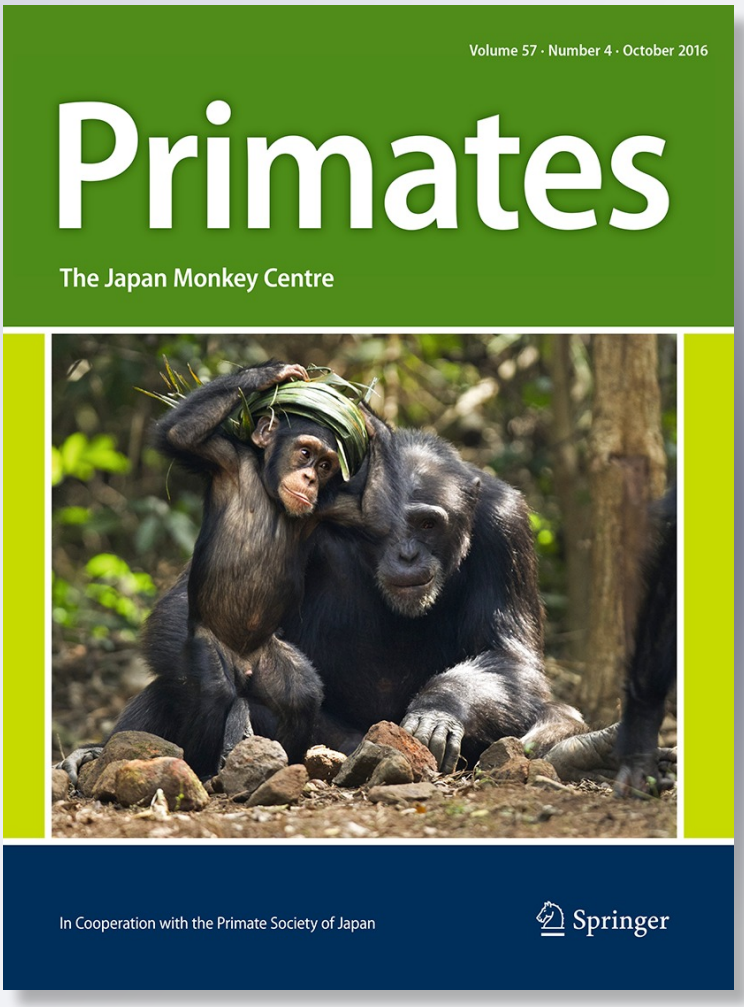
Hormonal correlates of life history characteristics in wild female Colobus vellerosus

J. V. Vayro, L. M. Fedigan, T. E. Ziegler, A. Crotty, R. Ataman, R. Clendenning, E. Potvin-Rosselet, E. C. Wikberg & P. Sicotte

Primates

ISSN 0032-8332
Volume 57
Number 4

Primates (2016) 57:509-519
DOI 10.1007/s10329-016-0551-1



Springer

Primates (2016) 57:509–519
DOI 10.1007/s10329-016-0551-1

ORIGINAL ARTICLE

Hormonal correlates of life history characteristics in wild female *Colobus vellerosus*

J. V. Vayro[1] · L. M. Fedigan[1] · T. E. Ziegler[2] · A. Crotty[1] · R. Ataman[1] · R. Clendenning[1] · E. Potvin-Rosselet[1] · E. C. Wikberg[1] · P. Sicotte[1]

Received: 30 January 2016 / Accepted: 16 June 2016 / Published online: 27 June 2016

Abstract Documenting primate life history characteristics is important because it provides information about traits that affect the timing and rate of reproduction in these long-lived species. This study describes the hormonal correlates of female reproductive events and quantifies for the first time key life history variables for *Colobus vellerosus,* using hormonal and observational data. This study also biologically validates that the reproductive events determined in the hormone profiles correspond to observed reproductive events for each female. We collected behavioural data on 18 females in our four study groups during 12 months (May 2012–2013) at the Boabeng-Fiema Monkey Sanctuary, using 10-min continuous focal and *ad libitum* sampling. We concurrently collected faecal samples ($n = 1866$) every 2–3 days from these 18 females (prepubescent $n = 2$, cycling $n = 2$, lactating $n = 12$, pregnant, $n = 7$, and post-reproductive $n = 1$) and extracted oestrogen (E2) and progesterone (P) metabolites in the field using solid-phase extraction cartridges. We created a hormone profile for each female by analyzing 1586 of our samples for E2 using radio-immuno assays, and P using enzyme-immunoassays at the Wisconsin National Primate Research Center. Mean ovarian cycle length was 24 days ± 1 ($n = 2$ cycles). 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: 17.75 months, range = 8–20.75 months, $n = 11$ vs. IBI infant death: 11.89 months, range = 8–18.5 months, $n = 9$). The values for most life history traits reported in this study are similar to those documented in other similarly sized colobine species. 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. This may be due to *C. vellerosus*' smaller body size, or their limited access to higher quality food resources.

Keywords Colobines · Female reproductive hormones · Life history · Inter-specific variation

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

✉ J. V. Vayro
jvvayro@ucalgary.ca

[1] Department of Anthropology and Archaeology, University of Calgary, Calgary, AB, Canada

[2] National Primate Research Center, University of Wisconsin, Madison, WI, USA

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 should also 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 twofold. 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-ha 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 10-minute focal samples, scan samples and ad libitum data from 6am to 2 pm, 6 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 h of focal observation were collected (mean 30.8 h per female; range 20.4–45.6 h; $n = 18$). 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 alpha level to 0.05, and the test was one-tailed.

We collected faecal samples each morning between 6am and 12 pm. No samples were collected after 12 pm 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 8 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 coefficients 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, ten were in or entered lactational amenorrhea, two were prepubescent, and one was post-reproductive (Table 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 ($n = 10$, two females, two cycles, range 3.12–7.89 ng/g). Mean baseline *P* concentrations were 161.81 ng/g ($n = 10$, two females, two 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, two 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 ($n = 8$, two females, two ovulations, mean 20.83 ng/g) and 70.58–2579.07 ng/g for *P* ($n = 8$, two females, two 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 (Figs. 1 and 2).

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, $n = 8$ females) and for E2 were 24.07 ng/g (range 1.17–96.94 ng/g, $n = 8$ females). The first 4 months of pregnancy were characterized by lower mean *P* values (544.40 ng/g, range 41.93–6102.83 ng/g, $n = 6$) and E2 values (13.63 ng/g, range 3.11–96.94 ng/g, $n = 6$ females) 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, $n = 7$ females) and 33.09 ng/g for E2 (range 3.82–154.21 ng/g, $n = 7$ females). We determined pregnancy from the hormone profiles for eight 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 ($n = 9$ females, 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 (Figs. 1 and 2). Six females were pregnant prior to the start of the study, and they experienced parturition and then lactational amenorrhea during the study. Figure 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, these females were probably prepubescent (Fig. 4). One female experienced two possible ovulations that fit an atypical pattern, with 12 weeks between

Table 1 Hormone steroid values for study females

ID	Samples analyzed	Reproductive state	Mean E2 lact. amen. (SD)	Mean *P* lact. amen. (SD)	Presumed ovulations (*n*)	Mean peak ovulatory E2 (SD)	Mean peak ovulatory *P* (SD)
BE	101	Pregnant; lactational amenorrhea	5.31 ng/g (3.17)	184.7 ng/g (116.42)	–	–	–
BL	114	Cycling? pregnant?[a]	–	–	–	–	–
FV	102	Cycling? pregnant?	–	–	–	–	–
JI	106	Prepubescent	–	–	–	–	–
SU	68	Pregnant; lactational amenorrhea	4.19 ng/g (1.74)	274.58 ng/g (179.51)	–	–	–
TR	115	Cycling? pregnant?	–	–	–	–	–
CT	105	Lactational amenorrhea	5.26 ng/g (6.93)	217.94 ng/g (142.32)	–	–	–
SA	78	Lactational amenorrhea; cycling?	6.13 ng/g (3.47)	291.22 ng/g (193.87)	–	–	–
SE	98	Lactational amenorrhea	5.99 ng/g (13.53)	226.67 ng/g (161.21)	–	–	–
VE	43	Pregnant; lactational amenorrhea	4.16 ng/g (1.71)	210.23 ng/g (155.65)	–	–	–
XE	100	Lactational amenorrhea	4.81 ng/g (2.05)	241.02 ng/g (162.48)	–	–	–
IS	91	Cycling; pregnant	–	–	1	27.535 ng/g (15.41)	678.51 ng/g (463.57)
VM	97	Prepubescent	–	–	–	–	–
XY	98	Pregnant; lactational amenorrhea	4.65 ng/g (1.77)	271.88 ng/g (381.64)	–	–	–
CR	60	Pregnant; lactational amenorrhea	5.43 ng/g (4.01)	491.30 ng/g (387.09)	–	–	–
IT	59	Pregnant; lactational amenorrhea	4.50 ng/g (1.87)	390.57 ng/g (302.46)	–	–	–
JN	75	Cycling; pregnant	–	–	1	14.12 ng/g (10.92)	1794.23 ng/g (790.56)
ML	75	Post-reproductive?	–	–	–	–	–

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

[a] ? denotes that the hormone profile did not fit the criteria for any reproductive state; however, reproductive state was determined using observational data

possible ovulations. Based on our long-term records, she was at least 18 years old and therefore was probably post-reproductive (Fig. 5). Three females fit no criteria for cycling, pregnancy or lactational amenorrhea, because their hormone profiles showed no clear patterns (Fig. 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 1 presents a summary of reproductive state and calculated reproductive parameters for each female.

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 data set). For females whose infants survived to nutritional independence, the mean inter-birth interval was significantly longer

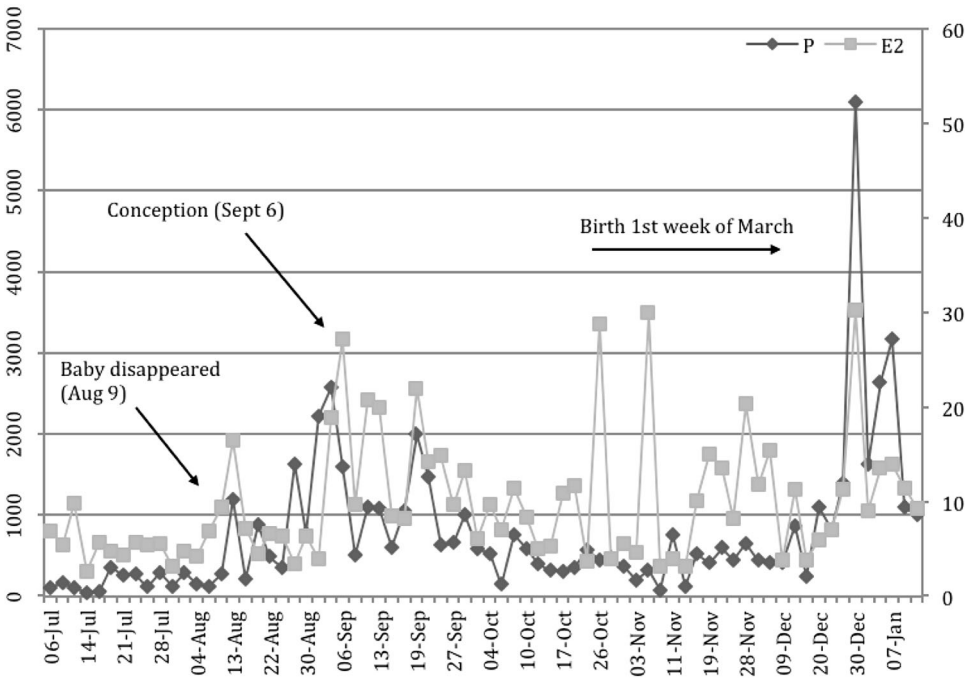


Fig. 1 Hormone profile for female JN showing E2 and *P* levels throughout the study period. The date of first infant disappearance, day of conception and duration of pregnancy are noted

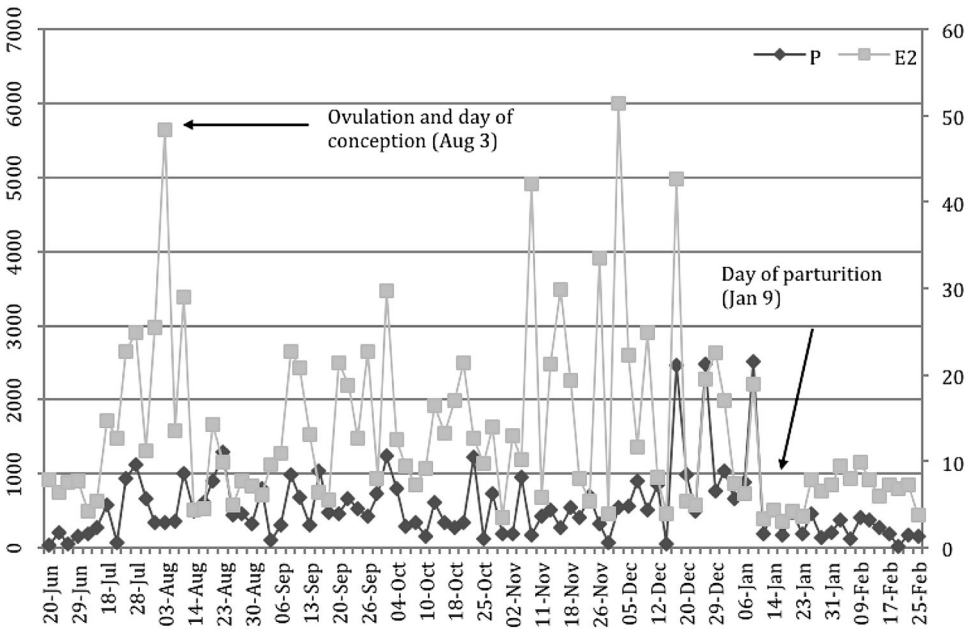


Fig. 2 Hormone profile for female IS showing E2 and *P* levels throughout the study period. The day of conception, duration of pregnancy and day of parturition are noted

(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) ($U = 14.5$, $p = 0.006$). The shortest inter-birth interval in our sample was linked to the disappearance of an infant (approximately 2 months old) on 9 August 2012. An extra-group male had wounded this infant during an incursion. The mother resumed cycling (and ovulated) 27 days later on 6 September 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

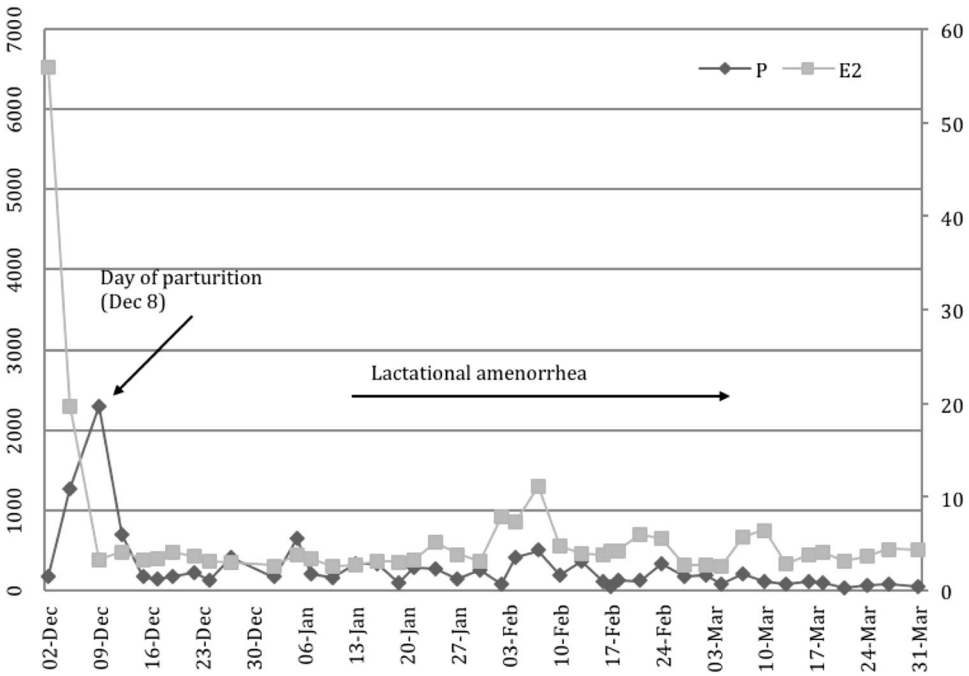


Fig. 3 Hormone profile for female VE showing E2 and *P* levels throughout the study period. The day of parturition and lactational amenorrhea are noted

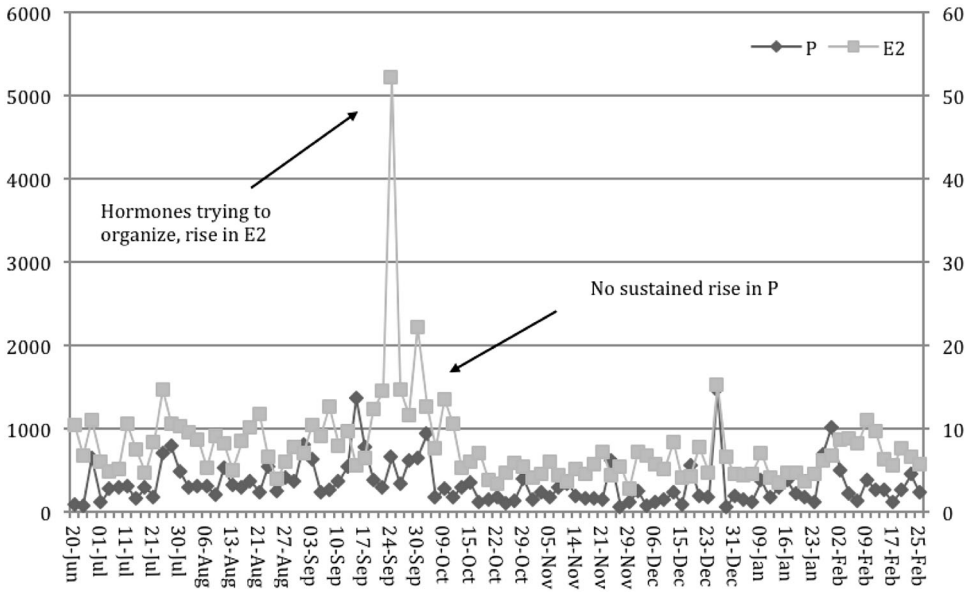


Fig. 4 Hormone profile for female XY showing E2 and *P* levels throughout the study period. This profile shows a pattern beginning to conform to a typical ovulatory cycle, but the conditions for ovulation were not met; hence, she was presumed prepubescent

pregnancy, day of parturition, and lactational amenorrhea. Table 2 presents a summary of key life history characteristics associated with each female.

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 data set) (Table 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.

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 (Figs. 2 and 3).

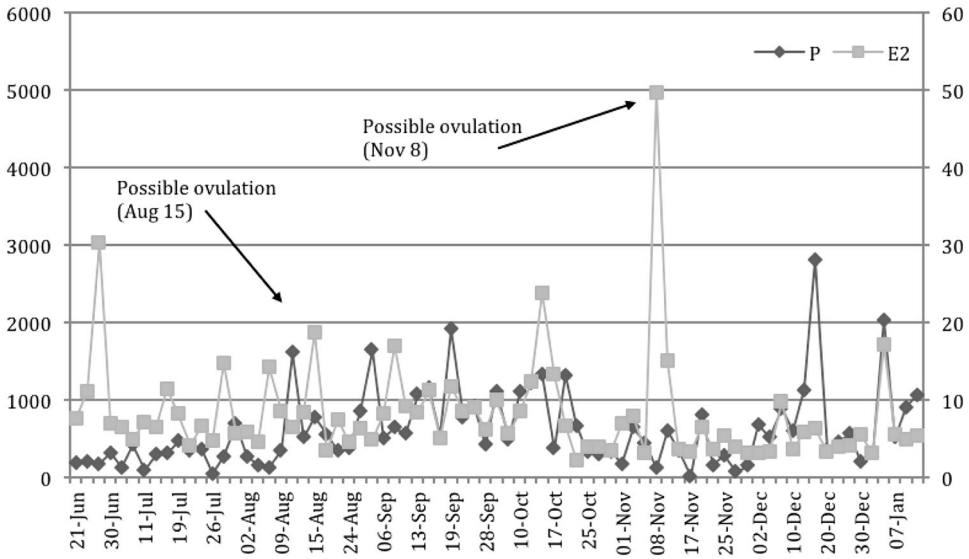


Fig. 5 Hormone profile for female ML showing E2 and *P* levels throughout the study period. This is an atypical profile with 12 weeks between possible ovulations; hence, she was presumed post-reproductive

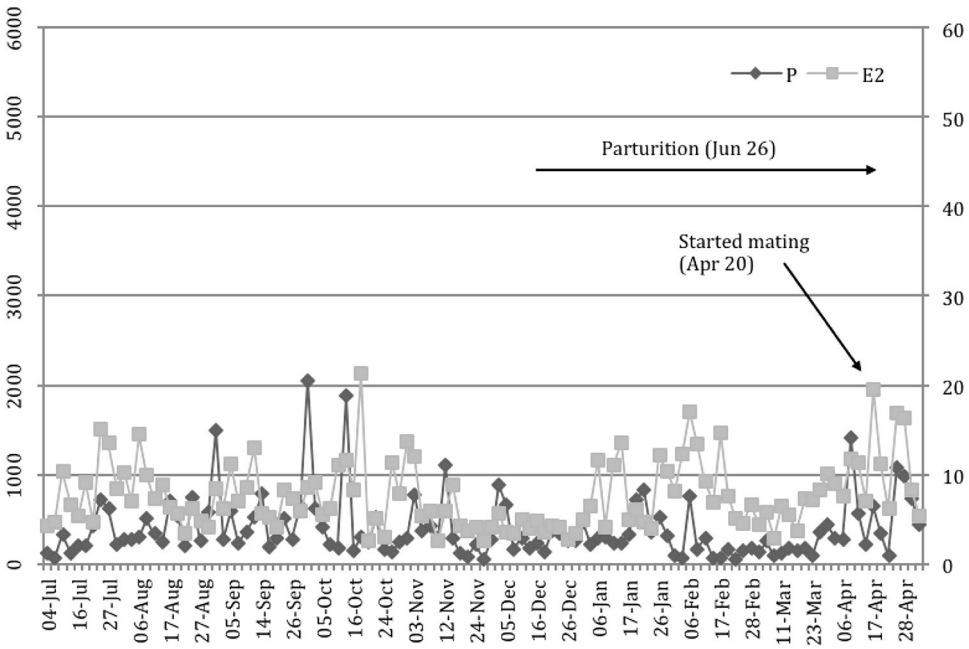


Fig. 6 Hormone profile for female TR showing E2 and *P* levels throughout the study period. The profile fit no criteria for cycling, pregnancy or lactational amenorrhea because there is no clear E2 and *P* pattern

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 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 3). However, several of these studies are based on observational data. This limits our ability to quantify life history variables (e.g. cycle length

Table 2 Life history characteristics for study females

ID	Cycle length (days)	Gestation length (weeks)	Interbirth interval w/infant survival (months)	Interbirth interval w/infant death (months)	Age at first reproduction (years)
BE	–	–	8 months[a]	–	4.66 years[b]
BL	–	–	14.88 months[b]	–	–
FV	–	–	18.75 months[b]	–	–
JI	–	–	–	–	–
SU	–	–	20 months[b]	–	5.50 years[b]
TR	–	–	16 months[b]	–	–
CT	–	–	17 months[b]	–	5.66 years[b]
SA	–	–	–	14.75 months[a]	5.66 years[b]
SE	–	–	20.75 months[b]		–
VE	–	–	–	10.75 months[b]	–
XE	–	–	20.25 months[b]		–
IS	23 days[a]	5.25 months[a]	–	18.5 months[b]	6.58 years[a]
VM	–	–	–	–	7.08 years[a]
XY	–	–	11.5 months[b]	–	5.33 years[a]
CR	–	–	15.25 months[b]	–	–
IT	–	–	16 months[b]	–	6.16 years[b]
JN	25 days[a]	5.85 months[a]	–	4.25 months[a]	–
ML	–	–	–	–	–

Values found in this study for each female including cycle length, gestation length, inter-birth interval, and age at first reproduction

[a] Denotes data from this study

[b] Denotes data from long-term data

Table 3 Cross species comparison of life history characteristics

Species	IBI w/infant survival (months)	IBI w/infant death (months)	Cycle length (days)	Gestation length (months)	Age at first reproduction (years)
C. vellerosus	**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**
C. guereza	**22[3] *n* = 6**	**6.8[4] *n* = 1**	**25[3] *n* = 5**	**5.75[3] *n* = 1**	–
C. polykomos	24[5] $n = 4$	–	–		–
P. badius	29.4[6] $n = 10$	–	–	5.25[6] $n = 4$	4.2[6] $n = 4$
P. nemaeus	–	–	**26[7] *n* = 5**	**7.5[8] *n* = 1**	–
P. rufomitratus	25.3[9] $n = 13$; 27.5[10] $n = 2$	14–17[9] $n = 13$; 9–18[11] $n = 2$	–	–	–
P. thomasi	26.8[12] $n = 36$	17.7[12] $n = 36$	–		5.4[12] $n = 9$
R. bieti	23.3[13] $n = 12$	14[13] $n = 5$	**29[14] *n* = 3**	**7.25[14] *n* = 3**	–
R. roxellana	23.3[15] $n = 36$	11.5[15] $n = 5$			5–6[15] $n = 5$
S. entellus	**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] $n = 12$
T. obscurus phayre	21.3–24.5[20] $n = 8$–15	–	–	–	–

IBI inter-birth interval

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, those in **bold** indicate studies in which observational and hormonal data were used

(1) This study, (2) Teichroeb and 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 and 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)

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).

Although *C. vellerosus* fits within the typical range for colobine species (Table 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 8.4–13.5 kg 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 live in a habitat that may contain lower quality food resources than those available to other colobine populations (Wong et al. 2006). 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 analyzed, 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 results 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 a 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 throughout 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.

Acknowledgments We would like to thank the Ghana Wildlife Division and the management committee of the Boabeng-Fiema Monkey Sanctuary for permission to conduct this research. Funding was provided by NSERC and the University of Calgary. Research assistance was provided by Robert Koranteng and Charles Kodom. This manuscript benefitted from helpful comments from Mackenzie Bergstrom and Stephanie Fox, and two anonymous reviewers. Data collection methods complied with the rules of the University of Calgary's Animal Care Committee and with the laws of Ghana.

References

Asquith PJ (1989) Provisioning and the study of free-ranging primates: history, effects, and prospects. Am J Phys Anthropol 32:129–158

Borries C (1997) Infanticide in seasonally breeding multimale groups of Hanuman langurs (*Presbytis entellus*) in Ramnagar (South Nepal). Behav Ecol Sociobiol 41:139–150

Borries C, Koenig A, Winkler P (2001) Variation of life history traits and mating patterns in female langur monkeys *Semnopithecus entellus*). Behav Ecol Sociobiol 50:391–402

Borries C, Larney E, Lu A, Ossi K, Koenig A (2008) Costs of group size: lower developmental and reproductive rates in larger groups of leaf monkeys. Behav Ecol 19:1186–1191. doi:10.1093/beheco/arn088

Borries C, Gordon AD, Koenig A (2013) Beware of primate life history data: a plea for data standards and a repository. PLoS ONE. doi:10.1371/journal.pone.0067200

Buchanan KL, Goldsmith AR (2004) Noninvasive endocrine data for behavioural studies: the importance of validation. Anim Behav 67:183–185

Butynski TM, Grubb P, Kingdon J (2013) *Procolobus Badius* western red colobus (Bay Colobus). In: Butynski TM, Kingdon J, Kalina J (eds) Mammals of Africa, vol 2. Bloomsbury Publishing, London, pp 128–133

Carnegie SD, Fedigan LM, Ziegler TE (2005) Behavioral indicators of ovarian phase in white-faced capuchins (*Cebus capucinus*). Am J Primatol 67:51–68

Cui LW, Sheng AH, He SC, Xiao W (2006) Birth seasonality and interbirth interval of captive *Rhinopithecus bieti*. Am J Primatol 68:457–463

Dasilva GL (1989) The ecology of the western black and white colobus (*Colobus polykomos*) on a riverine island in Southeastern Sierra Leone. D. Phil. thesis, University of Oxford

Fargey PJ (1991) Assessment of the conservation status of the Boabeng-Fiema Monkey Sanctuary. Final report to the flora and fauna preservation society, University of Science and Technology, Kumasi

Harris TR, Monfort SL (2003) Behavioral and endocrine dynamics associated with infanticide in a black and white colobus monkey (*Colobus guereza*). Am J Primatol 61:135–142

Harris TR, Monfort SL (2006) Mating behavior and endocrine profiles of wild black and white colobus monkeys (*Colobus guereza*): toward an understanding of their life history and mating system. Am J Primatol 68:383–396

Hawkes K, O'Connell JF, Blurton Jones NG (2002) Human life histories: primate trade-offs, grandmothering socioecology, and the fossil record. In: Kappeler PM, Pereira ME (eds) Primate life histories and socioecology. The University of Chicago Press, Chicago, pp 204–232

He Y, Pei Zou R, Ji W (2001) Changes of urinary steroid conjugates and gonadotropin excretion in the menstrual cycle and pregnancy in the Yunnan snub-nosed monkey (*Rhinopithecus bieti*). Am J Primatol 55:223–232

Heistermann M (2010) Non-invasive monitoring of endocrine status in laboratory primates: methods, guidelines and applications. Adv Sci Res 5:1–9

Heistermann M, Ademmer C, Kaumanns W (2004) Ovarian cycle and effect of social changes on adrenal and ovarian function in *Pygathrix nemaeus*. Int J Primatol 25:689–708

Heistermann M, Palme R, Ganswindt A (2006) Comparison of different enzyme-immunoassays for assessment of adrenocortical activity in primates based on fecal analysis. Am J Primatol 68:257–273

Hodges JK, Heistermann M (2011) Field endocrinology: monitoring hormonal changes in free-ranging primates. In: Setchell JM, Curtis DJ (eds) Field and laboratory methods in primatology: a practical guide. Cambridge University Press, Cambridge, pp 353–370

Lee P, Kappeler PM (2003) Socioecological correlates of phenotypic plasticity of primate life histories. In: Kappeler PM, Pereira ME (eds) Primate life histories and socioecology. The University of Chicago Press, Chicago, pp 41–65

Leigh SR (1994) Ontogenetic correlates of diet in anthropoid primates. Am J Phys Anthropol 94:499–522

Lippold LK (1981) Monitoring female reproductive status in the douc langur at San Diego Zoo. Int Zoo Yearb 21:184–187

Marsh CW (1979) Comparative aspects of social organization in the Tana River red colobus, *Colobus badius rufomitratus*. Z Tierpsychol 51:337–362

Murray CM, Heintz MR, Lonsdorf EV, Parr LA, Santymire RM (2013) Validation of a field technique and characterization of fecal glucocorticoid metabolite analysis in wild chimpanzees (*Pan troglodytes*). Am J Primatol 75:57–64

Nagle CA, Denari JH (1983) The cebus monkey (*Cebus apella*). In: Hearn JP (ed) Reproduction in new world primates. Springer, Netherlands, pp 39–67

Purvis A, Webster A, Agapow PM, Jones K, Issac N (2003) Primate life histories and phylogeny. In: Kappeler PM, Pereira ME (eds) Primate life histories and socioecology. The University of Chicago Press, Chicago, pp 25–40

Qi X, Li B, Ji W (2008) Reproductive parameters of wild female *Rhinopithecus roxellana*. Am J Primatol 70:311–319

Ruggiero LF, Hayward GD, Squires JR (1994) Viability analysis in biological evaluations: concepts of population viability analysis, biological population, and ecological scale. Conserv Biol 8:364–372

Saj TL, Sicotte P (2005) Male takeover in *Colobus vellerosus* at Boabeng-Fiema monkey sanctuary, Central Ghana. Primates 46:211–214

Saj TL, Sicotte P (2013) Species profile for *Colobus vellerosus*. In: Butynski TM, Kingdon J, Kalina J (eds) Mammals of Africa, vol 2. Bloomsbury Publishing, London, p 109

Saj TL, Teichroeb JA, Sicotte P (2005) The population status of the ursine colobus (*Colobus vellerosus*) at Boabeng-Fiema, Ghana. In: Paterson JD, Wallis J (eds) Commensalism and conflict: the human primate interface. American Society of Primatologists, Norman, pp 350–375

Saj TL, Mather C, Sicotte P (2006) Traditional taboos in biological conservation: the case of *Colobus vellerosus* at the Boabeng-Fiema Monkey Sanctuary, Central Ghana. Soc Sci Inf 45:285–310

Saltzman W, Schultz-Darken NJ, Scheffler G, Wegner FH, Abbott DH (1994) Social and reproductive influences on plasma cortisol in female marmoset monkeys. Physiol Behav 56:801–810

Smith RJ, Jungers WL (1997) Body mass in comparative primatology. J Hum Evol 32:523–559

Sommer V, Srivastava A, Borries C (1992) Cycles, sexuality, and conception in free-ranging langurs (*Presbytis entellus*). Am J Primatol 28:1–27

Starin ED (2001) Patterns of inbreeding avoidance in Temminck's red colobus. Behaviour 138:453–465

Stearns SC (2000) Life history evolution: successes, limitations, and prospects. Naturwissenschaften 87:476–486

Strier KB, Ziegler TE (1997) Behavioral and endocrine characteristics of the reproductive cycle in wild muriqui monkeys, *Brachyteles arachnoides*. Am J Primatol 42:299–310

Strier KB, Lynch JW, Ziegler TE (2003) Hormonal changes during the mating and conception seasons of wild northern muriquis (*Brachyteles arachnoides hypoxanthus*). Am J Primatol 61:85–99

Struhsaker TT, Leland L (1985) Infanticide in a patrilineal society of red colobus monkeys. Z Tierpsychol 69:89–132

Struhsaker TT, Pope TR (1991) Mating system and reproductive success: a comparison of two African forest monkeys (*Colobus badius* and *Cercopithecus ascanius*). Behaviour 117:182–205

Teichroeb JA, Sicotte P (2008) Social correlates of fecal testosterone in male ursine colobus monkeys (*Colobus vellerosus*): the effect of male reproductive competition in aseasonal breeders. Horm Behav 54:417–423

The IUCN Red List of Threatened Species. Version 2015-4. http://www.iucnredlist.org. Accessed 20 April 2016

van Schaik CP, Deaner RO (2002) Life history and cognitive evolution in primates. In: de Waal FBM, Tyack PL (eds) Animal social complexity. Harvard University Press, Cambridge

Wich SA, Steenbeek R, Sterck EH, Korstjens AH, Willems EP, Van Schaik CP (2007) Demography and life history of *Thomas langurs* (*Presbytis thomasi*). Am J Primatol 69:641–651

Wong SN, Saj TL, Sicotte P (2006) Comparison of habitat quality and diet of *Colobus vellerosus* in forest fragments in Ghana. Primates 47:365–373

Ziegler TE, Wittwer DJ (2005) Fecal steroid research in the field and laboratory: improved methods for storage, transport, processing, and analysis. Am J Primatol 67:159–174

Ziegler TE, Savage A, Scheffler G, Snowdon CT (1987) The endocrinology of puberty and reproductive functioning in female cotton-top taarins (*Saguinus oedipus*) under varying social conditions. Biol Reprod 37:618–627

Ziegler TE, Scheffler G, Wittwer DJ, Schultz-Darken NJ, Snowdon CT, Abbott DH (1996) Metabolism of reproductive steroids during the ovarian cycle in two species of callitrichids, *Saguinus oedipus* and *Callithrix jacchus*, and estimation of the ovulatory period from fecal steroids. Biol Reprod 54:91–99

Ziegler T, Hodges K, Winkler P, Heistermann M (2000) Hormonal correlates of reproductive seasonality in wild female *Hanuman langurs* (*Presbytis entellus*). Am J Primatol 51:119–134

Ziegler TE, Strier KB, Van Belle S (2009) The reproductive ecology of South American primates: Ecological adaptations in ovulation and conception. In: Garber PA, Estrada A, Bicca-Marques JC, Heymann EW, Strier KB (eds) South American Primates: comparative perspectives in the study of behaviour, ecology and conservation. Springer Science, Berlin, pp 191–210. doi:10.1007/978-0-387-78705-3_8