Reproductive behaviour, testis size and faecal androgen metabolite concentrations in the African lesser bushbaby

Juan Scheun[1,2,3], Nigel C. Bennett[2], Julia Nowack[4,5], Andre Ganswindt[1,2,3]

[1] Endocrine Research Laboratory, Department of Anatomy and Physiology, University of Pretoria, Onderstepoort 0110, South Africa.

[2] Department of Zoology and Entomology, University of Pretoria, Pretoria 0028, South Africa.

[3] National Zoological Gardens of South Africa, Pretoria, 0001, South Africa.

[4] Department of Animal Ecology and Conservation, Biocentre Grindel, University of Hamburg, 20146 Hamburg, Germany.

[5] School of Environmental & Rural Science, Zoology, University of New England, 2351 Armidale, NSW, Australia.

Corresponding author: J Scheun,Jscheun@zoology.up.ac.za

Short title: Reproductive endocrinology of male *Galago moholi*

Abstract

Periods of reproduction are linked to changes in male behaviour, physiology and physical parameters. Although high androgen concentrations hold numerous advantages, especially during reproductive periods, chronically elevated androgen concentrations over long periods may be costly and thus need to be regulated. As such seasonal breeders will display temporary elevated androgen concentrations, increased testis levels and mating behaviour only during important reproductive periods. We studied a captive as well as a free-ranging population of the polygamous strepsirrhine primate, the African lesser bushbaby (*Galago moholi*), to clarify the link between androgen concentration, reproductive behaviour and testis size and the importance of the two mating periods observed in the species. To monitor androgen patterns we used faecal sampling and quantification of faecal androgen metabolites (fAM). We additionally collected testicular measurements and behavioural data. *G. moholi* displayed a strong degree of reproductive seasonality, with maximum fAM concentration, testicular volume (TV), and behavioural activity focused on the mating periods. In contrast to

other studies, TV increased prior to fAM, with reproductive activity being initiated only when fAM concentrations reached high levels. Changes in TV and fAM concentrations were not significantly different between both mating periods. Based on the absence of a significant difference between mating seasons, it is likely that male *G. moholi* attempt to maximize their reproductive success by utilizing both mating periods equally. This study is the first to describe the reproductive endocrine pattern linked to physical changes and mating behaviour in any male galago species, increasing our understanding of the reproductive biology of nocturnal, polygamous primates.

Keywords: Reproduction; behaviour; androgens; testis volume; strepsirrhine primate

Introduction

Androgens play an important role in regulating many aspects of male reproduction, including the development of reproductive anatomy, spermatogenesis and the initiation of reproductive behaviour (Beehner *et al.,* 2006). An increase in androgen concentrations is often temporarily limited to respective developmental stages during adolescence or to periods of reproductive activity (Ostner, Kappeler & Heistermann 2008). A prolonged elevation of androgen concentrations might increase the risk of injury due to increasing male-male competition, lead to disproportional energy expenditure due to excessive home range monitoring and mate guarding, or result in the suppression of an individual's immune response, all factors subsequently reducing reproductive success (Wingfield, Lynn & Soma 2001). The possible cost-benefit trade-off of elevated androgen concentrations might well explain why androgen concentrations only increase during important life stages or crucial periods of reproductive activity (Goymann, Landys & Wingfield 2007). To avoid the cost of prolonged elevated androgen concentrations, seasonal breeding males will display elevated androgen concentrations only during defined reproductive periods to facilitate mating with numerous females (Wingfield*et al.,* 2001; Hirschenhauser & Oliveira 2006). As sperm production is an androgen-dependent function of the male testes, the increase in androgen concentrations

prior to and during reproductive periods result in increased sperm quantity and quality in seasonally mating species (Goeritz *et al.,* 2003). High rates of sperm production correlate with an increase in sperm-producing tissue, visible in the enlargement of the testes, which is assumed to be a reliable index of sperm competition (Harcourt, Purvis & Liles 1995). To understand better the often interlinked behavioural, physical and physiological changes associated with male reproductive activity, longitudinal endocrine monitoring can be a useful approach (Heistermann 2010). In this regard the use of faeces as a hormone matrix has been shown to be satisfactory for a wide range of species (Schwarzenberger 2007).

The African lesser bushbaby, *G. moholi,* is a small (~200 g) nocturnal strepsirhine primate found throughout southern Africa (Bearder 1987). According to behavioural studies, *G. moholi* is a non-gregarious species, with males and females having separate, but overlapping home ranges (Pullen, Bearder & Dixson 2000). As a result, the mating system has been defined as polygamous where males attempt to mate with various females found within their home range (Bearder 1987). Male mating behaviour, including mounting and intromission events, is elicited by female receptivity, which occurs during behavioural oestrous, lasting between one and four days (Lipschitz, Galpin & Meyer 2001; Scheun *et al.,* 2016). Two periods of reproductive activity have been observed in the species (May, September; Nekaris & Bearder 2007). Female gestation length varies from 123 to129 days and females can give birth to twins once or twice a year (Scheun et al. 2016). As the conception rate has been found to be higher in May than September, the latter has previously been described as a subsidiary mating event in females (Pullen *et al*., 2000; Scheun *et al.,* 2016). However, no attempt has been made to define the importance of both periods in male *G. moholi*.

In an attempt to better understand the reproductive endocrine patterns and behaviour in male members of the species, we studied captive and free-ranging populations of *G. moholi* and hypothesised that (1) an increase in testis size in *G. moholi* will be limited to periods of reproductive activity and coincide with an increase in androgen concentration. Furthermore

we expected that (2) males will use both mating periods equally, indicated by a non-significant difference in both androgen concentration and testis volume level between both reproductive periods.

Materials and methods

Study site and animals

We conducted the study between March and November 2013 at Ithumela Primate Sanctuary (Buffelsdrift Conservancy, Buffelsdrift, Pretoria, South Africa, 25°35'55.79"S, 28°19'30.82"E). In general, the study site has a hot, wet season from October to March and a cold, dry season from April to September. We studied seven captive males, each paired with a single adult female at the beginning of the study, at Ithumela Primate Sanctuary, as well as 14 males and 12 females from the surrounding wild, free-ranging population of Buffelsdrift Conservancy.

Pairs were housed in separate enclosures at Ithumela Primate Sanctuary. All individuals were in good body condition and sexually mature (> 8.5 months, Nekaris & Bearder 2007). We designed enclosures to allow easy separation of pairs during periods of sample collection. Captive individuals were fed a combination of fresh fruit, yoghurt and cat pellets, with water available *ad libitum*. To evaluate if findings from our captive study set-up are representative of wild *G. moholi*, we additionally collected faecal samples and body measurements from free-ranging males in the surrounding area. Sherman traps (7 x 7 x 30.5 cm, H. B. Sherman Traps, Florida, North America) and walk-in live traps, constructed by the researchers, were baited three times a week with honey, peanut butter and banana and placed throughout a 200 ha area at sunset. Traps were checked hourly until 5am to assess the presence of any individuals caught. Trapped individuals were handled for less than 15 min, in order to collect body measurements, before they were released at the site of capture. Free-ranging individuals were marked with subcutaneously injected passive identification transponders (ID11 Trovan, EURO I.D. Usling GmbH, Weilerswist, Germany) for individual identification. Respective data on captive and wild female *G. moholi* can be found in Scheun

et al., (2016). The entire study was performed with approval of the University of Pretoria Animal Use and Care Committee (Reference EC056-12).

Faecal sample and data collection

Captive G. moholi

We separated captive mating-pairs three times a week after they emerged from their sleeping boxes. As defecation did not always occur immediately following separation, individuals were left separated for a maximum of 30 min (collection success rate within 30 min: 100%). Cages were continuously monitored for fresh faecal samples and pairs reintroduced when samples were collected. Once a week captive individuals were removed from their sleeping boxes early in the morning (06h00-08h00) to measure body weight and length, as well as testis size and to identify female reproductive status (see below). We measured width and breath of the left testis in captive and free ranging males with the use of an electronic calliper. Males were handled without the use of general anaesthesia, and for no longer than 5 min before being placed back in their sleeping box. A total of 631 faecal samples (range: 87-94 samples per individual) were collected during the study period from the seven captive males.

Wild G. moholi

We collected faecal samples, as well as body measurements, including body weight, length and testis size whenever free-ranging individuals were trapped. In total 39 faecal samples were collected from 14 free-ranging males over the study period (range: 1-6 samples per individual; recapture rate: < 6%). Additionally we determined reproductive status of trapped females. Vaginal opening, which indicates oestrous in the species (Lipschitz 1996), was found in May (n=4) and September (n=5), respectively.

Behavioural observations

To assess the presence of both reproductive as well as aggressive behaviour, we performed nightly observations (20h00-04h00, *ad libitum* sampling, Altmann 1974) of captive individuals three times a week with the use of red filtered lights. We positioned cages to allow the

observation of all seven males simultaneously. During the study, reproductive behaviour occurred solely during end of May until beginning of June and mid- until end of September, aligning well with oestrous in wild subjects (as indicated above). We classified reproductive behaviour in captive individuals as high levels of pair-grooming, males continuously following females, vaginal sniffing and licking, grabbing and intense grooming of females, mounting and ultimately intromission, and used the behavioural data to define reproductive periods. Aggressive behaviour was classified as one individual chasing another, usually with high degrees of vocalization, culminating in grabbing, which may be accompanied by biting in extreme cases (see Lipschitz 1997; Lipschitz *et al.,* 2001).

Hormone extraction and analysis

Sample preparation

Individual faecal material was placed into 1.5 ml microcentrifuge tubes and stored at – 20 °C. Frozen samples were then lyophilized, pulverized and sieved through a thin mesh to remove any remaining fibrous material (Fieß, Heistermann & Hodges 1999). Following this, we extracted 50 – 55 mg of faecal powder by vortexing for 15 minutes with 1.5 ml of 80 % ethanol. Subsequently, we centrifuged each sample for 10 min at 1500 *g*, and supernatants were transferred into a new microcentrifuge tube and stored at – 20 °C until further analysis.

Biological validation of the enzyme immunoassay

The enzyme immunoassay (EIA) used during the study (Epiandrosterone) was biologically validated by demonstrating its ability to distinguish between male maturation stages in terms of immunoreactive faecal androgen metabolite (fAM) concentrations of captive male individuals. From the 27th of March to the 29th of April 2013 a total of 67 faecal samples were collected from sub adult males individually housed at Ithumela Primate Sanctuary. Faecal sample collection commenced at 19h00 for sub adult males, with faecal samples collected within 15 min of each defecation event. Date-matched samples from sub adult and adult males were used to compare fAM concentrations between age categories. This time period was

chosen to avoid the effect of mating season reproductive activity and increases in fAM. For the biological validation process we compared fAM concentrations of non-reproductively active adult (> 2 years; n=5 individuals; n=13-14 samples per animal) and sub adult males (< 6 months; n = 5 individuals; n=6-14 samples per animal). A two sample t-test indicated that fAM concentrations of adult males (median: 7.48 µg/g DW, range: 5.15-15.12 µg/g DW) were significantly higher than that of sub adult males (median: 0.96 µg/g DW, range: 0.62-1.70 µg/g DW; $t_{(112)} = 9.45$, $p < 0.001$). The Epiandrosterone assay is therefore able to discriminate between fAM concentrations of different maturation stages of male *G. moholi*.

Enzyme immunoassay analysis

We measured faecal extract for immunoreactive androgen metabolites using an EIA for 5α-Androstan-3β-ol-17-on (Epiandrosterone). Details of the EIA, including cross-reactivities of the antibody used, are described by Palme and Möstl (1993). The sensitivity of the assay was 7.5 ng/g faecal dry weight. Serial dilutions (1:500, 1:750, 1:1000, 1:1500, 1:2000) of two male and one female extracted faecal sample gave displacement curves that were parallel to the respective standard curve (correlation between the optical density of the standard and respective sample curves, n=3, was r=0.99 in all cases). Fifty microliters of each extract was used for the EIA analysis. The intra- and inter-assay coefficients of variation, determined by repeated measurements of high and low value quality controls (Epiandrosterone, Steraloids, Newport, USA), ranged between 8.7 % and 12.0 %. The assay was performed on microtiter plates as described by Ganswindt *et al.,* (2012) and conducted at the Endocrine Research Laboratory at the Faculty of Veterinary Science, University of Pretoria.

Data analysis

We calculated testicular volume (TV) from a linear dimension with a formula for an ellipsoid, shown to be robust for the calculation of testicular volume (Bercovitch 1989):

$$\frac{\pi w^2 L}{6}$$

Where *w* is the width and L the length of a single testis measured.

Individual testis volume (TV) baseline values were obtained by calculating median scores for each male, while periods of elevated TV were defined as the occurrence of two or more consecutive samples exceeding the respective baseline value. We calculated individual baseline fAM concentrations using an iterative process described by Brown *et al.,* (1999). All individual fAM concentrations exceeding the mean plus 2 standard deviation (SD) were removed, the average recalculated, and the process repeated until no values exceeded mean plus 2 SD, yielding the baseline value. Periods of elevated fAM concentrations were defined as the occurrence of two or more consecutive samples exceeding the individual baseline value.

All values are reported as mean ± SD. Analytical statistics were performed using R, v 3.2.0 (R Development Core Team 2013). We adjusted fAM concentration data to account for the 12-14 hour gut passage time delay (i.e hormone production to hormone metabolite excretion) in *G. moholi* (Caton, Lawes & Cunningham 2000; Scheun *et al.,* 2015). We ran Shapiro-Wilk tests to test for normality for all data sets. For testing biological validity of the EIA, respective hormone data have been log-transformed to assure normal distribution prior to a two sample t-test analysis. The duration of the study (March-October) was divided into four periods, based on the presence or absence of reproductive activity as described by previous research (Doyle, Andersson & Bearder 1971) as well as our own observations. The two months of May and September were classified as reproductive periods (R_1 and R_2, respectively). In addition, the periods March-April, June-Aug and October were categorized as non-reproductive periods (NR_1 and NR_2). In the absence of a significant difference between both reproductive seasons, as well as between both non-reproductive seasons, for TV (t-test; R_1 - R_2: $t = -1.12$, $p = 0.30$; $NR_1 – NR_2$: $t = -0.22$, $p = 0.83$) as well as for fAM concentration (t-test; R_1 - R_2: $t = -2.21$; $p = 0.07$; $NR_1 – NR_2$: $t = -0.56$; $p = 0.60$), we pooled the data into a single reproductive and non-reproductive period. We used a Pearson product-moment correlation to test the relationship between fAM concentration and testis volume. All tests were two-tailed, with the α-level of significance set at 0.05.

213 ## Results

214 **Faecal androgen metabolite concentrations and testicular volumes**

215 All seven captive males showed a similar pattern in fAM and TV alteration during the study
216 period (Fig 1). Androgen concentrations remained at individual baseline levels during both of
217 the non-reproductive periods. During the first reproductive period in May, increases in
218 individual fAM concentrations ranged from 190-470 % (mean: 282 ± 97 %), while fAM
219 concentrations increased by 117-527 % (mean: 312 ± 143 %) during the second reproductive
220 period in September (Tab 1). Similarly, TV remained at individual baseline for much of the
221 study period, increasing by 111-155 % (mean: 143 ± 15.0 %) and 130-153 % (mean: 142.34
222 ± 7.62 %) during the May and September reproductive periods, respectively (Tab 1). We found
223 a significant correlation between fAM concentration and TV patterns at the population (Fig. 1;
224 r^2 = 0.64, p = 0.025) as well as individual level (r^2-range: 0.54-0.69, $p < 0.05$) for all of our
225 seven captive males.

226 In the captive setup a temporal relationship existed between fAM concentration, TV
227 and occurrence of male reproductive behaviour (Fig 1). In all cases (May: N = 7/7; September:
228 N = 7/7), increase in TV preceded elevated fAM concentrations by 3-15 days. Similarly, the
229 majority of the individuals (May: N = 6/7; September: N = 5/7) showed an increase in fAM
230 concentration 3-9 days prior to the initiation of mating activity. Both fAM concentration and TV
231 remained elevated for a period of 3-11 days following the conclusion of mating activity.

232 Four of the seven captive males impregnated their paired female during the May
233 reproductive period (1st mating event, Tab. 1). The mean increase of fAM concentration for
234 these four males during the May mating period was comparatively lower than the increases
235 observed during the September mating period (Tab. 1; 216.8 ± 21.8% vs. 389.8 ± 131.6%
236 respectively). One of the seven captive males only impregnated his paired female during the
237 September mating period (Tab. 1). Similarly, the increase in fAM concentration was
238 comparatively lower during the September mating period, when impregnation occurred, than
239 the initial mating period in May (Tab 1; 470% vs. 117% respectively).

A significant difference in mean fAM concentrations was found between reproductive and non-reproductive periods (Paired t-test; NR: 10.92 ± 4.32 µg/g dry weight; R: 27.25 ± 6.66 µg/g dry weight, t = -9.99, df = 12, p < 0.01, Fig. 2). Similarly, we found a significant difference in mean TV between reproductive and non-reproductive periods (Paired t-test; NR: 0.77 ± 0.06 m^3; R: 1.05 ± 0.11 cm^3, t = 9.61, df = 12, p < 0.01; Fig. 3).

Although we were unable to use analytical statistics, the fAM and TV data collected from free-ranging animals supported the respective patterns revealed for the captive males. We found fAM concentrations to be on average 60-270 % higher during reproductive periods (May: 21.3 ± 4.6 µg/g DW, N = 5; September: 12.0 ± 4.0.5 µg/g DW, N = 5) compared to the non-reproductive periods (March-April: 7.4 ± 1.9 µg/g DW, N = 13; June-August: 5.8 ± 2.1 µg/g DW, N = 16). Similarly, we found TV to increase by about 25-38 % during reproductive periods (May: 1.1 ± 0.1 cm^3, N = 5; September: 1.0 ± 0.1 cm^3, N = 5) compared to the non-reproductive periods (March-April: 0.8 ± 0.1 cm^3, N = 13; June-August: 0.8 ± 0.1cm^3, N = 16).

Behavioural observations in captivity

In May, male *G. moholi* showed an increase in reproductive activity. Two weeks prior to the mating event males increased pair grooming and followed females more often than observed before. One week prior to the mating event, male *G. moholi* were observed frequently sniffing and licking female genitalia, while attempting unsuccessful mounts, which resulted in aggressive interactions between pairs. Observed vaginal opening (lasting 4.71 ± 0.49 days, Scheun *et al.,* 2016) allowed all seven males to mate with their paired females at the end of May. Following vaginal cornification, males attempted mounting bouts for another night, which resulted in aggressive interactions with females often chasing and biting males. No male reproductive behaviour was recorded between June and August 2013. During the second mating period in September, three of the seven males, all paired with non-pregnant females, showed similar reproductive activity, as observed in May, two and a half weeks prior to the mating event. Male mating activity coincided with female behavioural oestrous and lasted 4.67 ± 0.58 days. The four males paired with then already pregnant females also attempted to

initiate reproductive activity during this period, but were unsuccessful in all cases, with frequent aggressive interactions and high levels of vocalization occurring.

Discussion

The androgen secretion and testicular volume patterns found in the present study support the idea that *G. moholi* is a seasonal breeder, with two distinct periods during which the androgen concentrations and TV increase. Furthermore, we observed a significant correlation between androgen secretion and changes in testicular volumes, with an increase in both parameters occurring prior to mating activity.

The reproductively-related pattern in testicular volume found in *G. moholi* is comparable to that found for a range of primate species (*reviewed by* Harcourt *et al.,* 1995). Polygamous males from multi-male societies had significantly larger testis sizes during their reproductive periods. In line with our hypothesis, testis volumes increased to peak levels during mating periods. The increase in testis size during reproductive periods is an important factor in a multi-male mating scenario, presumably supporting sperm competition. The relative number of sperm produced can be a primary determinant of success in polygamous species with analogous competition between males and can therefore fundamentally enhance reproductive success (Parker 1990). Additionally, as it is often difficult to observe mating activity in nocturnal species, an increase in testis size may be a robust indicator of reproductive state (mating vs non-mating) in nocturnal, seasonal, polygamous breeders. This is supported by findings of testis volume increases prior to and during female oestrous and mating activity in a number of nocturnal mouse lemur species (see Wrogemann, Radespiel & Zimmermann 2001; Schwab 2000).

The role of androgens in the development of secondary sexual characteristics has been documented previously (Beehner *et al.,* 2006). Dixson (1976) and Bullard (1984) highlighted the importance of androgens in the development of secondary sexual characteristics in the greater galago (*G. crassicaudatus crassicaudatus*), which included the

development of the sternal cutaneous gland (used in scent marking) and penile spines. Similarly, we found a significant correlation between androgen concentrations and TV levels. However, unlike most studies, our data show that TV increased prior to a temporal elevation in fAM concentrations and not *vice versa.* Although this appears relevant and of importance, the baseline values calculated for both TV and androgen concentration are mathematical discrimination tools rather than fixed values based on biological significance. It is thus possible that increased androgen concentrations, while still below our defined baseline level, could be sufficient to initiate testicular growth and heightened spermatogenesis. This has been confirmed previously by McLachlan *et al.,* (1996) who showed that testosterone concentrations at 20% of the optimum sperm producing levels would be sufficient in initiating spermatogenesis in males.

As hypothesised, androgen concentrations increased to above baseline levels during periods of reproductive activity alone. FAM concentrations, like TV, remained at comparatively low levels during both non-reproductive periods, showing maximum increases during the reproductive periods, coinciding with frequent occurrence of pair interactions such as grooming and following. Such a seasonal pattern in androgen secretion has been demonstrated for a range of seasonal breeding species, including muriquis (*Brachyteles arachnoides*, Strier, Ziegler & Wittwer 1999), tufted capuchin monkeys (*Cebus apella,* Lynch, Ziegler & Strier 2002), and the strepsirrhine primates, the grey mouse lemur (*Microcebus murinus*, Aujard & Perret 1998) and the redfronted brown lemur (*Eulemur rufifrons*, Ostner *et al.,* 2008). The pattern of androgen secretion and testis volume found in *G. moholi* changes support the challenge hypothesis (Wingfield *et al.,* 1990), where males within a multi-male breeding system are proposed to show aggression and androgen increases to the maximum physiological limit only during periods of reproductive activity.

Despite the fact that low androgen concentrations may be sufficient in initiating testicular growth, the activation of male mating activity may require comparatively higher androgen concentrations. From the findings of this study it is clear that a distinct elevation in

androgen concentration accompanies the start of male mating activity within the species. These findings support previous data on the importance of androgens in controlling reproductive activity (Wallen 2005). Despite the apparent need for elevated androgen concentration, in order to activate mating behaviour and the development of secondary sexual characteristics, our results indicate that fAM concentrations should not exceed moderate levels to ensure optimal reproductive success (Tab 1). Male *G. moholi*, who successfully managed to impregnate their females during either one of the mating periods, did so when fAM concentrations were moderately increased (~ 50% of maximum individual fAM concentrations). Elevated androgen concentration above a certain threshold (> 50 %) may thus hinder the ability of males to successfully breed as a result of increased aggression and territorial defence (Wingfield *et al.,* 2001). Additional factors may contribute to a decrease in reproductive success in such cases, including negative pair interactions (Marler 1976), irregular ovarian cyclicity (Schwartz 2000) and suboptimal housing conditions (Wielebnowski *et al.,* 2002).

Although the two reproductive periods are so far defined as primary and subsidiary periods in the literature (Pullen *et al.,* 2000), the absence of distinct differences in the fAM and TV pattern, as well as the numerous observed mating attempts from males during both mating periods, rather indicate that the two reproductive periods are of equal importance to male *G. moholi.* Finally, an interesting finding of our study relates to the increase in androgen concentrations and TV of males paired with pregnant, non-receptive females during the September mating period. The data indicate that male reproductive activity is independent of the receptiveness of their paired female partner. This is of special interest as it confirms that *G. moholi* does not have a monogamous, but rather a polygamous mating system.

Acknowledgements

The research conducted was accomplished with the financial assistance of the Deutsche Forschungsgemeinschaft (DFG; DA 1031/3-1/2) and the DST-NRF SarchI Chair of Mammal Behavioural Ecology and Physiology from the Department of Science and Technology and the National Research Foundation, South Africa.

References

Altmann, J. (1974) Observational study of behavior: sampling methods. *Behavior,* **49,** 227

Aujard, F. & M. Perret (1998) Age-related effects on reproductive function and sexual competition in the male prosimian primate, *Microcebus murinus*. *Physiol Behav,* **64,** 513.

Bearder, S. K., (1987) Lorises, bushbabies, and tarsiers: diverse societies in solitary foragers. In: *Primate societies*: 11. B. B. Smuts, D. L. Cheney, R. M. Seyfarth, R. W. Wrangham & T. T. Struhsaker (Eds.). University of Chicago Press, Chicago.

Bearder, S. K. & R. D. Martin, (1979) The social organization of a nocturnal primate revealed by radio tracking. In: *A Handbook on biotelemetry and radio tracking*: 633. C. J. Amlaner Jr & W. MacDonald (Eds.). Pergamon, Oxford.

Beehner, J. C., T. J. Bergman, D. L. Cheney, R. M. Seyfarth & P. L. Whitten (2006) Testosterone predicts future dominance rank and mating activity among male chacma baboons. *Behav Ecol Sociobiol,* **59,** 469.

Brown, J., D. Schmitt, A. Bellem, L. Graham & J. Lehnher (1999) Hormone secretion in the Asian elephant (*Elephas maximus*): characterization of ovulatory and anovulatory luteinizing hormone surges. *Biol Reprod,* **61,** 1294

Bullard, S. C. (1984) Effects of Testosterone upon the Chest-Rubbing Behavior of *Galago crassicaudatus umbrosus*. *Folia Primatol,* **42,** 70.

Caton, J. M., M. Lawes & C. Cunningham (2000) Digestive strategy of the south-east African lesser bushbaby, *Galago moholi*. *Comp Biochem Physiol A,* **127,** 39.

Dixson, A. F. (1976) Effects of testosterone on the sternal cutaneous glands and genitalia of the male greater galago (*Galago crassicaudatus crassicaudatus*). *Folia Primatol,* **26,** 207.

Doyle, G. A., A. Andersson & S. K. Bearder (1971) Reproduction in the lesser bushbaby (*Galago senegalensis moholi*) under semi-natural conditions. *Folia Primatol,* **14,** 15.

Fieß, M., M. Heistermann & J. K. Hodges (1999) Patterns of urinary and fecal steroid excretion during the ovarian cycle and pregnancy in the African elephant (*Loxodonta africana*). *Gen Comp Endocrinol,* **115,** 76.

Ganswindt, A., A. S. W. Tordiffe, E. Stam, M. J. Howitt & F. Jori (2012) Determining adrenocortical activity as a measure of stress in African bufallo (*Syncerus caffer*) based on faecal analysis. *Afr Zool,* **47,** 261.

Goeritz, F., M. Quest, A. Wagener, M. Fassbender, A. Broich, T. B. Hildebrandt, R. R. Hofmann & S. Blottner (2003) Seasonal timing of sperm production in roe deer: interrelationship among changes in ejaculate parameters, morphology and function of testis and accessory glands. *Theriogenology,* **59,** 1487.

384 Goymann, W., M. M. Landys & J. C. Wingfield (2007) Distinguishing seasonal androgen
385 responses from male–male androgen responsiveness—revisiting the Challenge
386 Hypothesis. *Horm Behav,* **51,** 463.
387 Harcourt, A. H., A. Purvis & L. Liles (1995) Sperm competition: mating system, not breeding
388 season, affects testes size of primates. *Funct Ecol,* **9,** 468.
389 Heistermann, M. (2010) Non-invasive monitoring of endocrine status in laboratory primates:
390 methods, guidelines and applications. *Adv Sci Res,* **5,** 1.
391 Hirschenhauser, K. & R. F. Oliveira (2006) Social modulation of androgens in male
392 vertebrates: meta-analyses of the challenge hypothesis. *Anim Behav,* **71,** 265.
393 Lipschitz, D. L. (1997) Effects of estradiol-17B and progesterone on mating behaviour in
394 female lesser bushbabies (*Galago moholi*). *Horm Behav,* **32,** 73.
395 Lipschitz, D. L., J. S. Galpin & D. Meyer (2001) Reproductive behavioral changes during the
396 ovarian cycle of lesser bushbabies (*Galago moholi*) in captivity. *Am J Primatol,* **55,**
397 101.
398 Lynch, J. W., T. E. Ziegler & K. B. Strier (2002) Individual and seasonal variation in fecal
399 testosterone and cortisol levels of wild male tufted capuchin monkeys, *Cebus apella*
400 *nigritus*. *Horm Behav,* **41,** 275.
401 Marler, P. (1976) On animal aggression: The roles of strangeness and familiarity. *Am Psychol,*
402 **31,** 239.
403 McLachlan, R., N. Wreford, L. O'Donnell, D. De Kretser & D. Robertson (1996) The endocrine
404 regulation of spermatogenesis: independent roles for testosterone and FSH. *J*
405 *Endocrinol,* **148,** 1.
406 Nekaris, A. & S. K. Bearder, (2007) The lorisiform primates of Asia and mainland Africa:
407 diversity shrouded in darkness. In: *Primates in perspective*: 24. C. J. Campbell, A.
408 Fuentes, K. C. Mackinnon, M. Panger & S. K. Bearder (Eds.). Oxford University Press,
409 New York.
410 Ostner, J., P. Kappeler & M. Heistermann (2008) Androgen and glucocorticoid levels reflect
411 seasonally occurring social challenges in male redfronted lemurs (*Eulemur fulvus*
412 *rufus*). *Behav Ecol Sociobiol,* **62,** 627.
413 Palme, R. & E. Möstl (1993) Biotin-streptavidin enzyme immunoassay for the determination of
414 oestrogens and androgens in boar faeces. *Adv Steroid Anal,* **93,** 111.
415 Parker, G. A. (1990) Sperm competition games: sneaks and extra-pair copulations. *Proc R*
416 *Soc Lond B: Biol Sci,* **242,** 127.
417 Pullen, S. L., S. K. Bearder & A. F. Dixson (2000) Preliminary observations on sexual behavior
418 and the mating system in free-ranging lesser galagos (*Galago moholi*). *Am J Primatol,*
419 **51,** 79.

R Development Core Team, 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Scheun, J., N. Bennett, A. Ganswindt & J. Nowack (2015) The hustle and bustle of city life: monitoring the effects of urbanisation in the African lesser bushbaby. *Sci Nat,* **102,** 1.

Scheun, J., J. Nowack, N. Bennett & A. Ganswindt (2016) Female reproductive activity and its endocrine correlates in the African lesser bushbaby, *Galago moholi*. *J Comp Physiol B,* **186,** 255.

Schwab, D. (2000) A preliminary study of spatial distribution and mating system of pygmy mouse lemurs (*Microcebus myoxinus*). *Am J Primatol,* **51,** 41.

Schwartz, N. B., (2000) Neuroendocrine regulation of reproductive cyclicity. In: *Neuroendocrinology in physiology and medicine*: 135. P. M. Conn & M. Freeman (Eds.). Humana Press.

Schwarzenberger, F. (2007) The many uses of non-invasive faecal steroid monitoring in zoo and wildlife species. *Int ZooYearb,* **41,** 52.

Strier, K. B., T. E. Ziegler & D. J. Wittwer (1999) Seasonal and social correlates of fecal testosterone and cortisol levels in wild male muriquis (*Brachyteles arachnoides*). *Horm Behav,* **35,** 125.

Wallen, K. (2005) Hormonal influences on sexually differentiated behavior in nonhuman primates. *Front Neuroendocrinol,* **26,** 7.

Wielebnowski, N. C., K. Ziegler, D. E. Wildt, J. Lukas & J. L. Brown (2002) Impact of social management on reproductive, adrenal and behavioural activity in the cheetah (*Acinonyx jubatus*). *Anim Conserv,* **5,** 291.

Wingfield, J. C., R. E. Hegner, A. M. Dufty Jr & G. F. Ball (1990) The" challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am Nat,* **136,** 829.

Wingfield, J. C., S. Lynn & K. K. Soma (2001) Avoiding the 'costs' of testosterone: ecological bases of hormone-behavior interactions. *Brain, Behav Evol,* **57,** 239.

Wrogemann, D., U. Radespiel & E. Zimmermann (2001) Comparison of reproductive characteristics and changes in body weight between captive populations of rufous and gray mouse lemurs. *Int J Primatol,* **22,** 91.

Table legends

Table 1. Individual male baseline values for faecal androgen metabolite (fAM) concentration and testis volume (TV) for the seven study males. Additionally, the percentage increase in fAM concentration and TV levels during both periods of reproductive activity are shown. The timing of conception for the females housed with the study males are indicated.

	Faecal androgen metabolites (fAM)			**Testis volume (TV)**			
	Baseline (µg/g DW)	1st Reproductive period (% Increase)	2nd Reproductive period (% Increase)	Baseline (cm^3)	1st Reproductive period (% Increase)	2nd Reproductive period (% Increase)	Conception date (May or September)
Male 1	9.94	470	117	0.75	155	149	September
Male 2	13.58	241	527	0.81	153	140	May
Male 3	15.87	227	297	0.9	119	142	May
Male 4	25.46	324	286	0.77	149	130	N/A
Male 5	11.34	317	225	0.88	124	153	N/A
Male 6	14.20	191	476	0.93	145	148	May
Male 7	17.70	208	259	0.84	153	144	May
Population	15 ± 5	282 ± 97	312 ± 143	0.84	143 ± 15.0	142.34 ± 7.62	

Figure legends

Figure 1. Longitudinal profile of faecal androgen metabolite (fAM) concentration and testis volume (TV) derived from all seven male *G. moholi* monitored over 8 months. Grey circles represent overall mean fAM concentrations, while open circles represent overall mean TV (error bars represent respective standard deviations). The grey and black lines represent overall population baseline fAM concentration and testis volume levels. Grey blocks indicate the reproductive periods during the study period.

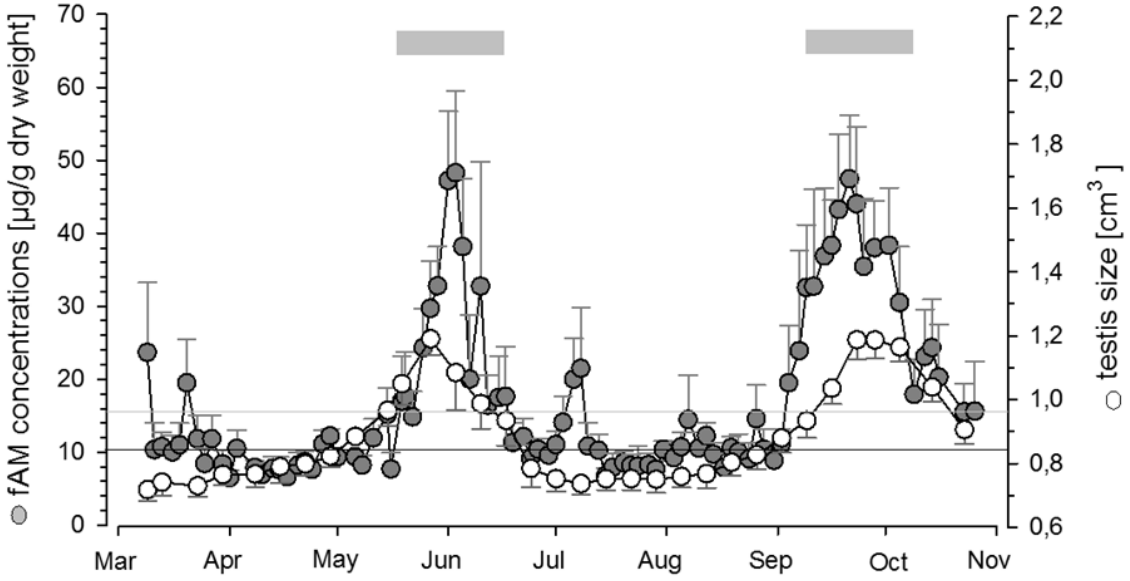
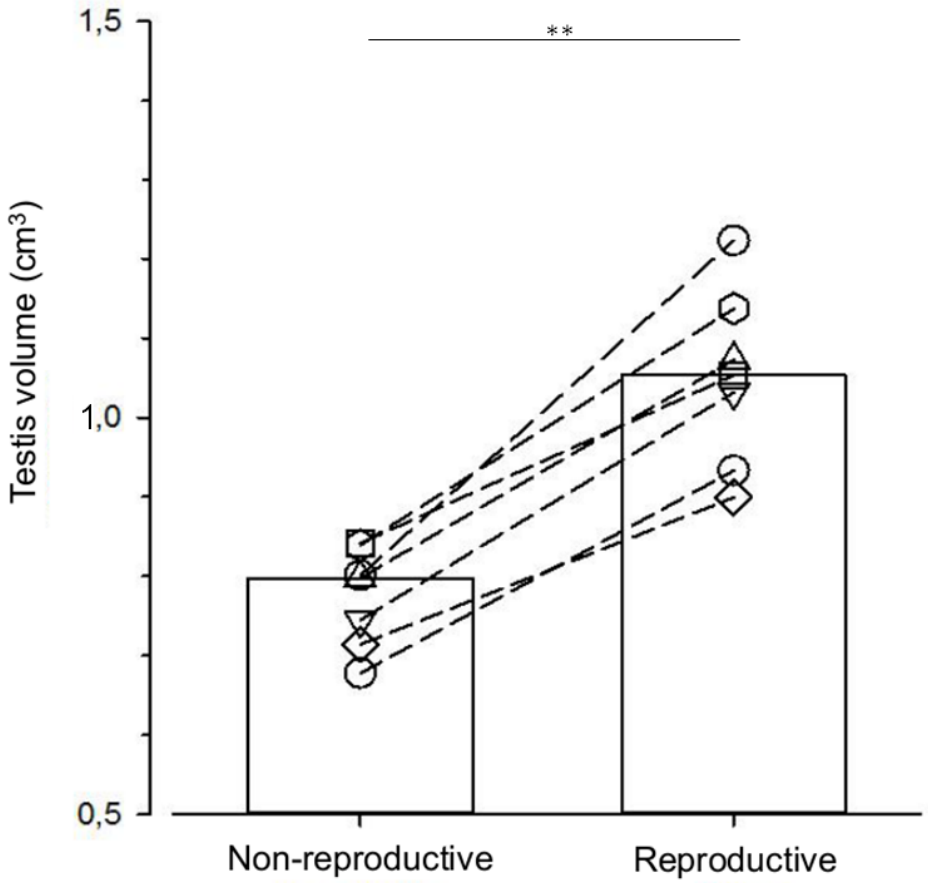
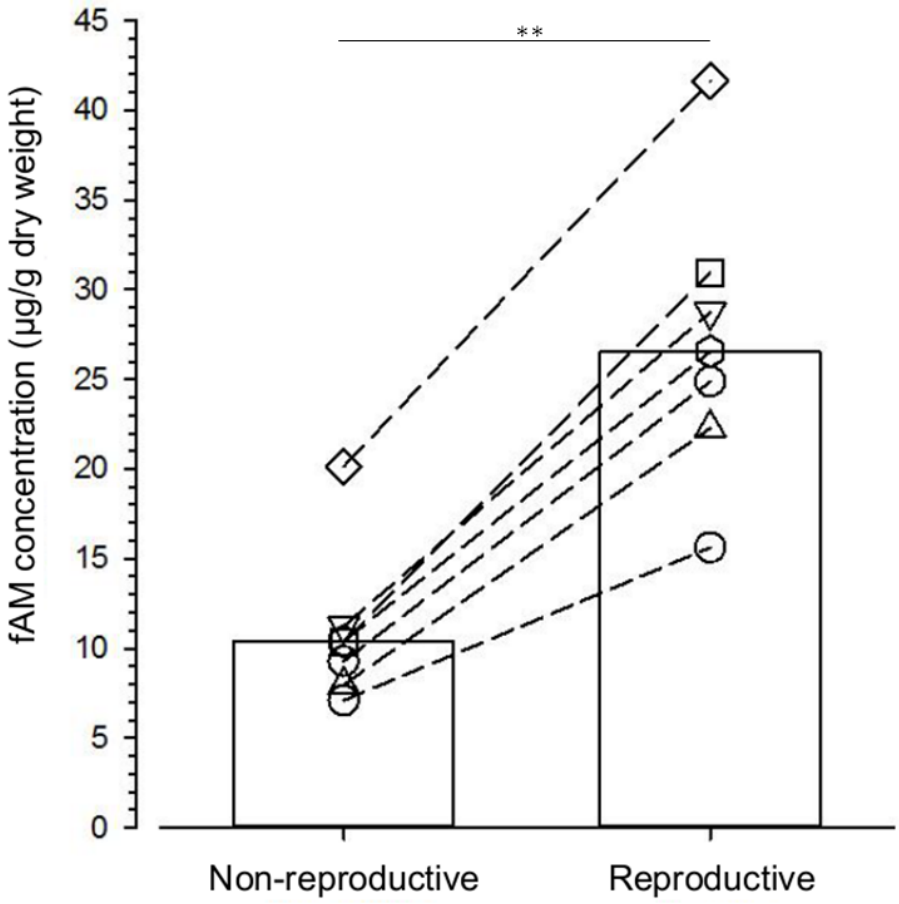


Figure 2. Comparison of individual mean faecal androgen metabolite (fAM) concentrations during non-reproductive and reproductive periods in male *G. moholi*. The seven symbols represent the respective mean fAM concentration for each male. A statistically significant difference between periods was found ($p < 0.01$)



469 **Figure 3**. Comparison of individual mean testicular volume (TV) levels during non-
470 reproductive and reproductive periods in male *G. moholi*. The seven symbols represent the
471 respective mean TV level for each male. A statistically significant difference between periods
472 were found ($p < 0.01$)



473