Unique Lemur Traits: Proximate and Ultimate Perspectives

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

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Abstract

Members of the lemur clade are known to exhibit a number of traits that are unusual in the context of living primates. Here, I investigate the basis of three of these traits: sexual size monomorphism, female dominance over males, and rapid rates of growth and attainment of adult body mass. My research focuses on multiple lemur species from the current and historic colony residing at the Duke Lemur Center in Durham, NC. Using long-term morphological data on 18 lemur species, I determine that rapid growth and early attainment of adult body mass do not constrain the development of sexual size dimorphism in lemurs. Next, I conduct behavioural observations and hair hormone analysis on ring-tailed lemurs (Lemur catta), revealing that receiving aggression leads to chronic stress in males and females and that female dominance and high rates female of aggression in females are potentially mediated by sex steroid hormone concentrations. Finally, using growth records and hair hormone analysis in Coquerel’s sifakas (Propithecus coquereli), I identify a negative relationship between chronic stress and relative body mass in early life, and demonstrate a potential carryover effect of low body
mass in early life on relative body mass in adulthood. Uncovering the proximate and ultimate causes of these unique lemur traits can advance our understanding of the factors underlying trait variation in primates and other mammals.
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This dissertation is dedicated to Chelsea and Benny Tennenhouse.
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Chapter 1
Introduction

1.1 Theoretical Basis of Common Primate Traits

It is common among animals for members of one sex to compete more intensely over access to mates and develop more extreme secondary sexual traits than the other sex. Such traits are products of both intrasexual and intersexual selection (Darwin, 1871). Through experiments on fruit flies, Bateman (1948) first showed that these differences between males and females in competition over mates stemmed from the fact that mating with multiple females constitutes a reproductive advantage for males, whereas additional mates do not translate to increased reproductive success for females. Males are therefore often driven to compete over access to a limited number of potential mates, and competition triggers the evolution of physical and behavioural traits that confer competitive advantages in males (Clutton-Brock, 2007; Emlen and Oring, 1977). These traits may be under simultaneous selection via female preference, which further promotes the evolution of traits that demonstrate male quality and will increase offspring fitness (Andersson and Iwasa, 1996; Arnqvist and Rowe, 2005). As a result of these strong selective pressures operating on males, sex differences in body size, presence and size of weapons such as canine teeth, and competitive behaviour have evolved in many species.
Trivers (1972) elaborated on the theoretical basis for the patterns identified by Bateman by emphasizing the different degrees to which males and females invest in their offspring. At the most basic level, eggs are more costly to produce than sperm, and these sex differences in the cost of producing gametes alone are enough to induce more intense male-male competition (Parker et al., 1972). Gestation constitutes an additional energetic cost for females, although mammals typically buffer this cost by building up fat reserves (Gittleman, 1988). However, lactation has been shown to be the most energetically expensive part of parental investment for female mammals (Clutton-Brock et al., 1989; Gittleman, 1988). By contrast, male parental care is rare among mammals (Kleiman and Malcolm, 1981). These differences in parental investment between males and females explain why female reproduction is more constrained than that of males in mammals. The primate order represents an extreme example of this greater maternal investment in offspring (Drea, 2005). This can be attributed to the fact that primate females have long gestation periods and produce highly encephalized offspring that have prolonged lactation and dependence (Harvey et al., 1987). Due to these heavy costs associated with reproduction, primate females produce markedly fewer offspring per litter given their body size than other mammals (Charnov and Berrigan, 1993). These factors underlie the slow life histories that are characteristic of the primate order, and elucidate other key primate traits. For example, in concert with slow growth, primates exhibit an extended juvenile period (Pereira and Fairbanks, 1993).
1.2 Unique Lemur Traits

Members of the Lemuriformes clade have been evolving in isolation on the island of Madagascar since their original colonization in the Eocene (Yoder et al., 1996). It is therefore not surprising that a number of unique traits that are uncommon in primates and other mammals are observed in lemurs (Wright, 1999). Researchers have been seeking proximate and ultimate explanations for these traits for decades (Dewar and Richard, 2007; Kappeler and Fichtel, 2015; Wright, 1999). One such trait is widespread female dominance over males (Eichmueller et al., 2013), which is in stark contrast to the typical mammalian pattern whereby greater intrasexual competition in males promotes the evolution of traits that confer male dominance over females (French et al., 2013). In contrast to most primate species, in which variation in the degree of sexual size dimorphism can usually be explained by the strength of intrasexual selection operating in a particular species, many lemur species are sexually monomorphic despite often having polygynous mating systems (Kappeler, 1990). Additionally, while haplorhine primates generally have slow rates of infant and juvenile growth compared to other mammals, lemurs experience comparatively rapid growth (Vinicius and Mumby, 2013). This dissertation investigates these puzzling lemur traits from novel perspectives by implementing comparative analyses, using long-term data, and investigating their associations with lemur physiology.
1.3 Rationale for Research Methods

With ample data on many primate species becoming increasingly available, it is an ideal time to synthesize these data in order to gain new insights into evolutionary trends. Indeed, employing a comparative approach has led to many recent advances in primatology (e.g., Bronikowski et al., 2011; Griffin and Nunn, 2012; MacLean et al., 2013; Nunn et al., 2014; Sandel et al., 2011; Shultz et al., 2011). Comparative studies on strepsirhines are particularly warranted, as long-term data on 27 strepsirhine species have recently been made publicly available (Zehr et al., 2014). Such cross-species comparative studies are an excellent tool, but require special statistical considerations to properly control for potential phylogenetic non-independence among species (Felsenstein, 1988). Therefore, current phylogenetic comparative methods are used in this dissertation to accurately synthesize long-term data on multiple lemur species (Chapter 2).

Studying physiological factors can provide a window into the proximate causes of behavioural and developmental traits, as well as their effects. Numerous primatological studies demonstrate that inter-individual (e.g., Cavigelli and Pereira, 2000; Higham et al., 2013; Maestripieri et al., 2009; Muller and Wrangham, 2004; Nunes et al., 2001; Onyango et al., 2008; Pappano and Beehner, 2014; Sapolsky, 1992), inter-sexual (Resko and Roselli, 1997; Sannen et al., 2003; von Engelhardt et al., 2000; Wallen, 2005; Drea, 2007), and even inter-species (Abbott et al., 2003; Fourie et al., 2015; Muehlenbein and Bribiescas, 2005; Sannen et al. 2003) variation in traits can be associated with corresponding differences in hormone
concentrations. Investigations into hormonal mechanisms can be highly complex, as many hormones and tissues may be involved in a single pathway and hormones operate on various timescales. In this dissertation, the simultaneous effects of multiple hormones, and their interactions, as well as the correspondence between the time period represented by a hormone sample and the period over which the trait of interest is examined, are all taken into consideration (Chapters 3 and 4).

1.4 Dissertation Goals and Overview

The overarching goal of this dissertation is to derive novel insights into some of the unique traits that have been observed in the lemur clade. Among strepsirhine primates, formal comparative studies of body mass ontogeny in lemurids (Leigh and Terranova, 1998), and galagos and lorises (O’Mara et al., 2012; Schaefer, 2012) have given insight into the contributions of ontogeny to trait variation, and have shown how social and ecological variables may influence growth. However, O’Mara et al. (2012) point out that many questions remain, some of which might be clarified by focusing on indriid and cheirogaleid species. With this research, I expand on these past studies by including new species in the analyses of lemur ontogeny, and employing a comparative approach to understand how lemur growth has influenced lemur traits such as sexual size monomorphism (Chapter 2). Furthermore, by examining hormone profiles in a large sample of lemurs, this research helps to clarify the physiological basis for female dominance over males and intrasexual social relationships in lemurs (Chapter 3). Finally, this research adds a new
perspective to the study of morphological ontogeny by identifying the potential role that hormones play in lemur growth, demonstrating the interplay between hormone exposure in early growth and adult body size, and revealing the processes underlying growth pattern variability (Chapter 4).

1.5 References


Chapter 2
The Influence of Growth Patterns on Sexual Size Monomorphism in Lemurs

Erica M. Tennenhouse

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2.1 Abstract

The lack of sexual size dimorphism among lemurs is puzzling given the high degree of polygyny in this clade. It has been proposed that the unique ecological conditions of Madagascar favour rapid completion of growth, limiting the opportunities for bimaturism and sexual size dimorphism in lemurs. Using recently compiled large datasets on many species across the lemur clade, I examined the prevalence of sexual size monomorphism of body mass among lemurs and tested the hypothesis that limited growth durations constrain sexual size dimorphism. I used segmented regression analyses to accurately model growth in each species. The majority of species analyzed exhibited a period of rapid growth followed by a distinct period of slow growth prior to attainment of adult body mass. While the first period of growth
was constrained by the need to attain the majority of adult body mass prior to the onset of the infant’s first dry season, the subsequent period of slow growth was unconstrained and sufficiently long to promote sexual bimaturism. Sex differences in the duration and rate of growth during this second growth phase appeared to account for the sexual size dimorphism exhibited by three lemur species. Therefore, constraints on growth processes do not limit sexual size dimorphism in lemurs, and other explanations for the prevalence of sexual size monomorphism in this clade should be examined. The importance of considering ontogeny in future investigations of sexual size monomorphism in lemurs is highlighted.

2.2 Introduction

Male-biased sexual dimorphism in body mass is widespread in mammals, with the most dimorphic taxa including members of the orders Pinnipedia, Proboscidea, Carnivora, Artiodactyla, and Primates (Abouheif and Fairbairn, 1997; Moors, 1980; Mysterud, 2000; Ralls, 1977; Weckerly, 1998). Ultimate explanations for high levels of dimorphism in these taxa are founded in sexual selection theory (Darwin, 1871). Because female mammals have lower potential rates of reproduction than do males, males often compete intensely over access to females (Clutton-Brock and Parker, 1992; Kvarnemo and Ahnesjo, 1996; Trivers, 1972). Sexual selection theory predicts that sexual dimorphism is a consequence of intrasexual competition, which favours larger, more competitive males (Isaac, 2005).
The extreme variation in sexual size dimorphism among members of certain taxa is often explained by differences in the strength of sexual selection. For example, much of the variation in degree of sexual dimorphism in body mass among haplorhine primate species, which ranges from extreme male bias in some Old World monkey species to completely lacking in some new world monkeys (Ford, 1994; Smith and Jungers, 1997), can be explained by degree of polygyny, operational sex ratio, and levels of inter-male competition, all of which are thought to reflect the strength of intrasexual selection (Clutton-Brock et al., 1977; Lindenfors and Tullberg, 1998; Mitani et al., 1996; Plavcan and van Schaik, 1992, 1997). Lemurs are unique among primates in that they have been shown to generally lack sexual size dimorphism (Kappeler, 1991) despite often having polygynous mating systems (Kappeler and Schaffler, 2008; Wimmer and Kappeler, 2002) and intense male-male competition during the breeding season (Cavigelli and Pereira, 2000; Gould and Ziegler, 2007; Kappeler, 1997a,b; Ostner et al., 2008; Pereira and Weiss, 1991; Sauther, 1991), both of which should result in strong intrasexual selection favouring large male body size. Furthermore, in contrast to expectations, the low levels of sexual size dimorphism that have been detected in some lemur species tend to be female-biased (Glander et al., 1992).

Explanations for the causes of sexual size monomorphism in lemurs despite circumstances favouring large male body size have been proposed. The fecundity selection hypothesis states that because lemur females have unusually high costs associated with reproduction, selection has favoured an increase in female size,
which reduces these costs and results in larger, healthier infants (Kappeler, 1990).

Alternatively, selective pressures on large male body size may be relaxed in lemur males as passive mate guarding strategies reduce the need for physical competition (Dunham and Rudolf, 2009). It has also been proposed that intersexual selection promotes sexual size monomorphism in lemurs through female preference for smaller, more compliant males who will not challenge females or their infants for food (Richard, 1992) or through female preference for more agile males (Kappeler, 1991). Rensch’s rule, which attempts to explain variation in sexual size dimorphism among primates as a correlated response to body size (Leutenegger, 1978; Leutenegger and Cheverus, 1982), fails to explain variation in dimorphism among strepsirrhines (Gordon, 2006; Lindenfors and Tullberg, 1998; Smith and Cheverud, 2002), many of which are relatively large but still lack dimorphism (Godfrey et al., 1993; Kappeler, 1991). Finally, sexual size monomorphism in lemurs has been attributed to phylogenetic inertia (Cheverud et al., 1985); however, this explanation does not address the cause of the origins of sexual size monomorphism (Lindenfors and Tullberg, 1998).

One hypothesis that has received much attention proposes that the unique ecological conditions of Madagascar have placed selective pressures limiting the growth processes that may lead to sexual size dimorphism in lemurs (Leigh and Terranova, 1998). At a proximate level, sexual size dimorphism arises from differences in growth patterns between the sexes (Badyev, 2002). A primary means by which sexual size dimorphism is produced in primates is through sex differences
in growth durations, termed bimaturism (Wiley, 1974). Differences between the sexes in growth duration require a sufficiently long growth period, which may be selectively favoured in most primate species because growing individuals risk starvation when feeding competition is high, but can reduce their metabolic costs by growing slowly over a longer period of time (Janson and van Schaik, 1993; Leigh, 2001). The resulting long growth periods commonly exhibited by haplorhine primates allow for bimaturism, and subsequently sexual size dimorphism, to develop.

By contrast, duration of growth in strepsirhine primates, such as lemurs, tends to be short (Leigh and Terranova, 1998). As a result, unlike many primate clades in which interspecific variation in body size results from differences in a combination of growth rates and duration (Leigh, 1992; Leigh and Shea 1995; O’Mara et al., 2012), variation in size among lemurid (Leigh and Terranova, 1998; O’Mara et al., 2012) and indriid (Ravosa et al., 1993) species appears to result primarily from variation in growth rates, indicating that growth durations may be constrained in lemurs. Selective factors restricting the length of the growth period in lemurs are therefore thought to have limited the evolution of sexual size dimorphism by restricting bimaturism.

Several factors may target lemur growth durations. Strong seasonal fluctuations in food availability may selectively favour the attainment of foraging independence prior to an individual’s first dry season (Leigh and Terranova, 1998;
Pereira, 1993). However, many other primate species inhabit highly seasonal environments (Dunham et al., 2013; Kamilar, 2009) and it has been demonstrated that environmental factors are not associated with sexual size dimorphism across primate species (Dunham et al., 2013). Thus, the fact that lemurs respond with shortened growth durations while haplorhine primates respond by adopting a risk aversion strategy that lengthens the growth period requires consideration. Life history theory predicts that when infant mortality rate is so extreme that there is a low probability of survival to reproductive age, fast growth is expected (Berrigan and Koella, 1994; Case, 1978; Stearns and Koella, 1986; Stearns, 1992). Reportedly high infant mortality in lemurs compared to haplorhine species (Wright, 1999) may indicate that lemurs are particularly sensitive to seasonality and that extended growth periods are simply not viable in lemurs. Alternatively, the energetic demands of rapid growth in early life may lead to high infant mortality, in accordance with the metabolic risk aversion hypothesis (Janson and van Schaik, 1993; Leigh, 2001). Breeding seasonality in lemurs is another factor that can affect growth and sexual size dimorphism. Most lemurs exhibit strong breeding seasonality (Rasmussen, 1985; van Schaik and Kappeler, 1993), likely as a response to seasonal availability of food resources (Pereira, 1993). Selection may favour reduced growth durations in lemurs because extension of the period of growth could cause seasonal breeders to forego a breeding season (Leigh, 1992), which would reduce lifetime reproductive output.
It has been argued that lemurs are subject to unusually high levels of energetic stress due to severe droughts, cyclones, poor soil, and seasonal food scarcity in Madagascar (Wright, 1999). In particular, the predictable stress brought on by the harsh winter season is cited as a strong driver of lemur life history traits (Pereira, 1993). By contrast, Dunham et al. (2013) showed that lemurs do not experience stronger seasonality than other primate species, but rather that inter-annual variability in seasonality is unusually high for lemurs. Indeed, although there is variation in the degree to which lemur populations experience seasonal fluctuations (Dewar and Richard, 2007; Gordon et al., 2013; Lehman et al., 2005), extreme inter-annual variability in seasonality within a single habitat leads to unpredictable phenological cycles (Wright et al., 2005). For example, a single site in Madagascar is reported as lacking a dry season in one year (Hemingway, 1998) and again as having distinct wet and dry seasons in another (Tecot, 2010). Consequently, even those lemur species traditionally considered to inhabit environments with low resource seasonality are frequently faced with stresses associated with challenging environments.

Potential ontogenetic constraints on sexual size dimorphism in lemurs have previously received little attention in the literature due to availability of small ontogenetic datasets for a limited number of species. Recently compiled large datasets on many species across the lemur clade provide the opportunity to test the relationships among life history variables, growth parameters, and sex and species differences in body size in lemurs. First, I examine the prevalence of sexual size
monomorphism and dimorphism in 18 species of lemur. Next, I test the hypothesis that limited growth durations reduce the opportunity for bimaturism and constrain sexual size dimorphism in lemurs. If short phases of growth preclude bimaturism in lemurs, durations of growth are expected to exhibit reduced variability among species and between the sexes. Reduced variability in growth durations may be balanced by high interspecific variability in growth rates. If lemurs respond to reduced resource availability by limiting growth durations, ultimately leading to a lack of sexual size dimorphism, strong associations between the life history variables that are likely to be constrained by resource availability (e.g. weaning age and age of first reproduction) and growth durations are expected.

2.3 Methods

2.3.1 Datasets

Data on chronological age and body mass were obtained from the Duke Lemur Center’s long-term records (Zehr et al., 2014) and from King et al. (2011) for 1,365 individuals (45,466 ontogenetic data points) of captive *Cheirogaleus medius*, *Eulemur albifrons*, *Eulemur collaris*, *Eulemur coronatus*, *Eulemur flavifrons*, *Eulemur fulvus*, *Eulemur macaco*, *Eulemur mongoz*, *Eulemur rubriventer*, *Eulemur rufus*, *Eulemur sanfordi*, *Lemur catta*, *Microcebus murinus*, *Mirza zaza*, *Propithecus coquereli*, *Varecia rubra*, and *Varecia variegata*, and wild *Propithecus edwardsi* (Appendix 2.1; see King et al., 2005 and 2011 for age estimation methods in the wild population). Studies have found tight correlations between wild and captive primate weights, including those measured at the Duke Lemur Center (Leigh, 1994; Strum,
Terranova and Coffman, 1997). Leigh (1994) reported that across 53 haplorhine species, variation in body weights between wild and captive individuals of the same species was no greater than the variation among wild body weights reported by different sources. Furthermore, Strum (1991) proposed that the weight differences between wild and captive individuals are similar to differences observed among wild populations of a given species, or even differences within one population among years. Captive individuals of some haplorhine species exhibit adolescent growth spurts similar to those exhibited by wild individuals of the same species (Hamada et al., 1996; Leigh, 1995; Leigh, 1996; Setchell et al., 2001). Additionally, the growth velocities of species in captivity generally match with expectations for the same species in the wild (Leigh and Shea, 1996).

Full ontogenetic data are not available for each individual in the dataset; therefore, the dataset was treated as mixed-longitudinal. One limitation to this approach is that in cannot reveal information about individual variability in growth patterns (Leigh, 1992; Tanner, 1986). While longitudinal data are considered suitable for tracking individual growth patterns, cross-sectional and mixed-longitudinal data are appropriate for comparisons among groups of individuals (Tanner, 1986). Consequently, mixed-longitudinal datasets are commonly used in cross-species comparative studies of primate growth (Bernstein et al., 2007; Garber and Leigh, 1997; Leigh, 1992; Leigh and Shea, 1995; Leigh and Terranova, 1998; O’Mara et al., 2012). In order to exclude potentially unhealthy individuals, I removed measurements taken on individuals within 60 days prior to
their date of death (Zehr et al., 2014). Additionally, measurements on females known to be pregnant were excluded. Plotting body weight against age revealed declines in body mass in old individuals consistent with senescence. Therefore, I removed body mass measurements taken on the oldest 20% of individuals of each sex and species prior to analysis. Individuals with body mass greater than two standard deviations above or below the mean for their sex and species were excluded from analyses.

2.3.2 Growth Parameter Estimation

All statistical analyses were conducted in R statistical environment (R Core Team, 2004). In previous studies, growth parameters for primates have been obtained from two-piece regression models, consisting of a period of rapid growth and a phase in which asymptotic body mass is reached, where iterative procedures are used to find the breakpoint that minimizes the residual squared error of the corresponding model (Leigh, 1995; Leigh and Terranova, 1998; O’Mara et al., 2012). When the resulting parameters for species in the Galagidae family are superimposed on scatterplots of the data (O’Mara et al., 2012), it is apparent that in a few species, more points fall above the asymptotic body mass line than below, indicating that the two-piece models underestimate the duration of growth in those species. By contrast, Schaefer (2011) employed a Gompertz model in estimation of growth parameters for some of the same species. It was noted that the Gompertz model fit the data better during the earlier phase of growth than during later growth, which resulted in consistent overestimation of growth rates (Schaefer, 2011).
Assessment of body mass plotted against age indicated that for the majority of species in this analysis, the growth period can be split into a period of rapid growth followed by a distinct period of slow growth prior to attainment of asymptotic body mass (see example in Fig. 2.1). Thus, a three-piece model, consisting of two separate phases of growth followed by a final phase of no growth, is appropriate for most of the species analyzed. Exceptions were *E. albifrons*, *E. sanfordi*, and *E. fulvus*, for which two-piece growth models fit the data better than three-piece models. For these three species I employed two-piece iterative models procedures, which involved sequentially selecting all possible breakpoint values in 0.25-year increments and fitting each resulting model to the data. The model with the lowest residual standard error was selected and the growth rate and growth duration in the first phase of growth were calculated from that model.

Three-piece regression models were implemented using the ‘segmented’ package (Muggeo, 2008). I specified a search for two breakpoints, defined as points where the linear relationship changes. From initial breakpoint estimates, an iterative search was implemented to find the two breakpoints that correspond to the model with the lowest residual squared error; the inclusion of initial breakpoint estimates increases the efficiency of the search procedure. In piecewise regression models, the likelihood landscape is often not concave, which increases the difficulty of finding the globally optimal model. To enhance the model search procedure, I varied the initial breakpoint estimates. As a starting point, I used visual assessment of cubic smoothing splines to make initial breakpoint estimates. From each of those
initial breakpoints, I moved up and down in 0.25-year intervals to select eight additional initial estimates for each breakpoint. Every combination of the first and second initial breakpoint estimates was sequentially included as the two initial breakpoint estimates in a segmented regression. Of the resulting 81 models, the one with the lowest residual standard error was selected as the model from which growth rates and durations in the first two phases of growth were calculated. Standard errors were calculated according to the Delta method (Oehlert, 1992).

2.3.3 Inter-Sex Comparisons

Adult body mass for each sex and species was calculated first by averaging body mass measurements occurring after the last breakpoint for each individual, and then averaging the resulting values for all individuals of a given sex. Mean adult body masses of males and females of each species were then compared using Welch’s unpaired two-sample t-tests. Samples that were not normally distributed according to a Shapiro-Wilks test were log-transformed to achieve normality. An index of sexual size dimorphism (ISD) was calculated for each species by dividing male adult body mass by female adult body mass. To determine the contributions of male and female growth parameters to each species’ degree of sexual size dimorphism I implemented regression analyses. To avoid multi-collinearity among predictors within a model, only models with variance inflation factors below 5 for each predictor were tested. The effects of sex differences in rate and duration of the first phase of growth on ISD across species were tested in one model, and the effects
of sex differences in rate and duration of the second phase of growth on ISD across species were tested in a separate model.

2.3.4 Inter-Species Comparisons

The contributions of growth parameters to adult body mass variation among species were examined separately for males and females using least squares multiple regressions. Due to correlations among some predictors, two models were tested for each sex - one containing duration and rate of growth in phase one as predictors, and the other containing duration and rate of growth in phase two as predictors of adult body mass.

2.3.5 Life History Variables

Species values for weaning age and female age of first reproduction were obtained from the literature (Wright, 1995; Wright, 1999; Kappeler & Pereira, 2003; Ostner & Kappeler, 2004; Tarnaud, 2004; Vasey, 2007; see Appendix 2.2). When different values for a given species were reported by multiple sources, the average value was used. Due to a strong positive correlation between weaning age and female age of first reproduction across species ($r=0.897, P=0.001$), simple regression models were used to test the effects of each of the life history variables on the durations of the first and second growth phases for each sex.
2.3.6 Phylogenetic Comparative Methods

To control for phylogenetic non-independence due to shared ancestry among species (Felsenstein, 1988), the same inter-sex and inter-species associations were re-tested with phylogenetic generalized least squares (PGLS) regressions, using the ‘caper’ package (Orme et al., 2013). Along with estimating the regression parameters, I also made maximum likelihood estimates of Pagel’s lambda using the ‘geiger’ package (Harmon et al. 2008), and implemented the appropriate lambda transformation of the phylogeny prior to incorporating it into the regression models. Lambda measures phylogenetic signal in the data, and the associated transformation involves multiplying internal branches by lambda (Pagel, 1999). The phylogeny I used is a consensus tree based on a Bayesian inference of 10,000 primate genetic phylogenies (10kTrees, Arnold et al., 2010), which I pruned to reflect only the species included in these analyses. PGLS regressions were not run on life history regressions because sample sizes did not provide sufficient power for lambda to detect phylogenetic dependence in the data (Freckleton et al., 2002).

2.4 Results

Growth parameters and average adult body masses for each sex and species are reported in Appendix 2.1. Growth rate during the first phase of growth ranged from 0.38-14.37g/day, while growth rate during the second phase of growth ranged from 0.2-2.39g/day. Duration of the first phase of growth ranged from 0.26-1.61 years and duration of the second phase of growth ranged from 0.85-6.03 years. The oldest age at which growth was complete was 6.89 years. Adult body mass ranged
from 77.1g in *M. murinus* to 5641.19g in *P. edwardsi*. In species for which three-piece growth models were run, approximately two-thirds (range: 55.6-84.2%) of adult body mass was attained by the end of the first growth period.

Results of least squares multiple regressions testing the effects of growth parameters on ISD and species-specific adult body mass did not differ from results of corresponding PGLS regressions, as lambda was never significantly different from zero. Therefore, only results of least squares multiple regressions are presented.

### 2.4.1 Inter-Sex Comparisons

Sexual size dimorphism in adult body mass was significant in *L. catta* ($t_{123}=-6.446, P<0.001, \text{ISD}=1.107$), *P. coquereli* ($t_{566}=2.984, P=0.005, \text{ISD}=0.939$), *E. mongoz* ($t_{544}=2.164, P=0.035, \text{ISD}=0.961$), *E. macaco* ($t_{945}=3.153, P=0.003, \text{ISD}=0.929$), *E. rubriventer* ($t_{845}=5.412, P<0.001, \text{ISD}=0.835$), *M. murinus* ($t_{130}=3.36, P=0.001, \text{ISD}=0.927$), and *C. medius* ($t_{90.8}=4.12, P<0.001, \text{ISD}=0.899$) (see Fig. 2.2). *Lemur catta* was the only one of these species in which significant sexual size dimorphism was male-biased. There were no significant associations between any growth parameters and ISD across species, which is likely due to the low variation in ISD. The majority of species examined exhibited either no difference in body mass between males and females or slightly larger females than males, but the difference between the sexes is less than 10% (see Appendix 2.3). However, the three most extreme species in terms of ISD were *E. rubriventer*, in which females are 19.7% larger than males, *C. medius*, in which females are 11.3% larger than males, and *L. 
catta, in which males are 10.7% larger than females. The duration of the second growth phase was higher in females than males (4.4 years longer than for females than males) in E. rubriventer, and higher in males than females (1.3 years longer for males than females) in L. catta. In C. medius, the growth rate during the second phase of growth was substantially higher for females than for males (approximately ten times higher for females than males).

2.4.2 Inter-Species Comparisons

The duration and rate of growth in phase one, and the duration and rate of growth in phase two, were all positively associated with species-specific adult body mass in males and females (results summarized in Table 2.1).

2.4.3 Life History Variables

Positive relationships between weaning age and the duration of the first growth phase (females: $b=0.1034, t_{1,9}=6.96, P<0.001, r^2=0.843$; males: $b=0.915, t_{1,9}=6.24, P<0.001, r^2=8.12$), and between age of female’s first reproduction and the duration of the first growth phase (females: $b=1.263, t_{1,8}=5.79, P=0.001, r^2=0.808$; males: $b=1.074, t_{1,8}=3.90, P=0.005, r^2=0.656$) were detected (Fig. 2.3). There were no significant relationships between weaning age and duration of the second growth phase (females: $b=0.656, t_{1,8}=1.88, P=0.097, r^2=0.306$; males: $b=0.0184, t_{1,8}=0.10, P=0.925, r^2=0.001$) nor between female age at first reproduction and duration of the second growth phase (females: $b=0.680, t_{1,7}=0.560, P=0.17, r^2=0.255$; males: $b=0.065, t_{1,7}=0.28, P=0.791, r^2=0.011$).
2.5 Discussion and Conclusion

Analysis of sexual dimorphism in adult body mass requires detailed information about ontogeny in order to define the age at which adult body mass is reached and determine how growth parameters relate to measures of sexual size dimorphism. Using detailed growth data and flexible models, sexual size dimorphism and growth parameters of 18 lemur species were estimated with a high degree of accuracy. Overall, sexual size dimorphism was low in lemurs compared to other primate clades, and sex differences in body mass were mainly female-biased, in agreement with other studies (Godfrey et al., 1993; Kappeler, 1991). Ford (1994) considered a sex difference in body size of at least 10% as a relevant cut-off for establishing sexual size dimorphism in primates. Therefore, while *L. catta*, *P. coquereli*, *E. mongoz*, *E. macaco*, *E. rubriventer*, *M. murinus*, and *C. medius* all exhibited statistically significant sex differences in body mass, only *E. rubriventer*, *L. catta*, and *C. medius* have sex differences exceeding 10%, indicating that sexual size dimorphism in these species may be biologically significant. Indeed, the degree of dimorphism in *E. rubriventer*, where females were almost 20% larger than males, is similar to values reported for sexually dimorphic galagos (O’Mara et al., 2012), though sex-reversed. Differences between this result and those reported in previous studies (Kappeler, 1991; O’Mara et al., 2012) may be attributed to higher sample sizes in the present study and different methods for estimation of the age of adult body mass acquisition. Other studies have shown some evidence of sexual size dimorphism in *P. coquereli*, *P. edwardsi* (Ravosa et al., 1993), *Propithecus diadema* and *M. murinus* (Kappeler, 1990), although dimorphism in the latter may actually
fluctuate throughout the year according to independent changes in male and female
body mass (Schmidt and Kappeler, 1998). Although the captive environment does
not affect adult body mass in lemurs (Terranova and Coffman, 1997), it is unknown
whether captivity influences ontogenetic trajectories. However, O’Mara et al. (2012)
note that while absolute growth parameters of captive lemurs may differ from their
wild counterparts, relative comparisons among species and between the sexes of a
given captive population presumably do not differ from comparisons made on wild
populations.

Nonetheless, the overall low level of sexual size dimorphism among lemurs
reported in this and previous studies is puzzling given their mating systems and
social organization. The ecological constraints hypothesis proposes that the extreme
ecological conditions of Madagascar have placed limits on sexual size dimorphism in
lemurs (Wright, 1999). Leigh and Terranova (1998) specifically suggest that
ecological factors target growth processes in lemurs, resulting in short growth
durations that preclude bimaturism as a pathway to sexual size dimorphism.

However, I find evidence indicating that constraints on growth durations are
insufficient to limit sexual size dimorphism in lemurs. Growth durations were found
to contribute to differences in body size both between males and females and
among lemur species. Additionally, based on examination of life-history variables
thought to be associated with resource availability, I infer that sensitivity to
resource availability does not constrain growth durations in lemurs. These
arguments are discussed in greater detail below.
Examining the growth period as two separate phases reveals that sex differences in adult body mass in the most dimorphic lemur species result from sex differences in the duration and rate of growth in the second phase of growth. Specifically, the second phase of growth in *E. rubriventer*, which exhibits female-biased sexual size dimorphism, is substantially longer for females than for males. Conversely, in male-biased sexually dimorphic *L. catta*, the second phase of growth is substantially longer for males than for females. Higher growth rates in females than in males during the second growth phase appear to be responsible for sex differences in adult body size in *C. medius*. Similarly, in other primate clades, both growth durations and rate variation lead to sexual size dimorphism (Leigh, 1992; Leigh and Shea, 1995; O’Mara et al., 2012). Even in sexually monomorphic lemur species, differences between the sexes in growth parameters during the second phase of growth were apparent, indicating that these sex differences do not always result in sexual size dimorphism. By contrast, the duration of the first phase of growth exhibited almost no variation between males and females, and limited variation among species (almost all species complete this phase of growth in their first year of life). Given that lemurs attain approximately two thirds of adult body mass by the end of the first growth phase, it is possible that selective pressures stemming from sensitivity to environmental factors and unusually high infant mortality rates in lemurs (Wright, 1999) favour the rapid attainment of the majority of adult body mass, but not necessarily completion of growth. Indeed, clear associations between infant mortality rate and growth rate have been demonstrated. Members of fast-growing mammalian clades have higher mortality
rates than members of slower-growing clades (Case, 1978). Even among small-scale human societies, low juvenile survival leads to fast growth and reduced growth spurts (Walker et al., 2006).

It is possible that significant drops in mortality rate following the end of the first growth period allow lemurs to switch growth strategies at this time. Mortality risk certainly decreases as lemurs age (Koyama et al., 2001; Pochron et al., 2004), but unfortunately data on changes in mortality risk prior to and after the end of the first growth phase are lacking. Pereira (1993) showed that ring-tailed lemur infants exhibit uncommonly high growth rates prior to their first dry season, and then reduce metabolic rate and grow slowly during the dry season, likely as a metabolic risk aversion strategy (Janson and van Schaik, 1993). My findings show that a similar pattern of rapid growth followed by a period of slow growth extends to additional lemur species. Regardless of the strategies underlying these growth patterns, such long total growth durations, which in some cases approach the growth period lengths reported for sexually dimorphic haplorhine species (e.g. Cheverud et al., 1992; Fragazy and Adams-Curtis, 1998; Schillaci and Stallman, 2005; Setchell et al., 2001), undoubtedly provide sufficient time for bimaturism and by extension, the development of sexual size dimorphism.

Furthermore, variation in growth duration was found to be a strong predictor of adult body size among species, which contrasts with previous findings showing that body size differences among species are only a product of differences
in growth rates (Leigh and Terranova, 1998; O'Mara et al., 2012; Ravosa et al., 1993). Aside from differences in sample size and growth parameter estimation methods, another important difference between this and previous studies is that previous studies only examined size variation among lemurids (Leigh and Terranova, 1998; O'Mara et al., 2012) or among indriids (Ravosa et al., 1993). By including both indriids and cheirogaleids in the analysis, which respectively contain the largest and smallest lemur species, along with lemurids, interspecific body size variation in this study was dramatically higher than in previous studies. The finding that both variations in rates and durations of growth contribute to body size variation among lemurs parallels findings in other primate clades (Leigh, 1992; Leigh and Shea, 1995; O'Mara et al., 2012), and again contradicts the idea that growth durations in lemurs are heavily constrained. Even in the absence of long growth durations, growth rate differences between the sexes can clearly support the development of sexual size dimorphism in lemurs. The high prevalence of compensatory growth, in which animals that have experienced delayed growth switch to accelerated growth when conditions improve, is well documented among animal taxa (Metcalfe and Monaghan, 2001), and demonstrates the inherent flexibility of growth rates.

Consistent with unconstrained growth durations in lemurs, life history variables likely to be moderated by ecological factors are not tightly associated with the durations of the second phase of growth across species. Vulnerability to the risks of starvation increases during weaning (Janson and van Schaik, 1993);
therefore, timing weaning to correspond to the limited period of increased fruit abundance is an important strategy for lemurs (Wright, 1999; Wright et al., 2005). Once weaned, lemurs have a short period of time to independently increase body size to a point that allows them to safely reduce metabolic rate and growth during the nutritionally stressful dry season (Pereira, 1993). Thus, weaning age was a strong predictor of the duration of the first growth phase in both males and females across lemur species, in partial agreement with the hypothesis proposed by Leigh and Terranova (1998); however, weaning age did not predict the duration of the second phase of growth in males or females. The long and highly variable period of slow growth that follows weaning probably diminishes any limits to sexual size dimorphism imposed by a shortened period of rapid growth. Likewise, the age at which females first reproduce did not predict the duration of the second phase of growth in males or females, which is not surprising considering that lemur females can reproduce prior to reaching adult body mass (King et al., 2011). However, the age at which females first reproduce was a significant predictor of the duration of the first phase of growth in both males and females. Because weaning age and age at female’s first reproduction were strongly correlated, it is difficult to determine which of these life-history traits places a stronger constraint on the duration of the first phase of growth. Weaning age and age at the end of the first period of growth are closely matched across species, although the end of this period of growth consistently occurs slightly after weaning, in agreement with the idea that rapid growth continues for a short period post-weaning, while foraging conditions are still optimal. On the other hand, females reproduce for the first time well after the end of
the first phase of growth. Therefore, it is likely that early weaning and pressure to attain the majority of adult body mass prior to one’s first dry season represents more direct limits to the duration of the first phase of growth in lemurs.

In summary, the hypothesis that constraints on growth processes have limited the development of sexual dimorphism in lemurs is not supported. Although sexual size dimorphism is generally low for lemurs, significant sex differences in adult body mass are found in some species. Furthermore, while the duration of the first phase of growth appears to be inflexible and constrained by pressure to attain the majority of adult body mass prior to weaning, the distinct period of slow growth that follows, and growth rate differences in this period, are sufficient to drive not only body size variation among species, but also between the sexes. The mechanisms leading to low levels of sexual size dimorphism in lemurs, despite their having mating systems that typically promote sexual size dimorphism, therefore remain unresolved. While a number of potential mechanisms have been proposed, they are generally unsupported (Gordon, 2006; Lindenfors and Tullberg, 1998; Smith and Cheverud, 2002) or untested (Kappeler, 1990; Kappeler, 1991; Richard, 1992). Multiple factors may simultaneously temper male body size and promote large female body size, making direct tests of hypotheses challenging (Plavcan, 2011). Recent comparative studies suggest that environmental factors may play a relatively minor role in the development of sexual size monorphism in lemurs, and instead highlight the importance of selection targeting male traits involved in post-copulatory competition (e.g. copulatory plugs) rather than male body size (Dunham
and Rudolf, 2009; Dunham et al., 2013). Researchers should continue to explore this and other hypotheses regarding the pathway to body size differences and similarities between male and female lemurs, and such studies would greatly benefit from considering the ontogenetic contributions to sexual size dimorphism and monomorphism. The results of this study demonstrate that contrary to traditional assumptions, sexual size dimorphism is present in some lemur species and can be facilitated by ontogenetic divergences between the sexes.

2.6 Acknowledgements

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2.7 References


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Duration1 = Duration (years) of growth prior to the first breakpoint.

Duration2 = Duration (years) between the first and second breakpoints.

Rate1 = Growth rate (grams/day) during the first growth period (SE).

Rate2 = Growth rate (grams/day) during the second growth period (SE).
**Figure Legends**

**Figure 2.1.** (a) Two-piece model and (b) three-piece model regression fit to growth data for female *P. coquereli*. Lines illustrate the appropriateness of three-piece regressions for modeling growth in this species.

**Figure 2.2.** Segmented regression estimates for seven lemur species. Solid lines show best fits for male (blue) and female (red) growth data. Dashed vertical lines indicate first and second break points for males (blue) and females (red). The final phase of growth has an intercept set to connect to the final breakpoint and a slope set to zero for presentation purposes.

**Figure 2.3.** Effects of weaning age (a, b) and age of female’s first reproduction (c, d) on the duration of the first growth phase in females (a, c) and males (b, d) across lemur species. All slopes are significant (*P*<0.05).
Figure 2.2

Lemur catta

Propithecus coquereli

Eulemur mongoz

Eulemur rubriventer

Eulemur macaco

Cheirogaleus medius

Microcebus murinus
Figure 2.3

(a) Log(Duration of First Growth Phase) vs. Log(Weaning Age)

(b) Log(Duration of First Growth Phase) vs. Log(Weaning Age)

(c) Log(Duration of First Growth Phase) vs. Log(Age of Female's First Reproduction)

(d) Log(Duration of First Growth Phase) vs. Log(Age of Female's First Reproduction)
Chapter 3
The Relationship Between Steroid Hormones in Hair and Social Behaviour in Ring-tailed Lemurs (*Lemur catta*)
Erica M. Tennenhouse, Sarah Putman, Nicole P. Boisseau, Janine L. Brown

3.1 Abstract

Relationships between the hypothalamic-pituitary adrenal (HPA) and hypothalamic-pituitary gonadal (HPG) axes and social behaviour in primates are complex. By using hair to quantify steroid hormones, one can obtain retrospective estimates of long-term free hormone levels from a single sample. In this study, hair was used to quantify long-term levels of cortisol, testosterone, and estradiol among members of a colony of ring-tailed lemurs (*Lemur catta*) to explore associations between intra- and intersexual levels of these hormones and social behaviour between the breeding and birthing seasons. Males and females receiving high rates of aggression over the course of the study period had elevated hair cortisol levels, reflecting heightened stress. While there was no relationship between sex steroid concentrations and intrasexual social interactions, high rates of aggression in females over the study period coincided with females exhibiting the same average concentrations of testosterone as males. It is therefore concluded that being the recipient of aggression might be more stressful than being aggressive in ring-tailed lemurs, and that testosterone potentially mediates female-dominance in this
species. We suggest that further investigation of hair hormones and behaviour in additional primate species could provide a useful comparative framework to guide interpretation of these novel findings.

3.2 Introduction

Certain features of animal social behaviour suggest involvement of the hypothalamic-pituitary adrenal (HPA) axis, which controls the stress response, and the hypothalamic-pituitary gonadal (HPG) axis, which is involved in regulation of the reproductive and immune systems. For example, glucocorticoids are produced when animals are faced with stressors, such as those that might result from social interactions (Creel, 2001; Sapolsky, 2005; Tilbrook and Clarke, 2006). Production of sex steroids can influence a range of social behaviours, particularly those involved in reproduction and aggression (Adkins-Regan, 2009; Brain and Haug, 1992; Soma et al., 2008). Because many primate species are highly social, gleaning information about the interplay between these hormones and social behaviour is often a goal in primatological studies (Anestis, 2010; Bernstein et al., 1983; Michael and Zumpe, 1993; Sapolsky, 2005).

Social relationships can have strong influence on physiological stress in primates. It has been proposed that individuals in subordinate positions have the highest stress because they experience a lack of control and predictability in their social interactions (Creel, 2001). Indeed, subordinates often have higher stress, as measured by circulating glucocorticoid levels, than dominant individuals (Abbott et
al., 2003; Sapolsky, 1983); however, this does not hold true in a number of species (Bales et al., 2006; Bercovitch and Clarke, 1995; Stavisky et al., 2001; Steklis et al., 1986). Furthermore, the negative association between dominance rank and cortisol concentration is normally apparent when the hierarchy is stable, but often disappears in unstable hierarchies (Coe et al., 1979; Keverne et al., 1982; Shively and Kaplan, 1984). Sapolsky (1992) suggested that high-ranking individuals in stable hierarchies experience control and predictability, which reduce psychological stress. In unstable hierarchies, on the other hand, high-ranking individuals face rank challenges, which have been shown to induce high levels of stress (Engh et al., 2006; Sapolsky, 1992). Nonetheless, dominant female ring-tailed lemurs (Lemur catta; Cavigelli 1999) and male Japanese macaques (Macaca fuscata; Barrett et al., 2002) and chimpanzees (Pan troglodytes schweinfurthii; Muller and Wrangam, 2004) were found to have higher cortisol levels than subordinates during periods of social stability. It has been proposed that in these cases, dominant individuals experienced significant metabolic stress due to the energetic costs of aggression (Barret et al., 2002; Cavigelli, 1999; Muller and Wrangham, 2004).

Social status in ring-tailed lemurs is neither linear nor stable (Nakamichi and Koyama, 1997) and females attain high rank through aggression (Kappeler, 1990). Analysis of dominance rank and cortisol concentration in ring-tailed lemurs revealed that the best predictor of elevated cortisol among high-ranking females was the number of aggressive interactions they initiated, whereas being a recipient of many aggressive attacks was the best predictor of elevated cortisol among low-
ranking females (Cavigelli, 2003). However, studies on the relationship between dominance rank and cortisol concentration in this species have yielded mixed results with some groups demonstrating a positive relationship and others exhibiting no relationship (Cavigelli, 1999, 2003; Pride, 2005; Starling et al., 2010). These different findings are not completely unexpected given the non-linear and unstable dominance hierarchies characteristic of this species, as different social groups may experience vastly different social challenges (Starling et al., 2010).

Whereas aggression potentially induces stress, affiliation may temper the stress response. Primate social bonds are thought be physiologically beneficial, and participation in grooming in particular has been shown to reduce stress (Aureli et al., 1999; Ehmke, 2010; Shutt et al., 2007; Wittig et al., 2008). Evidence supports both grooming others (Ehmke, 2010; Shutt et al., 2007) and receiving grooming (Aureli et al., 1999; Schino et al., 1988) as leading to stress reduction in primates. Like many primate species, ring-tailed lemurs regularly groom one another (Nakamichi and Koyama, 1997); however, the influence of grooming on physiological indices of stress has not been explicitly evaluated in this species.

It is well established that androgens can influence aggressive behaviour in primate males. Experimental increases in androgen levels in rhesus macaques, *Macaca mulatta*, have been clearly shown to result in rises in the amount of aggressive behaviour individuals perform (Cochran and Perachio, 1977; Dixson, 1980; Gordon et al., 1979). In the wild, rates of aggression tend to rise during
periods of increased testosterone (Barrett et al., 2002; Brockman et al., 1998; Cavigelli and Pereira, 2000; Girard-Buttoz et al., 2009; Gould and Ziegler, 2007; Kraus et al., 1999; Muller and Wrangham, 2004; Muroyama et al., 2007) and individual levels of aggression have been linked to testosterone levels (Anestis et al., 2006; Beehner et al., 2006; Mohle et al., 2002; Ostner et al., 2011; Teichroeb and Sicotte, 2008). Several studies have further clarified that the relationship between testosterone and aggression is dependent on stability of the dominance hierarchy, with a positive relationship only becoming apparent during unstable periods (Beehner et al., 2006; Cavigelli and Pereira, 2000; Gould and Ziegler, 2007; Mohle et al., 2002; Sapolsky, 1983; Teichroeb and Sicotte, 2008). Species engaged in permanently unpredictable social relationships may maintain this positive relationship between testosterone and dominance rank outside of periods of increased aggression (Gesquiere et al., 2011; Mendonca-Furtado et al., 2014; Muehlenbein et al., 2004; Muller and Wrangham, 2004; Setchell et al., 2008).

Evidence suggests that androgens may have particularly strong effects when individuals are in the midst of acquiring higher rank (Beehner et al., 2006; Ostner et al., 2011; Wickings and Dixson, 1992). Additionally, elevated testosterone coincides with aggressive intergroup encounters in some species (Schoof and Jack, 2013; Teichroeb and Sicotte, 2008). By contrast, species exhibiting permanently low levels of aggression tend not to exhibit any consistent relationship between androgen level and aggressive behaviour (Sannen et al., 2004; Strier et al., 1999). For ring-tailed lemurs, positive associations between testosterone and aggression in males have
been observed specifically during periods of social instability and heightened aggression (Cavigelli and Pereira, 2000; Gould and Ziegler, 2007).

The associations between sex steroids and aggression in primate females have been less well studied. Female intrasexual aggression has been shown to increase with androgen level in some primate studies (Beehner et al., 2005; Cochran and Perachio, 1977), but not in others (Altmann et al., 1995; Batty et al., 1986; Sannen et al., 2004; von Engelhardt et al., 2000). More consistent evidence of a positive relationship between circulating estradiol level and female aggression has been revealed across primate species (Birch and Clark, 1946, 1950; Coleman et al., 2011; Drea, 2007; Michopoulos et al., 2011; Wasser, 1996), although more studies are required on how individual variation in estradiol levels might explain individual differences in aggression. For ring-tailed lemurs, estradiol in females was shown to increase during periods of elevated aggression (Drea, 2007).

In addition to effects on intrasexual social interactions, sex steroids potentially play a significant role in the development and maintenance of intersexual dominance relationships. Males are socially dominant over females in the majority of primate species (Kappeler and van Schaik, 2002), a social system presumably maintained in part by high androgen levels in males and corresponding high levels of aggression. By contrast, female dominance over males characterizes many lemur species (Curtis and Zaramody, 1999; Digby and Kahlenberg, 2002; Digby and Stevens, 2007; Kappeler, 1990; Marolf et al., 2007; Meyer et al., 1999;
Pereira et al., 1990; Pochron et al., 2003; Pollock, 1979; Radespiel and Zimmerman, 2001; Ramanankirahina et al., 2011; Rendall, 1993), and potentially bonobos (Pan paniscus; Parish, 1994; Parish et al., 2000; Stevens et al., 2007; Vervaecke et al., 2000, but see Vervaecke and de Waal, 1999; White and Wood, 2007). Prenatal exposure to sex steroids has been implicated in both the development of masculinized genitalia in lemurs (Drea, 2011; Ostner et al., 2003), and general masculinization in some other primates (Wallen, 2005; Wallen and Hassett, 2009). Circulating sex steroids in adulthood have also been proposed to facilitate the female-dominant social system exhibited by most lemurs (Drea, 2007; von Engelhardt et al., 2000). However, the ratio of male to female serum testosterone in ring-tailed lemurs (Drea, 2007), and female-dominant Eulemur species (Petty and Drea, 2015), was not found to differ from expectations derived from male-dominant mammalian species, with males sustaining significantly higher baseline testosterone levels than females. Nonetheless, fecal testosterone levels in female-dominant brown mouse lemurs, Microcebus rufus, did not differ between males and females (Zohdy et al., 2014).

Results from studies attempting to establish relationships between steroid hormone concentrations and primate social behaviour are clearly mixed. Importantly, quantification of steroid hormones from different sample types may yield varying results, as each substrate provides information about hormone concentrations over a specific time frame (Whitten et al., 1998). Most studies focus on acute endocrine changes through examination of hormones in blood, urine, and
feces, all of which are subject to short-term fluctuations. On the other hand, because steroid hormones are lipophilic substances, they are constantly incorporated into the hair shaft as it grows (Cone, 1996), providing an accurate record of average hormone levels over the period of hair growth (Anestis 2010; Stalder and Kirschbaum, 2012). Indeed, correlations between concentrations of cortisol, testosterone, and estradiol in hair and concentrations in blood plasma or saliva samples averaged over the period of hair growth have been previously demonstrated in mammals, including primates (D’Anna-Hernandez et al., 2011; Davenport et al., 2006; Koren et al. 2006; Yang et al., 1998). Recently, hair has been used to quantify steroid hormones in several haplorhine primate species (rhesus macaques, Davenport et al., 2006; Dettmer et al., 2012; Kapoor et al., 2014; vervet monkeys, Chlorocebus pygerythrus, Guinea baboons, Papio papio, Fourie and Bernstein, 2011; orang-utans, Pongo spp., Carlitz et al., 2014). A better understanding of hormonal correlates of social behaviour in lemurs could derive from a study of steroid hormones in hair.

In the present study we examine the concentrations of cortisol, testosterone, and estradiol in hair samples in relation to intra- and intersexual social relationships in ring-tailed lemurs, a highly social species in which females are socially dominant over males (Jolly, 1966). Based on past findings, it is expected that high rates of aggression are associated with high cortisol levels, while high rates of affiliation reduce cortisol levels. The relationship between testosterone and aggression in males is predicted to depend on the stability of the dominance
hierarchy, with no relationship expected if the hierarchy is stable, and a positive relationship expected if the hierarchy is unstable. Additionally, we explore the effects of both testosterone and estradiol on aggressive behaviour in females. Finally, we examine whether intersexual differences in hormone concentrations underlie the female-dominant social system that characterizes ring-tailed lemurs.

3.3 Methods

3.3.1 Animals

Subjects for this study included 34 ring-tailed lemurs (16 males and 18 females) between the ages of 0.5 – 31 years (average ± SE = 10.02 ± 1.41 years), housed at the Duke Lemur Center (DLC) in Durham, NC. Ring-tailed lemurs are seasonal breeders (Pereira, 1991; Sauther, 1991) and in the Northern Hemisphere the breeding season is approximately 6 months out of phase with the breeding season in Madagascar (Rasmussen, 1985). At the DLC, ring-tailed lemurs breed in November and give birth between March and April (Pereira, 1991). This study was conducted between the birthing and breeding seasons, in order to examine baseline hormone levels and behaviours. All research protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Duke University (protocol #A134-14-05) and the Local Animal Care Committee (LACC) of the University of Toronto Scarborough Campus (protocol #20010916).
3.3.2 Behavioural Observations

Between July 1 and September 1, 2014, a single observer collected behavioural observations on a subset of the subjects included in this study (23 individuals; 10 males and 13 females) belonging to four semi-free ranging social groups in the DLC outdoor natural habitat enclosures. Each group contained between 1-3 males and 2-4 females. During this period, observations were conducted five days per week from 0830 to 1630 hr. Data on social behaviour were collected using 15-min continuous focal observations and ‘all-occurrences’ sampling of aggression (Altmann, 1974). Similar to Sbeglia et al. (2010), aggressive (contact fight, displace, bite, chase, take food, cuff, attack, and tail wave) and affiliative (groom, mutual groom, play) behaviours were recorded. Individuals were identified with the aid of uniquely coloured collars, physical characteristics, and shaving patterns.

Individual rates of initiating aggression and receiving aggression were calculated by counting the number of aggressive acts for which the focal individual was an actor and a recipient, respectively, and dividing those values by the number of hours for which that individual was observed during the study period. To calculate individual rates of affiliation, only grooming and mutual grooming were considered, as play behaviour is age-dependent (unpublished data). Individual rates of grooming given and grooming received were calculated by dividing the amount of time that the focal individual spent grooming and receiving grooming (mutual grooming was counted as both grooming given and grooming received for both
participants), respectively, by the number of hours for which that individual was observed during the study period.

3.3.3 Hair Sampling and Hair Growth

Hair samples were collected from all subjects by DLC staff between July and August 2014, with the majority of samples from the subjects of the behavioural observations collected at the end of August. Lemurs were either caught briefly in a net or samples were taken opportunistically during annual veterinary check-ups. Hair samples weighing >0.1 g were shaved (using an electric shaver) from the right hip of each subject. All hair samples were stored in labeled paper envelopes at ambient temperature.

Six individuals (five males and one female) were re-captured several weeks after initial hair samples were collected (average ± SE = 42.167 ± 1.815 days) for weighing or collection of samples unrelated to this study. During these re-captures, the length of re-grown hair was measured to the nearest millimetre with a tape measure. To determine average daily rate of hair growth, the length of hair re-growth was divided by the number of days between hair sample collections.

3.3.4 Hormone Analysis

Hair samples were placed in 16 x 125 mm glass tubes, 10 ml of isopropanol was added, and the samples were shaken on a multi-tube vortexer (pulse rate 1/s; Glas-Col, Terre Haute, IN, USA) at a speed of 1300 rpm for 3 min; this wash
procedure was repeated twice. Samples were dried in a fume hood under directed
air for 24 h (Davenport et al., 2006; Fourie et al., 2015). Each sample was then
ground into a fine powder using a Retsch ball mill (mixer mill MM 200; 10-ml
stainless steel grinding jars; single 12-mm stainless steel grinding balls; Retsch
GmbH, Haan, Germany) for 4 min at 25 Hz (Davenport et al., 2006). Approximately
100 mg of each sample was placed in a 16 x 125 mm glass tube and 4 ml of methanol
was added. Samples were capped, vortexed for 30 s at a speed of 1300 rmp and then
incubated on a shaker for 24 h at room temperature at a speed of 800 rpm.

Following extraction, tubes were centrifuged at 1150 x g at 4°C for 20 min
(Sorval RC 3C Plus, Kendro Laboratory Products, Newtown, CT), and the extract was
aliquoted into a new set of tubes. Methanol (4 ml) was added to the initial tubes and
shaken for 30 s at 1300 rmp. The tubes were centrifuged following the same
procedure, and the extracts were combined with the first batch of supernatant. The
extracts were dried down in a fume hood under directed air and resuspended in 2
ml of methanol. Resuspended extract (500 µl) was dried down under directed air
and reconstituted in 500 µl of phosphate-buffered saline, vortexed, and sonicated
while the remaining extract was dried down again and stored at -20°C.

Hair hormones were quantified using enzyme-immunoassays. These
included human salivary kits for cortisol and testosterone (ALPCO Diagnostics,
Salem, NH, USA), and an assay that relied on a polyclonal antibody to estradiol-17β
(R4972; C.J. Munro). For more details about assays and validations, see Appendix
3.3.5 Statistical Analyses

All statistical analyses were performed in R statistical environment (R Core Team, 2014). Hair hormone concentrations greater than three standard deviations from the mean were considered outliers and removed from the analyses \((n = 1)\). For models with behavioural predictors, samples include only the subset of subjects on which behavioural observations were conducted, while tests of sex differences in hormone concentrations include all subjects; for this reason, different statistical tests are associated with difference sample sizes.

The influence of behaviour on hair cortisol concentration was tested separately for each sex using a stepwise backward elimination regression procedure, which eliminated predictors and calculated Akaike Information Criterion (AIC) values associated with each new model. The full behaviour model included as predictors: rate of aggression, rate of aggression received, rate of affiliation, rate of affiliation received, and age included as a quadratic term. To avoid multi-collinearity among predictors within a model, only final models with variance inflation factors below five for each predictor were tested. The final models were those that minimized the AIC values. For males, the final model included as predictors: rate of aggression initiated, rate of aggression received, rate of affiliation, and age. For females, the final model included as predictors: rate of aggression received and rate of affiliation.
Next, the effects of testosterone, estradiol, and the ratio of testosterone to estradiol concentration on both rates of aggression given and received, as well as age, were examined using simple regressions. Two-tailed t-tests were used to examine sex differences in hormone concentrations (cortisol, testosterone, estradiol, and testosterone to estradiol ratio) and rates of aggression and aggression received in adults. A significance level of $\alpha = 0.05$ was used for all tests.

3.4 Results

Ring-tailed lemur hair grew at an average rate of 0.29 mm (range: 0.20 - 0.37 mm) per day. Assuming a constant and steady rate of growth, this is equivalent to a growth rate of 0.86 cm (range: 0.60 - 1.1 cm) per month. The average length of hairs in collected samples was 2.45 cm (range: 1.5 - 3.6 cm). Therefore, if one assumed synchronous hair growth, the hormones extracted from these samples would represent accumulation over 2-3 months prior to sampling. However, in humans and several non-human primate species, asynchronous hair growth has been documented (Ebling, 1987; Fourie, 2012; Harkey, 1993), whereby different hairs are in different phases of growth (anagen – active growth, catagen – maturation, telogen – resting) at any given time. A sample of human scalp hair typically contains 15% of hairs in the telogen phase and 85% in either the anagen or catagen phases (Ebling, 1987; Harkey, 1993), while the percentages of hairs in different growth phases for other primate species are not known. Assuming asynchronous hair growth in ring-tailed lemurs, 2-3 months would represent the minimum period represented by the samples in this study. It is likely that the period represented by these samples
extends to several months prior to behavioural data collection, potentially including part of the birthing season, which could introduce noise into my analyses.

Results from statistical tests on hormones and social behaviour including individuals of all ages did not differ from results of tests that only included adults; therefore, only results from models including individuals of all ages are presented. There was a positive relationship between rate of receiving aggression and hair cortisol concentration in females ($b = 5.977, r^2 = 0.503, F_{3,8} = 3.01, P = 0.037$), while none of the behavioural measures significantly predicted hair cortisol level for males. Nonetheless, for both males and females, the individuals on the receiving end of the most aggression exhibited higher hair cortisol levels than those that received the least aggression (Fig. 3.1). When the association between age and hair cortisol concentration was tested in a separate model, a significant polynomial relationship was revealed ($r^2 = 0.192, F_{2,30} = 3.559, P = 0.041$; Fig. 3.2).

There was no relationship between testosterone, estradiol, or the ratio of testosterone to estradiol and intrasexual rates of aggression initiated or received for males or females; however, the male with the highest testosterone level also exhibited the highest rate of initiated aggression among males (Appendix 3.2). Age was unrelated to sex steroid concentrations in males or females.

Adult males and females did not differ significantly in cortisol, estradiol, or testosterone concentrations, or the ratio of testosterone to estradiol (Fig. 3.3).
Females exhibited significantly higher ($t = 3.18, P = 0.006$) levels of initiating aggression (mean = 3.592, SD = 2.96) than males (mean = 0.784, SD = 1.012) and males received significantly higher ($t = -4.41, P < 0.001$) levels of aggression (mean = 4.391, SD = 2.285) than females (mean = 0.950, SD = 1.064; Fig. 3.4). Moreover, females performed all types of aggressive behaviour at higher average hourly rates than did males, with the exception of the male-specific tail waving display (Table 3.1).

3.5 Discussion

This study examined how steroid hormones quantified from hair samples were related to social behaviour in ring-tailed lemurs. While previous studies have examined similar questions in this species, they have relied on quantification of hormones from fecal (Cavigelli et al., 1999; Cavigelli and Pereira, 2000; Gould and Ziegler, 2007; Pride, 2005; Starling et al., 2010; von Engelhardt et al., 2000) or serum (Drea, 2007) samples. By examining hormone levels in hair, this study differs from these studies in two important ways. Because steroid hormones are continuously incorporated into hair as it grows (Cone, 1996), given the measurements of hair re-growth in this species, hormone levels estimated in this study represent an individual’s average hormone levels accumulated over the 2-3 months prior to sampling, while previous studies on ring-tailed lemurs provide estimates of short-term changes in hormone levels. Additionally, only the unbound fraction of steroid hormones is incorporated into hair (Raul et al., 2004), while some previous studies measure both unbound and bound hormones (but see Touma and
Palme, 2005). According to the free hormone hypothesis, hormone activity is determined by its unbound rather than total concentration (Mendel, 1989); therefore, it is the unbound concentration of a hormone that is expected to be the most relevant to behaviour. With these considerations, it is not surprising that the results of the present study differed from previous findings in some key aspects, which are discussed below.

We found that individuals receiving the highest rates of aggression had the highest cortisol levels, suggesting that being the recipient of aggression activates the endocrine stress response. For females, this positive association between cortisol concentration and rate of aggression received was statistically significant, while there was a clear positive trend between these variables that was not statistically significant for males. Being on the receiving end of aggression can be physiologically and/or psychologically stressful, and is associated with high cortisol levels in other primate species (Abbott et al., 2003; Gust et al., 1993; Steklis et al., 1986). However, no relationship was found between rate of aggression initiated and cortisol level in this study. This finding contrasts with past research on female ring-tailed lemurs showing that dominant individuals had the highest cortisol levels, which were attributable to higher rates of aggression (Cavigelli et al., 1999, 2003). Importantly, this relationship was only present in groups having high levels of aggression (Cavigelli et al., 2003); therefore, it is possible that such a relationship would have been found in the present study had aggression levels been higher. On the other hand, Starling et al. (2010) found that social status was a poor predictor of cortisol
level in ring-tailed lemurs that were provisioned and therefore not nutritionally stressed. Similarly, Pride (2005) found no consistent relationship between rates of intragroup aggression given or received and cortisol concentration. Clearly, results have been mixed; nonetheless, the positive association between aggression received and cortisol concentration in the present study might indicate that low-ranking ring-tailed lemurs are in fact more stressed than high-ranking individuals, as has been shown to be the case for other species (Abbott et al., 2003; Sapolsky, 1983).

It is well established that an important function of primate grooming is the strengthening of social bonds (Di Bitetti, 1997; Dunbar, 1991; Henzi and Barrett, 1999). Indeed, ring-tailed lemurs are highly social (Jolly, 1966), and frequently engage in grooming behaviour (Hosey and Thompson, 1985; Nakamichi and Koyama, 1997). The fact that no relationship between performing affiliative acts and cortisol level was found in either sex, in contrast to the expectation that affiliation would decrease cortisol levels, may indicate that social bonding in ring-tailed lemurs does not translate to reduced stress. Alternatively, those individuals with the highest initial cortisol levels may be more likely to engage in affiliative behaviours like grooming as a stress-reducing strategy, which would eliminate an observable relationship between affiliation and cortisol concentration after affiliation has taken place. Finally, it is possible that rather than having a social function, grooming in ring-tailed lemurs functions more strongly as a hygienic practice, as has been suggested for some primate species (Akinyi et al., 2013; Tanaka and Takefushi, 1993; Zamma, 2002).
The lack of association between testosterone and aggression in this study may be attributable to the fact that it was conducted during the non-breeding season. The challenge hypothesis posits that aggression is regulated by testosterone during periods of social challenge, which occur most commonly in a reproductive context when males compete intensely over access to receptive females (Wingfield, 1990). Indeed, previous studies have shown that the positive relationship between male testosterone levels and dominance rank in ring-tailed lemurs is only apparent during the mating season (Cavigelli and Pereira, 2000; Gould and Ziegler, 2007). However, the male exhibiting the highest rate of aggression initiated in this study also had the highest testosterone concentration, which may indicate testosterone influences aggression only above a certain threshold level even outside of the mating season. Indeed, testosterone is associated with aggressive behaviour outside of the mating season in other lemur species (Brockman et al., 1998; Kraus et al. 1999).

For females, on the other hand, the birthing season is sometimes associated with heightened aggression (Pereira and Weiss, 1991; Vick and Pereira, 1989). There is some evidence that estradiol may play a larger role than testosterone in promoting female intrasexual aggression in primates (Birch and Clark, 1946, 1950; Coleman et al., 2011; Drea, 2007; Michopoulos et al., 2011; Wasser, 1996). Nonetheless, there was no relationship between either estradiol or testosterone and intrasexual aggression among females in this study, which may reflect the fact that
this study took place between the birthing and mating seasons, a period when the dominance hierarchy tends to be stable and rank reversals rarely occur in ring-tailed lemurs (Jolly, 1966; Pereira and Kappeler, 1997).

Previous studies have demonstrated that mammalian males have higher hair testosterone levels than females (Bryan et al., 2013; Bryan et al., 2015; Gleixner and Meyer, 1997). A notable exception is a study by Koren et al. (2006), which showed that rock hyrax (Procavia capensis) females, thought to be socially dominant over males (Koren, 2000), also exhibited higher hair testosterone levels than males. In contrast to most primates, many lemur species, including ring-tailed lemurs, exhibit female dominance over males (Kappeler, 1990; Wright, 1999). It has been suggested that sex steroids might be involved in promoting this unusual social system (Drea, 2007; von Engelhardt et al., 2000). For example, although mammalian males are expected to have substantially higher levels of circulating testosterone than females due to the presence of testes, both von Engelhardt et al. (2000) and Drea (2007) predicted that ring-tailed lemur females, which are dominant over males, would exhibit heightened androgen levels compared to males. Although the results of those two studies did not support this prediction (testosterone in males was found to be significantly higher than that of females), in the present study, testosterone levels did not differ between males and females. It is likely that this intersexual testosterone ratio would change in the breeding season, as breeding season baseline testosterone levels for males must be sufficiently high to promote spermatogenesis, expression of some secondary sexual traits, and reproductive behaviour (Goymann
et al., 2007; Wingfield et al., 1990). Nonetheless, the fact that non-breeding season levels of testosterone are similar between males and females might provide a proximate explanation as to why females exhibit such high levels of aggression relative to males and ultimately, why females are socially dominant over males in this species. Although directionality of the association between testosterone and aggression can be challenging to establish (Mazur, 1985), this adds to a growing list of studies demonstrating similar levels of testosterone between males and females in female dominant mammals (Koren et al., 2006; Zohdy et al., 2014).

The results of this study could reflect either unusually high levels of free testosterone in females or low levels of free testosterone in males. Other studies have attempted to address similar questions by comparing intersexual hormone levels among closely related species (Petty and Drea, 2015; Sannen et al., 2003). Future studies of hair hormones in additional lemur species could therefore provide a comparative framework within which to examine whether free testosterone levels in ring-tailed lemurs are relatively high in females or relatively low in males.

Mammalian studies have rarely quantified estradiol in both males and females of the same species. Therefore, the fact that estradiol levels did not differ between ring-tailed lemur males and females might not be unusual. Indeed, both Djungarian (Phodopus campbelli) and Siberian (Phodopus sungorus) dwarf hamster males were found to have serum estradiol levels within the range of adult cycling females (Schum & Wynne-Edwards 2005).
Although cortisol concentration was associated with rate of aggression received within each sex, there was no difference between the sexes in average cortisol concentrations. Given that males were found to receive significantly higher rates of aggression than females, a corresponding elevation in cortisol concentration would have been expected in males. Nonetheless, Starling et al. (2010) similarly reported that fecal glucocorticoid concentrations did not differ between male and female ring-tailed lemurs. Importantly, that study spanned the breeding and non-breeding seasons, whereby confirming that the lack of sex differences in cortisol concentration observed in the present study did not simply reflect the fact that it was conducted outside of the breeding season.

In addition to the relationships between hormone concentrations and behaviour, and intersexual differences in hormone concentrations, this study also explored age-related changes in hormone concentrations. We found that young ring-tailed lemurs exhibit high levels of hair cortisol, which decline with age and level off in adulthood. This finding closely matches with ontogenetic patterns of adrenal output that have been established in haplorhine taxa (Castracane et al., 1981; Dettmer et al., 2014; Fourie and Bernstein, 2011; Fourie et al., 2015; Gesquiere et al., 2005; Laudenslager et al., 2012; Pryce et al., 2002). In marmosets, consistently high levels of cortisol among neonates are associated with large adrenal glands (Pryce et al., 2002); a similar ontogenetic pattern of adrenal size could explain the age-related changes in cortisol concentration observed in ring-tailed lemurs. Additionally,
testosterone is expected to increase around puberty in primates (Abbott and Hearn, 1978; Castracane et al., 1986; McCormack, 1971; Resko, 1967). Because ring-tailed lemurs reach puberty at an early age (approximately 16 months; Pereira, 1993), the vast majority of males in this study were past puberty. Indeed, the youngest male in this study was 1.4 years of age. Therefore, the sample was likely not sufficient to demonstrate these typical age-related changes in testosterone levels among males.

3.6 Conclusions

The usefulness of hair to examine endocrine correlates of behaviour has been demonstrated in only a few primate species (rhesus macaques, Davenport et al., 2006; Dettmer et al., 2012; orang-utans, Carlitz et al., 2014). The present study measured the concentrations of several steroid hormones in hair collected from ring-tailed lemurs and related these concentrations to patterns of social behaviour and intersexual dominance. While accurate assessment of hormone levels using traditional substrates requires many samples to be collected from the same individual over a given period of time, a single hair sample provides estimates of average hormone accumulation over the period of hair growth, making it useful as a retrospective calendar of hormone levels (Stalder and Kirschbaum, 2012). Individuals receiving high rates of aggression from other group members were found to exhibit elevated hair cortisol levels, reflecting chronically high stress. While steroid hormone concentrations did not significantly influence intrasexual social interactions in the present study, high rates of aggression in females and the fact that females exhibited the same concentrations of testosterone as males are
potentially related to the female-dominant social system exhibited by ring-tailed lemurs. These findings suggest that subordinate positions might be more stressful than dominant positions in this species, and that female-dominance is potentially mediated by female testosterone. Future studies of hair hormones and behaviour in additional lemur species could provide a broader context to enhance the interpretation of these novel findings.

3.7 Acknowledgements

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3.8 Author Contributions

Conceived and designed the study: EMT. Collected samples: EMT. Performed lab work: EMT, SP, NPB. Contributed reagents/materials/analysis tools: EMT, SP, NPB, JLB. Analyzed the data: EMT. Wrote the paper: EMT. All authors edited the manuscript.
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Table 3.1. Comparison of the average rates (per hour) at which male and female ring-tailed lemurs performed various aggressive behaviours

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Male rate (per hour)</th>
<th>Female rate (per hour)</th>
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</thead>
<tbody>
<tr>
<td>Contact fight</td>
<td>0.165</td>
<td>0.188</td>
</tr>
<tr>
<td>Displace</td>
<td>0.206</td>
<td>1.813</td>
</tr>
<tr>
<td>Bite</td>
<td>0.0206</td>
<td>0.0625</td>
</tr>
<tr>
<td>Chase</td>
<td>0.0412</td>
<td>0.234</td>
</tr>
<tr>
<td>Take food</td>
<td>0.0618</td>
<td>0.313</td>
</tr>
<tr>
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$^1$Tail waving is a male-specific behaviour in ring-tailed lemurs.
Figure Legends

**Figure 3.1.** Relationship between rate of aggression received and hair cortisol concentration in (a) female and (b) male ring-tailed lemurs.

**Figure 3.2.** Cubic smoothing spline fitted to age and hair cortisol concentration data for ring-tailed lemurs.

**Figure 3.3.** Comparisons of concentrations of (a) testosterone, (b) estradiol, (c) testosterone/estradiol, (d) cortisol (mean ± SE) in hair between adult male and female ring-tailed lemurs.

**Figure 3.4.** Sex differences in (a) rates of aggression, and (b) rates of aggression received (mean ± SE) in ring-tailed lemurs.
Figure 3.1

(a) Hair cortisol (pg/mg) vs. rate of aggression received (per hour)

- $b = 5.977$, $P = 0.0373$
- $R^2 = 0.503$

(b) Hair cortisol (pg/mg) vs. rate of aggression received (per hour)

- $b = 3.645$, $P = 0.12$
- $R^2 = 0.757$
Figure 3.2

$R^2 = 0.192, P = 0.041$
Figure 3.3
Figure 3.4

(a) Rate of aggression (per hour)
F: \( n = 13 \)  
M: \( n = 10 \)

\[ t = 3.18, P = 0.006 \]

(b) Rate of aggression received (per hour)
F: \( n = 13 \)  
M: \( n = 10 \)

\[ t = -4.41, P < 0.001 \]
Chapter 4
Growth and Relative Body Mass in Coquerel’s Sifakas
(Propithecus Coquereli): The Effects of Cortisol and Testosterone

Erica M. Tennenhouse, Sarah Putman, Nicole P. Boisseau, Michael A. Schillaci, Janine L. Brown

4.1 Abstract

Chronic stress leads to prolonged elevations in circulating cortisol, which can have detrimental effects on growth and development. Considerable evidence exists linking maternal stress during gestation to reduced offspring birth weight and postnatal body mass, as well as the detrimental effects of postnatal stress on growth and body mass. Additionally, there is evidence that testosterone, which is thought to stimulate growth, is inhibited by elevated cortisol. The aim of this study was to examine the effects of free cortisol and testosterone levels on growth and relative body mass in Coquerel’s sifakas (Propithecus coquereli). By assessing hormone concentrations in hair samples, we were able to demonstrate a significant negative relationship between hair cortisol concentration and relative body mass among growing individuals, but no relationship between these factors was observed among grown individuals. There was no relationship between hair testosterone and growth rate. Hair cortisol concentrations were not correlated with hair testosterone concentrations. These findings support the idea that chronic stress during
development can lead to deficits in body mass in non-human primates. The relevance to adult body mass of deficits produced during growth by high levels of cortisol was assessed by examining the relationship between individual weights-for-age in early and late growth stages. We found links between individual weights-for-age in early and late growth, suggesting that low relative body mass in early life has carry-over effects into adulthood. Future long-term studies investigating the direct effects of hormone levels in early life on relative body mass in adulthood are warranted.

4.2 Introduction

Production of the hormone cortisol is triggered by stress, which activates the hypothalamic-pituitary-adrenal (HPA) axis. Acute stressors typically lead to short-term increases in cortisol (Dickerson, 2004; Smyth et al., 1998). Chronic stress, on the other hand, can lead to prolonged elevations in circulating cortisol, which can have detrimental effects on health (Chrousos, 2009; Miller et al., 2007). One such health-related consequence of chronic stress is the inhibition of growth and development (Chrousos, 2009).

High basal levels of cortisol interfere with many of the body's somatic processes including growth, development, reproduction, and immunity (Munck and Guyre, 1991, Orth et al., 1991). The inhibition of growth during chronic stress is a result of chronic activation of the HPA axis, which leads to suppression of growth hormone (Stratakis et al., 1995). There is considerable evidence linking maternal
stress during gestation to reduced offspring birth weight and postnatal body mass in rodents (Emack et al., 2008; Guo et al., 1993), non-human primates (Schneider et al., 1999), and humans (Rondo et al., 2003). Additionally, postnatal stress has been shown to inhibit growth in children growing up in a range of stressful environments (e.g. Dabrova-Krol et al., 2008; Kertes et al., 2008; Montgomery et al., 1997).

Although cortisol and testosterone are typically examined separately, there is evidence that elevated cortisol inhibits testosterone production (Ferin, 1999; Johnson et al., 1992; Rivier and Rivest, 1991; Tilbrook et al., 2000), with one established mechanism being that cortisol reduces gonadal sensitivity to luteinizing hormone, thus reducing gonadal production of sex steroids (Whirledge and Cidlowski, 2010). Cortisol has been shown to have catabolic effects on human muscle protein (Brillon et al., 1995; Gelfand et al., 1984; Gore et al., 1993). Additionally, testosterone is known to have anabolic effects, which include stimulating growth (Cassorla, 1984; Lorentzon et al., 2005; Preece et al., 1984; Tanner, 1976). Inhibition of testosterone by cortisol could therefore potentially contribute to the negative relationship between cortisol level and relative body mass that has been observed in mammals.

Early deficits in growth are known to lead to low adult body mass in non-human primates (Altmann and Alberts, 2005; Setchell et al., 2001) and humans (Stein et al., 2010; Sterling et al., 2012). Therefore, the endocrine factors influencing growth and body mass in early life potentially have enduring effects. By contrast,
compensatory growth, whereby growth is accelerated following a period of growth deficit, often through hyperphagia, has been shown to occur widely among both invertebrates and vertebrates (Metcalfe and Monaghan, 2001). Such compensatory growth would serve to reduce the degree to which hormone levels during growth determine body mass in adulthood.

Short-term changes in hormone levels are often measured in blood, saliva, urine, and fecal samples. Quantification of long-term hormone levels, on the other hand, can be challenging because it requires frequent sampling of individuals over an extended period. However, unbound steroid hormones are continuously incorporated into the hair shaft as it grows, making hair an ideal substrate for assessment of endocrine activity over long periods of time (Stalder and Kirschbaum, 2012). Additional advantages of hair hormone analysis include non-specific storage requirements of hair samples, stability of steroid hormones in hair over long periods of time, and sufficiency of a single hair sample to reflect long-term hormone levels (Stalder and Kirschbaum, 2012). Indeed, this method is increasingly being used to obtain integrated estimates of chronic stress (e.g. Carlitz et al., 2014; Davenport et al., 2006; Dettmer et al., 2014; Gow et al., 2010; Russell et al., 2012).

Rarely are sufficient growth data available on individuals of a given species to accurately measure individual growth rates and relative body masses. However, recent publication of long-term data from the Duke Lemur Center (Zehr et al., 2014) has enabled the detailed analysis of growth patterns in several lemur species.
(Tennenhouse 2015). Building on this previous work, the aim of the present study is to examine the relationship between steroid hormone levels measured in hair, and growth and relative body mass in Coquerel’s sifakas (*Propithecus coquereli*). Their long growth period relative to other lemur species (Richard et al., 2002; Dewar and Richard, 2007) makes sifakas an ideal taxon in which to examine questions about the factors influencing growth. Elevated hair cortisol is expected to reduce growth rates and relative body mass of growing individuals, while hair testosterone is expected to have the opposite effect on growth and relative body mass. We also investigate the degree to which early growth deficits carry over into adulthood in this species by testing whether an individual’s relative body mass in early life predicted their relative body mass in adulthood.

### 4.3 Methods

#### 4.3.1 Animals

Subjects for this study included 33 Coquerel’s sifakas (17 males and 16 females) between the ages of 0.13 – 27 years (average ± SE = 7.46 ± 1.26 years), housed at the Duke Lemur Center (DLC) in Durham, NC. The animals at DLC receive daily supplemental provisions of primate chow and fresh fruits and vegetables. The Coquerel’s sifakas were housed in groups of 2-8 individuals, similar to the group size range of 3-10 individuals that has been recorded for their wild counterparts (Mittermeier et al., 2013). From mid-May to mid-November, or when ambient temperature is above 5 °C, the Coquerel’s sifakas are given access to large forested enclosures. During sample collection, low temperatures dictated that groups were
housed in separate, large (1575 ft³/individual) indoor/outdoor pens. All research protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Duke University (protocol #A134-14-05) and the Local Animal Care Committee (LACC) of the University of Toronto Scarborough Campus (protocol #20010916).

4.3.2 Body Mass

To calculate individual relative body mass, we first used data on chronological age and body mass obtained from the Duke Lemur Center’s long-term records (Zehr et al., 2014) to create segmented regression models of the relationship between body mass and age, separated by sex (see Tennenhouse 2015 for further details about these models). The Coquerel’s sifaka growth models consisted of three phases – a rapid early growth phase, a slow growth phase, and an asymptotic adult body mass phase where body mass remained constant (Fig. 4.1). Based on these models, we determined each subject’s predicted weight given their age and sex. Standard weights-for-age were then calculated by dividing an individual's recent weight (measured within 18 days of hair sample collection) by their predicted weight and log transforming the result. Negative values indicate an individual is light-for-age, while positive values indicate an individual is heavy-for-age.

Next, we used the DLC long-term database to test the associations between individual relative body masses across different growth phases. Only individuals
that were weighed during either the first or second growth phase as well as the third growth phase were included in this analysis ($N = 37$). Using the same approach as above, we generated a predicted weight corresponding to each age for the actual weight measurements, and used these values to calculate standard weights-for-age. These values were then averaged for each individual within each growth phase.

4.3.3 Growth Rate

Two weight measurements for each subject were used to calculate current growth rate – a recent measurement (see above), and a measurement taken approximately 2-3 months prior. Current growth rate (grams per day) for a given individual was calculated as the difference between the two weight measurements, divided by the number of days between weighings. Next, individual relative growth rates were calculated by subtracting the mean growth rate for a given sex and growth phase from an individual’s current growth rate, and dividing the result by the standard deviation of the mean growth rate.

4.3.4 Hair Sampling

Hair samples were collected from all subjects by DLC staff between January and February 2015. This sampling period was between the birthing and breeding seasons for Coquerel's sifakas at the DLC. Lemurs were either caught briefly in a net or samples were taken opportunistically during annual veterinary check-ups. Hair samples weighing $>0.1$ g were shaved (using an electric shaver) from the right hip of
each subject. All hair samples were stored in labeled paper envelopes at ambient temperature.

4.3.5 Hormone Analysis

Hair samples were placed in 16 x 125mm glass tubes, 10 ml of isopropanol was added, and the samples were shaken on a multi-tube vortexer (pulse rate 1/s; Glas-Col, Terre Haute, IN, USA) at a speed of 1300 rpm for 3 min; this wash procedure was repeated twice. Samples were dried in a fume hood under directed air for 24 h. Each sample was then ground into a fine powder using a Retsch ball mill (mixer mill MM 200; 10-ml stainless steel grinding jars; single 12-mm stainless steel grinding balls; Retsch GmbH, Haan, Germany) for 4 min at 25 Hz. Approximately 100 mg of each sample was placed in a 16 x 125mm glass tube and 4 ml of methanol was added. Samples were capped, vortexed for 30 s at a speed of 1300 rpm and then incubated on a shaker for 24 h at room temperature at a speed of 800 rpm.

Following extraction, tubes were centrifuged at 1150 x g at 4°C for 20 min (Sorval RC 3C Plus, Kendro Laboratory Products, Newtown, CT), and the extract was aliquoted into a new set of tubes. Four millilitres of methanol was added to the initial tubes and shaken for 30 s at 1300 rpm. The tubes were centrifuged following the same procedure, and the extracts were combined with the first batch of supernatant. The extracts were dried down in a fume hood under directed air and resuspended in 2 ml of methanol. 500 µl of resuspended extract was dried down under directed air and reconstituted in 500 µl of phosphate-buffered saline,
vortexed, and sonicated while the remaining extract was dried down again and stored at -20°C.

Hair hormones were detected using enzyme-immunoassays for human salivary cortisol and testosterone (ALPCO Diagnostics, Salem, NH, USA). Samples were diluted (1:6 or 1:10) when required so they could be read at an optimal range on the standard curve. For full details about assays and validations, see Appendix 4.1.

4.3.6 Statistical Analyses

Females known to be pregnant at any time within four months prior to hair sample collection were excluded from the analyses ($N = 4$). Additionally, hair hormone concentrations greater than 2 standard deviations above or below the mean for one's sex were excluded ($N = 1$). Multiple least squares regression analyses and odds ratio calculations were conducted in R statistical environment (R Core Team, 2014). An alpha level of 0.05 was adopted for all tests. Odds ratios were statistically significant at alpha=0.05 if the 95% confidence interval did not contain the value 1.

The influence of hormone concentrations on relative growth rate and relative body mass among growing individuals in the first or second growth phases (see Tennenhouse, 2015), and grown individuals (i.e., third growth phase) was tested in separate multiple regression models for each response variable. A stepwise
backward elimination regression procedure was applied to these models, which eliminated predictors and calculated Akaike Information Criterion (AIC) values associated with each new model. The full models included as predictors: hair cortisol concentration, hair testosterone concentration, and age. The final models with the lowest respective AIC values were as follows: (1) hair cortisol concentration as a predictor of relative body mass in growing individuals; (2) hair cortisol concentration as a predictor of relative body mass in grown individuals; and (3) hair testosterone as a predictor of relative growth rate in growing individuals.

We also explored the potential correlation between hair cortisol and hair testosterone levels. Because Coquerel’s sifakas exhibit extremely low levels of sexual size dimorphism, and males and females follow similar growth trajectories during the first and second phases of growth (Tennenhouse, 2015), male and female data were pooled for these analyses.

We calculated the odds ratio of being light-for-age or heavy-for-age in a given growth phase for individuals who were either light-for-age or heavy-for-age in previous growth phases. For each 2 x 2 contingency table, cells containing zero were set to 0.5 (Fleiss, 1981).

### 4.4 Results

We found a significant negative relationship between hair cortisol concentration and relative body mass among growing Coquerel’s sifakas ($b = -0.004$, $t_{1,12} = -2.323$, $P = 0.0386$, $r^2 = 0.31$; Fig. 4.2), but this relationship was not observed
among grown individuals \( (P = 0.635, r^2 = 0.0136) \). Additionally, the relative growth rates of growing individuals were not correlated with hair testosterone concentration \( (P = 0.450, r^2 = 0.0484) \), and hair cortisol and testosterone concentrations were not correlated among growing individuals \( (P = 0.610, r^2 = 0.0235) \). There was no significant linear or polynomial association between age and hair cortisol concentration. Nonetheless, visual assessment of a cubic smoothing spline suggests that young individuals have slightly elevated hair cortisol, which levels off into adulthood (Fig. 4.3).

The odds of being light-for-age in growth phase two was 5.5 times higher for individuals who were light-for-age in growth phase one, and these increased odds were statistically significant \((OR = 5.5, 95\% CI = 1.073-28.30)\). Additionally, the odds of being light-for-age in growth phase three was 26.71 times higher for individuals who were light-for-age in growth phases one and two, and these increased odds were statistically significant \((OR = 26.71, 95\% CI = 1.39-513.12)\). Being heavy-for-age in growth phases one and two led to an 8.75 times higher odds of being heavy-for-age in growth phase three \((OR = 8.75, 95\% CI = 1.75-43.60)\).

4.5 Discussion

This study examined the long-term effects of cortisol and testosterone on growth and relative body mass in Coquerel’s sifakas. We obtained estimates of long-term cortisol and testosterone levels by measuring these hormones in hair samples. Additionally, by using long-term data available from the Duke Lemur Center, we
were able to accurately measure individual growth rates and relative body masses. We found that growing individuals with high hair cortisol concentrations tended to have relatively low body mass, and that individuals who were light-for-age in early life were also light-for-age in adulthood, while individuals who were heavy-for-age in early life remained heavy-for-age in adulthood.

The finding of a negative trend between cortisol concentration and relative body mass among growing individuals is in agreement with research on anubis baboons (Fourie, 2012) and humans (Kertes et al., 2008). Cortisol is secreted as part of the stress response, which mobilizes energy by increasing blood glucose levels (Romero and Butler, 2007) and diverting energy from digestion, reproduction, growth, and tissue repair, allowing animals to survive acute challenges (Sapolsky, 2000). However, chronic stress can have adverse effects on growth and body size (Chrousos, 2009; Sapolsky, 2000). Indeed, chronically elevated cortisol is known to have catabolic effects on muscle (Brillon et al., 1995; Gelfand et al., 1984; Gore et al., 1993). An extreme example of the consequences of chronic stress is the phenomenon of psychosocial short stature, which is a term describing severe short stature in childhood or adolescence associated with psychosocial stress (Khadikar et al., 1998). Although hair cortisol concentration in Coquerel’s sifakas shows a negative trend with age in early life, there is still considerable variation in hair cortisol concentration among individuals at any given age. By comparing hair cortisol concentration to a standard measure of body mass that controlled for age,
the present findings support the idea that chronic stress during development can contribute to deficits in relative body mass in non-human primates.

Contrary to expectation, we found no association between hair testosterone concentrations and relative growth rate. As an anabolic steroid hormone, testosterone theoretically promotes growth. However, previous studies examining the effects of testosterone on growing primates have focused on the development of secondary sexual characteristics (Castracane et al., 1986; Emery-Thompson et al., 2012; Soliman et al., 1995; Setchell and Dixson, 2001; Terasawa and Fernandez, 2001; Wickings and Dixson, 1992), rather than growth per se. It is therefore possible that the effects of testosterone on primate growth and body mass are negligible. Alternatively, Coquerel’s sifakas may be unique in terms of the lack of relationship between testosterone and growth. Further research on growth rates, relative body mass, and testosterone in other primate species are required to resolve this question.

Individuals in a given weight-for-age category (light or heavy) in the early phases of growth tended to remain in that same category in later phases, which is consistent with previous studies on non-human primates (Altmann and Alberts, 2005; Setchell et al., 2001) and humans (Stein et al., 2010; Sterling et al., 2012). The link between individual relative body masses during these periods suggests that low relative body mass in early life has carry-over effects, and indicates that compensatory growth (Metcalfe and Monaghan, 2001) does not occur in this
species. Although compensatory growth is a widespread phenomenon, a period of rapid growth can carry significant costs (Metcalfe and Monaghan, 2001). Early growth rates in lemurs are high relative to other primates (Vinicius and Mumby, 2013; Tennenhouse, 2015); the cost of an additional period of rapid compensatory growth may therefore be physiologically unfeasible or too costly in terms of exposure to predators due to increased foraging.

Together, these findings suggest that chronic elevations in cortisol levels during early life may influence not only relative body mass during growth, but also in adulthood. A limitation of this study was that subjects for which hormone levels during early growth were assessed were not followed into adulthood, as this would have required a long-term study. However, because the subjects of this study are continually being monitored and regularly weighed, it will eventually be possible to conduct a follow-up study directly assessing how the hormone levels of individuals during growth, which have been documented here, are associated with those same individuals’ relative body mass in adulthood.

4.6 Acknowledgements
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Society of Primatologists Small Research Grant, the University of Toronto, and a Natural Sciences and Engineering Research Council of Canada Postgraduate Scholarship to E.M.T.

4.7 Author Contributions

Conceived and designed the study: EMT and MAS. Collected samples: EMT. Performed lab work: EMT, SP, NPB. Contributed reagents/materials/analysis tools: EMT, SP, NPB, JLB. Analyzed the data: EMT. Wrote the paper: EMT. All authors commented on and edited the manuscript.

4.8 References


Figure Legends

**Figure 4.1.** Segmented regressions for Coquerel’s sifakas, *Propithecis coquereli*, taken from Tennenhouse (2015). Solid lines show best fits for male (blue) and female (red) growth data. Dashed vertical lines indicate first and second break points for males (blue) and females (red), separating the first, second, and third growth phases. The final phase of growth has an intercept set to connect to the final breakpoint and a slope set to zero for presentation purposes.

**Figure 4.2.** Least squares regression showing relationship between hair cortisol (pg/mg) and standard weight in growing Coquerel’s sifakas, *Propithecus coquereli*.

**Figure 4.3.** Cubic smoothing spline fitted to age and hair cortisol concentration data for Coquerel’s sifakas.
Figure 4.1
Figure 4.2

\[ b = -0.004, \ P = 0.0386 \]

\[ R^2 = 0.31 \]
Figure 4.3

![Graph showing the relationship between age and hair cortisol levels. The graph includes a trend line with the notation $R^2 = 0.134$, $P = 0.21$, and $n = 24$. The x-axis represents age (years), and the y-axis represents hair cortisol (ng/mg).]
Chapter 5
Conclusion

5.1 Summary of Findings

Although the unusual traits found in lemurs have been the focus of many studies (e.g. Drea, 2007; Dunham, 2008; Dunham and Rudolf, 2009; Eichmueller et al., 2013; Gordon et al., 2013; Kappeler and Fichtel, 2015; Kappeler and Schaeffler, 2008; Leigh and Terranova, 1998; Petty and Drea, 2015; Wright, 1999), they remain poorly understood. Therefore, the aim of this dissertation was to gain new insights into some of these traits by implementing cross-species comparative methods and endocrinological analyses. The main conclusions concerning the evolution of sexual size monomorphism, the relationships between hormones and social behaviour, including intersexual dominance relationships, and the contributions of hormones to growth patterns and body size, are outlined below.

In Chapter 2, I tested the extensively cited hypothesis that widespread sexual size monomorphism in lemurs stems from their unusually short periods of growth (Leigh and Terranova, 1998). By considering not only the dichotomy of the early rapid growth and asymptotic body mass phases, but also an additional period of slow growth that occurs in between, I was able to accurately model growth in 18 species of lemurs. Further analysis of the resulting growth parameters clearly demonstrated that the distinct period of slow growth that I identified is sufficiently
long to promote sexual bimaturism and sexual size dimorphism. Additionally, three of the species analyzed exhibited substantial sexual size dimorphism. Together, these findings indicate that the constraints on growth imposed by Madagascar’s extreme seasonality likely do not limit sexual size dimorphism in lemurs.

The focus of Chapter 3 was the relationship between hormones, intrasexual social behaviour, and intersexual dominance relationships in ring-tailed lemurs. By quantifying steroid hormone levels from hair samples, I was able to investigate the social causes and consequences of long-term average hormone levels. I found that an individual’s rate of receiving aggression was a better predictor of chronic stress than the rate at which they directed aggression toward others in both males and females. Furthermore, females were found to have the same average concentration of testosterone as males over the long term. Relatively high levels of testosterone potentially mediated high rates of female aggression, and might underpin the female dominant social system exhibited by this species.

In Chapter 4, I investigated the hormonal basis of growth in Coquerel’s sifakas, using long-term growth records. Long-term cortisol concentrations, quantified from hair samples, were found to negatively predict relative body mass of growing individuals, indicating that chronic stress in early development can lead to body size deficits. Moreover, an individual’s relative body mass in early life was strongly related to their relative body mass in later life. Together, these findings
suggest that the adverse effects of chronic stress in early life on relative body mass potentially carry over into adulthood.

5.2 Contributions

The research presented in this dissertation provides contributions to methodology and theory in the field of primatology. Through my analysis of growth patterns across lemur species, I make the important observation that growth models in the species should not be constrained to the common two-phase growth models. Future studies involving modeling of growth in primates will undoubtedly benefit from following the methods presented in Chapter 2. Additionally, Chapters 3 and 4 provide details of hair hormone analysis procedures for ring-tailed lemurs and Coquerel’s sifakas. While hair cortisol has successfully been quantified in ring-tailed lemurs (Fourie and Bernstein, 2012), this is the first example to my knowledge of extraction and measurement of sex steroid hormones in ring-tailed lemur hair samples, and of and hair hormone analysis being performed on Coquerel’s sifaka samples. As the measurement of hormones from hair samples becomes increasingly common in primatology (Carlitz et al., 2014; Davenport et al., 2006; Dettmer et al., 2012; Fourie and Bernstein, 2011; Kapoor et al., 2014), these procedures will likely be replicated in future research.

The evidence provided in Chapter 2 indicating that short growth durations likely do not constrain sexual size dimorphism in lemurs will encourage a shift toward tests of alternate theories that have been proposed to explain widespread
sexual size monomorphism among lemurs (Tennenhouse, 2015). In Chapter 3, I present a potential physiological mechanism underlying the rare social dominance of females over males that is commonly observed in lemurs. A common mechanism underlying the occurrence of female dominance over males across mammalian species has not yet been identified; thus, it is possible that the hormonal profile that I suggest underpins this trait in lemurs could potentially extend to other species. Finally, my findings in Chapter 4 contribute to a growing body of evidence linking early life physiology in mammals to traits in adulthood (e.g. Dantzer et al., 2013; Dufty et al., 2002; Wallen, 2005).

5.3 Future Directions

Though this dissertation resolves some key questions about lemur behaviour, life history, and development, my findings also raise a number of new questions. For example, with the new understanding that sexual size monomorphism in lemurs is not the result of reduced growth periods, it now becomes necessary to return to the fundamental question of what factors underlie sexual size monomorphism in lemurs. Additionally, the knowledge that some lemur species exhibit high levels of sexual size dimorphism warrants research that addresses the variation in sexual size dimorphism within the lemur clade. For example, further investigation into the effectiveness of copulatory plugs in conferring paternity for multiple lemur species could provide insights into this variation. The finding that ring-tailed lemur males and females have similar baseline testosterone concentrations, and that this coincided with higher rates of aggression
in females than in males, requires that replicated studies be conducted on different ring-tailed lemur populations, and on other male-dominant and female-dominant primate species for comparison. Finally, an important next step will be to determine if there is a direct link between elevated cortisol in early life and relative body mass in adulthood in Coquerel’s sifakas through longitudinal studies.

There are few rules in nature that are without exception, and exploring the exceptional cases can provide insight into the mechanisms underlying patterns that are typically observed. Lemurs are exceptional with respect to many traits, including female dominance over males, sexual size monomorphism, and rapid growth and development. They are therefore a valuable taxon on which to focus future research, in order to better our understanding about the basis of some of these traits, and to better understand the factors underlying the variation in these traits that exists in primates and other mammals.

5.4 References


## Appendix 2.1. Growth parameter and adult body mass estimates for 18 lemur species

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<td>(0.589)</td>
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<td>2.752</td>
<td>3.767</td>
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<td>0.867</td>
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<td>(0.137)</td>
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<td>Tail (cm)</td>
<td>Ear (cm)</td>
<td>Snout (cm)</td>
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<td>Femur (cm)</td>
<td>Hum (cm)</td>
<td>MNH</td>
<td>MRW</td>
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<td>Eulemur rubriventer</td>
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<td>6.890</td>
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<td>0.228</td>
<td>0.673</td>
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<td>4.990</td>
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<td>0.364</td>
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<td>Mirza zaza</td>
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<td>1.180</td>
<td>1.503</td>
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<td>0.722</td>
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<td>(0.064)</td>
<td>(0.123)</td>
<td>(0.037)</td>
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<tr>
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<td>M</td>
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<td>1.564</td>
<td>1.515</td>
<td>0.201</td>
<td>0.721</td>
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<td>(0.096)</td>
<td>(0.019)</td>
<td></td>
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<tr>
<td>Varecia variegata</td>
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<td>6.160</td>
<td>14.367</td>
<td>0.539</td>
<td>0.814</td>
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<td>(0.378)</td>
<td>(0.952)</td>
<td>(0.056)</td>
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141
<table>
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<th>Species</th>
<th>Gender</th>
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<th>13.233</th>
<th>1.015</th>
<th>0.947</th>
<th>3430.723</th>
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<th>699</th>
<th>17.44</th>
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<td>(0.308)</td>
<td>(0.075)</td>
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<td>(0.502)</td>
<td>(0.198)</td>
<td>(344.794)</td>
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<tr>
<td></td>
<td>M</td>
<td>(0.016)</td>
<td>(0.445)</td>
<td>(0.083)</td>
<td>(243.704)</td>
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<tr>
<td>Propithecus edwardsi</td>
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<td>13.263</td>
<td>2.389</td>
<td>0.884</td>
<td>3567.024</td>
<td>435</td>
<td>1410</td>
<td>29.76</td>
</tr>
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<td>(0.063)</td>
<td>(0.502)</td>
<td>(0.198)</td>
<td>(344.794)</td>
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</tr>
<tr>
<td></td>
<td>M</td>
<td>(0.149)</td>
<td>(0.445)</td>
<td>(0.083)</td>
<td>(243.704)</td>
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<td></td>
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<tr>
<td>Microcebus murinus</td>
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<td>1.5</td>
<td>0.485</td>
<td>0.065</td>
<td>0.667</td>
<td>83.200</td>
<td>1134</td>
<td>1665</td>
<td>31.17</td>
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<tr>
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<td>(0.049)</td>
<td>(0.016)</td>
<td>(0.001)</td>
<td>(12.300)</td>
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<td></td>
<td>M</td>
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<td>0.433</td>
<td>0.028</td>
<td>0.675</td>
<td>77.900</td>
<td>1852</td>
<td>2080</td>
<td>37.52</td>
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<tr>
<td>Cheirogaleus medius</td>
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<td>2.036</td>
<td>0.124</td>
<td>0.496</td>
<td>237.000</td>
<td>410</td>
<td>2347</td>
<td>38.83</td>
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<tr>
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<td>(0.079)</td>
<td>(0.016)</td>
<td>(0.001)</td>
<td>(9.980)</td>
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</tr>
<tr>
<td></td>
<td>M</td>
<td>0.193</td>
<td>1.45</td>
<td>2.036</td>
<td>0.124</td>
<td>237.000</td>
<td>410</td>
<td>2347</td>
<td>38.83</td>
</tr>
</tbody>
</table>
| Species          | Sex | Mass | Phenotypic Score | Males | Females | Troop Size | Sex Ratio | Age | Genotype | Number
|------------------|-----|------|------------------|-------|---------|------------|-----------|-----|----------|--------|
| Eulemur albifrons| F   | 5.088| 0.856            | 2149.766 | 8 | 129 | 27.4
|                  |     | (0.387) |          | (149.632) |   |   |   |
|                  | M   | 6.255| 0.592            | 2008.709 | 5 | 162 | 13.92
|                  |     | (1.256) |          | (159.989) |   |   |   |
| Eulemur sanfordi | F   | 3.877| 0.284            | 2164.025 | 5 | 452 | 50.78
|                  |     | (0.615) |          | (156.700) |   |   |   |
|                  | M   | 3.638| 0.581            | 2008.709 | 9 | 307 | 31.60
|                  |     | (0.464) |          | (159.989) |   |   |   |
| Eulemur fulvus   | F   | 5.477| 0.724            | 2596.139 | 13 | 185 | 18
|                  |     | (0.654) |          | (286.110) |   |   |   |
|                  | M   | 6.134| 0.880            | 2381.895 | 12 | 106 | 5.13
|                  |     | (0.698) |          | (375.341) |   |   |   |
Sample sizes are given for the number of data points in the growth phases ($n$ growing) and adult phases ($n$ adult) of the growth models.

Break1 = Age (years) at which breakpoint one occurs (SE).

Break2 = Age (years) at which breakpoint two occurs (SE). NA in cases where two-piece models were run.

Rate1 = Growth rate (grams/day) during the first growth period (SE).

Rate2 = Growth rate (grams/day) during the second growth period (SE). NA in cases where two-piece models were run.

ABM = Average adult body mass (g) (SD).

Weights/Individual = Average number of weight measurements taken per individual.
**Appendix 2.2.** Species-specific life history variables obtained from the literature and growth durations

<table>
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<tr>
<th>Species</th>
<th>Weaning</th>
<th>♂ Reproduction</th>
<th>♀ Duration1</th>
<th>♂ Duration2</th>
<th>♀ Duration1</th>
<th>♂ Duration2</th>
</tr>
</thead>
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<td>2.297</td>
<td>0.684</td>
<td>1.157</td>
<td>0.908</td>
<td>2.457</td>
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<td><em>Eulemur fulvus</em></td>
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<td>2.550</td>
<td>1.137</td>
<td>NA</td>
<td>1.013</td>
<td>NA</td>
</tr>
<tr>
<td><em>Eulemur macaco</em></td>
<td>0.371</td>
<td>2.140</td>
<td>0.593</td>
<td>1.157</td>
<td>0.820</td>
<td>2.203</td>
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<td><em>Varecia variegata</em></td>
<td>0.349</td>
<td>2.003</td>
<td>0.491</td>
<td>5.669</td>
<td>0.510</td>
<td>2.349</td>
</tr>
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<td><em>Cheirogaleus medius</em></td>
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<td>1.295</td>
<td>0.292</td>
<td>NA</td>
<td>0.258</td>
<td>NA</td>
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<td><em>Propithecus coquereli</em></td>
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<td>4.200</td>
<td>1.008</td>
<td>2.172</td>
<td>1.045</td>
<td>2.387</td>
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<tr>
<td><em>Eulemur mongoz</em></td>
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<td>2.510</td>
<td>0.847</td>
<td>1.905</td>
<td>0.663</td>
<td>1.567</td>
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<td><em>Microcebus murinus</em></td>
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<td>1.000</td>
<td>0.380</td>
<td>NA</td>
<td>0.302</td>
<td>NA</td>
</tr>
<tr>
<td><em>Propithecus edwardsi</em></td>
<td>0.794</td>
<td>4.000</td>
<td>1.286</td>
<td>4.211</td>
<td>1.613</td>
<td>4.278</td>
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<td>0.527</td>
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<td><em>Eulemur rubriventer</em></td>
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<td>0.860</td>
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<td>0.608</td>
<td>4.382</td>
<td>0.422</td>
<td>2.093</td>
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</table>

*Weaning = Average weaning age (years). Values obtained from: Kappeler & Pereira (2003); Ternaud (2004); Vasey (2007); Wright (1999).*
♀ Reproduction = Average age at female's first reproduction (years). Values obtained from: Kappeler & Pereira (2003); Ostner & Kappeler (2004); Wright (1995).

♀ Duration1 = Duration of females’ first growth phase (years).

♀ Duration2 = Duration of females’ second growth phase (years). NA in cases where two-piece models were run.

♂ Duration1 = Duration of males’ first growth phase (years).

♂ Duration2 = Duration of males’ second growth phase (years). NA in cases where two-piece models were run.
Appendix 2.3

**Eulemur coronatus**

**Eulemur collaris**

![Graph of Eulemur coronatus growth over age]

![Graph of Eulemur collaris growth over age]

![Graph of Eulemur coronatus growth over age]

![Graph of Eulemur collaris growth over age]
**Appendix 2.3.** Segmented regression estimates for 11 lemur species. Solid lines show best fits for male (blue) and female (red) growth data. Dashed vertical lines indicate first and second break points for males (blue) and females (red). The final phase of growth has an intercept set to connect to the final breakpoint and a slope set to zero for presentation purposes.
Appendix 3.1. Assays and validations for hair hormone analyses in ring-tailed lemurs

Cortisol

The manufacturer reported the following crossreactivities of the antibody with steroids: cortisol = 100%, prednisolone = 13.6%, corticosterone = 7.6%, deoxycorticosterone = 7.2%, progesterone = 7.2%, cortisone = 6.2%, deoxycortisol = 5.6%, prednisone = 5.6%, and dexamethasone = 1.6%. The sensitivity of this assay was 1.0 ng/ml. Fifty ul of reconstituted sample was run in duplicate according to the assay protocol on two plates. The mean inter-assay and intra-assay coefficients of variation were 4.36% and 2.24%, respectively. Analysis of covariance revealed no significant interaction between absorbance and group (standard curve based on kit calibrators and serial dilutions of samples), indicating parallelism for females ($F = 0.189, P = 0.693$) and males ($F = 0.17, P = 0.701$).

Testosterone

The manufacturer reported the following crossreactivities: testosterone = 100%, dihydrotestosterone = 5.2%, androstenedione = 1.4%, androstanediol = 0.8%, progesterone = 0.5%, and androsterone = 0.1%. The sensitivity of this assay was 1.0 pg/ml. One hundred ul of reconstituted sample was run in duplicate according to the assay protocol on a single plate. The mean intra-assay coefficient of variation was 2.83%. Analysis of covariance revealed parallelism between the standard curve generated using kit calibrators and serial dilutions of samples for females ($F = 4.62$, P = 0.031).
$P = 0.084$) and males ($F = 3.02, P = 0.14$).

**Estradiol**

The estradiol antiserum (R4972; Coralie Munro, UC California, Davis, CA) was raised in rabbits and had crossreactivities of 100% with estradiol, 3.3% with estrone, 0.8% with progesterone, 1.0% with testosterone and androstenedione, and <1% with cortisol and dihydrotestosterone. Twenty ul of reconstituted sample was run in duplicate according to the assay protocol on two plates. The mean inter-assay and intra-assay coefficients of variation were 2.9% and 4.01%, respectively. Analysis of covariance revealed parallelism between the standard curve generated using kit calibrators and serial dilutions of samples for females ($F = 0.04, P = 0.85$) and males ($F = 1.21, P = 0.30$).
Appendix 3.2. Scatterplot showing the association between hair testosterone concentration and rate of aggression in male ring-tailed lemurs.
**Appendix 3.3.** Data on rates of aggression and hair hormone concentrations in ring-tailed lemurs

<table>
<thead>
<tr>
<th>Name</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Aggression Given (rate per hour)</th>
<th>Aggression Received (rate per hour)</th>
<th>Cortisol (pg/mg)</th>
<th>Testosterone (pg/mg)</th>
<th>Estradiol (ng/mg)</th>
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Appendix 4.1. Assays and validations for hair hormone analyses in Coquerel’s sifakas

**Cortisol**

The manufacturer reported the following cross-reactivities of the antibody with steroids: cortisol = 100%, prednisolone = 13.6%, corticosterone = 7.6%, deoxycorticosterone = 7.2%, progesterone = 7.2%, cortisone = 6.2%, deoxycortisol = 5.6%, prednisone = 5.6%, and dexamethasone = 1.6%. Fifty ul of reconstituted sample was run in duplicate on a single plate according to the assay protocol. The intra-assay coefficient of variation was 2.64%. Analysis of covariance revealed no significant interaction between absorbance and group (standard curve based on kit calibrators and serial dilutions of samples), indicating parallelism for females ($F = 0.225, P = 0.660$) and males ($F = 0.541, P = 0.503$).

**Testosterone**

The manufacturer reported the following cross-reactivities: testosterone = 100%, dihydrotestosterone = 5.2%, androstenedione = 1.4%, androstanediol = 0.8%, progesterone = 0.5%, and androsterone = 0.1%. One hundred ul of reconstituted sample was run in duplicate according to the assay protocol. All samples were run on single plates and the intra-assay coefficient of variation was 1.71%. Analysis of covariance revealed parallelism between the standard curve generated using kit calibrators and serial dilutions of samples for females ($F = 1.903, P = 0.226$) and males ($F = 3.684, P = 0.113$).
### Appendix 4.2. Data on body mass, growth, and hair hormone concentrations in Coquerel’s sifakas

<table>
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<th>Testosterone (pg/mg)</th>
<th>Mass (g)</th>
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Growth phases are based on age categories defined in Tennenhouse (2015).

Growth rates were only calculated for individuals in growth phases one and two.

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1 Growth phases are based on age categories defined in Tennenhouse (2015).

2 Growth rates were only calculated for individuals in growth phases one and two.