we used to calculate mean interbirth interval and mean age at first birth

Validation of reproductive events using observational data

Based on observed reproductive events, we confirmed that the rise in P and E2 correspond to pregnancy and birth. The observed day of parturition shows a distinct decrease in both E2 and P in the females' hormone profiles (Figure 2-2 and 2-3). Additionally, following parturition, when females are nursing and are in lactational amenorrhea, E2 and P show a “flatline” whereby levels of both hormones decrease sharply and remain low for several months. Both conditions are consistent with expected hormone patterns (Ziegler *et al.* 1996, Harris and Monfort 2006) and the observed events reflect these patterns.

Discussion

This study describes the hormonal correlates of female reproductive events and quantifies for the first time key life history variables for *C. vellerosus*, using hormonal and observational data. It also confirms that the reproductive events determined in the hormone profiles correspond to observed reproductive events for each female. The key life history variables documented in this study are ovarian cycle length (24 days), gestation length (5.75 months), inter-birth interval for females whose infants survive to nutritional independence and for those whose infants that do not survive to nutritional independence (17.75 months vs. 11.89 months), and age at first reproduction (5.8 years).

Many studies have documented life history parameters for other colobines (Table 2-3). However, several of these studies are based on observational data. This limits our ability to quantify life history variables (e.g., cycle length and gestation length) and leaves gaps in our understanding of these characteristics for colobines. The mean ovarian

cycle length of 24 days that we report here is within a day or two of what is reported for most colobines, but is at the lower end of the range (24-29 days). The mean age at first reproduction of 5.82 years is within the range of other colobine species (3.5-6.7 years). The mean gestation length of 5.75 months is the same as that for *C. guereza* (Harris and Monfort 2006), but shorter than that found in other colobines (e.g. Borries *et al.* 2001, Starin 2001). The inter-birth interval for *C. vellerosus* when an infant survived to nutritional independence is 17.75 months, slightly shorter than that in other colobine species. The mean inter-birth interval when an infant died before nutritional independence is 11.89 months, which is within the range for other colobine species under similar conditions (9-18 months).

Table 2-3 Cross species comparison of life history characteristics. Cross species comparison of inter-birth interval with infant survival to nutritional independence (IBI w/infant survival), and infant death before nutritional independence (IBI w/infant death), cycle length, gestation length, and female age at first reproduction in colobines. Values and references in regular font indicate studies in which only observational data were used, whereas those in bold indicate studies in which observational and hormonal data were used.

Species	IBI w/ infant survival (months)	IBI w/ infant death (months)	Cycle length (days)	Gestation length (months)	Age at first reproduction (years)
<i>C. vellerosus</i>	18.5 ¹ n=11	12 ¹ n=4; 10 ² n=5	24 ¹ n=2	5.75 ¹ n=2	5.8 ¹ n=8
<i>C. guereza</i>	22 ³ n=6	6.8 ⁴ n=1	25 ³ n=5	5.75 ³ n=1	-
<i>C. polykomos</i>	24 ⁵ n=4	-	-	-	-
<i>P. badius</i>	29.4 ⁶ n=10	-	-	5.25 ⁶ n=4	4.2 ⁶ n=4
<i>P. nemaus</i>	-	-	26 ⁷ n=5	7.5 ⁸ n=1	-
<i>P. rufomitratu s</i>	25.3 ⁹ n=13; 27.5 ¹⁰ n=2	14-17 ⁹ n=13; 9-18 ¹¹ n=2	-	-	-
<i>P. thomasi</i>	26.8 ¹² n=36	17.7 ¹² n=36	-	-	5.4 ¹² n=9
<i>R. bieti</i>	23.3 ¹³ n=12	14 ¹³ n=5	29 ¹⁴ n=3	7.25 ¹⁴ n=3	-
<i>R. roxellana</i>	23.3 ¹⁵ n=36	11.5 ¹⁵ n=5	-	-	5-6 ¹⁵ n=5
<i>S. entellus</i>	28.9 ¹⁶ n=24; 16 ¹⁷ n=2; 16.7 ¹⁸ n=113	13.3 ¹⁶ n=72; 11 ¹⁷ n=72; 7 ¹⁸ n= not reported	24.1 ¹⁸ n=161	7.25 ¹⁹ n=6	6.7 ¹⁶ n=26; 3.5 ¹⁸ n=12
<i>T. obscurus phayre</i>	21.3-24.5 ²⁰ n=8-15	-	-	-	-

(1) This study (2) Teichroeb & Sicotte 2008 (3) Harris and Monfort 2006 (4) Harris and Monfort 2003 (5) Dasilva 1989 (6) Starin 2001 (7) Heistermann *et al.* 2004 (8) Lippold 1981 (9) Marsh 1979 (10) Struhsaker and Leland 1985 (11) Struhsaker & Pope 1991 (12) Wich *et al.* 2007 (13) Cui *et al.* 2006 (14) He *et al.* 2001 (15) Qi *et al.* 2008 (16) Borries *et al.* 2001 (17) Borries 1997 (18) Sommer *et al.* 1992 (19) Ziegler *et al.* 2009 (20) Borries *et al.* 2008.

Although *C. vellerosus* fits within the typical range for colobine species (Table 2-3), cycle (24 days) and gestation length (5.75 months) for *C. vellerosus* in this study are shorter than those found for most colobines of similar size (range 24-29 days; 5.25-7.5 months). *C. vellerosus* weigh on average 6.9 kg for females and 8.5 kg for males (Saj and Sicotte 2013), which is on the lower end of the range for colobines [between 5-10 kg for

females and 8.4-13.5kg for males (Smith and Jungers 1997, reviewed in Butynski *et al.* 2013)]. Typically, larger species have slower life histories, mature later, have longer gestation, wean later and at a larger size, and have longer gaps between births (Purvis *et al.* 2003). *C. vellerosus*' size could account for their slightly shorter gestation and cycle length, relative to other colobines. In addition, diet and substrate use affect life history pace (Leigh 1994, van Schaik and Deaner 2002, Wich *et al.* 2007). Although all colobines are leaf eaters, different populations vary in their specific diet and access to high quality food resources, which in turn could have influenced their life history pace (reviewed in Asquith 1989). Our population may live in a habitat that contains lower quality food resources than other colobine populations (Wong *et al.* 2007). More detailed comparisons among wild populations with known diets and life history parameters are necessary to assess this relationship.

Previous research on *C. vellerosus* used behavioural data to estimate some life history variables (e.g. inter-birth interval after infanticide, Teichroeb and Sicotte 2008). Our study corroborates these findings, confirming that some life history characteristics can be reliably determined using observational data alone. We were thus able to use observational data to determine some life history traits for those females with hormone profiles that do not show any clear patterns.

Validation of hormone analyses is important to ensure accuracy of the results. One of the validation procedures we used in this study is a series of 'standards' for each plate/assay of samples analysed, to measure inter- and intra-assay variability. From these 'standards' we calculated the coefficient of variation (CV) within and between each set of samples. Our CV values for P are high compared to other studies. For some steroid

hormones, such as cortisol, even very slight changes in the quantity of a hormone can indicate a change in the stress level of an individual (Heistermann *et al.* 2006). In these cases, having very low inter- and intra-assay variability is important because it is otherwise not possible to determine if observed variation between amounts of steroid hormones result from variation in the assay process or actual physiological changes in the subject. For other steroid hormones, such as E2 and P, the variation needed to determine a female's reproductive state is very high (i.e. E2 levels increase by a mean of 15.78 ng/g and P by a mean rate of 956.36 ng/g during pregnancy compared to lactational amenorrhea. The range of values within lactational amenorrhea for E2 is 4.16-6.13 ng/g and for P is 210.23-491.30 ng/g, whereas the range during pregnancy for E2 is 1.17-96.94 ng/g and for P is 41.93 - 3168.15 ng/g). Therefore, despite our high CV values, the variation between assays in this study should not affect the determination of reproductive events in females' lives, because the changes in quantity of E2 and P are so distinct that ovulation, conception, and lactational amenorrhea can be determined even with some variability between assays.

Despite a large data collection team, a large number of focal females and faecal samples, and high number of contact hours, the number of hormonally determined ovulatory cycles in this study is low. As is often the case with primates who are aseasonal breeders, many of the females were pregnant or lactating during the study period. In addition, some of our subjects were prepubescent, and one was probably post-reproductive. Two females cycled but conceived early in the study after one cycle, and two were excluded from analysis because their hormone profiles met no criteria for ovulation, pregnancy or lactational amenorrhea. It is likely that the possible range of

variation between females is not represented in our small sample. Our conclusions should thus be taken as preliminary. The information presented here on reproductive cycle and state is nevertheless one of the most complete pictures of wild colobine life history characteristics to date.

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**CHAPTER 3 THE INFLUENCE OF REPRODUCTIVE HORMONES AND
MALE GROUP MEMBERSHIP ON POST-CONCEPTIVE MATING IN
FEMALE *COLOBUS VELLEROSUS*.**

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Introduction

In many mammals, female mating activity is under strict hormonal control (reviewed in Prendergast *et al.* 2002) and is restricted to a short period surrounding ovulation (Ziegler *et al.* 2009). This period, called “oestrus”, was first defined as a specific period in which female mammals show sexual desire, are sexually receptive to males, and are fertile (Heape 1900). Many primate females, however, do not conform to a typical oestrus pattern because they can and do mate outside the fertile period, and at times when they are not ovulating (e.g., Nunn 1999, Deschner *et al.* 2004, Barelli *et al.* 2008, Van Belle *et al.* 2009). In particular, females of many primate species mate post-conceptively (reviewed in Dixson 2012, Hrdy and Whitten 1987, Martin 1992). Post-conceptive mating is characterized by female sexual solicitation and/or morphological signalling (e.g. sexual swellings) that are typically associated with the fertile period (Hrdy 1977). Because females display these behaviours and signals at a time when they are not fertile, post-conceptive mating has been referred to as “abnormal oestrus” (Heape

1900) or ‘pseudo-oestrus’ (Hrdy 1974, 1977). Although post-conceptive mating is prevalent among many primate species (for example: *Leontopithecus rosalia*, Kleiman and Mack 1977, *Macaca sylvanus*, Kuester and Paul 1984, *Cercopithecus torquatus atys*, Gordon *et al.* 1991, *Semnopithecus entellus*, Sommer *et al.* 1992, *Cebus capucinus*, Manson *et al.* 1997, *Cercopithecus mitis*, Pazol 2003, *Macaca fascicularis*, Engelhardt *et al.* 2007), studies aimed at understanding this behaviour are lacking (Engelhardt *et al.* 2007).

One proximate mechanism to explain post-conceptive mating is that it is linked to hormonal fluctuations during pregnancy. During a typical ovulatory cycle motivation to mate, and mating itself, are usually associated with fluctuating levels of oestradiol (E2) and progesterone (P), and their relative ratio (Zehr *et al.* 1998, Aujard *et al.* 1998, Engelhardt *et al.* 2005). In many species (for example: *Macaca mulatta*, Bielert *et al.* 1976, Wilson *et al.* 1982, Zehr *et al.* 1998, *Propithecus verreauxi*, Brockman *et al.*, 1995, *Brachyteles arachnoides*, Strier and Ziegler 1997, *Macaca nemestrina*, Risler *et al.* 1987, *Papio cynocephalus*, Wasser *et al.* 1991) solicitations and mating are linked to high E2 and low P levels (Baum *et al.* 1977, Beach 1976, Zehr *et al.* 1998, Aujard *et al.* 1998, Engelhardt *et al.* 2005). Whereas, in a few species female solicitations and mating are associated with low levels of E2 and high levels of P (e.g. *Macaca arctoides*, Nieuwenhuijsen *et al.* 1986), or there is no association of solicitations and mating with either hormone (*Papio anubis*, Higham *et al.* 2009). In the same way that hormones motivate females to mate during the fertile period, post-conceptive mating may be a by-product of fluctuating or elevated E2 and P levels (Saymann 1975), or the result of variable E2:P ratios during pregnancy (Engelhardt *et al.* 2007). Although there is

variability in whether E2 or P is the hormone driving mating, most studies point to an association between relative levels of E2 and P and female mating.

An alternative hypothesis is that females' access to multiple males during pregnancy may be driving post-conceptive mating. Males typically use cues given by females, particularly female proceptive behaviours, but also visual, olfactory, and hormonal cues gained from inspection of female anogenital areas (reviewed in Dixson 1998, Deschner *et al.* 2003, Mohle *et al.* 2005, Cerda-Molina *et al.* 2006, Heistermann *et al.* 2008), to concentrate mating around the fertile period (Young *et al.* 2013). However, these cues are often imprecise and can occur over extended periods of time (Deschner *et al.* 2004, Carnegie *et al.* 2005, Engelhardt *et al.* 2005, reviewed in Hrdy and Whitten 1987) making it difficult for males to definitively discern the fertile period (Young *et al.* 2013). As such, males may benefit from mating whenever presented with proceptive behaviours from females. Female proceptive behaviours that occur outside the fertile period can extend the mating period, particularly when females have access to multiple males. In group-living species that contain several males and females, exposure to multiple males can lead to longer mating periods for females (Cords 1984, Takahata 1980) and females can mate with more than one male over this extended period of receptivity (Borries *et al.* 1999, 2001). Indeed, females living in multi-male male groups, or those with access to multiple males, mate post-conceptively (Nunn, 1999, Deschner *et al.* 2004, Barelli *et al.* 2008, van Belle *et al.* 2009). Because it becomes increasingly time and energy expensive for one male to mate guard a potential mating partner when there are many other potential mates and competitors, the ability of one male to monopolize mating tends to correlate negatively with the number of females and males in a group

(Hausfater 1975, Cowlshaw and Dunbar 1991). Post-conceptive mating may thus increase a female's opportunity to mate with multiple males because it extends the mating period and may help to free females from monopolization by a single male (especially the dominant male).

Post-conceptive mating with multiple males may be an important counter-strategy to infanticide by confusing paternity certainty (Hrdy 1974, Wolff and MacDonald 2004). If males are unable to definitively discern the fertile period, and they use mating as a cue to paternity (Hrdy 1979, Palombit *et al.* 1997, Palombit 2000, van Schaik *et al.* 2000), post-conceptive mating may spread some paternity certainty among all the males in the group. Male infanticide has been documented in a range of mammals (reviewed in van Schaik and Janson 2000) and typically occurs when a male enters a group or rises in rank within a group, and attacks and kills unrelated infants. Infanticide is extremely costly to females and therefore there should be strong selective pressure for females and males (the sire of the infant) to adopt reproductive strategies against infanticide (Hrdy 1974, Wolff and MacDonald, 2004, van Schaik, 2000). Boyko and Marshall (2009) suggest that there are two different reproductive strategies that could be occurring to reduce the risk of infanticide depending on the group type and strength of the male(s) within the group. One is for a male to gain 100% paternity probability of the infants born in his group by excluding other males from the group and from mating access to females. The other, in groups where one male is not able to exclude other males, is to concede some mating opportunities to other males and spread paternity certainty among these males, to reduce the risk they will kill infants born in the group. In addition, by conceding some reproductive opportunities to non-dominant males, a dominant male may also ensure that

the group contains other fathers who may also help protect against infanticidal attacks (Henzi *et al.* 2010). Females may also employ these two reproductive strategies, whereby those females in uni-male groups may concentrate paternity certainty in one male who could protect infants, while those in multi-male groups may spread paternity certainty among many males.

The goal of this study is to test whether or not post-conceptive mating in female *Colobus vellerosus* is a by-product of fluctuating and/or elevated E2 and P levels during pregnancy or if it is a result of females' access to multiple males. We also consider whether or not patterns of female post-conceptive mating in *C. vellerosus* support the infanticide avoidance hypothesis. We first present data showing that wild female *C. vellerosus* mate post-conceptively. To test the effect of female reproductive hormones on post-conceptive mating, we determine if E2 and P show a pattern within and/or between the three stages of pregnancy in female *C. vellerosus*. We compare E2 and P levels to rates of solicitation and rates of copulation to determine if there is an association between post-conceptive mating and hormone levels during pregnancy. If fluctuating hormone levels affect post-conceptive mating, we expect to see an association between levels of E2 and/or P and the rate of solicitation and/or rate of copulation. To test the effect of male group membership on post-conceptive mating, we look at rates of solicitation and copulation relative to group-type, number of males in a group, and male rank. If females mate post-conceptively as a result of access to multiple males, we expect a higher overall rate of female directed solicitation and copulation in multi-male groups compared to those in uni-male groups. If females mate with multi-males simply because these males are present, we expect no difference in rates of female sexual behaviours across male

dominance ranks and/or between individual males. We also look at rate of solicitation received from males to determine if males solicit females at a different rate relative to group-type, number of males and male dominance status. If females mate post-conceptively because males are soliciting them, then we expect males to solicit females more than females solicit males. We discuss the possibility that these two proximate mechanisms (hormonal influence and male membership) are linked, and are both driving female post-conceptive mating. Because female post-conceptive mating is often associated with infanticide avoidance (Hrdy 1974, 1979, Agrell *et al.* 1998), we also discuss our results in the context of female post-conceptive mating as a possible mating strategy to confuse paternity and reduce the risk of male infanticide (Hrdy 1974, 1979, Agrell *et al.* 1998).

C. vellerosus is an ideal species to investigate the occurrence and the pattern of post-conceptive mating because females show no external morphological signs of ovulation and they cycle asynchronously throughout the year (Saj and Sicotte 2013). Group composition is fluid and can shift over time. A multi-male group can become uni-male if one male becomes dominant and ousts the other males, or a uni-male group can become multi-male through male immigration (Teichroeb *et al.* 2011, Sicotte *et al.* 2015). In multi-male groups, females mate polyandrously and mating skew appears low (Teichroeb and Sicotte, 2010). In uni-male groups, observational and genetic data suggest that females mate primarily with the resident male (Teichroeb *et al.* 2013, Wikberg unpublished data), although extra-group copulations have been documented (Teichroeb *et al.* 2005). Male mating competition is intense and infanticide occurs regularly in this population (Teichroeb & Sicotte 2008, Teichroeb *et al.* 2012).

Methods

Study Site and Study Groups

This study was conducted at the Boabeng-Fiema Monkey Sanctuary (BFMS) in Central Ghana (7° 43' N and 1° 42' W), a 192 hectare forest fragment. BFMS is a dry semi-deciduous forest consisting of primary and secondary forest (regenerating farmland), woodland savannah, and riverine forest. The colobus monkeys at BFMS have been the subjects of on-going study since 2000, under the direction of Dr. P. Sicotte at the University of Calgary (for more details on the site and on its colobus population, see Saj *et al.* 2005, Sicotte *et al.* 2015, Teichroeb *et al.* 2009, 2011, Wong & Sicotte 2006).

We studied three multi-male groups, one uni-male group and one all-male band (AMB) (see Table 3-1 for group makeup and variation in male group membership). Males in multi-male groups were classified as either dominant or non-dominant. We determined male dominance using the direction and the outcome of male-male agonistic interactions. Aggressive behaviours included biting, hitting, chasing, and displacing. Submissive behaviours included avoiding, fleeing, submissive presenting, and cowering. The male who was the “winner” of all or most interactions relative to all other males in the group was considered dominant. In groups with regular male incursions (Sicotte & MacIntosh 2004) the resident male was always considered dominant, because the incurring male(s) were not established members of the group, did not sleep with the group, and often came and went throughout any given day. If an outside male remained, ranged and slept with a group for more than 1 month (Teichroeb *et al.* 2011, Sicotte *et al.* 2015), he was considered a group member and we took agonistic interactions into

consideration to establish dominance status. We did not determine 2nd, 3rd, etc. rank, but rather classified all males other than the dominant male as “non-dominant”.

Table 3-1 The number of adult females and non-dominant males in each study group. Each group had only one dominant male. Group types are classified as Stable Uni-Male (SUM), Stable Multi-Male (SMM), or Unstable Multi-Male (UMM). When two group types are given for the same group, it indicates that a change occurred during the study period. In groups that changed group types during the study period, we considered the two group types separately for statistical analysis. AMB = All-male band

Group	# Adult females	# Non-dominant males	Reason for variation in male membership	Group-type
SP	5	4-8	AMB present/Male death	UMM
RT	6	4	N/A	SMM
WT	3	0-4	AMB present/Death of dominant	UMM
WW	4	0-1	Male disappeared	UMM/SUM

We classified groups as stable uni-male (SUM), stable multi-male (SMM) and unstable multi-male (UMM) (Table 3-1). SUM groups were those that had one resident male and did not experience male incursions or (attempted) takeovers for the length of this study. SMM groups were those in which several males resided, with a clear dominant male, and the groups did not experience male incursions or (attempted) dominance takeovers for the length of this study. UMM groups were those in which several males resided, but where dominance relationships were not settled, so there were regular bidirectional agonistic interactions between the resident males and/or the incoming males. We had no unstable uni-male groups (UUM), which are uni-male groups that experienced male incursions or (attempted) dominance takeovers, with the incurring

male(s) staying less than one month. One group (WW) changed from multi-male to uni-male during data collection so the two periods were treated separately for analysis. Male membership for all other groups remained the same for the duration of data collection.

Female group membership did not change throughout the study period. Fourteen females were parous, and four were nulliparous at the start of the study period. Twelve out of 18 females were pregnant during the study period as previously determined from hormonal and observational data (Vayro *et al.* 2016). Females ranged in age from seven to 18 years old and were all considered “adults” (See Wikberg 2012 for details on *C. vellerosus* age classification). Of these 18 females, two were prepubescent, 15 were cycling and/or pregnant and/or in lactational amenorrhea, and one was post-reproductive (Vayro *et al.* 2016).

Data Collection

I led a team of seven assistants, and we spent 5820 total contact hours with our four study groups from May 2012 to May 2013. All members of the study groups were individually recognizable based on eyebrow shape, black cap and white ruff shape, distinct scars, and tail shape and length. We collected ten minute focal samples (Altmann 1974) from 6 am to 2 pm, six days per week. We collected a total of 562 hours of focal observation (mean 30.8 hours per female; range 20.4-45.6 hours; n=18). We documented demographic changes and infant injuries. In addition, we collected all occurrences of mating events, mating solicitations, and agonistic behaviours. We included socio-sexual and sexual behaviours from our all occurrence data because these are rare behaviours and were difficult to capture during our focal follows alone. When socio-sexual or sexual

behaviours occurred within a focal, we usually captured the entire series of events, unless the event was truncated (i.e. the sequence had already started at the beginning of the focal). However, in the all occurrence data, we sometimes did not capture the subtle solicitation behaviour that occurred at the beginning of a sexual event. "Approaches" can be considered as solicitations, but this can be imprecise because approaches are not always associated with sexual behaviours. If we included all approaches as solicitations, we would run the risk of overestimating the number of solicitations occurring between individuals. We thus decided to include only the more distinct indicators of solicitation, such as a male "hip touch" or a female "sexual present".

We sampled all females opportunistically, and our goal was to ensure that each female was sampled for the same amount of time on a weekly basis. We also collected behavioural data from the all-male band when they were in the study area and/or interacting with any of the study groups, because in the event of male incursions or takeovers these males were potential mates for the females in our study groups (Teichroeb *et al.* 2005, 2011).

Our behavioural data collection focused on socio-sexual and sexual behaviours. We called proceptive behaviours (Beach 1979) "solicitations directed by a female" and included events of female presentation of posterior to a male. We called behaviours indicative of attractivity (Beach 1979 "female received solicitations" and included events of a male hip grabbing a female, which could occur in conjunction with male sniffing, touching and/or licking a female's genitals. We called behaviours indicative of receptivity (Beach 1979) "copulations" and we defined copulation as a male mounting a female with intromission and thrusting (Kuster and Paul 1984). We could sometimes

infer ejaculation when a male showed an ejaculatory pause immediately before the end of a copulation (Kuster and Paul 1984), but this is not always a reliable indicator in this species so we did not include it in the analyses.

We collected faecal samples in a sterile collection vial each morning between 6 am and 12 pm. We did not collect samples after 12 pm to avoid the potential effects of diurnal variation (Hodges and Heistermann 2011). We collected a total of 1866 faecal samples from 18 focal females, with a mean of 104 samples per female (range 75-120; Vayro *et al* 2016). We extracted oestradiol and progesterone metabolites at the field station within eight hours of collection using the techniques outlined in Strier and Ziegler (1997) and Ziegler and Wittwer (2005). We processed 1586 samples at the Wisconsin National Primate Research Centre (WNPRC) at the University of Wisconsin-Madison. I assayed faecal oestradiol metabolites using radio-immunoassay (RIA) and progesterone metabolites using enzyme immunoassay (EIA) (Ziegler *et al.* 1987, Saltzman *et al.* 1994).

We validated the assays using quality controls for each plate (for P) and each assay (for E2). The mean intra- and inter-assay coefficient of variation (CVs) for P were 7.03 and 25.22 for low pools, and 5.33 and 28.76 for high pools. The mean intra- and inter-assay CVs for E2 were 7.37 and 15.43 for low pools, and 4.43 and 11.75 for high pools.

Behavioural Data Analysis

Socio-sexual and sexual behaviours in *C. vellerosus* were relatively rare, therefore we combined copulations and solicitations from focal and all occurrence data. Because groups of black and white colobus monkeys tend to be quite cohesive, and given the large

number of observers with the groups on a daily basis, we are confident that we captured and recorded most sexual and socio-sexual events during contact hours for our study females. Additionally, we spent a similar number of contact hours with each group to reduce bias of any particular group or female. For analysis, we used rates per 100 hours to standardize the number of behaviours relative to contact time.

We calculated rates of solicitation and copulation by dividing the number of copulations between male-female dyads by the contact hours for that dyad during periods when the female was pregnant. All reported rates are per 100 contact hours. We included all pregnant females in the analysis, including those who were never observed copulating or soliciting. We did not differentiate between copulations with or without ejaculation, and used both for analysis. We considered copulations distinct when a series of mounts and dismounts were separated by at least one hour (Teichroeb and Sicotte 2010). We tested all data using a Generalized Estimating Equation (GEE) in SPSS statistical software (Version 22, 2013) to account for repeated measures within our data set. The distribution of data was treated as normal because our variables were in the form of rates, which are continuous data (Tak Fung, personal communication). We chose our correlation type [auto-regression (AR1) or unstructured] based on the “goodness of fit” of the data to the model and used the form “smallest-is-better”. If multiple post-hoc analyses were performed on the same dataset, we applied Bonferroni corrections to the required significance threshold. We set the alpha level to 0.05.

There is a 24-48 hour time lag between when hormones are excreted and when they become present in faeces (Bahr *et al.* 2000, Heistermann 2010, Wasser *et al.* 1994). When looking at hormone levels relative to a behavioural event to determine if the two

were associated, we accounted for the time lag by considering mean hormone levels for 3 days after the event, not including the day of the event (Carosi *et al.* 1999).

Pregnancy lasts a mean of 22.2 +/- 1.92 weeks (n = 2; Vayro *et al.* 2016) or 168.5 +/- 13.44 days (n = 2 pregnancies) (Chapter 4). We plotted the occurrence of solicitation and copulation for the 12 focal females who were pregnant during our study period over 168 days. We aligned pregnancy and gestation by day of conception for the two females for whom we had hormonal data indicating the day of conception, for the other 10 females we aligned pregnancy and gestation by subtracting 168 days from the day of parturition. Upon visually inspecting the data (Jayaratne 1979) we found that there was a distinct pattern whereby copulations were high in the beginning of pregnancy, low in the middle, and absent at the end. Solicitations were low in the beginning of pregnancy, high in the middle and almost absent at the end of pregnancy. Based on this distribution, we divided pregnancy into three equal stages (56 days each): early, mid and late pregnancy (Wallis and Goodall 1993, Borries *et al.* 2001, Beehner *et al.* 2006, Roberts *et al.* 2012). We used these three categories in subsequent analyses to test if there was a difference in mean hormone levels, or rates of copulation and solicitation, throughout pregnancy.

To test whether or not post-conceptive mating in female *C. vellerosus* is a by-product of fluctuating and/or elevated E2 and P levels during pregnancy, we conducted three GEE analyses. First, to determine if hormone levels varied throughout pregnancy, we tested mean levels of P, E2, and their ratios between early, mid, and late pregnancy. Second, to determine if rates of solicitation and copulation varied throughout pregnancy, we tested mean rates of solicitation and copulation between early, mid, and late pregnancy. And third, to determine if hormone levels affected solicitation and copulation

we tested whether or not there was an effect of mean levels of P, E2, and their ratios on solicitation and/or copulation within three days of the solicitation or copulatory event. “Copulation/solicitation within the next three days” did not include the day of copulation or solicitation.

To test whether or not female post-conceptive mating is a result of females’ access to multiple males we used a GEE to determine if solicitation or copulation rates were affected by 1) group-type (SUM, SMM, or UMM), 2) number of males in the group, or 3) male dominance rank. Solicitations directed by a female to a male and received from a male to a female were treated as separate variables. To determine if there are differences between multi-male and uni-male groups we compared mean rates of solicitation directed and received, and copulation with either the resident male of the uni-male group or the dominant male of a multi-male group. To look further at the effect of 1) group type, 2) male rank, and 3) the interaction between group-type and rank on rates of mating and solicitation we excluded SUM groups and looked only at SMM and UMM groups, because there are no non-dominant males in uni-male groups.

Results

C. vellerosus post-conceptive mating patterns

Nine of the 12 pregnant females directed and received solicitations, and copulated after conception. We recorded a total of 57 post-conceptive copulations, 17 post-conceptive received solicitations, and 49 post-conceptive directed solicitations. Not all solicitations resulted in copulation, with 35% of solicitations received and 39% of solicitations directed resulting in copulation. Post-conceptive copulations occurred

between days 4 and 115 of pregnancy (n= 52 copulations), and directed and received solicitations between days 13 and 132 (n = 43 solicitations). Rates of copulation and solicitation per pregnant female for all males with whom she directed and received solicitation, and copulated are presented in Table 3-2. Possible and actual dyads per female are presented in Table 3-3. Rates of copulation and solicitation for each pregnant female-male dyad per group are shown in Figures 3-1 – 3-5.

Table 3-2 Total rates of copulation and solicitation per pregnant female for all males with whom she directed and received solicitation, and copulated for each group. Rate of solicitation received and directed, and rate of copulation are given per 100 contact hours. “Individual Contact Hours” are the contact hours for each female in which the female was pregnant and the male was present in the group. n = 12 females.

Female	Group	Individual Contact Hours	Received solicitation rate	Directed solicitation rate	Copulation rate
BE	RT	309.22	0	0	0.16
BL	RT	514.5	0.07	0.07	0.13
FV	RT	519.6	0	0	0.31
SU	RT	337.36	0	0	0
TR	RT	318.95	0.11	0	0.31
SA	SP	73.14	0	0	0
VE	SP	1160.8	0	0.07	0.03
IS	WT	1215.8	0.08	0.20	0.20
XY	WT	1154.25	0.05	0.22	0.24
CR	WW	198.63	0	0.61	0
JN	WW	596.07	0.17	0.42	0.17
IT	WW	210.43	0	0	0

Table 3-3 Possible and actual dyads per female for each group. We calculated the percent of actual dyads by dividing the actual mating dyads by the possible mating dyads in each group.

Group	Group-type	Possible Mating Dyads	Actual Mating Dyads	Percent of actual dyads
RT	SMM	25	10	40%
SP	UMM	20	4	20%
WT	UMM	14	11	79%
WW	UUM	6	2	33%
WW	SUM	3	1	33%

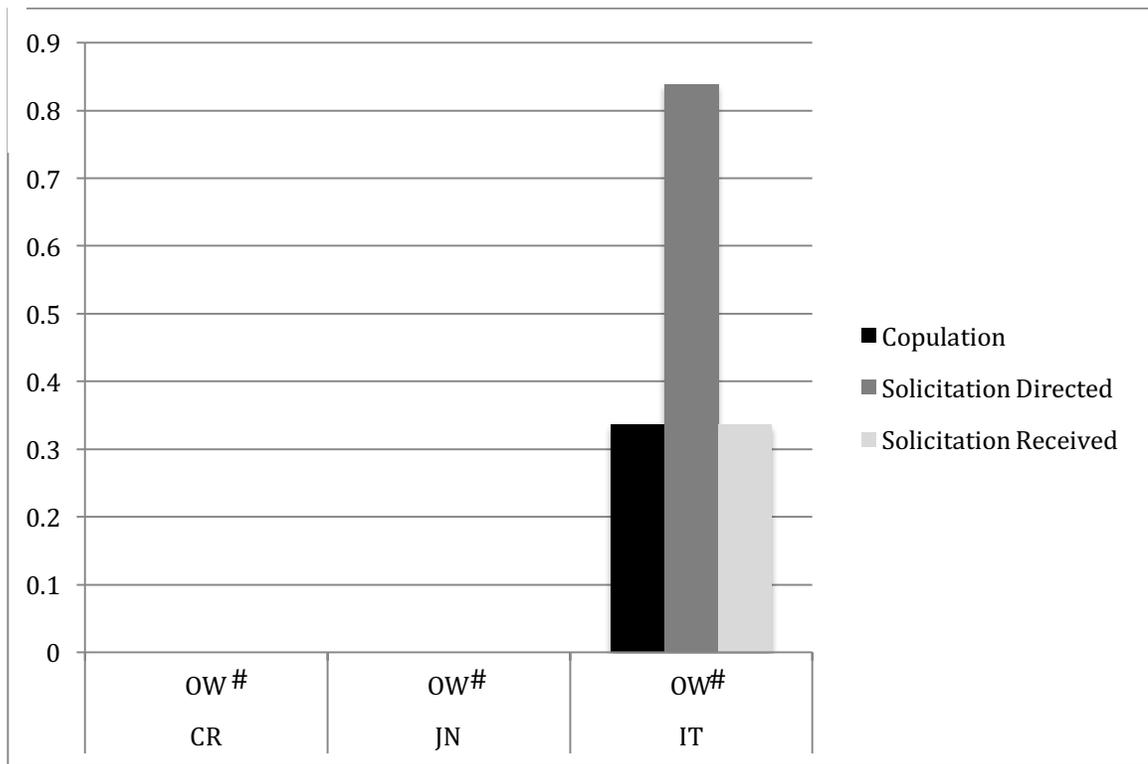


Figure 3-1 Rates of copulation, solicitation directed, and solicitation received per dyad in Wawa group when it was a stable uni-male group. The Y-axis shows mean rate per 100 contact hours that both the male and female were present while the female was pregnant. The X-axis shows each female- (two initials at the bottom) male (two initials at the top) dyad. The dominant male is indicated with a #. n = 1 male, 3 females.

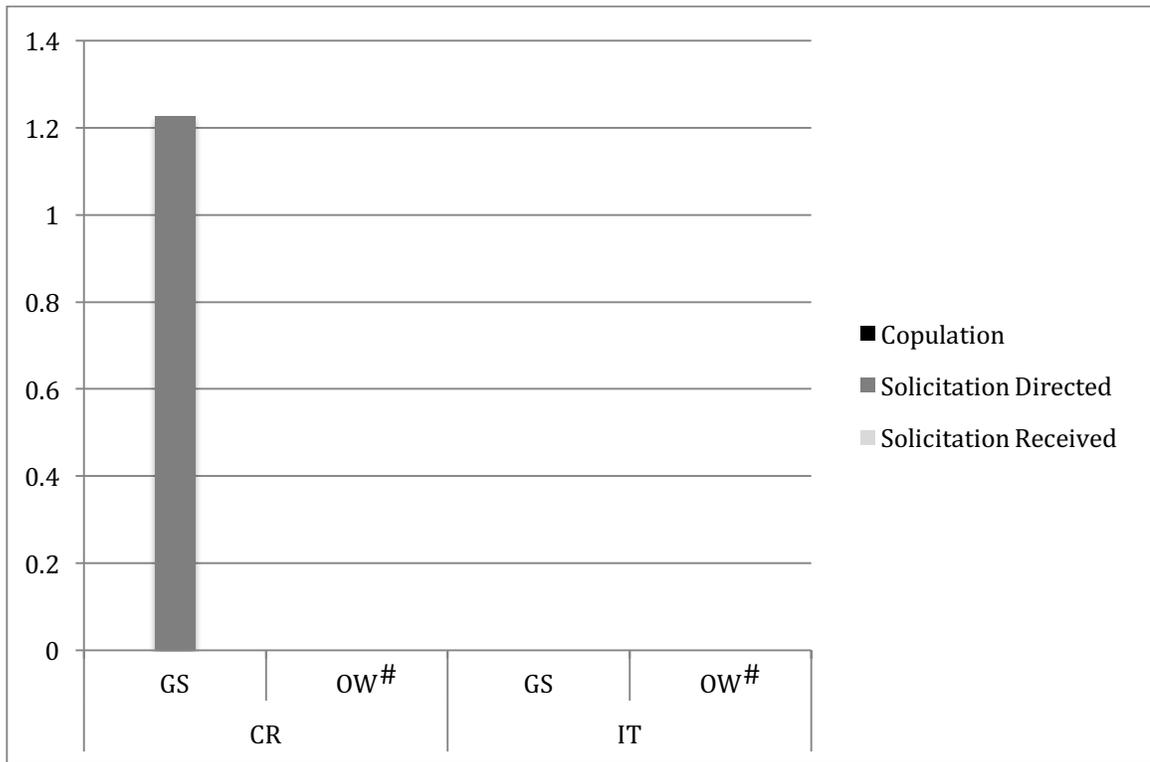


Figure 3-2 Rates of copulation, solicitation directed, and solicitation received per dyad in Wawa group when it was an unstable multi-male group. The Y-axis shows mean rates per 100 contact hours that both the male and female were present while the female was pregnant. The X-axis shows each female- (two initials at the bottom) male (two initials at the top) dyad. The dominant male is indicated with a #. n = 2 males, 2 females.

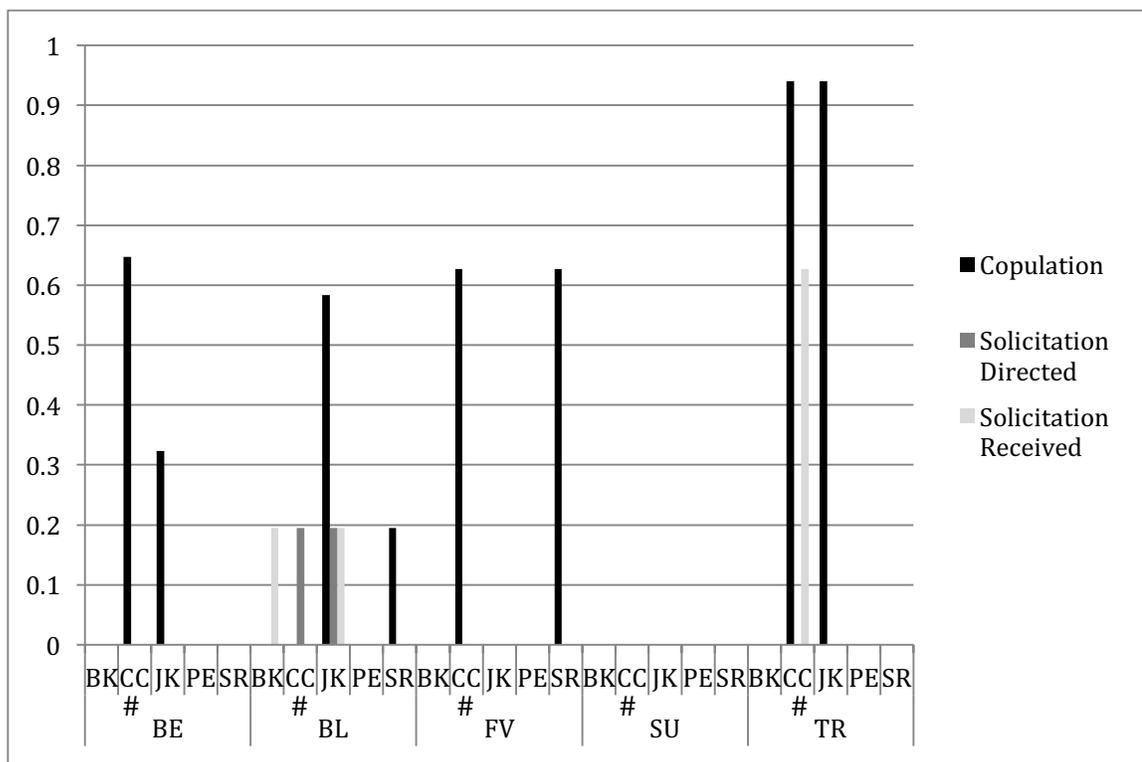


Figure 3-3 Rates of copulation, solicitation directed, and solicitation received per dyad in Redtail, a stable multi-male group. The Y-axis shows mean rates per 100 contact hours that both the male and female were present while the female was pregnant. The X-axis shows each female- (two initials at the bottom) male (two initials at the top) dyad. The dominant male is indicated with a #. n = 5 males, 5 females.

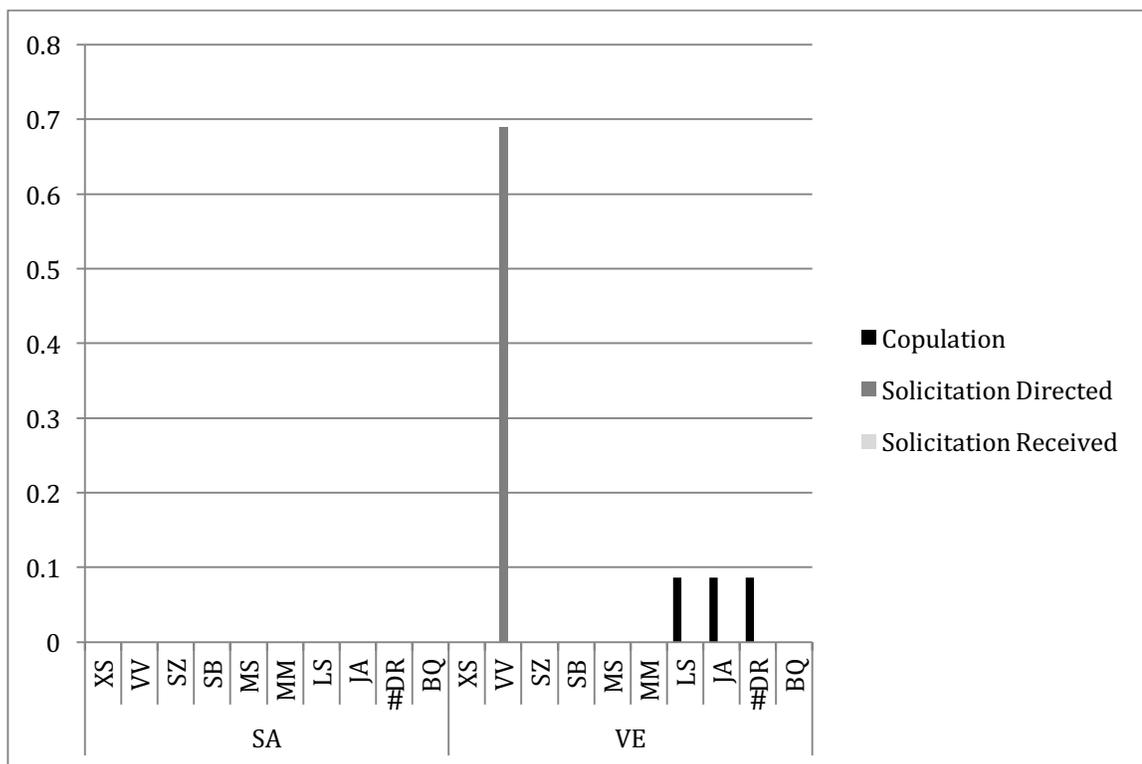


Figure 3-4 Rates of copulation, solicitation directed, and solicitation received per dyad in Splinter group, an unstable multi-male group. The Y-axis shows mean rates per 100 contact hours that both the male and female were present while the female was pregnant. The X-axis shows each female- (two initials at the bottom) male (two initials at the top) dyad. The dominant male is indicated with a #. n = 10 males, 2 females.

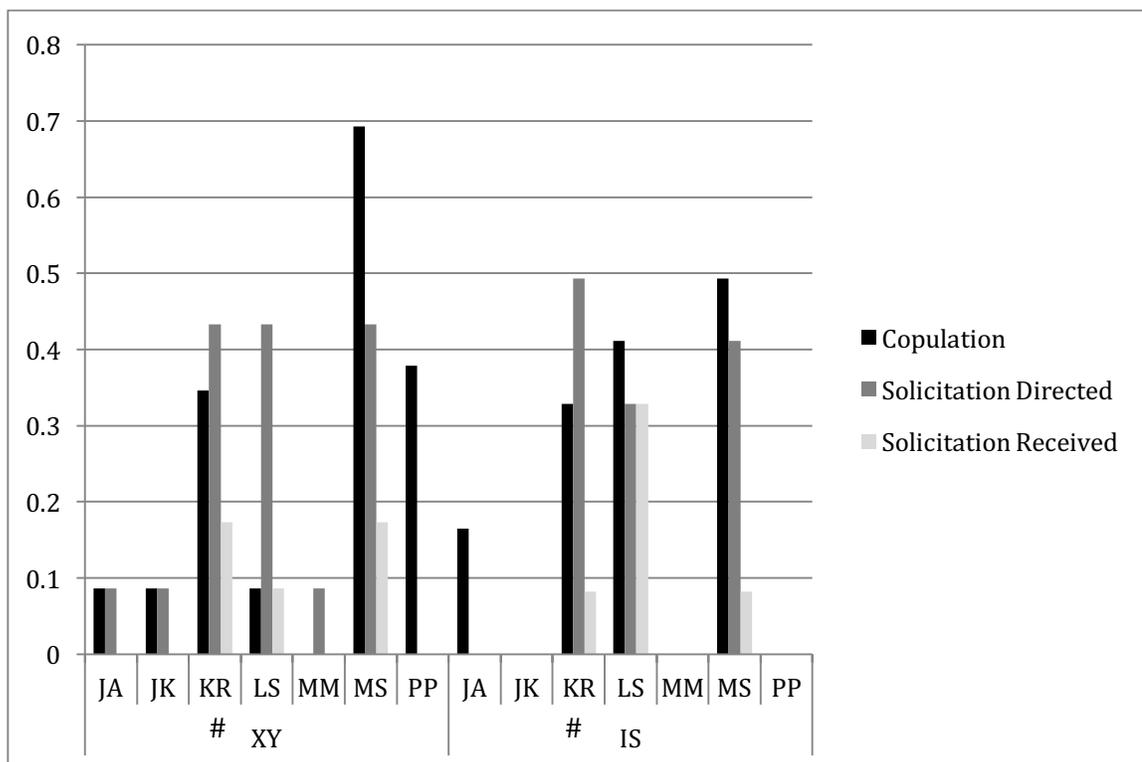


Figure 3-5 Rates of copulation, solicitation directed, and solicitation received per dyad in Winter group, an unstable multi-male group. The Y-axis shows mean rates per 100 contact hours that both the male and female were present while the female was pregnant. The X-axis shows each female- (two initials at the bottom) male (two initials at the top) dyad. The dominant male is indicated with a #. n = 6 males, 2 females.

Hormone patterns across pregnancy

Pregnant female P levels were significantly different across early, mid, and late pregnancy. Pairwise comparisons (Figure 3-6) showed P was significantly higher between early and late pregnancy, and mid and late pregnancy ($p < 0.001$). E2 levels were significantly different across early, mid, and late pregnancy. Pairwise comparisons (Figure 3-7) showed E2 levels were significantly different between early and mid pregnancy ($p < 0.001$), mid and late pregnancy ($p < 0.001$) and early and late pregnancy

($p < 0.001$). The ratio of E2:P was not significantly different across pregnancy stages. See Table 3-4 for detailed model results.

Table 3-4 Mean levels of progesterone, oestradiol and the ratio of oestradiol:progesterone in early, mid, and late pregnancy. Table summarizes the Wald's χ^2 value with degrees of freedom and the significance level. Lines in *italics* indicate a significant difference. n = 10 females, n = 401 data points

Dependent Variable	Independent Variable	Wald's χ^2 (df)	p value
<i>P</i>	<i>Pregnancy stage</i>	74.703(2)	< 0.001
<i>E2</i>	<i>Pregnancy stage</i>	31.734(2)	< 0.001
E2:P	Pregnancy stage	2.780(2)	0.249

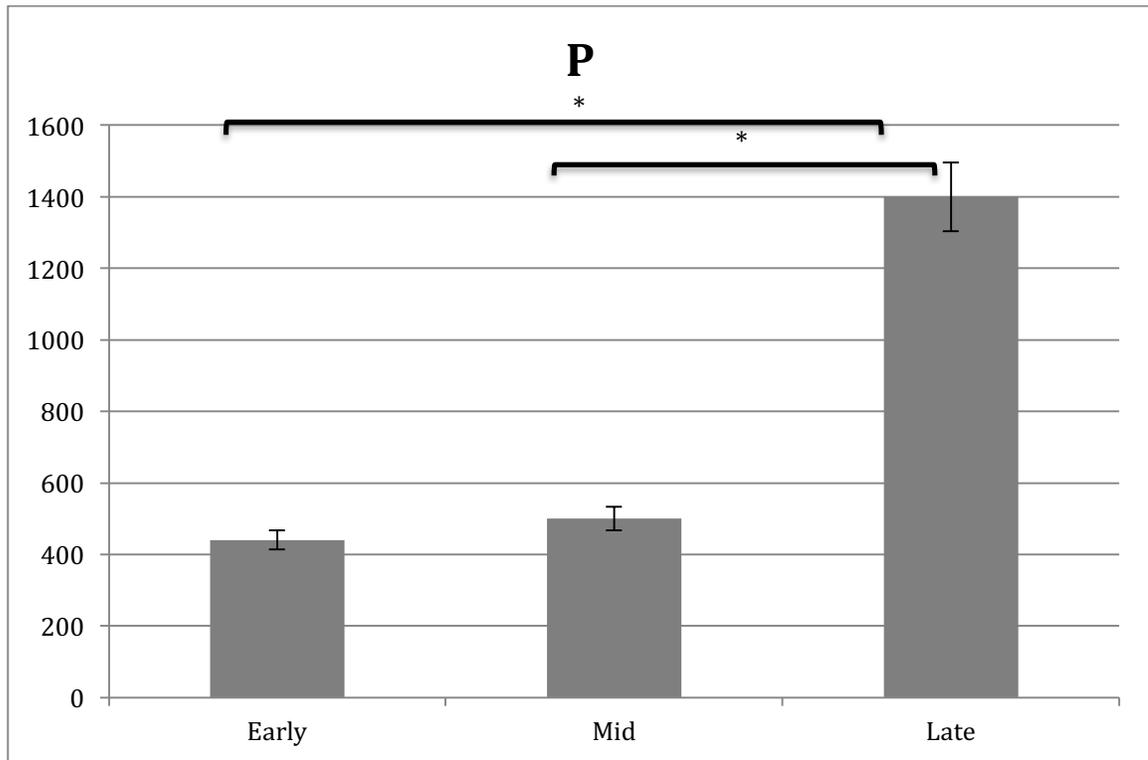


Figure 3-6 Mean female progesterone (P) levels in early, mid, and late pregnancy with one standard error. The Y-axis shows mean hormone levels for P (ng/g). The X-axis shows early, mid, and late pregnancy. Significant results are denoted by an asterisk (*). n = 10 females, n = 401 data points

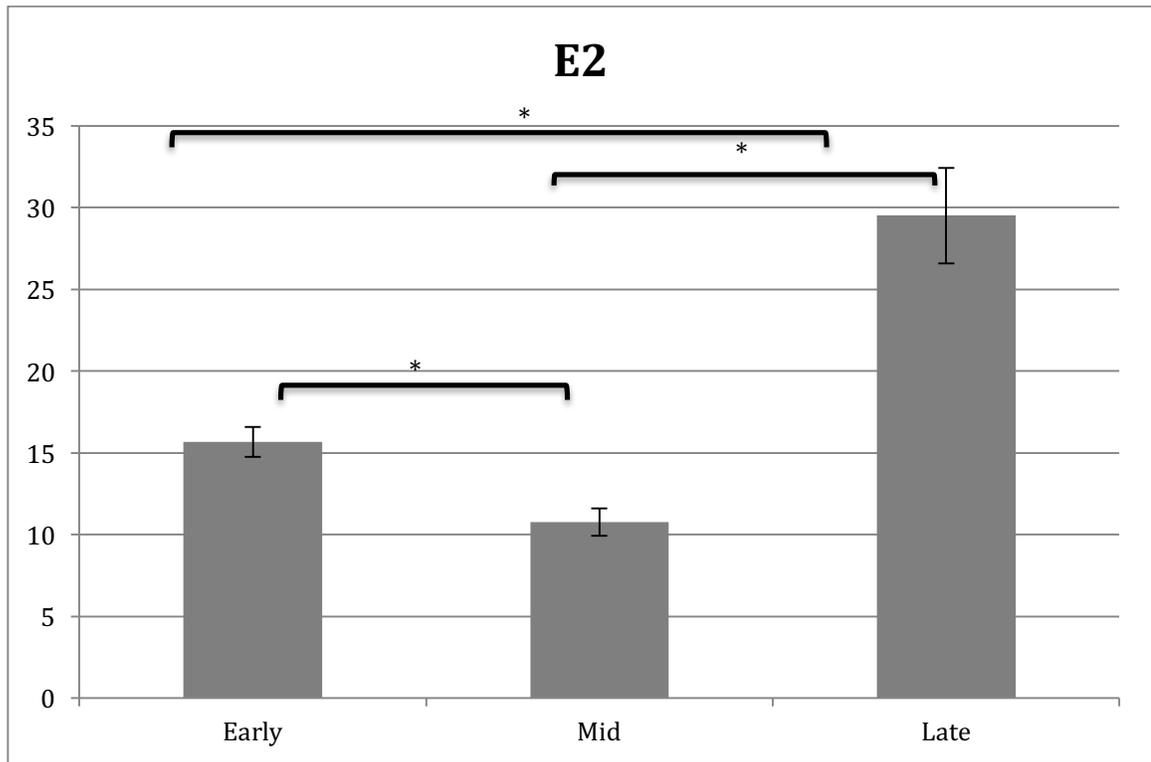


Figure 3-7 Mean female oestradiol (E2) levels in early, mid, and late pregnancy with one standard error. The Y-axis shows mean hormone levels for E2 (ng/g). The X-axis shows early, mid, and late pregnancy. Significant results are denoted by an asterisk (*). n = 10 females, n = 401 data points

Mating patterns relative to hormone levels

Progesterone levels were significantly higher in samples collected three days after copulation, which represent levels on the day of copulation. E2:P ratios were significantly lower during the three days after copulation. No other behavioural variable was significantly affected by P, E2 or E2:P ratios (Table 3-5).

Table 3-5 Progesterone, oestradiol, and oestradiol:progesterone levels within three days of solicitation directed and received, and copulation, compared to levels outside the three days following a solicitation or copulation. Table summarizes the Wald's χ^2 value with degrees of freedom and the significance level. Lines in *italics* indicate a significant difference. n = 10 females, n = 420 data points

Dependent Variable	Independent Variable	B	S.E.	Wald's χ^2 (df)	p value
Solicitation Directed	P	0.000	0.0002	0.925 (1)	0.336
	E2	0.007	0.0057	1.704(1)	0.192
	E2:P	5.768	3.0079	3.677(1)	0.055
Solicitation Received	P	0.001	0.0005	1.722(1)	0.189
	E2	0.017	0.0106	2.518(1)	0.113
	E2:P	-1.049	0.7005	2.241(1)	0.134
Copulation	<i>P</i>	<i>0.001</i>	<i>0.0003</i>	<i>9.294(1)</i>	<i>0.002</i>
	E2	0.004	0.0021	3.679(1)	0.055
	<i>E2:P</i>	<i>-1.732</i>	<i>0.6413</i>	<i>7.291(1)</i>	<i>0.007</i>

Mating patterns across pregnancy

The rate of solicitation directed by females was significantly different across early, mid, and late pregnancy (Table 3-6). Pairwise comparisons showed that the rate of female directed solicitation was significantly higher in mid pregnancy compared to late pregnancy ($p = 0.018$) and there were no differences between the other stages (early and mid pregnancy: $p = 0.544$; early and late pregnancy: $p = 0.144$; Figure 3-8). Rate of copulation across early, mid, and late pregnancy was significantly different. Pairwise comparisons showed that rate of copulation was significantly higher in early compared to late pregnancy ($p = 0.003$) (Figure 3-8).

Table 3-6 Mean rates of solicitation directed, solicitation received, and copulation in early, mid, and late pregnancy. Table summarizes the Wald's χ^2 value with degrees of freedom and the significance level. Lines in *italics* indicate a significant difference. n = 12 females, n = 28 data points.

Dependent Variable	Independent Variable	Wald's χ^2 (df)	p value
<i>Solicitation Directed</i>	<i>Pregnancy stage</i>	<i>11.724(2)</i>	<i>0.003</i>
Solicitation Received	Pregnancy stage	0.309(2)	0.857
<i>Copulation</i>	<i>Pregnancy stage</i>	<i>15.389(2)</i>	<i><0.001</i>

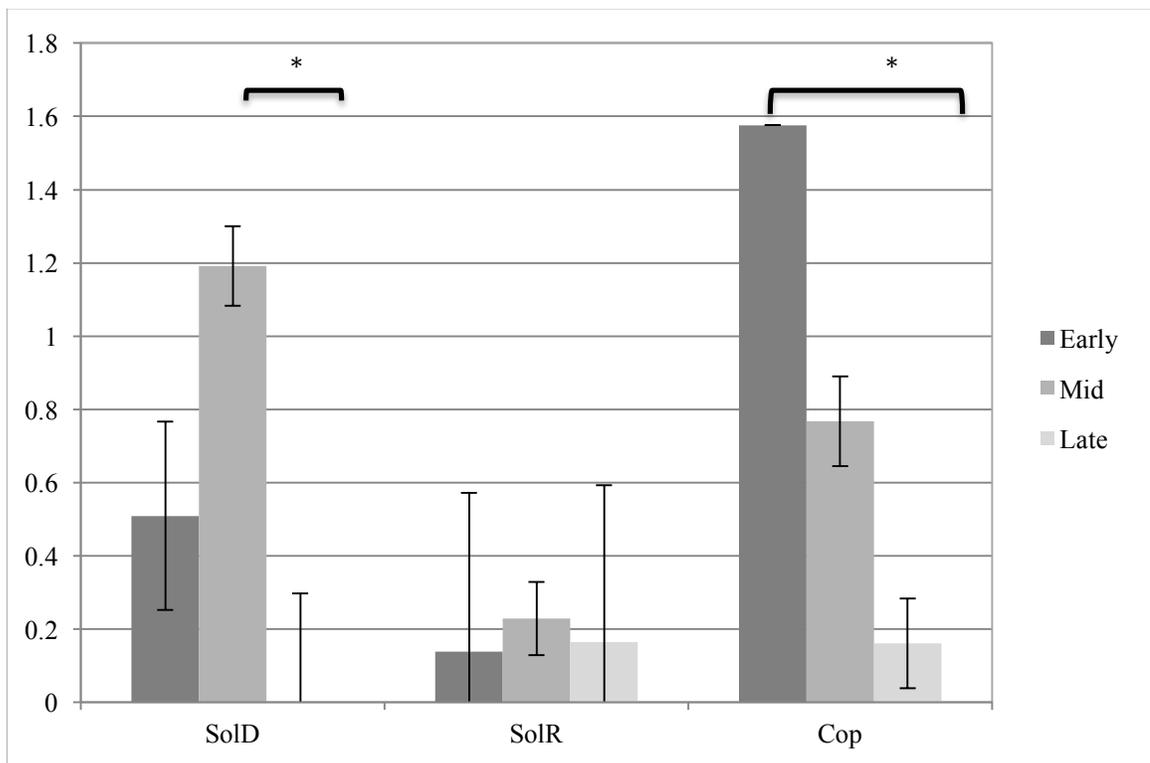


Figure 3-8 Mean rates of solicitation directed (SolD), solicitation received (SolR), and copulation (Cop) in early, mid, and late pregnancy with one standard error. Significant results are denoted by an asterisk (*). n = 12 females, n = 28 data points.

Rates of copulation and solicitation relative to group-type, number of males, and male rank for the three groups types

Rate of female directed solicitations was significantly different among group-types (Table 3-7, Figure 3-9), but pairwise comparisons revealed no significant differences between the group types once it was corrected using Bonferroni. The number of males in the group and male rank did not affect the rate of solicitation directed. There was no effect of group-type, number of males, or male rank on the rate of solicitation received or the rate of copulation (Table 3-7).

Table 3-7 Mean rates of solicitation directed, solicitation received, and copulation between the three group-types, number of males, and male rank (dominant or non-dominant). Table summarizes the Wald's χ^2 value with degrees of freedom and the significance level. Lines in *italics* indicate a significant difference. n = 12 females, n = 72 data points.

Dependent Variable	Independent Variable	Wald's χ^2 (df)	p value
Solicitation Directed	<i>Group-type</i>	<i>7.289(2)</i>	<i>0.026</i>
	Number of males	0.163(1)	0.686
	Male Rank	1.701(1)	0.192
Solicitation Received	Group-type	0.942(2)	0.624
	Number of males	1.781(1)	0.182
	Male rank	1.740(1)	0.187
Copulation	Group-type	1.483(2)	0.476
	Number of males	1.609(1)	0.205
	Male rank	3.160(1)	0.075

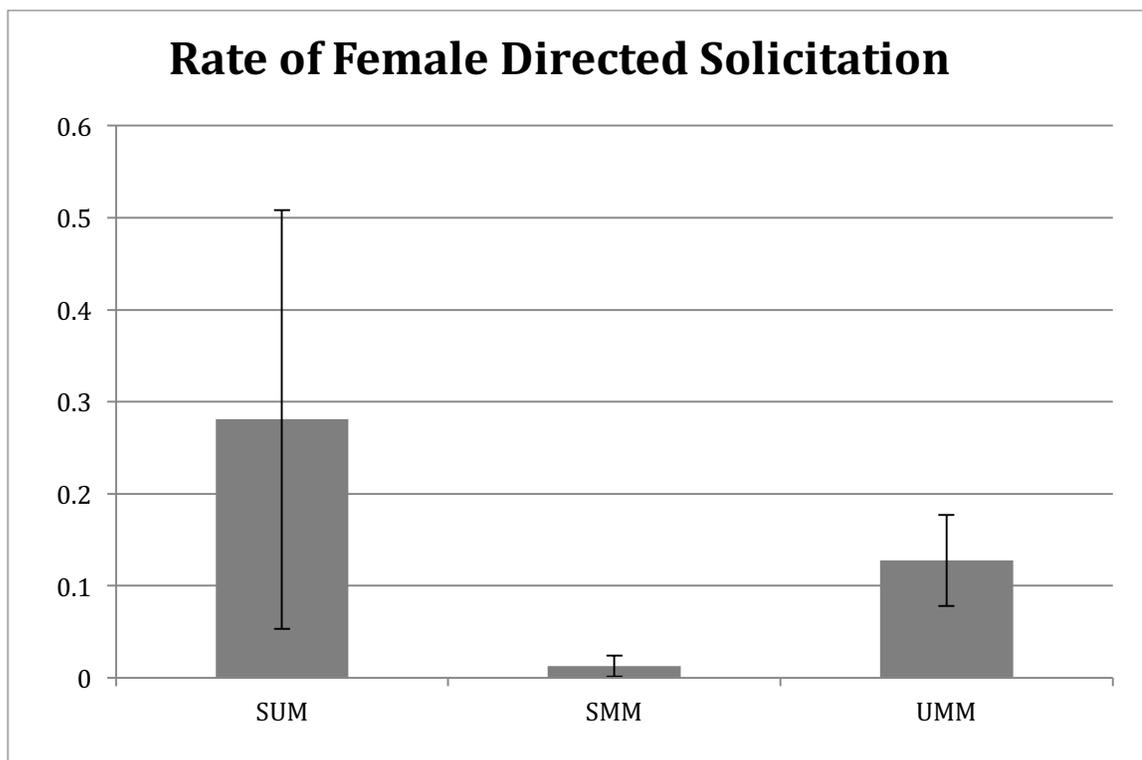


Figure 3-9 Estimated marginal means for the rate of female directed solicitations for stable uni-male (SUM), stable multi-male (SMM), and unstable multi-male groups (UMM) with one standard error. Significant results are denoted by an asterisk (*). n = 12 females, n = 72 data points.

Rates of copulation and solicitation to dominant or resident male between the three group-types

We compared rates of solicitation directed and received, and copulation with the dominant or, in SUM groups, the resident male, between the three group-types to determine if females solicit or copulate with the dominant/resident male at a higher rate in different group-types. There was no significant difference in rate of solicitation directed to the dominant/resident male (Wald's $\chi^2(2)$: 4.154, $p = 0.125$), solicitation received from him (Wald's $\chi^2(2)$: 1.508, $p = 0.471$), or rate of copulation with him (Wald's $\chi^2(2)$: 3.170, $p = 0.205$) between the three group-types.

Rates of copulation and solicitation between dominant and non-dominant males in stable multi-male and unstable multi-male groups only

The rate of solicitation directed was significantly different between the two group types, with females in UMM groups directing solicitations more than females in SMM groups. Male rank, and the interaction between group-type and rank had no effect on rate of solicitation directed (Table 3-8). The rate of solicitation received was not affected by group-type, male rank, or the interaction between group-type and male rank (Table 3-8). The rate of copulation was significantly different between dominant and non-dominant males, with females copulating more with dominant males. Group type, and the interaction between group-type and rank had no effect on rate of copulation (Table 3-8).

Table 3-8 Mean rates of solicitation directed, solicitation received, and copulation for stable multi-male and unstable multi-male groups between group-type, male rank and the interaction between group-type and male rank. Table summarizes the Wald's χ^2 value with degrees of freedom and the significance level. Lines in *italics* indicate a significant difference. n = 11 females, n = 69 data points.

Independent Variable	Dependent Variable	B	S.E	Wald's χ^2 (df)	p value
Solicitation Directed	<i>Group-type (SMM and UMM)</i>	<i>-0.094</i>	<i>0.0596</i>	<i>9.511(1)</i>	<i>0.002</i>
	Male Rank	0.125	0.1097	1.939(1)	0.164
	Group-type*Male Rank	-0.091	0.1138	0.636(1)	0.425
Solicitation Received	Group-type (SMM and UMM)	-0.009	0.0205	0.551(1)	0.458
	Male Rank	0.013	0.0147	1.221(1)	0.269
	Group-type*Male Rank	0.102	0.1158	0.779(1)	0.377
Copulation	Group-type (SMM and UMM)	0.047	0.0581	2.721(1)	0.099
	<i>Male Rank</i>	<i>0.031</i>	<i>0.0369</i>	<i>4.368(1)</i>	<i>0.037</i>
	Group-type*Male Rank	0.258	0.1533	2.843(1)	0.092

Discussion

In this study we investigated whether or not post-conceptive mating in female *C. vellerosus* is a by-product of fluctuating and/or elevated E2 and P levels during pregnancy or if it is a result of females' access to multiple males. Over the course of one year, despite 5820 contact hours and 562 focal hours on 18 female *C. vellerosus* (and 1866 faecal samples), the number of solicitations and copulations was still relatively small. Furthermore, we had to break these data down to answer individual research questions, so our results are based on relatively small sample sizes. Despite these small

samples sizes, our data allowed us to determine that: 1) Female progesterone and oestradiol levels increase throughout pregnancy. 2) Females solicit males more in mid than late pregnancy, copulate more in early than late pregnancy, and males solicit females consistently across all stages of pregnancy. 3) Progesterone levels are significantly higher during the three days following a copulation, and the ratio of oestradiol:progesterone levels are significantly lower during the three days following a copulation. 4) Female directed solicitations were significantly different among the three group types. Although pairwise comparison (corrected using Bonferroni) showed no significant difference between the groups, rates of solicitation directed were highest in stable uni-male groups and lowest in stable multi-male groups. There were no other differences between the three group types for solicitation directed, received, or copulation relative to male rank, number of males or group-type. 5) When we compared only stable multi-male groups and unstable multi-male groups to investigate the difference between copulations and solicitations between dominant and non-dominant males, the rate of copulation was significantly higher for dominant males than for non-dominant males. Finally, females directed solicitations significantly more in unstable multi-male groups than in stable multi-male groups.

Female progesterone and oestradiol levels remained elevated throughout pregnancy and, with the exception of oestradiol in mid-pregnancy, increased from conception to the end of pregnancy, as is typical of primates (Kent 2000). In most species, oestradiol rises consistently throughout pregnancy, while in our study the mean oestradiol level was lowest in mid-pregnancy. This may be a result of the variability in our consecutive hormone values between samples. It has been suggested that plant

produced oestrogens (e.g. phytoestrogens) can affect hormone levels in humans and primates (e.g. Wasserman *et al.* 2012, Uehara *et al.* 2000), and this interference of phytoestrogens may explain the variability we see in the hormone profiles between days. Despite this variability, the pattern of increased oestradiol and progesterone from early to late pregnancy are consistent with expected patterns.

Females mated more at the beginning of pregnancy than at the end, as has been seen in other species (e.g. *Semnopithecus entellus*, Borries *et al.* 2001). Females copulated more in early than late pregnancy but solicited more in mid than late pregnancy, which implies that females may be motivated to mate for long periods during pregnancy but as female hormone levels increase, males may be less motivated to continue mating (perhaps as they begin to receive hormonal cues that the female is pregnant, or not fertile). Although we do not have enough data to test this, it is an area of future research that merits further investigating.

In *C. vellerosus*, elevated progesterone levels seem to be linked to copulation (as was reported for *Macaca arctoides*, Nieuwenhuijsen *et al.* 1986). Progesterone levels were highest within three days of copulation and the ratio of oestradiol:progesterone was lowest. This pattern is different from reports in other primate species, where motivation to mate and mating are closely tied to high levels of oestradiol (e.g. *Macaca mulatta*, Bielert *et al.* 1976, Wilson *et al.* 1982, Zehr *et al.* 1998, *Propithecus verreauxi*, Brockman *et al.* 1995, *Brachyteles arachnoides*, Strier and Ziegler 1997, *Macaca nemestrina*, Risler *et al.* 1987, *Papio cynocephalus*, Wasser *et al.* 1991). Further study is necessary to more fully document the link between hormone levels and the occurrence of post-conceptive mating. It is interesting to note that progesterone levels were

significantly higher three days after copulation, but levels were not higher three days after directed or received solicitations. This may be because solicitations were not hormonally driven. However, it is more likely that this is a product of our data. We were not always able to see the start of copulation, but rather once we saw a copulation begin, we recorded the actors and behaviours that followed. To look further at the link between hormones and solicitation we need more behavioural data on the solicitor of copulation.

Nine of our study subjects directed and received mating solicitations, and/or copulated after conception during the study period. Most of those females with access to multiple males mated with more than one male. Of the three females who did not mate or solicit post-conceptively, one was in a stable uni-male group, one in a stable multi-male group, and one in an unstable multi-male group. Female rates of solicitation and copulation were very different for the two unstable multi-male groups (Figures 3-4 and 3-5). These differences among females may be the result of variation in individual behaviour, and may reflect the variability that females show in their expression of particular behaviours. Alternatively, these differences may be a reflection of the stage of pregnancy that these females were in at the time of data collection. In Splinter group, female SA was in the late stages of pregnancy upon the start of the study. Female VE in Splinter group, along with females XY and IS in Winter group were all in early and mid-pregnancy. Based on our result that rates of copulation decline over pregnancy, it is likely that female SA was copulating less because she was in the late stages of pregnancy.

We predicted that if females mate post-conceptively because they have access to multiple males, then females in multi-male groups should solicit copulation and copulate post-conceptively more than those in uni-male groups. However, we found the opposite:

females in uni-male groups directed solicitations more than those in multi-male groups. We also expected no difference in rates of female socio-sexual and sexual behaviours across male dominance ranks or between individual males, but our results did not support this prediction. Females in unstable multi-male groups directed solicitations more than those in stable multi-male groups, and in multi-male groups (both stable and unstable) females copulated more with dominant than non-dominant males. We also considered whether females mate with multiple males post-conceptively because they receive solicitations from these males, but rates of solicitation received from males were stable for all groups over the study period, and did not differ between any of the group types, between male ranks, or relative to the number of males in the group. Therefore, our second hypothesis that females mate with multiple males post-conceptively because they have access to these males is unlikely. However, the majority of our study females mated post-conceptively, which supports the idea that females may be motivated to extend the mating period (Borries *et al.* 1999, 2001). To better understand why females may be trying to extend the mating period, we now consider if post-conceptive mating could be a reproductive strategy to reduce the risk of infanticide.

Our result that females in uni-male groups solicited post-conceptive mating more than those in multi-male groups supports the idea that females may be trying to concentrate paternity certainty in the resident male in uni-male groups. Extra-group copulations have been reported in colobines (Fashing 2001, Teichroeb *et al.* 2005), and, by soliciting post-conceptively with the resident male, females in uni-male groups may be trying to increase the resident male's paternity certainty (Clarke *et al.* 2009). When the

probability of paternity is high, male defence of offspring can benefit both the male and female (van Schaik 1996, Palombit *et al.* 1997).

Females in both stable multi-male and unstable multi-male groups mated more with the dominant male than non-dominant males, but females in unstable multi-male groups solicited all males more than those in stable multi-male groups. These results support the paternity confusion hypothesis. In groups with multiple males, females may be trying to concentrate paternity in the dominant male (who is at that time the best positioned to defend his offspring), while also diluting paternity probability among the rest of the males in the group. By simultaneously spreading paternity probability among males in the group, females may decrease the risk of attack in the case any of these males rise in rank and become dominant (Clarke *et al.* 2009). The level of stability among males in each group type may explain the difference in solicitation rates between stable and unstable multi-male groups. In unstable multi-male groups, the level of instability among males may be a cue for females to try to mate with all the males in the group because any one of these males could rise in rank and take over the dominant breeding position. By mating with (or trying to mate with) non-dominant males when the probability of conception is low, females can raise a non-dominant male's paternity probability (Clarke *et al.* 2009) and decrease the likelihood that he will kill an infant should he rise in rank (van Schaik 2000). Whereas in stable multi-male groups, the level of stability among males may somewhat reduce the motivation for females to solicit and mate with multiple males because the probability of an immediate dominance takeover is lower. Females will still be motivated to spread paternity probability, but the intensity of female motivation may be lessened by the stability among the males in the group.

In conclusion, our results point to a combination of hormonal and behavioural factors influencing female post-conceptive mating in *C. vellerosus*. Hormonal fluctuations may be a proximate mechanism that partially drives female post-conceptive mating. If hormones alone were the driver for post-conceptive mating, we would expect to see no difference in rates of solicitation and copulation between females or group types. Given that we do see variability in the expression of this trait among females, if the offspring of those females who mate post-conceptively survive better than those who do not, then there will be selection for this hormonal drive to mate post-conceptively. The presence of multiple males and the stability or instability of male interactions within the group may also affect female motivation to solicit and mate. Both these proximate mechanism may be working together and may be selected for if indeed females who are hormonally motivated to mate post-conceptively, and do so with multiple males in unstable groups, spread paternity certainty and ultimately reduce the risk of infanticide.

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CHAPTER 4 FACTORS INFLUENCING THE TIMING AND OVERLAP OF REPRODUCTIVE EVENTS IN WILD FEMALE *COLOBUS VELLEROSUS*.

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Introduction

Female mammals invest heavily in their offspring, both directly and indirectly, over long periods of time through gestation, lactation, carrying, protection from predators and conspecifics, food sharing, and providing learning opportunities (reviewed in van Noordwijk *et al.* 2013). Some species are able to optimize their reproductive success by overlapping investment in consecutive offspring, which van Noordwijk (2012) termed ‘stacking’ investment. In this chapter we extend the definition of stacked investment to include conception and gestation of a new offspring while having an infant in nipple contact, and explore whether infant stacking occurs in a wild colobine. We also investigate what social and ecological conditions may lead to variation in the degree of stacking among conspecific females.

Among diurnal, group-living primates the association between a mother and her offspring is often longer than gestation and lactation (reviewed in van Noordwijk 2012). Once an offspring is weaned and has become nutritionally independent, females may stack investment by directly investing in a new offspring while continuing to invest indirectly in their current offspring (reviewed in van Noordwijk 2012). In some species a female can begin investment in a second offspring before her current infant is weaned if she becomes pregnant while she is still lactating (for example: *Papio anubis*, Rowell 1966, Nicolson 1982, *Papio cynocephalus*, Altmann 1980, *Pan troglodytes*, Pusey 1983, *Ateles paniscus*, Symington 1987, *Trachypithecus francoisi*, Gibson and Chu 1992, *Macaca fuscata*, Tanaka 1992, *Semnopithecus spp.*, Borries *et al.* 2001, *Pongo pygmaeus*, van Noordwijk *et al.* 2013). For this paper, we have adapted van Noordwijk's (2012) definition of stacked investment to include direct female investment in two offspring at the same time. Whether or not a female can stack investment may be constrained by physiological (hormonal and energetic constraints), ecological (food availability and level of birth seasonality), and social factors (access to high-quality resources and stress).

The most energetically costly form of female investment in mammals is lactation (Boyce 1988, Altmann and Samuels 1992, reviewed in Wade and Schneider 1992). When suckling an infant, most mammals go through a temporary period of postnatal infertility (lactational amenorrhea), whereby ovulation is suppressed and conception is not possible (McNeilly 1994, reviewed in McNeilly 2001). Two mechanisms are at play in this suppression: one hormonal and one energetic. Hormonal suppression of ovulation is driven by suckling stimulation that suppresses gonadotropin hormone secretion and prevents normal follicular development and ovulation (Short 1976, McNeilly 1994,

reviewed in McNeilly 2001). Energetic suppression of ovulation is driven by the high energetic requirements of lactation that signal to the body that there is not enough energy to sustain a pregnancy (Lee 1987, reviewed in von Borell *et al.* 2007). While there is still some debate as to which mechanism is *the* driver of lactational amenorrhea, it is likely that both are acting together.

Because energetics can play a role in the length of lactational amenorrhea, females that have access to high-quality food resources may also have improved body condition (Strum 1991), allowing them to potentially resume cycling and conceive earlier (reviewed in Asquith 1989, reviewed in van Noordwijk 2012). The level of flexibility females express in the timing of their resumption to cycling, conception, and birth is influenced by the level of breeding seasonality their species (or population) exhibits (Brockman and van Schaik 2005, reviewed in van Noordwijk 2012). Generally, primates follow two breeding strategies: they are either “income” or “capital” breeders (Brockman and van Schaik 2005). Income breeding females use their current energy intake to sustain reproductive events. This strategy results in more seasonal breeding, because these females have limited fat stores and therefore gestation must be timed to precede periods of high food availability (Brockman and van Schaik 2005). This strategy is advantageous because it ensures that lactation and weaning coincide with predictable periods of high food availability and increases chances of infant survival (Brockman and van Schaik 2005, Beehner and Lu 2013). Capital breeders, on the other hand, rely heavily on stored nutrients (e.g., fat stores) to support their reproduction, and tend to be aseasonal breeders (Brockman and van Schaik 2005, Beehner and Lu 2013). Capital breeders tend to be more flexible in the timing of their reproductive events, rates of conception, and levels of

investment because they are not constrained by timing their births to a specific period of optimal ecological conditions (Lee 1996, Brockman and van Schaik 2005). If females have access to high quality food resources and are in good physical condition, they can potentially conceive again while lactating (Janson and Verdolin 2005), which could allow for stacked investment of successive offspring.

Access to high quality resources can depend on both ecological availability and on female rank. High-ranking females tend to have preferential access to food compared to lower ranking females (Whitten 1983, Harcourt 1987, Silk 1987, Isbell *et al.* 1999) and typically have higher reproductive success (Lycett *et al.* 1998, Borries *et al.* 2001, Cheney *et al.* 2006, Zhao *et al.* 2008, Emery Thompson *et al.* 2012). Dominance rank can also affect female stress levels (Sapolsky 2005). Stress can negatively affect the functioning and response of the endocrine, reproductive and immunological systems (Berne & Levy, 1998, Carlstead, 1996, Sapolsky, 2002). High stress levels can have a direct effect on female rates of reproduction and levels of investment in offspring because they can cause irregularities in the menstrual cycle and pregnancy failure in some primate species (Abbott *et al.* 1986, Adams *et al.* 1985, Wasser and Starling 1988, Sapolsky 2002, Harcourt 1987, Cameron 1997), and hormones associated with stress can affect body condition, milk production, and the timing of reproductive events (reviewed in Varley and Stedman 1994, reviewed in Xiao and Ferin 1997, Sapolsky, 2002, Kalantaridou *et al.* 2004, Dobson *et al.* 2012). Female primates living in a social group can experience psychosocial stress (Sapolsky 2005) as a result of the threat of aggression or actual aggression received from other group members (reviewed in Dixson 2012, e.g. Wasser and Starling 1988). Stress is not exclusively higher in low-ranking females

(reviewed in Pusey 2012), and which rank is most affected is species-specific (Sapolsky 2005, reviewed in DeVries *et al.* 2016, Abbott 1984, Barton and Whiten 1993, Kappeler and Pereira 2002). However, high rank is typically associated with higher birth rates (*Theropithecus gelada*, Dunbar and Dunbar 1977, *Semnopithecus spp.*, Borries *et al.* 1991), higher offspring survival (*Macaca fascicularis*, van Noordwijk and van Schaik 1999, reviewed in Pusey 2012) and faster offspring development (*Macaca mulatta*, Drickamer 1974, *Papio cynocephalus*, Altmann and Alberts 2005, *Lophocebus albigena*, Arlet *et al.* 2015).

Females can also experience stress as a result of the males in their group. Male-male competition for access to fertile females can be intense, and during periods of male immigration, unstable male membership, or male dominance rank fluctuations, females may experience high stress levels (Alberts *et al.* 1992, Sapolsky 1993, Palombit *et al.* 2000). This effect can be the result of male aggression directed at the female or the threat of infanticide that these males pose (Busse and Hamilton 1981, Collins *et al.* 1984, Bulger and Hamilton 1987, Tarar 1987, Palombit *et al.* 2000, Beehner *et al.* 2005). When new males immigrate, females sometimes exhibit elevated faecal glucocorticoid levels (Alberts *et al.* 1992, Sapolsky 1993, Palombit *et al.* 2000, Carnegie *et al.* 2011), which is indicative of psychological stress (Beehner *et al.* 2005) and can lead to abortion (Pereira 1983, Beehner *et al.* 2005, Roberts *et al.* 2012) and disruption of ovulatory cycles (Goodall 1986, Manson and Perry personal communication, cited in Smuts and Smuts 1993). Females living in groups with unstable male group-membership may spend long periods of time assessing the potential threat these males pose (Maestriperi 1993). The energy and stress associated with this extra vigilance can result in longer times to post-

partum recuperation (Mas-Rivera and Bercovitch 2008) and prevent females from investing in two offspring simultaneously.

This study aims to use hormonal and observational data to determine whether female *Colobus vellerosus* can stack investment in offspring by conceiving while the previous infant is still in nipple contact, using nipple contact as a proxy for suckling (reviewed in Borries *et al.* 2014). We look at the timing and overlap of reproductive events (cessation of nipple contact, resumption of cycling, and conception) in relation to the social conditions under which they occur, paying particular attention to male group membership and the stability of the group. We present the distribution of these events relative to one another, and explore what factors may be influencing their overlap (or lack thereof). In particular, we explore which variables may affect females' inter-birth intervals and their ability to invest in more than one offspring at a time. To do so, we ask six main questions:

- (1) Do female *C. vellerosus* conceive a subsequent offspring when they still have a previous infant in nipple contact?
- (2) If so, how long is the period of overlap between conception and last nipple contact?
- (3) Do any characteristics of the infant in nipple contact affect female stacked investment (infant age, infant sex, proportion of time spent in nipple contact per day)?
- (4) Does female age affect female stacked investment?
- (5) Do male group membership and group stability affect female stacked investment?

(6) Does food availability affect female stacked investment?

Colobus vellerosus present an ideal setting in which to test these questions because females in our study population show considerable flexibility in the duration of nipple contact with their infants (range: 275-640 days, mean: 409.8 days, median: 436 days, Crotty 2016) and in the length of their inter-birth intervals (range: 8-20.8 months, mean: 17.75 months when infants survive to nutritional dependence; and range: 8–18.5 months, mean: 11.89 months when infants did not survive to nutritional independence, Vayro *et al.* 2016). The social context in which females live also varies because *C. vellerosus* live in both uni-male and multi-male groups (Wong and Sicotte 2006) and male-takeovers are common (Sicotte *et al.* 2015). Females sometimes disperse to other bi-sexual groups when their group is experiencing unstable male-membership (Teichroeb *et al.* 2009) or male takeover (Sicotte *et al.* 2015). When dispersal is not an option, females can spend considerable time in unstable groups where multiple males fight over dominance status or extra-group males try to enter the group and oust the resident male(s) (Sicotte *et al.* 2015). These takeovers often result in infanticide (Teichroeb and Sicotte 2008). In unstable multi-male groups, the increased threat of aggression and/or infanticide may increase female stress levels, and may result in disruption of female reproduction and levels of investment.

Predictions

We predict that if infant age affects female stacked investment, then females with older infants will stack investment more. We also predict that if proportion of nipple

contact affects female stacked investment, then females with a higher proportion of nipple contact will stack investment less. As infants age and become more nutritionally independent, suckling patterns change, and infants suckle less frequently or at a slower rate until they are completely nutritionally independent and weaned (Gomendio 1989). The reduction in suckling stimulus inhibits the suppression of gonadotropin releasing hormone and normal follicular development can occur (McNeilly 1994). This also means that less energy is being expended by the mother and she can transfer that energy into cycling, and potentially gestation of her next offspring (Oftedal 1984, Lee 1996, Ross and Jones 1999).

If infant sex affects a mother's ability to stack investment, then we expect mothers with female infants to stack investment more. Among primates, male infants grow faster and attain larger body size early in life while they are still nutritionally dependent on their mother's milk (Bercovitch *et al.* 1998, 2000, Silk *et al.* 1993, Garcia *et al.* 2009, reviewed in Smith and Leigh 1998). First-born males tend to have lower rates of survival than do first-born females, which suggests that rearing males is more costly than rearing females (Bercovitch *et al.* 1998, Hinde 2007). If rearing male infants requires greater maternal investment, then mothers raising males are likely to show greater maternal depletion, reduced fertility, and longer inter-birth intervals (Bercovitch and Berard 1993, Garcia *et al.* 2009, Mas-Rivera and Bercovitch 2008). Additionally, in our population of *C. vellerosus*, male infants are at a higher risk of infanticide (Teichroeb & Sicotte 2008), and tend to transition coat color faster than female infants (Badescu *et al.* 2015), which suggests that males develop faster. Females may invest more heavily (but for shorter

periods) in male infants after birth in order to speed up development and reduce the period that these infants are at risk of infanticide.

If maternal age affects female stacked investment, we predict that middle-aged females will stack investment more than young and old females. Maintaining good maternal condition during lactation can reduce the time to conception of the next offspring (Emery-Thompson 2013). Young primiparous mothers often have to trade-off between investing in current reproduction and investing in their own continued growth (Stearns 1992). Because of these constraints, infants of very young females may be more costly investments (Hinde 2007) and young females may be unable to invest simultaneously in concurrent offspring. Once a female ages and has finished growing, she may be able to invest more in her offspring and less in her own growth, allowing her to stack investment. Similarly, older females, who are reaching the end of their reproductive lives, may not have future offspring and thus may invest more in a current offspring (reviewed in Pusey 2012). If middle-aged females are in better physical condition than young and old females, and are able to recover from gestation and lactation more quickly than young and old females, they may be able to cycle and conceive while an infant is in nipple contact, and overlap gestation and nipple contact among concurrent offspring.

If male group membership and group stability affect female stacked investment, we expect females in stable groups to stack investment more than those in unstable groups. Because instability in male group membership can affect female reproduction (e.g. Alberts *et al.* 1992, Sapolsky 1993, Palombit *et al.* 2000, Carnegie *et al.* 2011), and females in unstable groups may be experiencing high levels of stress, these females may

have longer times to post-partum recuperation (Mas-Rivera and Bercovitch 2008) and this may prevent them from investing in two offspring simultaneously.

We expect we will find little or no effect of food availability on female stacked investment. This is because *C. vellerosus* fall on the spectrum of capital breeders and show no strict birth seasonality (Teichroeb and Sicotte 2008). Because capital breeders tend to be more constrained by their maternal condition than by timing their births to optimal ecological conditions, these females can be more flexible in the timing of their reproductive events, rates of conception, and investment levels (Lee 1996, Brockman and van Schaik 2005). Therefore, the level of food availability should not have a large impact on whether or not females stack offspring investment.

Methods

With the help of six trained assistants, we collected data on one uni-male and three multi-male groups of wild ursine colobus monkeys at the Boabeng-Fiema Monkey Sanctuary (BFMS), a 192-hectare forest fragment in Central Ghana. The colobus monkeys at BFMS have been the subject of ongoing study since 2000, under the direction of Dr. P. Sicotte at the University of Calgary (for more details on the site and on its colobus population, see Saj *et al.*, 2005, Sicotte *et al.* 2015, Teichroeb *et al.* 2009, 2011, Wong and Sicotte 2006).

We spent 5820 contact hours and 562 focal hours with our 18 study females from May 2012 to May 2013. From 6am to 2pm, 6 days per week, we collected hourly scan samples. We collected faecal samples 2-3 times per week. Faecal data collection and analysis methods have been described elsewhere (Vayro *et al.* 2016, and Chapter 2 of this

dissertation). During hourly group scans we recorded a “nurse” event whenever an infant was in nipple contact with a female. We were not able to determine suckling or the intensity of suckling (nutritive vs. non-nutritive suckling, Tanaka 1992), so we used “nipple contact” as a proxy for suckling (reviewed in Borries *et al.* 2014). There is some debate as to whether nipple contact is a precise measure of investment in an infant, both because it is difficult to determine if time spent in nipple contact is actually representative of suckling rate, and because nipple contact does not give information about the amount of milk being transferred (reviewed in Borries *et al.* 2014). However, some studies have shown that milk transfer may continue as long as nipple contact occurs (Reitsema 2012) and faster growth rates are associated with higher rates of suckling (Lee and Bowman 1995), which suggests that nipple contact can provide a behavioural measure of female investment (reviewed in Borries *et al.* 2014). As such, we chose to use nipple contact as a proxy for suckling.

Data analysis

We calculated the duration of all life-history variable values (gestation length, inter-birth interval, and age at first birth) in days, and when necessary converted them to months by using 30.4 days in a month. We calculated one year as 12 months. The values we used to calculate mean inter-birth interval when an infant survived and when an infant died, and age at first birth are taken from this study, Teichroeb and Sicotte 2008, and our long-term BFMS dataset. Appendix IV provides a breakdown of the values and the sources of data. These values were previously reported in months (Vayro *et al.* 2016), but the current measure of days is more precise and therefore the values presented in this

chapter should be used for comparison between other species, as well as for reference for *C. vellerosus*. We determined pregnancy as outlined in Vayro *et al.* 2016, using both hormonal and observational data. For 14 females we calculated day of conception by subtracting the mean gestation length for *C. vellerosus* (168.5 days; this study) from the day of parturition. For the other two females we used the rise in E2 and P to determine ovulation and the day of conception, and the sharp decrease in E2 and P to determine day of parturition (Vayro *et al.* 2016). We categorized a female as having “stacked investment” if she had an infant in nipple contact during conception and gestation of her subsequent infant. We had one female whose last day of observed nipple contact was her estimated day of conception of the subsequent infant. We chose to be conservative and classify this female as not-stacking investment because she did not continue nipple contact into gestation.

We used approximate and exact birthdates to calculate infant age and female age at the time of the first infant’s last nipple contact. Since the year 2000, trained researchers who are present at the field site at least once per year have assigned approximate ages for all individuals who are seen for the first time. For infants, all approximate ages were given within 3 months or less of the infant’s birth and typically the age given is +/- one month. Since 2012 trained local assistants and researchers (when present) have recorded all infant births, and exact infant ages are assigned within 1-2 days. Our sample consists of nine infants with approximate ages and seven with exact ages (Table 4-1). For adult females, females under the age of 9 were first seen when they were either infants or juveniles and their ages were estimated based on their size and facial features. Females over 9 years were already adults when researchers first saw them and their ages were

estimated based on the female's size, fur patterns and facial features. We calculate a daily "proportion of nipple contact" by dividing the number of records of "nurse" per day by the total number of scans per day for each female. As infants get older, rates of infant suckling tend to decrease (Gomendio 1989). To account for differing proportions of expected nipple contact relative to infant age, we categorized infants into non-overlapping age groups (1: 0-2 months, 2: 3-5 months, 3: 6-8 months, 4: 9-11 months, 5: 12-14 months, 6: 15-17 months, 7: 18-20 months, 8: 21+ months) and compared proportion of nipple contact per day for infants within each age category.

We categorized group type as stable uni-male (SUM), stable multi-male (SMM) and unstable multi-male (UMM) as previously presented in Chapter Two. We categorized food availability around the time of conception (one month prior to conception) as "high food availability" or "low food availability". December to March contain the highest availability of high quality food resources (i.e. flowers, fruits, seedpods and young leaves), and April to the end of November contain the lowest availability of high quality food resources (Saj 2005, Saj and Sicotte 2007, Crotty 2016). We categorized food availability as high or low one month prior to the date of estimated conception for each female, and used this classification for analyses. We used food availability one month prior to conception because if females rely on high quality resources to have enough energy to cycle and conceive, then the availability of these resources prior to conception would be most important.

To test the effect of infant sex, infant age, female age, and food availability on stacked investment we ran multiple independent generalized estimating equations (GEE) with binomial distribution and logit link function. In this model we considered "group"

(Redtail, Splinter, Winter, and Wawa) to be a repeated measure with an “unstructured” correlation structure (Tak Fung, personal communication Feb. 2017). To test the effect of male group membership and group stability on stacked investment we used a Chi-square. We did not have enough different groups per group-type to use a GEE to test the effect of male group membership and group stability on stacked investment (Tak Fung, personal communication Feb. 2017). For the Chi-square we report exact significance levels (Fischer’s exact test, two-tailed) because our sample size is small (<5 expected samples per category; Tak Fung, personal communication Feb. 2017). To determine if proportion of nipple contact changes over time for infants within an age category (as infants get older) we tested proportion of nipple contact between each age category using a GEE with binomial distribution and a logit link function. To test the effect of proportion of nipple contact per day within each age category on female stacked investment we used a GEE with binomial distribution and a logit link function. We ran all statistical analyses in SPSS Statistics (Version 19.0). For all analyses, we used an alpha level of 0.05 as our significance threshold.

Results

Life-history variables

Gestation length was 159 days (± 0 days) and 178 days (± 3 days) respectively; mean gestation length was 168.5 days (range=159-178 days, n=2). For females whose infants survived to nutritional independence the mean inter-birth interval was 16.5 months (range=8.3-24.0 months, n=11). The mean inter-birth interval for females whose

infants did not survive to nutritional independence was 11.4 months (range=8.0-17.1 months, n=9). The mean age at first birth was 5.9 years (range=4.7-6.7, n=8).

Patterns of stacked investment

Out of 16 females that conceived during the study period, eight females were observed with an infant in nipple contact when they conceived and into pregnancy with the subsequent offspring. Seven females ceased nipple contact before their next conception. One female was observed with an infant in nipple contact on the estimated day of conception but not after the day of conception (Table 4-1).

Table 4-1 Summary of study subjects in relation to stacked investment. Female ID, group membership [Redtail (RT), Splinter (SP), Winter (WT), Wawa (WW)], group type at time of second conception [Stable uni-male (SUM), Stable multi-male (SMM), Unstable multi-male (UMM)], female age (years) at time of first infant’s last nipple contact (LNC), number of days between last nipple contact (LNC) of first infant and second conception, first infant age at last nipple contact (LNC), and food availability (high or low) one month before conception of the second infant. A negative number in the “Days between LNC and conception” column indicates that last nipple contact occurred before the day of next conception. A positive number in the “Days between LNC and conception” column indicates that last nipple contact occurred after the day of next conception. A # beside the Female ID indicates that the infant died/disappeared before weaning. Infant ages in bold are exact age. Infant ages in italics are approximate. n = 16 females.

Female ID	Group	Group type at time of 2nd conception	♀ Age at LNC (years)	Days between LNC and conception	1st Infant Age at LNC (months)	Infant Sex	Food availability
BE	RT	SMM	8.7	157	<i>10.5</i>	F	Low
BL	RT	SMM	18.3	135	<i>13.7</i>	M	Low
FV	RT	SMM	7.5	84	<i>17.1</i>	M	High
SU	RT	SMM	11.7	98	<i>16.8</i>	F	Low
TR	RT	SMM	15.3	134	<i>15.2</i>	F	High
CT	SP	UMM	8.3	0	<i>15.6</i>	F	Low
SA [#]	SP	UMM	7.4	-205	2.8	Unknown	High
SE	SP	UMM	16.8	-19	<i>18.9</i>	M	Low
VE [#]	SP	UMM	17.1	-70	2.2	M	High
XE	SP	UMM	13.7	-15	<i>19.0</i>	F	Low
IS [#]	WT	SUM	7.5	-55	9.8	M	Low
VM	WT	SUM	8.4	149	23.2	M	Low
XY	WT	SUM	7.0	-218	11.4	F	Low
CR	WW	SUM	18.7	129	12.8	F	Low
IT	WW	SUM	9.8	134	13.7	M	Low
JN [#]	WW	SUM	18.6	-28	<i>4.3</i>	M	Low

Infant characteristics affecting stacked investment

Infant sex was not a significant predictor of female stacked investment (B=0.287, S.E.=0.5411, Wald's χ^2 =0.282(1), p=0.595, n=15). Infant age was not a significant predictor of female stacked investment ((B= 0.144, S.E.=0.1054, Wald's χ^2 =1.855(1), p=0.173, n=16).

The proportion of time spent in nipple contact was significantly different between infant age categories (Wald's χ^2 =58.905(5), p<0.001, n=1217 scans, 15 infants, Figure 4-1). The linear trend line denotes a downward trend with the proportion of time spent in nipple contact decreasing as infant age increases. (See Appendix III for pairwise comparison of proportion of time spent in nipple contact between infant age categories).

Females that stacked investment had a significantly higher proportion of time with an infant in nipple contact (B=0.00000318, S.E. = 0.000001584, Wald's χ^2 =4.031(1), p=0.045, n=11217 scans, 15 females) than those that did not stack investment.

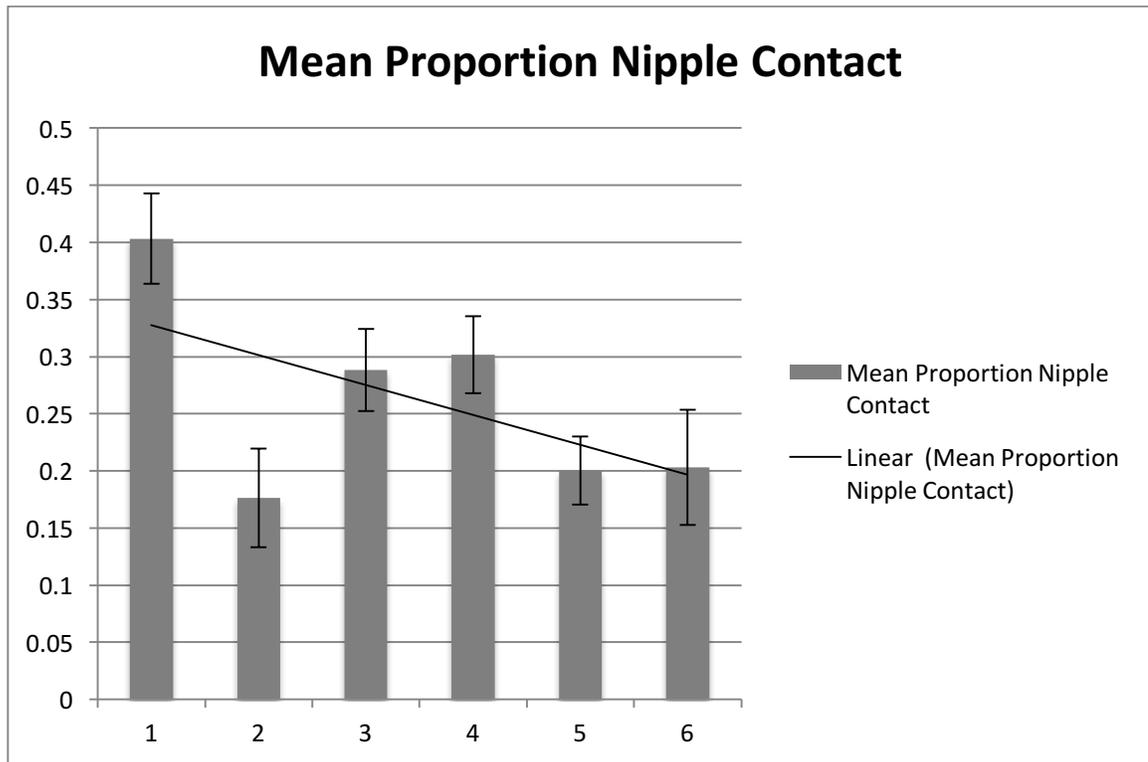


Figure 4-1 Mean proportion of time in nipple contact for 6 infant age categories. Includes a linear trend line for the mean proportion of time in nipple contact. The y-axis is the mean proportion of time an infant spent in nipple contact. The x-axis is the infant age category. n = 245 infant age category 1, n = 241 infant age category 2, n =305 infant age category 3, n =276 infant age category 4, n =134 infant age category 5, n =16 infant age category 6, n =1217 total, n =15 infants.

Effect of female age, male group membership and group stability, and food availability on stacked investment

Female age was not a significant predictor of female stacked investment ($B=-0.019$, $S.E.=0.0549$, Wald's $\chi^2=0.119(1)$, $p=0.730$, $n=16$). Male group membership and group stability were a significant predictor of female stacked investment (Fischer's Exact Test= $9.793(2)$, $p=0.006$, $n=16$, Table 4-2), with females in stable multi-male groups stacking investment significantly more than expected and females in unstable multi-male groups significantly less than expected. Females in stable uni-male groups did not differ

from expected chi-square frequencies. Food availability was not a statistically significant predictor of stacked investment ($B = 0.149$, $S.E. = 0.1515$, Wald's $\chi^2 = 0.970(1)$, $p = 0.325$, $n = 16$).

Table 4-2 Effect of male group membership and group stability on stacked investment χ^2 cross-tabulation. Presents the observed and expected frequency of females that stacked or did not stack investment for stable uni-male (SUM), stable multi-male (SMM), and unstable multi-male (UMM) groups.

Stacked investment	SUM	SMM	UMM
Yes	Observed: 3 Expected: 2.5	Observed: 5 Expected: 2.5	Observed: 0 Expected: 2.5
No	Observed: 3 Expected: 2.5	Observed: 0 Expected: 2.5	Observed: 5 Expected: 2.5

Discussion

This study used hormonal and observational data to determine whether female *C. vellerosus* stack investment in their offspring, by conceiving while the previous infant is still in nipple contact. During our study period, eight females stacked investment in concurrent offspring, and eight did not. Of the eight females that stacked investment, three were in a stable uni-male group, and five in a stable multi-male group. Of the eight females who did not stack investment, five were in unstable multi-male groups and three were in stable uni-male groups. Male group membership and group stability had a significant effect on female stacked investment. Females in multi-male groups stacked investment significantly more than expected and females in unstable multi-male groups stacked investment significantly less than expected. The proportion of daily nipple

contact was significantly different among age categories and tended to decrease with infant age. The proportion of nipple contact was higher in females that stacked investment than in those that did not.

Instability in male group membership can affect female reproduction in multiple ways. Females in unstable groups may be under greater stress and may experience an increase in the hormones associated with stress (i.e. cortisol) due to frequent male-male agonistic interactions, which can suppress ovulation (Goodall 1986, Manson and Perry personal communication, cited in Smuts and Smuts 1993, Beehner *et al.* 2005). Females may also experience reduced feeding time because they are being more vigilant or are displaced more often by these competing males, which can make it difficult for them to meet their daily nutritional requirement. This may lead to overall poor body condition and a subsequent reduction in reproductive capabilities. Females in unstable groups may have longer time to post-partum recuperation (Mas-Rivera and Bercovitch 2008) and this may prevent them from investing in two offspring simultaneously. Alternatively, females in stable groups may experience reduced stress levels and relatively uninterrupted feeding time, which could translate to better maternal condition, freeing energy that can be invested in future offspring. However, if male group membership and stability alone predicted female stacked investment, we would have expected females in stable uni-male groups to stack investment more. Yet there was no difference in expected versus observed counts of female stacked investment in stable uni-male groups. This could be an artefact of our small sample size. Or it could be that there are other variables affecting female stacked investment. Further investigation of the effect of male group membership and group stability, in addition to other factors, could help clarify these results.

Females that stacked investment showed a higher proportion of time with an infant in nipple contact than did those females that did not stack investment. This result contradicts what we expected, since high rates of nipple contact have been shown to suppress ovulation (Short 1983, 1984, McNeilly 1994). However, when we take all our results together, they point to female condition being a good predictor of female stacked investment. As such, females who stack investment may be in better maternal condition, which allows them to not only stack investment, but do so while an infant is in nipple contact at a relatively high rate. As previously discussed, maternal condition can determine the amount of energy and resources a female can put into reproduction. Our results suggest that females that stack investment are in better condition and may be able to afford the extra energy required to have an infant in nipple contact, cycle, and conceive simultaneously.

We considered earlier whether nipple contact is a suitable proxy for infant suckling and/or milk transfer. Although recent work in chimpanzees suggests that nipple contact is not always associated with milk transfer (Badescu *et al.* 2016), we chose to use this measure to investigate the effect of nipple contact on female stacked investment because other studies have shown a connection between nipple contact and levels of female investment (Lee and Bowman 1995), as well as nipple contact and milk transfer (Reitsema 2012). As such, our result that female stacked investment is affected by the proportion of time an infant spent in nipple contact is notable, but must be treated with caution, as these results are preliminary. Our results show that having an infant in nipple contact affects female stacked investment, but it is unclear if this effect is the result of the rate of suckling and/or amount of milk transferred, or if it is simply a result of having an

infant in close proximity, which can be energetically costly (in the form of lost feeding time or because the female is expending energy by carrying or holding the infant). To test directly the effect of intensity of suckling and/or milk transfer on female stacked investment, we need to collect behavioural data on actual suckling rates, and/or faecal samples from infants on which to do stable isotope analysis for milk concentration in faeces. This is an exciting area of future research that could help clarify the actual mechanism driving our result that there is an association between nipple contact and female stacked investment.

There was a significant difference in the proportion of time infants spent in nipple contact among infant age categories, with a trend toward declining proportions over time. However, there was variability in this trend among some infant age categories (Figure 4-1). Specifically, the proportion of time spent in nipple contact in category two (3-5 months) was much lower than categories one (0-2 months) and three (6-8 months). This variability may be explained by differences between mothers. Some females may be in better maternal condition, allowing infants higher rates of nipple contact, or simply may be more tolerant of infants in nipple contact. However, these data should be treated with caution. We used group scans to calculate proportion of time in nipple contact and it may be that we do not have enough samples of nipple contact to determine a clear pattern. The number of samples among age categories was different, which may have also contributed to the variability in our results. In order to look further at rates of nipple contact relative to infant age, we need more precise data with more individuals.

It is noteworthy that infant age did not affect female stacked investment. We would expect that females with younger infants would be less likely to stack investment,

because young infants require more maternal care and hence females would be investing in them more heavily, at the expense of future offspring. However, our data suggest this is not the case. It is possible that females who stack investment are in extremely good maternal condition and thus have high quality milk. These females may be able to afford the high costs associated with rearing a young infant, while at the same time commencing investment in a subsequent offspring. We must also consider that all of the infants that survived to nutritional independence, and whose mother stacked investment, in our sample size were 10.5 months old or older (Table 4-1), so it is likely that females with very young infants cannot or do not stack investment. More likely is that there is a threshold age, related to the amount of energy an infant requires and/or the rate of suckling, at which females can resume cycling and conceive again. Weaning age ranges from 9.1 months to 21.1 months in *C. vellerosus* (Crotty 2016) and the youngest infant (10.5 months, Table 4-1) in our study was nursed the longest into gestation. It may be that females who are in good enough condition to stack investment are nursing longer into gestation because they can, and because their young infants still require maternal investment in the form of milk. Future analysis controlling for other variables such as female age could shed light on the effects of infant age on female stacked investment. To look more closely at the effect of infant age, as well as the other variables we have tested, we need a larger sample size and more exact ages for infants. Future contributions to our long-term dataset could allow us to reanalyze these variables, while controlling for multiple other variables.

Colobines fall on the spectrum of capital breeders (Brockman and van Schaik 2005) and tend to be more flexible in the timing of their reproductive events, rates of

conception, and levels of investment because they are not constrained by timing their births to a specific period of optimal ecological conditions (Lee 1996, Brockman and van Schaik 2005). It is therefore unsurprising that female stacked investment was not linked to periods of high food availability in this study. Instead, based on our results, females living in stable groups seem to be in good physical condition with less stress, which allows them to stack investment in concurrent offspring.

This is the first study to highlight female stacked investment and explore what factors may be influencing females' ability to invest directly in more than one offspring at the same time. Further analysis, using a larger dataset, will allow us to investigate these variables further and start to uncover more completely how these and any other factors may be influencing female investment patterns.

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CHAPTER 5 DISCUSSION

In this dissertation I described life history characteristics of female *Colobus vellerosus* using both behavioural and hormonal data. I also documented female mating behaviour and female stacked investment in offspring, paying particular attention to the influence of male group membership and group stability. By using a combination of behavioural and endocrine data I have produced results that contribute to a growing body of work describing primate life history variables, mating systems, and female investment patterns.

In Chapter Two I described the hormonal correlates of female reproductive events and quantified key life history variables for *C. vellerosus*. I documented ovarian cycle length (24 days), gestation length (168.5 days), age at first birth (5.87 years), and inter-birth interval for females whose infants survived to nutritional independence and for those whose infants that did not survive to nutritional independence (16.5 months vs. 11.4 months). I used observed reproductive events to confirm the validity of the reproductive events that I determined in the hormone profiles for each female. Once I confirmed that the observed reproductive events and hormonal data were congruent, I concluded that some life history characteristics can be reliably determined using observational data alone (such as using date of birth to estimate day of conception). We were thus able to use observational data to determine some life history traits for those females with hormone profiles that did not show any clear patterns.

In Chapter Three I investigated whether or not post-conceptive mating in female *C. vellerosus* was a by-product of fluctuating and/or elevated oestradiol and progesterone

levels during pregnancy, or if it was a result of females' access to multiple males. I determined that female progesterone and oestradiol levels tended to increase throughout pregnancy. I also found that females solicited males more in mid than late pregnancy, copulated more in early than late pregnancy, and males solicited females consistently across all stages of pregnancy. I found that progesterone levels were significantly higher during the three days following a copulation, and the ratio of oestradiol:progesterone levels were significantly lower during the three days following a copulation. Female directed solicitations were significantly different across stable uni-male, stable multi-male and unstable multi-male groups. Pairwise comparison (corrected using Bonferroni) showed no significant differences among the groups, but rates of solicitation directed by females were highest in stable uni-male groups and lowest in stable multi-male groups. There were no differences among the three group types for solicitation directed, received, or copulation relative to male rank, number of males or group-type. However, when I compared stable multi-male groups to unstable multi-male groups only, to investigate the differences in rates of copulation and solicitation with dominant and non-dominant males, rate of copulation was significantly higher for dominant males than for non-dominant males. Finally, females directed solicitations significantly more in unstable multi-male groups than in stable multi-male groups. Our results pointed to a combination of hormonal and behavioural factors influencing female post-conceptive mating in *C. vellerosus* and both these mechanisms may help females spread paternity certainty among multiple males, thus reducing the risk of infanticide.

In Chapter Four I used hormonal and observational data to determine if female *C. vellerosus* stack investment in their offspring by conceiving a new offspring while a prior

infant is still in nipple contact. I found that eight out of 16 females stacked investment and females in stable multi-male groups stacked investment significantly more than those in unstable multi-male groups. I also found that females that stacked investment spent a higher proportion of time with an infant in nipple contact than did those that did not stack investment. Both results suggest that females living in stable groups may be in better condition due to reduced stress, which allows them to stack investment in concurrent offspring.

Taken together, our results highlight the importance of documenting female life history characteristics, mating patterns, and investment patterns while taking into consideration the social context in which females live. I also emphasize the effects of male group membership and the stability of the group on mating and investment patterns, which is especially interesting in the context of a species that exhibits male infanticide. I have shown that females vary in their mating and investment patterns, and that these patterns seem to be influenced not only by ecological factors, but also social factors. Females can be flexible and opportunistic, and the particular strategy a female employs will be dependent on both the ecological and social environment in which she lives.

Future Research Directions

The questions addressed in this dissertation have all served to create more questions. First and foremost, I would benefit greatly from more data on which to draw conclusions. In all three data chapters, a larger sample size would allow us to more thoroughly investigate our research questions. With more hormonal data, I could determine more precise values for cycle length and gestation length, as well as document

the variability among females for each of the life history characteristics. With such more precise data, I could also more clearly determine how *C. vellerosus* life history characteristics compare to other colobines of similar body size.

I noted in Chapter Three that female progesterone levels were significantly higher three days after a copulation, but not three days after a solicitation. To further investigate the relationship between solicitation and progesterone levels I would like to collect more data on who (females or males) solicits copulation. Using such data, I could more closely investigate the relationship between reproductive hormones and female motivation to mate, and potentially gain a clearer understanding as to whether or not increased levels of progesterone are driving females' motivation to mate. I would also like to further investigate the relationship between ignored solicitations (both female directed and received) and solicitations that result in a copulation. Looking at ignored solicitations could help us understand whether females are choosing particular males with whom to mate regardless of rates of solicitation, or vice versa, if males are being choosy about mating with females. These data could help us understand if females are choosing particular males with whom to mate regardless of how often females are solicited. It would also shed light on whether or not males are using cues (hormonal or otherwise) about female reproductive state, or if mating is simply a male response to female socio-sexual behaviours.

I would also like to use more hormonal and behavioural data to compare female post-conceptive mating patterns with female mating patterns during the fertile period. This would allow me to document female mating patterns and their associated hormone levels more completely, and determine if these patterns and hormone levels are the same

across female reproductive states. Looking at female mating patterns within the fertile period would also allow me to further investigate if females mate with multiple males to confuse paternity. By looking at mating within and outside the fertile period, I could document which males females mate with and when, and if the timing of mating events with particular males is associated with the fertile period or not.

Female stacked investment is of particular interest to me, and it was an unforeseen event that I documented during my field season. I would like to continue to explore female stacked investment because the implications and impact of females investing directly, through gestation and lactation, in more than one infant at a time are potentially far reaching. It has typically been understood that female mammals are unable to conceive while lactating, so compiling data that shows otherwise could change the way we study female investment and what we expect of female investment patterns. Over the past three years researchers have been adding more, precise data on mother/infant interactions to our long-term database. To look further at the factors affecting female stacked investment, I would like to add such long-term data to my own dataset. For example, our most recent demographic data shows that at least three females have stacked investment in the past two years. Of these, two of the infants were less than one year old when the female gave birth to a subsequent offspring. By including these recent data in future analyses (specifically for publication) I hope to explore further the factors affecting female stacked investment. This data would be particularly useful to further investigate whether infant age has an effect on stacked investment and if not, why. In addition, I would like to collect more precise data on milk transfer and/or suckling rates. A current Master's student (Tianna Rissling) is planning to look at faecal milk

concentrations using stable isotope analysis, and these data would give me concrete information on the proportion of milk being transferred to infants of different ages. I could then determine if, in the event of female stacked investment, the proportion of milk affects stacked investment. Proportion of milk transferred is a more direct measurement of maternal energy expenditure. In addition to milk transfer and rate of suckling data, I would like to look at baseline cortisol levels in my study females during my field season (May 2012-2013). I have 280 faecal samples in SPE cartridges that I did not extract at the WNPRC laboratory, in addition to 1586 vials of supernatant from the samples that I extracted for this dissertation. I would like to use these samples to look at the baseline cortisol levels in females to determine if there is a difference in cortisol levels between the three group types. This would allow me to determine if females living in unstable groups are more stressed than females living in stable groups.

Broader implications of the Research

This study is the first to look at female reproductive hormones, mating behaviour and investment patterns in wild *Colobus vellerosus* using hormonal data. Detailed study of a single species, can provide crucial insight into its life history, mating patterns, and investment patterns in offspring, as well as the subtle effects that male group membership and group stability can have on social relationships. By documenting female mating allocation and the hormonal correlates of mating behavior, I was able to investigate female post-conceptive mating, female partner preference, and whether post-conceptive mating is a strategy employed by females to confuse paternity and reduce the risk of infanticide.

Evolutionary theory provides a powerful framework to explain mating patterns in humans (Landolt et al. 1995). Early human mating systems are difficult to determine but were most likely characterized by polygynyandrous mating and extended periods of female receptivity, including post-conceptive mating. This extended mating period can lead to a “constant” receptivity by females. A long receptive period may have blurred the timing of conception and lead to paternity uncertainty. Understanding the use of polyandrous and post-conceptive mating in non-human primate species can provide insight into how female strategies may have shaped human mating systems and evolution. Reconstructions of early hominin mating systems have often assumed that females are monandrous and males range from monogamous to polygynous (reviewed in Hrdy 2000). However, human and non-human female primates may enhance their reproductive success by mating post-conceptively with multiple males relative to social context (Hrdy 2000). Looking at female mating patterns in relation to male group membership, and changing male membership, allows us to study females as flexible and opportunistic individuals (Hrdy 2000).

In addition, habitat fragmentation and destruction are a serious threat faced by many primate populations (Mittermeier *et al.* 2007) and high rates of infanticide may be due to a disequilibrium caused by these disturbances (Sterck 1998, Sterck *et al.* 1997, van Schaik 2000). If environmental disturbance leads to high rates of male changeover, or high densities of outside males, then the risk of infanticide may simultaneously increase (Janson and van Schaik 2000). Understanding female mating patterns can potentially contribute to future management strategies for threatened or endangered primates.

By enlarging the dataset, re-examining some of my questions, and asking new questions, I can contribute to our understanding of females as flexible and opportunistic individuals. Females vary in their behaviour patterns and investment levels, and continuing to document this variability will allow me to not only more thoroughly describe the variability itself, but also document more completely the patterns that exist.

APPENDIX I ALL MEMBERS OF STUDY GROUPS

Group	ID	Sex	Age Class	DOB	Precision of DOB	Death/ Disappearance
AMB	JA	M	S	01-Feb-07	Estimate	Dis 2-Mar-13
AMB	LS	M	S	01-May-07	Exact	Dis 18-Feb-13
AMB	MS	M	S	01-Jan-07	Estimate	
AMB	MX	M	S	02-Nov-08	Exact	
RT	BE	F	A	1-Mar-04	Estimate	
RT	BL	F	A	1-Jan-95	Estimate	
RT	FV	F	A	1-Jan-06	Estimate	
RT	SU	F	A	1-Jan-01	Estimate	
RT	TR	F	A	01-Jan-98	Estimate	
RT	CC	M	A	01-Jan-02	Estimate	
RT	JI	F	S	1-Dec-07	Estimate	Dis 10-Apr-13
RT	BK	M	S	1-Feb-08	Estimate	
RT	JK	M	S	26-Jun-06	Exact	
RT	PE	M	S	1-Feb-08	Estimate	
RT	SR	M	S	1-Mar-08	Exact	
RT	FT	F	J	16-Jan-11	Exact	
RT	SG	F	J	1-May-11	Exact	
RT	TM	F	J	16-Jan-11	Estimate	
RT	BD	M	J	26-Dec-10	Estimate	
RT	BY	M	J	16-Jan-11	Exact	
RT	FN	M	J	01-Apr-12	Estimate	
RT	BO	F	I	10-Dec-13	Exact	
RT	BG	F	I	15-Jan-12	Exact	
RT	TE	F	I	1-Apr-12	Estimate	
RT	BD2	M	I	1-Apr-12	Estimate	
RT	S9	M	I	2-Dec-12	Exact	
SP	CT	F	A	01-May-05	Estimate	
SP	SA	F	A	01-May-05	Estimate	
SP	SE	F	A	01-Jan-97	Estimate	
SP	VE	F	A	01-Jan-96	Estimate	
SP	XE	F	A	01-Jan-00	Estimate	
SP	DR	M	A	01-May-08	Exact	
SP	SB	M	S	1-Oct-07	Estimate	
SP	SZ	M	S	6-Feb-09	Exact	
SP	VV	M	S	11-Mar-09	Exact	
SP	XS	M	S	26-Dec-08	Exact	Died 17-Aug-12
SP	SR	F	J	01-Jan-09	Estimate	
SP	BQ	M	J	01-Jan-10	Estimate	

Group	ID	Sex	Age Class	DOB		Death/ Disappearance
SP	V9	M	I	08-Dec-12	Exact	Dis Feb 16 13
SP	CK	F	I	1-May-12	Estimate	
SP	XA	F	I	1-Feb-12	Estimate	
SP	SD	?	I	20-Jun-12	Estimate	Dis 07-Sep-12
SP	SW	M	I	1-Mar-12	Estimate	
WT	IS	F	A	19-May-06	Exact	
WT	VM	F	A	02-Jul-07	Exact	
WT	XY	F	A	1-Nov-06	Estimate	
WT	KR	M	A	01-Jan-06	Estimate	
WT	PP	M	A	01-Jul-05	Estimate	Died 19-Ju-12
WT	VN	F	I	19-Nov-12	Exact	
WT	I9	M	I	9-Jan-13	Exact	
WW	CR	F	A	1-Jan-95	Estimate	
WW	IT	F	A	01-Jan-01	Estimate	
WW	JN	F	A	01-Jan-93	Estimate	
WW	ML	F	A	01-Jan-94	Estimate	
WW	OW	M	A	01-Jan-00	Estimate	
WW	XM	M	A	Unknown		Dis 10-Jul-12
WW	CX	F	I	21-Aug-12	Exact	
WW	CG	F	S	01-Apr-09	Exact	
WW	WL	F	S	01-May-08	Exact	
WW	CN	M	J	23-Sep-10	Exact	
WW	MQ	M	J	01-Dec-09	Estimate	
WW	IB	M	I	07-Sep-12	Exact	
WW	JS	M	I	15-Apr-12	Estimate	Died 9-Aug-12

APPENDIX II ETHOGRAM

(Adapted from Wikberg 2008)

Type of data:

- A adlib interaction. Followed by behaviour code, actor, receiver, and then “other” ID (if it’s a triadic interaction)
- F dyadic interaction where focal is actor. Followed by behaviour, then recipient ID
- R dyadic interaction where focal is the recipient of the behaviour from other; followed by behaviour, then other ID
- M mutual initiation of interaction; followed by behaviour and other ID in focal follows
- S self-directed behaviour or non-directed behaviour by focal

Proximity classes:

- 0 In body contact
- 1 Within one tail length (approximately 1 meter)
- 3 Within three tail length
- 5 Within five tail length
- W In view – can be used during intergroup interactions, normally don’t do in view
- N Nursing in nipple contact
- V Ventral, no nipple contact face in view, or face out of view in mother’s chest

Proximity related behaviours:

- AL Follow
- AR Run towards
- A Approach
- L Leave
- D Dorsal approach/leave/follow

Self-directed and display behaviours:

- AB Defecating
- AE Piloerect
- AF Fecal rub
- AG Autogroom
- AH Touch
- AI Inspect
- AN Erection
- AP Autoplay
- AS Scratch
- AU Urinating
- AV Vigilant
- AY Yawn

Display behaviour

- DB Stiff leg, both legs on branch

DE Display end
DH Display hop
DI Stiff leg, one leg only
DJ Jump display
DL Stiff leg, one leg on branch
DO Open mouth
DR Run display/run through
DS Stiff leg, two legs

Affiliative behaviour:

FE End groom
FE S Grooming at end of follow
FF Play face
FG S Grooming at start of follow
FG Start groom
FH Hug
FI Inspect
FJ (Infant) jumps on top of someone else (often when males stiff leg)
FK Kiss, mouth to mouth, face sniff/inspect
FM Grooming open mouths, not as wide as normal open mouths
FO Over-the-head mount
FP Groom present
FR Reach for
FS Sniff
FT Touch
FU Friendly pull
FY Play

Aggressive and submissive

GA Avoid. The approached animal leaves before approaching animals comes within 1m.
The approaching animal does not take the spot of the approached animal
GB Bite
GC Chase
GD Displace. Includes approach of displacer to 2 m and a leave by the displaced animal
within 5 seconds of the approach. (F food, S social partner, R resting spot)
GE Aggressive end
GF Flee
GG Fear grin
GH Hit
GJ Bounce GL Lunge
GM Moving displace. One individual leaves before the second one approaches to one but
the second one takes the spot of the first one
GN Pinch
GO Cower
GP Pounce on

GQ Displacement but the displayed individual stays in one
GV Push, shove
GR Grab
GS Snap at
GU Pull
GW Swipe at
GX Contact fighting (when I don't see exact behaviours I will just put contact fighting.
GY delayed displace (both individuals at the same spot for more than 5 sec before one of them leaves)

Infant-related behaviours

IA Attempted transfer
IB Infant climb.
IC Carry
ID Infant dorsal
IE End nursing
IE S Nursing at end of follow
IF Failed nursing attempt or attempt to get to nursing position
II Ride invite
IL Infant waves its tail
IN Start nursing
IN S Nursing at start of follow
IO Try to get infant off nipple
IR Restrain, hold back, resist transfer, retrieve infant, i.e. pull infant to body contact.
IT Transfer infant (AITBLPOFI – BL is transferring FI from PO)
IV Infant in ventral position
IW Want to get infant off ventral/dorsal position

Social food related behaviours

MA Attempted theft
MC Co-feeding (feeding in the same spot, within 1 tail length or from same cluster of leaves/food patch)
MI Food interest
MO Tolerated theft
MS Steal food
MT Touch others food

Sexual behaviour:

SA Attempted mount
SD Dismount
SE Sex end
SJ Ejaculate
SG Sex grin
SH Hip touch
SI Inspect anogenital area

SM Mount
SN Sniff anogenital area
SP Present
SR Resist mount
SS Stop thrust without dismount
ST Mount with thrust
SW Watches sex, individual looks at couple copulating with or without interference

Triadic aggression

Same codes as aggressive but first letter is a T instead of a G

E.g. ATCSUBLJE (SU=aggressor, BL=recipient of aggression, JE=individual being supported)

Vocalizations

VA Click-alarm call
VC Click before loud call
VG Grunt
VF Fast grunt
VH Cough, the vocalization, not just coughing
VI Intense grunt
VK click when open mouth
VL Loud call (e.g. AVL16ED)
VP Pant grunt
VQ Squeal, normal, followed by space I for intense, and space W for weak
VR Fight roar
VS Scream
VT Intense pant grunt
VU Unhappy vocalization
VY Yelp
VX Unknown vocalization
If vocalization is coming from a distance AVQ D, or from a certain group AVQ SK (Skittish)

Food-related behaviours

B Bite
H Handle
I Ingest

Food items

A Sap
B Bark
D Water, drink
E Flower bud
F Fruit
G Grass

H Pith
I Stem of the fruit
K Stick
L Leaf
M Mature leaf
O Other
P Seed pod
Q Leaf bud
R Flower
S Seed
T Petiole
U Bud
V Vine
W Wall
X Unknown
Y Young leaf

Travel

TT Start travelling
TE End travelling
TD travel distance, e.g. TD5 for travelling 5 tail lengths
TTS Start travelling before start of follow
TES End travelling after the end of the follow

Group scans and point samples

X proximity data within focal samples, followed by distance code and all IDs within that category. Use a different line for each prox category (e.g. X0POBL, X1GI, X3WO, X5JD, XNPE). Type "X" if no one is in prox of the subject at that point sample.
Y activity at point sample

Activity codes for point samples

AA other self-directed behaviour
AG autogroom or scratch
FB foraging in bark
FD drink
FF foraging fruit
FL foraging leaf
FO foraging other food
FT foraging and locomoting simultaneously
OT other / unknown
RE rest lying or sitting, sleep, standing still
SA aggressive behaviour
SD social display
SF social friendly, affiliative interaction other than groom
SG groom

SM social food-related behaviour
SP play (social)
SS sexual behaviour
ST triadic/coalitionary behaviour
SU submissive behaviour
TT travelling
TV travelling and vigilant
VF visually foraging, scanning close range for food (conspecifics)
VV vigilant, scanning longer range

Conspecifics

XX unidentified
XM unidentified male
XF unidentified adult female
XJ unidentified juvenile
XB unidentified infant

Other animals

OO observer
OH other human besides observers
ZM mona monkey
ZB bird
ZS snake
ZP sheep
ZG pig

Other codes

F food
S social partner
R resting spot
I Infant
D in distance
R reply
M mutual
X sexual harassment
OV Out of view
OVF Face out of view
IV In view
IVF Face in view
D focal subject ignores whatever happened on the previous line
FAV focal subject looks at whatever happened on the previous line or looks at monkey if followed by ID code (e.g. FAVPO) or looks at jumps (FAV JUMP)

Intergroups

IS Intergroup start

ISS Intergroup started before observer arrived
IE Intergroup end
IES Intergroup still going on when observer leave
IL Location of encountered group or focal group if taking other location points than those scheduled on the hour
IW Winner
IR Contested resource
IY Activity before, during, after intergroup
IM Main participant
CI= all types of comments regarding intergroups or male/female incursions/excursions
E.g. IL SP 150DA1

APPENDIX III PAIRWISE COMPARISONS OF PROPORTION OF TIME SPENT IN NIPPLE CONTACT AMONG INFANT AGE CATEGORIES. SIGNIFICANT RESULTS ARE IN ITALICS

Pairwise Comparisons							
Infant age category	Infant age category	Mean Difference	Std. Error	df	Sig.	95% Wald Confidence Interval for Difference	
						Lower	Upper
1	2	<i>.22696^a</i>	.065372	1	.001	.09884	.35509
	3	.11485	.058677	1	.050	-.00016	.22985
	4	<i>.10155^a</i>	.050605	1	.045	.00236	.20073
	5	<i>.20302^a</i>	.049667	1	.000	.10568	.30037
	6	<i>.20035^a</i>	.063878	1	.002	.07515	.32555
2	1	<i>-.22696^a</i>	.065372	1	.001	-.35509	-.09884
	3	<i>-.11211^a</i>	.027986	1	.000	-.16697	-.05726
	4	<i>-.12541^a</i>	.053652	1	.019	-.23057	-.02026
	5	-.02394	.050998	1	.639	-.12389	.07602
	6	-.02661	.063876	1	.677	-.15181	.09858
3	1	<i>-.11485</i>	.058677	1	.050	-.22985	.00016
	2	<i>.11211^a</i>	.027986	1	.000	.05726	.16697
	4	-.01330	.040398	1	.742	-.09248	.06588
	5	<i>.08818^a</i>	.042718	1	.039	.00445	.17190
	6	.08550	.060004	1	.154	-.03211	.20311
4	1	<i>-.10155^a</i>	.050605	1	.045	-.20073	-.00236
	2	<i>.12541^a</i>	.053652	1	.019	.02026	.23057
	3	.01330	.040398	1	.742	-.06588	.09248
	5	<i>.10147^a</i>	.028007	1	.000	.04658	.15637
	6	.09880	.070978	1	.164	-.04031	.23791
5	1	<i>-.20302^a</i>	.049667	1	.000	-.30037	-.10568

	2	.02394	.050998	1	.639	-.07602	.12389
	3	-.08818 ^a	.042718	1	.039	-.17190	-.00445
	4	-.10147 ^a	.028007	1	.000	-.15637	-.04658
	6	-.00267	.078228	1	.973	-.15600	.15065
6	1	-.20035 ^a	.063878	1	.002	-.32555	-.07515
	2	.02661	.063876	1	.677	-.09858	.15181
	3	-.08550	.060004	1	.154	-.20311	.03211
	4	-.09880	.070978	1	.164	-.23791	.04031
	5	.00267	.078228	1	.973	-.15065	.15600
Pairwise comparisons of estimated marginal means based on the original scale of dependent variable xn_rate							
a. The mean difference is significant at the .05 level.							

APPENDIX IV: SOURCES OF DATA FOR INTER-BIRTH INTERVAL AND AGE AT FIRST BIRTH.

This table presents the sources of data used for calculations in Chapter 2 and 4 of mean inter-birth interval with infant survival and death, and mean female age at first birth. Please note that the values in the body of the table are the new updated values as calculated in Appendix V.

IBI with infant survival from this study (months)	IBI with infant death from this study (months)	IBI with infant death from Teichroeb and Sicotte 2008 (months)	Age at first birth from this study (years)	Age at first birth from long-term data (years)
24.01	14.80	11	6.65	4.72
24.01	10.99	11	6.44	5.50
21.09	9.93	11	6.06	5.72
10.66	17.11	9		5.72
18.36		8		6.17
8.32				
13.72				
17.30				
14.84				
14.08				

APPENDIX V: ERRATUM TO: HORMONAL CORRELATES OF LIFE HISTORY CHARACTERISTICS IN WILD FEMALE *COLOBUS VELLEROSUS*.

The authors would like to update the data published in the original article. In our original publication we used a conversion of seven days in a week and four weeks in a month for gestation length, inter-birth interval, and age at first birth. We also rounded the values up or down after converting to weeks, and in doing so, lost some precision in the data. For this update we calculated all values in days, and when necessary convert them to months by using 30.4 days in a month. We calculated one year as 12 months. Converting the values using 30.4 days in a month allowed us to calculate the variables more precisely and remove the imprecision of rounding up or down. The correction details for gestation length, inter-birth interval, and age at first birth are specified below and these values should be used for comparison between other species, as well as reference for *C. vellerosus*. All other values presented in Vayro *et al.* 2016 are correct and should be used for referencing purposes.

In the “Abstract” the sentences reading: “Mean gestation length was 23 weeks (range = 21-25 weeks, n = 2 complete pregnancies). For females whose infants survived to nutritional independence, the mean inter-birth interval (IBI) was significantly longer than for females whose infants died prior to reaching nutritional independence (Mann-Whitney U Test; $U = 14.5$, $p = 0.006$; IBI surviving infants: 17.5 months, range = 8-20.75 months, n = 11 vs. IBI infant death: 11.89 months, range = 8-18.5 months, n = 9).” should read as:

Mean gestation length was 168.5 days (range = 159-178 days, n = 2 complete pregnancies). For females whose infants survived to nutritional independence, the mean inter-birth interval (IBI) was significantly longer than for females whose infants died prior to reaching nutritional independence (Mann-Whitney U Test; $U = 20.5$, $p = 0.025$; IBI surviving infants: 16.5 months, range = 8.3-24.0 months, n = 11 vs. IBI infant death: 11.4 months, range = 8.0-17.1 months, n = 9).

In the “Abstract” the sentence reading: “Some values are on the lower end of the range for similarly sized colobines; *C. vellerosus* shows a cycle of 24 days and gestation length of 5.75 months vs. a range of 24–29 days for cycle length and 5.25–7.5 months for gestation length in other colobines.”

should read as:

Some values are on the lower end of the range for similarly sized colobines; *C. vellerosus* shows a cycle of 23-25 days and gestation length of 159-178 days vs. a range of 24–29 days for cycle length and 158-210 days for gestation length in other colobines.

In the “Results” section, under “Life history characteristics from hormonal data”, the sentence reading: “Gestation length was 23.4 weeks (± 5 days) and 21 weeks (± 5 days) respectively; mean gestation length was 22.2 weeks (range = 21–23.4 weeks, n = 2).”

should read as:

Gestation length was 159 days (± 0 days) and 178 days (± 3 days) respectively; mean gestation length was 168.5 days (range = 159-178 days, n = 2).

In the “Results” section, under “Life history characteristics from observational data”, the sentence reading: “For females whose infants survived to nutritional

independence the mean inter-birth interval was significantly longer (17.75 months, range = 8–20.75 months, n = 11) than the inter-birth interval for females whose infants did not survive to nutritional independence (11.89 months, range = 8–18.5 - months, n = 4 from this study, n = 5 from 2004–2011 dataset) (U = 14.5, p = 0.006).”

should read as:

For females whose infants survived to nutritional independence the mean inter-birth interval was significantly longer (16.5 months, range = 8.3-24.0 months, n = 11) than the inter-birth interval for females whose infants did not survive to nutritional independence (11.4 months, range = 8.0-17.1 months, n = 9, n = 4 from this study, n = 5 from 2004–2011 dataset) (U = 20.5, p = 0.025).

In the “Results” section, under “Age at first pregnancy”, the sentence reading: “The mean age at first pregnancy was 5.82 years (range = 4.66-7.08, n = 3 from this study, n = 5 from 2004-2011 data set).”

should read as:

“Age at first birth: The mean age at first birth was 5.87 years (range = 4.72-6.65, n = 3 from this study, n = 5 from 2004-2011 data set).”

In the “Discussion” section, the sentence reading: “The key life history variables documented in this study are ovarian cycle length (24 days), gestation length (5.75 months), inter-birth interval for females whose infants survive to nutritional independence and for those whose infants do not survive to nutritional independence (17.75 months vs.11.89 months), and age at first reproduction (5.8 years).”

should read as:

The key life history variables documented in this study are ovarian cycle length

(24 days), gestation length (168.5 days), inter-birth interval for females whose infants survive to nutritional independence and for those whose infants did not survive to nutritional independence (16.5 months vs. 11.4 months), and age at first birth (5.87 years)

In the “Discussion” section, the sentences reading: “The mean age at first reproduction of 5.82 years is within the range of other colobine species (3.5–6.7 years). The mean gestation length of 5.75 months is the same as that for *C. guereza* (Harris and Monfort 2006), but shorter than that found in other colobines (e.g. Borries *et al.* 2001, Starin 2001). The inter-birth interval for *C. vellerosus* when an infant survived to nutritional independence is 17.75 months, slightly shorter than that in other colobine species. The mean inter-birth interval when an infant died before nutritional independence is 11.89 months, which is within the range for other colobine species under similar conditions (9–18 months).”

should read as:

The mean age at first birth of 5.87 years is within the range of other colobine species (3.5–6.7 years). The mean gestation length of 168.5 days is similar to that for *C. guereza* (Harris and Monfort 2006), but shorter than those found in other colobines (e.g. Lippold 1981, He *et al.* 2001, Borries *et al.* 2001). The inter-birth interval for *C. vellerosus* when an infant survived to nutritional independence is 16.5 months, slightly shorter than that in other colobine species. The mean inter-birth interval when an infant died before nutritional independence is 11.4 months, which is within the range for other colobine species under similar conditions (6.1-18 months).

In the “Discussion” section, the sentence reading: “Although *C. vellerosus* fits within the typical range for colobine species (Table 2-3), cycle (24 days) and gestation

length (5.75 months) for *C. vellerosus* in this study are shorter than those found for most colobines of similar size (range 24–29 days; 5.25–7.5 months).”

should read as:

Although *C. vellerosus* fits within the typical range for colobine species (Table 2-3), mean cycle (24 days) and gestation length (168.5 days) for *C. vellerosus* in this study are on the shorter end of those found for most colobines of similar size (range of cycle lengths: 24–29 days; range of gestation lengths: 158-210 days).

In the “Results” section Table 2-2 is replaced by:

Table 2-2 Life history characteristics for study females.

Values found in this study for each female, including cycle length (days), gestation length (days), inter-birth interval (months) and age at first birth (years). Inter-birth intervals are reported in months in order to produce values comparable to other studies. (One month = 30.4 days; one year = 30.4*12). *denotes data from this study; ^denotes data from long-term data. Appendix IV provides a breakdown of the values and the sources of data.

ID	Cycle length (days)	Gestation length (days)	Inter-birth interval w/ infant survival (months)	Inter-birth interval w/ infant death (months)	Age at first birth (years)
BE	-	-	8.32*	-	4.72^
BL	-	-	13.72^	-	-
FV	-	-	17.30^	-	-
JI	-	-	-	-	-
SU	-	-	18.36^	-	5.50^
TR	-	-	14.84^	-	-
CT	-	-	21.09^	-	5.72^
SA	-	-	-	14.80*	5.72^
SE	-	-	24.01^	-	-
VE	-	-	-	9.93^	-
XE	-	-	24.01^	-	-
IS	23*	159*	-	17.11^	6.65*
VM	-	-	-	-	6.06*
XY	-	-	10.66^	-	6.44*
CR	-	-	14.08^	-	-
IT	-	-	14.80^	-	6.17^
JN	25*	178*	-	10.99*	-
ML	-	-	-	-	-

In the “Discussion” section Table 2-3 is replaced by:

Table 2-3 – Cross species comparison of life history characteristics.

Cross species comparison of inter-birth interval with infant survival to nutritional independence (IBI w/infant survival), and infant death before nutritional independence (IBI w/ infant death), cycle length, gestation length, and female age at first birth in colobines. Values and references in regular font indicate studies in which only observational data were used, whereas those in bold indicate studies in which observational and hormonal data were used. Values are presented as a mean (M) or median (Mdn) depending on the information reported in the original source. Range (R) is given when no mean or median was available.

Species	IBI w/ infant survival (months)	IBI w/ infant death (months)	Cycle length (days)	Gestation length (days)	Age at first birth (years)
<i>Colobus vellerosus</i>	<i>M:</i> 16.5 n=11¹	<i>M:</i> 13.2 n=4¹ ; <i>M:</i> 10 n=5²	<i>M:</i> 24 n=2¹	<i>M:</i> 168.5 n=2¹	<i>M:</i> 5.8 n=8¹
<i>Colobus guereza</i>	<i>M:</i> 22 n=6³	<i>M:</i> 6.1 n=1³	<i>M:</i> 25 n=5³	<i>Mdn:</i> 158 n=4³	-
<i>Colobus polykomos</i>	<i>M:</i> 24 n=4⁴	-	-	-	-
<i>Procolobus badius</i>	<i>M:</i> 29.4 n=10⁵	-	-	<i>M:</i> 159.6 n=4⁵	<i>M:</i> 4.2 n=4⁵
<i>Pygathrix nemaeus</i>	-	-	<i>M:</i> 26 n=5⁶	<i>M:</i> 210 n=1⁷	-
<i>Procolobus rufomitratu</i>	<i>M:</i> 25.3 n=13⁸ ; <i>M:</i> 27.5 n=2⁹	<i>M:</i> 14-17 n=13⁸ ; <i>R:</i> 9 -18 n=2¹⁰	-	-	-
<i>Presbytis thomasi</i>	<i>M:</i> 26.8 n=36¹¹	<i>M:</i> 17.7 n=36¹¹	-	-	<i>M:</i> 5.4 n=9¹¹
<i>bieti</i>	<i>M:</i> 23.2 n=12¹²	<i>M:</i> 14.1 n=5¹²	<i>M:</i> 29 n=3¹³	<i>M:</i> 203.7 n=3¹³	-
<i>Rhinopithecus roxellana</i>	<i>M:</i> 23.3 n=36¹⁴	<i>M:</i> 11.6 n=5¹⁴	-	-	<i>M:</i> 5-6 n=5¹⁴
<i>Semnopithecus entellus</i>	(shortest) 21.8 n=1¹⁵ ; <i>M:</i> 17.2 n=82¹⁶	(shortest) 13.2 n=1¹⁵ ; <i>M:</i> 15.4, n=32¹⁶	<i>M:</i> 24.1 n=113¹⁶	<i>M:</i> 209.4 n=17¹⁵ ; <i>M:</i> 200.3 n=31¹⁶ ; <i>M:</i> 211.6 n = 6¹⁷	<i>M:</i> 6.7 n=26¹⁵ ; <i>M:</i> 2.9 n=12¹⁶
<i>Trachypithecus phayrei</i>	<i>R:</i> 21.3 - 24.5 n=8-15¹⁸	-	-	-	-

(1) This study (2) Teichroeb & Sicotte 2008 (3) Harris and Monfort 2006, Harris and Monfort 2003 (4) Dasilva 1989 (5) Starin 2001 (6) Heistermann *et al.* 2004 (7) Lippold 1981 (8) Marsh 1979 (9) Struhsaker & Pope 1991 (10) Struhsaker and Leland 1985 (11)

Wich *et al.* 2007 (12) Cui *et al.* 2006 (13) **He *et al.* 2001** (14) Qi *et al.* 2008 (15) **Borries *et al.* 2001**: IBI with and without infant survival is combined in this paper and reported as *M*: 28, n=72, Borries 1997 (16) Sommer *et al.* 1992 (17) **Ziegler *et al.* 2000** (18) Borries *et al.* 2008.

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**APPENDIX VII PERMISSION TO STUDY OR CONDUCT RESEARCH WITHIN
WILDLIFE PROTECTED AREAS IN GHANA**



Receipt No. 0063289 of 8/5/12



FCWD/GH-01

**APPLICATION TO STUDY OR CONDUCT RESEARCH WITHIN WILDLIFE
PROTECTED AREAS IN GHANA**

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Information on proposed project

Title Female polyandrous mating as a counter-
strategy to infanticide in black & white colobus monkeys
Proposed Start Date May 10 2012 End Date April 15 2013

Specimen and/or samples will / will not be collected within wildlife protected areas in Ghana during the course of the proposed project

Name and region of proposed research sites in protected areas of Ghana

1. BOABENG - ELEMA MONKEY SANCTUARY
2. _____
3. _____
4. _____
5. _____

To: Executive Director, Wildlife Division, Forestry Commission, P.O. Box MB 239 Accra

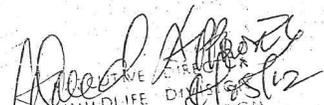
If I and my collaborators receive permission to carry out the proposed project, we will comply with any and all laws, regulations, stipulations, and agreements germane to this work, and, in addition, abide by any additional orders and requests made by government officials.

I have submitted the following documents with this application for your consideration:

1. project proposal
2. biographical sketches for each of the _____ (number of persons) collaborators
3. A photocopy of the signature page from the passport

Sincerely yours,


 Signature _____ date May 3 2012
(JOSIE VAIRO)
 Printed
 Project Leader


 EXECUTIVE DIRECTOR
 WILDLIFE DIVISION
 FORESTRY COMMISSION
 ACCRA

Official use only	
EX-1	LITIME DIRECTOR
WIL	Research project code
DATE	<u>10/10/12</u>
ACCRA	

This research/ study is approved under the following conditions:

- Pay a flat research fee of \$500 or its equivalent in cedis
- Pay for entrance fee, guide fees and other services that you may require
- Submit one (1) copy of thesis/report/document to the Executive Director.
- Provide three (3) copies of any publication emanating from the study.
- Obtain CITES export permit should there be the need to send specimen outside the country


 FOR: EXECUTIVE DIRECTOR
 X OAKA (OF AAKAKMAH)

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