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Reproductive Biology of Mouse and Dwarf Lemurs of Eastern Madagascar, With an Emphasis on Brown Mouse Lemurs (*Microcebus rufus*) at Ranomafana National Park, A Southeastern Rainforest

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REPRODUCTIVE BIOLOGY OF MOUSE AND DWARF LEMURS OF EASTERN
MADAGASCAR, WITH AN EMPHASIS ON BROWN MOUSE LEMURS
(*MICROCEBUS RUFUS*) AT RANOMAFANA NATIONAL PARK, A
SOUTHEASTERN RAINFOREST

A Dissertation Presented

by

MARINA BEATRIZ BLANCO

Submitted to the Graduate School of the
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of the requirements for the degree of

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Anthropology

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DEDICATION

To the unforgettable nocturnal lemurs of Madagascar, especially to mouse lemur female
“J” who (un)willingly shared so many estrous cycles and pregnancies during my field
work years.

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After years of academic endeavors, emotional turmoil, field work and collaborations I find it almost impossible to name everyone who has contributed in one way or another to support, help and make my almost perennial graduate career enjoyable.

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have learned from her to pay attention to the tiniest details as much as to explore big-picture questions, to think around driving hypotheses and be optimistic despite my nature. I will always remember to find the “hook”, check the flow and go full circle in every paper I write. Thank you Laurie, I cannot imagine my future academic career without you.

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ABSTRACT (ENGLISH)

REPRODUCTIVE BIOLOGY OF MOUSE AND DWARF LEMURS OF EASTERN
MADAGASCAR, WITH AN EMPHASIS ON BROWN MOUSE LEMURS
(*MICROCEBUS RUFUS*) AT RANOMAFANA NATIONAL PARK, A
SOUTHEASTERN RAINFOREST

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This dissertation investigates reproductive schedules of brown mouse lemurs at Ranomafana, using intensive trapping techniques. The reproductive condition of female mouse lemurs was recorded on the basis of vaginal morphology, vaginal smears, body mass gain profiles and nipple development. Testis size was measured in males throughout the reproductive season. The timing of the first seasonal estrus was determined in frequently captured females over multiple years and it showed individual periodicities close to 365 days, consistent with endogenous regulation and entrainment by photoperiod. The timing of estrus did not correlate with female age or body mass. Males showed testicular regression during the rainy season, although there was high inter-individual variation in testes size at any given point during the reproductive season. Furthermore, some individuals completed testicular regression earlier than others. Implications for polyestry are discussed.

For comparative purposes, mouse lemurs were also trapped at two study sites in the Tsinjoarivo area: one in a forest fragment and the other within continuous forest. These forests are higher in altitude than the main study area at Ranomafana. Trapping success for mouse lemurs was lower at Tsinjoarivo than Ranomafana. Albeit preliminary, data from Tsinjoarivo suggest that females have lower reproductive success than do females at Ranomafana. Nevertheless, mouse lemurs in the Tsinjoarivo forest fragment did not appear to be in “poorer” condition than those in the continuous forest. It had been reported in the literature that western gray mouse lemurs captured in secondary forests have lower body masses and lower recapture rates than those captured in primary forest; in fact, the opposite was true of the mouse lemurs at Tsinjoarivo.

I additionally collected data on a larger member of the family Cheirogaleidae, the dwarf lemurs (*Cheirogaleus*), which live in sympatry with *Microcebus* at Ranomafana and Tsinjoarivo. I analyzed the patterns of growth, development and reproduction in *Cheirogaleus* and *Microcebus* and compared dwarf and mouse lemurs to other similarly-sized prosimians which do not undergo torpor or hibernation. These comparisons draw attention to the unusual reproductive and metabolic strategies employed by cheirogaleids to cope with Madagascar’s unpredictable environments, which ultimately define their very unique life histories.

ABSTRACT (SPANISH)

BIOLOGÍA REPRODUCTIVA DE LOS LEMURES RATÓN Y ENANOS DEL ESTE DE MADAGASCAR, CON ÉNFASIS EN LEMURES RATÓN PARDOS (*MICROCEBUS RUFUS*) DEL PARQUE NACIONAL DE RANOMAFANA, UNA SELVA LLUVIOSA DEL SUDESTE

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Esta tesis investiga patrones reproductivos de los lemures ratón pardos utilizando técnicas de captura intensiva. La condición reproductiva de las hembras fue determinada a través de inspecciones e isopados vaginales, estado de desarrollo mamario y variaciones de peso. El primer estro estacional, que se pudo determinar en hembras capturadas con regularidad y durante varias estaciones reproductivas, mostró una periodicidad cercana a los 365 días, lo cual es consistente con una regulación endógena sincronizada por el fotoperiodo. No hubo correlación entre la fecha del estro y la edad o peso de las hembras. También se midió el tamaño testicular en machos y aunque todos los individuos redujeron el tamaño testicular durante la estación lluviosa, se observó una gran variación inter-individual especialmente al final del periodo reproductivo cuando la reducción testicular fue completa. Se discuten implicaciones sobre poliestría.

Lemures ratón pardos fueron también capturados en la selva de Tsinjoarivo, al centro-este de Madagascar: en un fragmento selvático y en selva primaria. Estos sitios

están a altitudes mayores que el área principal de muestreo en Ranomafana. Lemures ratón fueron capturados menos frecuentemente en Tsinjoarivo que en Ranomafana. Datos preliminares indican que las hembras tuvieron un menor éxito reproductivo en Tsinjoarivo en comparación con Ranomafana. Sin embargo, en Tsinjoarivo, las hembras capturadas en el fragmento selvático se encontraron en mejores condiciones que las capturadas en selva primaria. Previos reportes han señalado que hembras de lemures ratón grises capturadas en selvas fragmentadas tenían comparativamente menor peso corporal y que el número de individuos recapturados era también menor en fragmentos que en selva primaria. En este caso, los lemures ratón pardos de Tsinjoarivo mostraron la tendencia opuesta.

También se capturaron lemures enanos (*Cheirogaleus*) pertenecientes a la misma familia Cheirogaleidae, que viven en simpatría con *Microcebus* en Ranomafana y Tsinjoarivo. Perfiles de crecimiento, desarrollo y reproducción de *Cheirogaleus* y *Microcebus* fueron analizados y comparados con otros grupos de prosimios de tamaño similar pero que no exhiben letargo ni hibernación. Estas comparaciones destacan las estrategias reproductivas y metabólicas de los cheirogálidos para enfrentar las condiciones climáticas imprevisibles de Madagascar, y sus peculiares tácticas de supervivencia (“life histories”).

ABSTRACT (MALAGASY)

NY FANANAHANA ARA-BIOLOJIKAN'NY "TSIDY MENA" SY NY "MATAVY RAMBO" AO AMIN'NY FARITRA ANTSINANAN'I MADAGASIKARA, ASONGADINA MANOKANA NY TSIDY MENA AO AMIN'NY VALAN-JAVABOAHARIN'I RANOMAFANA.

MEY 2010

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Ity boky ity dia natao hampahalalana ny fotoana fananahan'ny tsidy mena miaina ao amin'ny valan-javaboaharin'i Ranomafana. Niainga tamin'ny alalan'ny fihazana ara-teknika nandritra ny andro maromaro nifanesy ny asa natao. Faritana amin'ny alalan'ny fisokafan'ny lalan-jaza sy ny fitombon'ny lohanono ny fahalonahan'ny vavy ary dinihana manampy izany ihany koa ny lanjany. Ny an'ny lahy kosa dia faritana amin'ny alalan'ny halavana sy ny haben'ny voan'ny filahiany. Hita ary fa mahatratra 365 andro ny elanelan'ny fahalonahana roa mifanesy eo amin'ny vavy. Io fotoana mahalonaka ny vavy io dia voamarika fa mifanaraka tsara amin'ny fifandrindrana ara-biolojikan'ny taovany sy ny hazavan'ny andro. Tamin'ny alalan'ny fandalinana ireo taova ireo kosa anefa dia voamarika fa ny fahalonahan'ny vavy dia tsy mifandray velively amin'ny taonany na koa ny lanjany. Ho an'ny lahy kosa na dia hita misongadina aza ny fihenana'ny voa'ny filahiany mandritra ny fotoam-pahavaratra, dia voamarika koa ny fiovana amin'izy ireo. Lasa aloha io fihenana io ho an'ny sasantsasany ary somary tara kosa ho an'ny hafa.

Natao koa ny fampitahana ny tsidy tao amin'ny alan'i Tsinjoarivo sy Ranomafana. Ny faritr'i Tsinjoarivo izay ahitana ala mitsinjarazara sy ala mifampitohy ary avoavo kokoa miohatra amin'ny alan'i Ranomafana. Hita ary fa vitsy kokoa ny biby ao amin'ny alan'i Tsinjoarivo nohon'ny biby ao amin'ny alan'i Ranomafana. Kelikely kokoa ny fahombiazana ara-pananahanan'ny vavim-biby ao Tsinjoarivo nohon'ny ao Ranomafana. Voamarika ihany koa fa ao Tsinjoarivo dia mavesa-danja ny biby ao amin'ny ala mitsinjarazara nohon'ny biby ao amin'ny ala mifampitohy. Ny boky anefa dia milaza fa tamin'ny alalan'ny fihazana ara-teknika dia vitsy ary maivan-danja kokoa ny tsidy ao amin'ny ala voatsinjarazara nohon'ny tsidy ao amin'ny ala mifampitohy. Mifanohitra tanteraka amin'io tranga eo ambony io kosa ny tsidy ao amin'ny alan'i Tsinjoarivo.

Natao koa ny fanadihadiana mikasika ny matavy rambo ao amin'ny alan'i Tsinjoarivo sy Ranomafana. Ny fandalinana dia nifantoka manokana tamin'ny fitombon'io biby io, ny fivelarany, ary ny fananahany. Natao ihany koa ny fampitahana azy ireo amin'ny biby hafa izay mitovy habe aminy, saingy tsy milevona kosa. Ny fanadihadiana dia maneho fa mampiavaka tanteraka ny fianakaviam-ben'ny "Cheirogaleidae" ny fomba fananahany, ny fandrindrana ara-biolojikan'ny vatany, ary ny fomba filevenany eo anatrehan'ny fahasarotan'ny tontolo manodidina iainany.

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CHAPTER I

INTRODUCTION

Since my early years in college when I first came across his name, I have cherished natural history stories by S. J. Gould. They usually begin telling a small, unusual and interesting tale that would later develop into a gracious example of evolution. They were a source of inspirational ideas about how nature is shaped by forces of evolution in a world subject to contingency. I also learned how scientific curiosity must obey rules, trial and error, verification and refutation, repetition and experimentation. The origin of a question, however, could be random and arbitrary. It was just like that, after hearing about the mouse lemurs and Madagascar that I became interested in their reproductive biology.

Mouse lemurs (*Microcebus*) are one of the five genera of the family Cheirogaleidae (along with *Allocebus*, *Cheirogaleus*, *Phaner* and *Mirza*), all comprised of small-bodied (~30g-500g) nocturnal lemurs. Mouse lemurs are interesting study subjects in that they are mouse-sized, omnivores and relatively easy to keep in captivity. At the same time they are primates, so they represent a biological system on which one can conduct biomedical research with greater potential for applications to humans than might research on other (non-primate) mammals. For example, some aged mouse lemurs in captivity express neurodegeneration resembling human Alzheimer's disease (Bons et al., 2006). This dual nature of being a primate but also a very distant relative which is well suited for "laboratory" conditions (e.g. long generation times, multiple offspring) has rendered mouse lemurs attractive targets of research. I would personally argue that their significant distance from humans in the primate tree (they look more like rodents than

like anthropoid primates) has allowed researchers to perform procedures that are more invasive than would be condoned for “higher” primates.

Captive studies on mouse lemurs, which now span more than three decades, have relied on information collected primarily on one species, the gray mouse lemur, *M. murinus*, and to a lesser extent, brown mouse lemurs (*M. rufus* and possibly *M. lehilahytsara*) (Wrogemann and Zimmermann, 2001; Wrogemann et al., 2001). This research explored a variety of topics including reproductive regulation (Perret and Aujard, 2001; Wrogemann et al., 2001), dynamics of female estrous synchrony (Radespiel and Zimmermann, 2001), behavioral correlates of female and male hormonal variation under different experimental conditions (Perret, 1986; Perret, 1992), the role of olfactory bulbs in photoperiodic responses (Perret and Schilling, 1993; Perret et al., 2003; Séguy and Perret, 2005), circadian regulation and aging (Aujard et al., 2001; Aujard et al., 2006; Perret and Aujard, 2005; Perret and Aujard, 2006; Schilling et al., 1999), among other topics.

Mouse lemurs are long day seasonal breeders and reproduction is entrained by photoperiod. In a ~2 year study, Perret and Aujard (2001) found that female mouse lemurs underwent estrus even under constant light conditions, whereas males required alternating short and long days in order for proper testicular development to occur. Estrous synchrony in female gray mouse lemurs was higher during the first seasonal estrus (and weakened from the first to the second) and synchronization did not appear to be socially induced (Radespiel and Zimmermann, 2001).

Field work has confirmed the seasonal nature of reproduction in mouse lemurs. However, studies showed variation in the time and duration of the reproductive season

between and within species at different sites across Madagascar (Atsalis, 2008; Eberle and Kappeler, 2004; Génin, 2008; Lahann et al., 2006; Radespiel, 2000; Randrianambinina et al., 2003; Schmelting et al., 2000; Schwab, 2000). Species diversity likely contributed to the observed reproductive variation. Captive studies were based primarily on a single species while field studies focused on multiple mouse lemur species. In the process of studying mouse lemurs in the wild, researchers ascertained that the taxonomic diversity of mouse lemurs had been underestimated. The number of recognized species of *Microcebus* was substantially transformed from ~5 species in the late 1990s to 18, and more may be described in the next few years (Louis et al., 2008; Olivieri et al., 2007; Radespiel et al., 2008).

After assessing data from captivity and field work, I was initially interested in investigating how much of the variation observed in female reproductive schedules between wild mouse lemur populations could be caused by species-level biological differences, and how much might respond in a plastic manner to habitat variation¹. I was also interested in the relationship between the number of litters a female may have per season (i.e., polyestry) and environmental variation (year to year, place to place). I considered several possible research strategies. First, it made sense to study a single population in different years during the reproductive season. It also made sense to study different eastern mouse lemur populations in the same year (e.g., at different altitudes). Ideally, one might like to sample sympatric species of cheirogaleids in single habitats to ascertain “options” that might be available to these animals in the same habitats.

Alternatively, if possible, one might study the same species in more and in less disturbed habitats, but in the same general location.

With these questions in mind, between October and December in 2004, I conducted a pilot study at Ranomafana National Park, a rainforest in southeastern Madagascar joining a collaborative team of researchers who were already studying mouse lemurs. A long term study of a population of brown mouse lemurs at this study site, describing their natural history, had been initiated by Atsalis in the mid 1990s (Atsalis, 1999a; 1999b; 2000; 2008), and I thought it would be interesting to follow the same animals over a period of several years (reproductive seasons). In 2004, I collected preliminary reproductive data, and assessed the reproductive status of females, mostly indirectly (e.g. presence of vaginal openings, possibly pregnant females, possibly lactating females) and measured testis size in males. I marked mouse lemurs with Avid microchips to be able to reliably identify them when recaptured. A year later, in 2005, trapping schedules were changed to accommodate more trapping nights and to minimize the disturbance of reproductive activities; now we trapped every day to maximize captures of females in estrus and we released all mouse lemurs later the same night at capture sites. In my subsequent trips to Ranomafana (2006, 2007, 2008) the same trapping area and the same protocols were used to maintain consistency.

Although some of my original research questions could be addressed through this research, others proved impossible because of logistic difficulties that I had to face in the field. I had feverishly hoped to capture mouse lemurs during the rainy season (December-

¹ A comparison of reproductive parameters between gray and brown mouse lemurs had showed differences in the duration of the reproductive period under captive conditions

March) at Ranomafana, a critical time of the year, when females were expected to wean their first litter, perhaps undergo a second estrus (assuming that they express normal or regular polyestry in the wild) or begin to fatten. Heavy rains and other factors (e.g. foraging activities may be reduced in time and space by both sexes) meant that few individuals entered traps during this period. This reduced capture success to the point that I could only speculate about certain aspects of the reproductive strategies of female brown mouse lemurs at Ranomafana given the little data I had. Whereas in the dissertation chapters that follow, I avoid speculation, I have permitted myself the freedom of speculation in my Conclusions. Many of the ideas mentioned there will require future research, if they are to be amply addressed.

Each of my dissertation chapters stands alone and is formatted as a paper to be submitted for publication. Together, they more or less follow a chronological journey: Chapters II and III are the core of the dissertation in that they discuss in detail the reproductive observations I made in mouse lemurs over the years since my first trip to Ranomafana in 2004. The fourth Chapter presents data on a second study site to which I was introduced by Dr. Mitchell Irwin in 2006, well after I had started data collection at Ranomafana. At this forest of Tsinjoarivo, I focused not merely on trapping mouse lemurs, but I also successfully trapped dwarf lemurs (*Cheirogaleus*) – animals that I had captured at Ranomafana in 2005 with only partial success. This shift in focus allowed me to begin to address additional questions (e.g., cross species comparisons in single habitats, the distribution of cheirogaleids across disturbed and undisturbed habitats). In

(Wrogemann et al., 2001, see also Chapter II).

Chapter V then, I present most of the data that I collected on dwarf lemurs and I compare them to their close relatives, the mouse lemurs, and other similarly-sized prosimians.

In sum, Chapter II reports reproductive observations in mouse lemur females. Results support the statement that female reproduction is endogenously regulated and entrained by photoperiod. Although Radespiel and Zimmermann (2001) considered variation in the seasonal first estrus to have an individual basis and Perret and Aujard (2001) suggested the endogenous nature of reproduction in gray mouse lemur females, no long term data were available describing the timing of estrus (as determined here by cytological characteristics in vaginal smears) in a wild population of mouse lemur females. This simple conclusion, that females undergo the first seasonal estrus regardless of body mass or age, has important implications for mouse lemur reproductive strategies. Chapter III discusses aspects of male reproduction, including variation in testis volume during multiple reproductive seasons at Ranomafana. Unlike females, for which energetic costs are high during pregnancy and particularly lactation, spermatogenesis in males and the maintenance of testicular development is energetically costly, so that individuals regress their testicular tissue outside the window of reproductive opportunities determined by the availability or presence of estrous females. Finally, I discuss variation in testis regression among brown mouse lemur males in relation to the probable expression of female polyestry, and the implications for the duration of the reproductive season. Chapter IV presents mouse lemur observations collected at two study sites within the Tsinjoarivo area, in one of the forest fragments and within continuous forest. Mouse lemurs are well known for living and thriving in very different habitats, even in highly disturbed environments where other lemur species may not be able to survive. However,

little is known about the long term viability of mouse lemur populations in highly degraded habitats. Preliminary studies showed that mouse lemurs inhabiting secondary forests in western Madagascar had lower body masses than their counterparts from primary forest and that recapture rates were also lower in secondary vs. primary forests (Ganzhorn and Schmid, 1998). Albeit preliminary, results from Tsinjoarivo presented in Chapter IV showed overall a higher percentage of females displaying reproductive “failure” or not reproducing at all compared to Ranomafana. Unlike western mouse lemurs, individuals from the forest fragment had higher body masses and were recaptured more often than mouse lemurs trapped in the continuous forest site. Chapter V takes a broader look at mouse and dwarf lemurs, the other members of the family Cheirogaleidae, specifically how they use metabolic strategies such as hibernation to cope with scarcity of resources during the dry season. As discussed in this chapter, the use of torpor and/or hibernation appears to profoundly shape the lives of mouse and dwarf lemurs. The last Chapter presents the conclusions.

Finally, data collected over the years with my local assistants, Malagasy students and other fellow researchers have provided the basis for several publications, usually in collaboration with others. The arguments made in these articles are not repeated in this dissertation although they derive from the research I conducted while doing my dissertation research in Madagascar. Field research almost always yields serendipitous results – i.e., answers to questions that were not even originally posed. This is part of the beauty and fun of doing science, as Dr. Stephen Gould would certainly say. One must of course identify the species of animal on which one is conducting research. This seemed simple in 2004: eastern mouse lemurs were *Microcebus rufus*, and dwarf lemurs in the

same forest were *Cheirogaleus major*. This is how they had been identified in prior publications by other researchers. However, in collecting my own morphometric data and in collaborating with others doing genetic research, I found that (1) the species of dwarf lemur living at Ranomafana (Talatakely) (and previously identified as *C. major*) is actually *Cheirogaleus crossleyi*, (2) two species of dwarf lemurs inhabit Tsingoarivo, one mostly in the continuous forest (*C. crossleyi*, but smaller than the population at Ranomafana) and another in fragmentary forests (*C. sibreei*). The latter species is today known **only** from this single forest, where it is likely highly endangered. I also found that mouse lemurs (likely *Microcebus lehilahytsara* and not *M. rufus* in the high mountains) are rare or difficult to capture in the highlands of Tsingoarivo, whereas dwarf lemurs (which hibernate) are readily captured at this forest. This was also unanticipated, and it demands explanation. Some of the fur samples that I collected were contributed to an island wide study of mouse lemur stable isotopes (carbon and nitrogen) spearheaded by Dr. Brooke Crowley who is now at the University of Toronto. This analysis provides an overview of how or whether mouse lemur isotopes signal variation in diet or habitat; addressing this question has important implications for the use of stable isotopes in reconstructing the diets or habitats of extinct lemurs from their tissue (bone, in the case of the extinct lemurs). Finally, in working with Dr. J. Meyer (U. Mass. Amherst, Department of Psychology) on laboratory tools for analyzing hormones from mouse lemur fecal pellets, we contributed to the methodological literature. My goal here was to conduct hormonal analysis of wild mouse lemurs as non-invasively as possible.

My peer-reviewed journal articles dealing with some of these issues are:

Blanco, M.B. 2008. Reproductive schedules of female *Microcebus rufus* at Ranomafana National Park, Madagascar. *International Journal of Primatology* 29 (2): 323-338.

Blanco, M.B. and Meyer, J.S. 2009. Assessing reproductive profiles in female brown mouse lemurs (*Microcebus rufus*) from Ranomafana National Park, southeast Madagascar, using fecal hormone analysis. *American Journal of Primatology* 71(6): 439-446.

Blanco, M.B., Godfrey, L.R., Rakotondratsima, M., Rahalinarivo, V., Samonds, K.E., Raharison, J.-L. and Irwin, M.T. 2009. Discovery of sympatric dwarf lemur species in the high altitude forest of Tsinjoarivo, Eastern Madagascar: Implications for biogeography and conservation. *Folia Primatologica* 80 (1): 1-17.

Groeneveld, L.F., **Blanco, M.B.**, Raharison, J.-L., Rahalinarivo, V., Rasoloarison, R.M., Kappeler, P.M., Godfrey, L.R., and Irwin, M.T. 2010. MtDNA and nDNA corroborate existence of sympatric dwarf lemur species at Tsinjoarivo, eastern Madagascar. *Molecular Phylogenetics and Evolution* [in galley].

Crowley, B.E., Thorén, S., Rasoazanabary, E., Barret, M.A., Zohdy, S., **Blanco, M.B.**, McGoogan, K.C., Arrigo-Nelson, S.J., Irwin, M.T., Vogel, E.R., Wright, P.C., Radespiel, U., Godfrey, L.R., Koch, P.L. and Dominy, N.J. (submitted). Geographic isotopic variation among mouse lemurs (*Microcebus*) populations. *Journal of Biogeography*.

Two papers are currently in preparation: one in collaboration with Anja Deppe, Andrea Baden and Patricia Wright on the effects of lunar cycles, temperature and rainfall on trapability of mouse lemurs; the second one with Sarah Zohdy, Jukka Jernvall, Patricia Wright, Stephen King and Laurie Godfrey on longevity in wild mouse lemurs.

CHAPTER II

REPRODUCTION IN WILD BROWN MOUSE LEMUR FEMALES AT RANOMAFANA NATIONAL PARK, SOUTHEASTERN MADAGASCAR

Onset of estrus, periodicities of inter-estrus intervals and expression of polyestry

Introduction

Reproductive seasonality is characteristic of environments where energy availability is not constant throughout the year and the probability of reproductive success is increased when the season of highest energetic demands can be coupled with periods of food abundance. Photoperiod is the most important environmental “zeitgeber” (entraining agent) that regulates circannual reproductive rhythms. The extent to which organisms respond to additional environmental or internal cues to adjust reproductive function varies extensively within the mammals, e.g., some female squirrels need to achieve a certain body weight in order to be able to reproduce (Huber et al., 1999).

Madagascar, one of the earth’s biodiversity hotspots, is situated at higher latitudes than other tropical islands which allows for greater day length variation between seasons. Its unique geography and climate gave rise to a variety of habitats, from dry, spiny forests in the west and south to rainforests in the east. Madagascar’s forests have been characterized as more unpredictable than comparable areas in continental Africa (Dewar and Richard, 2007). Unpredictability in the eastern forests is mostly dictated by great intra-annual variation in rainfall (i.e., similar annual amounts, but rainfall is differently distributed across months) whereas the main factor in the west, north and south is strong seasonality. Unpredictability in these regions relates to inter-annual variation (i.e., uncharacteristically “dry” or “wet” years; Dewar and Richard, 2007; Génin, 2008).

Weather variation in Madagascar has generated unique patterns of phenology wherein, for example, fruits may be absent for several months of the year. These patterns differentiate Madagascar from other tropical forests (Wright, 2006). In addition to climatic variation, human disturbance has been an undeniable contributor to environmental unpredictability. Human disturbance has had both direct and indirect impacts on the biology and reproduction of lemur species (Wright, 2006). The most dramatic effects are deforestation; more than 80% of forest cover has been lost. The consequences of deforestation are multifaceted: by removing food and structural resources from the forest and increasing fragmentation and edge habitats, predation rates are prone to increase as are population densities in restricted areas and resulting intra- and interspecies competition. Ecotourism has also been shown to increase predation rates in certain animal species (e.g. ground-roller birds at Ranomafana (Razafimahaimodison, 2004)) and this may indirectly affect lemur activity patterns.

Mouse lemurs (genus *Microcebus*) are small-bodied nocturnal prosimians that live in a variety of habitats in Madagascar. *Microcebus* and their close relatives, the dwarf lemurs (genus *Cheirogaleus*), are unique among primates in that they are heterothermic and undergo torpor or hibernation as energy saving strategies during the season of scarce resources (Génin and Perret, 2003). Although there is significant overall “physiological and behavioral flexibility” within mouse lemurs, a few species (e.g. *M. murinus*, *M. rufus*) have very broad geographical distributions while others have very restricted ranges (e.g. *M. myoxinus*, *M. sambiranensis*) (Radespiel, 2006). Differences in species’ distributions are likely correlated to variation in dietary requirements: some mouse lemurs have more “specialized” (e.g. *M. berthae*) rather than “generalized” (e.g. *M. murinus*)

diets (Dammhahn and Kappeler, 2008). Despite inter-specific differences, behavioral and physiological flexibility is characteristic of mouse lemurs as a group. For instance, members from the same species have been reported to have different reproductive strategies (e.g. one litter vs. two or more per year at different study sites) (Lahann et al., 2006) and individuals from the same population have been observed hibernating and experiencing daily torpor (Kobbe and Dausmann, 2009). Thus, it appears that habitat diversity in addition to unusually high levels of environmental unpredictability has favored a variety of opportunistic responses in small-bodied nocturnal lemurs.

Mouse lemurs are long-day seasonal breeders, and reproduction is mainly restricted to the rainy season, however, the actual duration of the reproductive season depends on the habitat and the species. Due to their small body size, relatively fast generation time, and generalist diet (omnivory), mouse lemurs are well suited for survival in captivity.

The gray (*M. murinus*) and brown (*M. rufus*) mouse lemurs have been bred well in captivity and they have showed similarities and differences in reproductive parameters when exposed to similar photoperiodic conditions. For example, both gray and brown mouse lemur females undergo between 1 and 4 estrous cycles, with an average of ~2 cycles per season (Wrogemann and Zimmermann, 2001; Wrogemann et al., 2001). Interestrous intervals are very variable in mouse lemurs, ranging from ~40-60 days (Glatston, 1979; Perret, 1986) and up to 66 for brown mouse lemur females or 68 for gray mouse lemur females (Wrogemann et al., 2001). This variation could be partly explained by early resorptions or abortions of the embryo that may pass undetected by researchers (Perret, 1982). Among differences between species, brown mouse lemur females have

shorter reproductive periods, i.e. undergo estrus within 6 months of the year vs. 8 months in gray mouse lemur females, and have shorter gestations than *M. murinus* females (57 vs. 62 days) (Wrogemann et al., 2001).

Under captive conditions gray mouse lemur females undergo the first seasonal estrus soon after they are exposed to long day lengths, and they do so even under constant photoperiodic conditions, suggesting the existence of an endogenous regulatory mechanism of reproduction (Perret and Aujard, 2001). The extent to which additional environmental or internal factors may affect the fine tuning of estrous onset in mouse lemur females has been investigated under experimental conditions. Radespiel and Zimmermann (2001) found no correlation between estrous onset (or estrous interval length) and parity, age or housing conditions. However, Perret (1995) had argued that presence of active males could enhance estrous synchrony among females and Génin (2007) suggested that estrous synchrony could be affected by female groupings and higher synchrony was expected in females who shared sleeping sites.

In the wild, reproductive observations cannot be obtained with the same resolution or accuracy, but several studies have described aspects of reproduction in mouse lemur populations. A study of *M. ravelobensis*, the golden brown mouse lemur at Ampijoroa, western Madagascar, led researchers to suggest that body condition, i.e., female body mass, may affect the timing of estrus, as heavier females showed a tendency to undergo estrus earlier than females who achieved the same weight later in the season (Randrianambinina et al., 2003). However, no correlation between body mass and day of estrus was found in brown mouse lemur females at Ranomafana (Blanco, 2008). A positive correlation between estrous onset and female proximity, measured as the distance

between female trapping locations, was found at the latter study site, but the cause of the association remained elusive: females who were trapped together were probably more likely to interact with each other (e.g., communicating through olfactory cues), but they were also more likely to be biologically related and thus to share genes that may be involved in expression of endogenous rhythms (Blanco, 2008).

Several field studies have demonstrated that female mouse lemurs show “moderate” estrous synchrony (i.e., some but not significant overlap in estrus onset), and these observations applied to different mouse lemur species (e.g., Blanco, 2008; Eberle and Kappeler, 2004a, b). It is worth mentioning, however, that the ~4-week estrous interval reported for gray mouse lemur females has been considered by some as “not significant” synchrony (Eberle and Kappeler, 2002) and a “high degree” of synchrony by others (Radespiel, 2000). The interpretation of reproductive data has implications for testing models of mating and social systems. In the context of socioecological models, for example, a low degree of estrous synchrony, is considered a necessary condition for male monopolization, i.e. benefits of active “desynchronization” (Eberle and Kappeler, 2002) whereas active “synchronization” can be beneficial for female alloparental care (Génin, 2008). However, an alternative hypothesis would render estrous synchronization a “passive” consequence of female individual variation.

Despite attempts to explain reproductive strategies in mouse lemur populations, hypothesis testing in the wild has been impaired by the lack of reliable dates of estrus, which are sometimes based on mating observations, but more often rely on the presence of vaginal openings. These observations may be problematic as vaginal openings can last for ~4 to 10 days during an estrous cycle, while estrus occurs in a single night (e.g.

Buesching et al., 1998) (see Results section below). Despite the existence of only partial reproductive data, it is indubitable that there is variation in the timing of estrus reported within and between mouse lemur species at different localities (Blanco, 2008).

The main goal of this study is to collect detailed reproductive observations of brown mouse lemur females during multiple reproductive seasons at Ranomafana National Park, in order to: 1) determine the timing of estrus (and its variation among females at this single locality); 2) assess additional reproductive conditions of individual females (i.e., are they gestating, lactating, non-reproductive?); and 3) determine whether polyestry is expressed at this study site. I designed this research so that I would be able to compare the dates of first estrus of the season in individual females across years, and assess their correlation with other variables, such as body mass and climate conditions. Finally, my goal was to integrate these data with those derived from other studies on mouse lemurs, in order to address broader comparative questions. Among specific questions I will address are: Does time of estrus among female *M. rufus* at Ranomafana occur consistently across years or does it possibly change according to non-photoperiodic factors? Is it possible to predict date of estrus based on prior reproductive observations in female mouse lemurs? Is there evidence of regular and/or rebound polyestry at Ranomafana?

Methods

Local assistants and I trapped brown mouse lemurs using intensive capture/mark/recapture techniques during 5 reproductive seasons (between 2004 and 2008) at Ranomafana National Park, a southeastern montane rainforest (21°15'50"S, 47°25'08" E, 1000 m). A rainy season, with higher temperatures and monthly

precipitation, is usually restricted between December and March (Atsalis, 1999). I confined the trapping area to ~9 ha within the Talatakely Trail System which includes ~5 km² of mapped trails. This part of the park had been selectively logged in 1986 and 1987 and, since the inauguration of the park in 1991, was frequently visited by tourists (Wright and Andriamihaja, 2002). Successful trapping nights, with at least one captured mouse lemur (the number of male or female captures per month indicated in parentheses), were as follows: in 2004: October (17), November (16), December (6); in 2005: October (26), November (23), December (17); 2006: January (3), October (27), November (11), December (16); and in 2007: January (14), October (20), December (5); 2008: January (2), February (5), September (2), and October (13). Recapture rates were estimated from individuals trapped in October-November, i.e., at the beginning of the reproductive season when trapping success is the highest, between 2004 and 2007, and trapping success was determined by the proportion of captures over total number of set traps.

My collaborators and I set up a maximum of 50 Sherman traps daily at ~17:00, each baited with a small piece of fresh banana. To obtain detailed observations and maximize recaptures, traps were placed on consecutive nights. They were set up in pairs at each side of selected trails and separated by between ~25-50 meters. We checked traps at ~19:30 and all captured mouse lemurs were brought back to the Centre ValBio research station. All non-primate captures were released immediately at the trapping site. All empty traps were brought back to the station and washed overnight. First-time captured mouse lemurs were temporarily immobilized with Telazol (5-6mg/kg) and marked with Avid microchips. All mouse lemurs were weighed and examined to document their reproductive condition. After processing, all individuals were released at the site of

capture around midnight on the same day to minimize disruptions in their reproductive schedules.

Reproductive condition of females was assessed by checking vaginal and nipple morphology. If females presented vaginal openings (a condition occurring only during estrous cycles and parturition), I collected vaginal smears and determined proestrous, estrous and metestrous conditions through microscopic cytological observations following published protocols (Blanco, 2008; Wrogemann and Zimmermann, 2001). I estimated inter-annual estrous intervals when days of estrus were available for the same females in multiple years. Advanced stages of pregnancy could be detected by careful palpation and by assessing weight gain profiles. For males, length and width of right and left testes were measured with calipers. Those results and analyses are described in Chapter III, this dissertation. For more details on field protocols see Blanco (2008).

To illustrate possible discrepancies between methods used as proxy for estrus, such as the presence of vaginal openings (e.g. Eberle and Kappeler, 2002; Radespiel, 2000; Schwab, 2000), or cytological examination of vaginal smears, I compare results using data from the same individual females captured in 2006.

I ranked individual females as being “successful” if reproductive observations after estrus indicated full-term gestations or signs of lactation, or “unsuccessful” if the individual exhibited a low weight gain profile during pregnancy, or signs of abortion or perinatal death of offspring. Particular attention was paid to females captured late in the season, i.e. after December, as they may show signs of reproductive activity associated with polyestry.

Climate data, e.g. monthly precipitation and temperature maxima and minima between 2005 and 2008 provided by the Centre ValBio staff were used to compare weather variation among years. Annual precipitation and rainfall during “rainy and dry” seasons were also compared.

Results

A total of 134 individual mouse lemurs (66 females, 68 males) were marked with Avid microchips and trapped ~1474 times between 2004 and 2008 (Table 1). Overall annual recapture rates ranged between ~29-52%. About 29% of mouse lemurs captured in 2005 were marked in 2004; 15.5 % of individuals captured in 2006 were marked in 2004 and 32.8% were marked in 2005; 6.6% of mouse lemurs captured in 2007 were marked in 2004, 18% were marked in 2005 and 27.9% in 2006. Trapping success was high in October (~30-45%) but decreased during the following months, November (~23-34%), December (~10-15%), January (~12% in 2007, but 0.6% in 2008), February (~1%).

I estimated day of estrus for 29 females in 47 occasions during the 2005-2008 reproductive seasons (Table 2). These data include day of estrus in 3 consecutive years for 3 females and 4 consecutive years for one individual (Figure 1). This last female (J) has been captured since 2005, and her day of estrus in 2008 was predicted to be around October 7th-8th based on prior individual records (Table 3). Female J was first captured in 2008 on October 6th, displaying a very swollen vagina and she was trapped again in October 8th, with a vaginal opening. Vaginal smears showed deeply stained, anucleated or fully cornified epithelial cells characteristic of the estrous condition. For female St, I estimated estrous onset within a range of 4 days or less for 5 seasons using a combination of reproductive observations (Figure 2). Gestation length in this population had been

estimated to be around 57 days, on the basis of reproductive data from three mouse lemur females, captured around time of estrus and parturition in 2005 (Blanco, 2008).

Estrous females were mostly captured in October but there was ~1-month window of variation. In 2005, dates were reported between October 11 and November 18, in 2006, captured females were in estrus between October 7 and November 7, in 2007, between October 14 and 23 and in 2008 between October 7 and 16 (but no trapping was conducted in November during the last two years and thus, probably several estrous females were missed from the population).

I calculated 16 inter-annual estrous intervals from 11 different females (Table 3). I found no significant correlations (Pearson r : 0.178, NS; Spearman r : -0.058, NS) between female body mass and time of estrus (Figure 3) and between female body mass 15 days prior to estrus and day of estrus (data partly shown in Table 4), but young females (~1 year old) are lighter than adults as is evidenced by their body mass records in consecutive years (Chapter V, this dissertation) (Figure 4), an observation also reported for captive gray mouse lemurs (Perret, 2005).

Differences in the assessment of the day of estrus using observations of vaginal openings or vaginal smears are shown in Figure 5. Ten estrous intervals were defined by the first and last capture of an individual female observed with a vaginal opening, and 10 days of estrus were determined for the same females via examination of vaginal smears.

Seventy-two to 84% of females were classified as being reproductively “successful”, after repeated captures allowed for determination of full time gestations and/or signs of lactation. On the other hand, a minimum of four females and as many as seven females showed “unsuccessful” signs of reproduction such as evidence of abortion,

low weight gain profiles and swollen vaginas possibly resulting from perinatal death of offspring (Table 5).

Due to the very low trapping success in late December, January and February, I could not determine whether or not regular polyestry (i.e. a second estrous cycle after weaning the first litter) or rebound polyestry (i.e. renewed estrus as a result of loss of prior offspring) occurs at Ranomafana. Very slightly whitish and swollen vaginas, suggestive of imminent vaginal openings, were observed in 5 out of 9 females captured in mid-January, after ~19 (female Me), ~30 (females Cl, I, Ke) and ~47 (female J) days after estimated parturition; only females frequently captured (within 10 days in between trappings) in December and January were included to make sure that vaginal swellings or openings, if present, were not missed (Table 6). Three vaginal swellings, one observed in mid-November (female Je) and two observed in mid-December (females Ag and K) indicated the possibility that renewed estrus had occurred due to loss of the first offspring (i.e., rebound polyestry) (Table 5). One female (Ps) captured in mid-January and one female (Q) captured in mid-February might have been pregnant based on their relatively high body masses, 70.5g and 71g respectively. They both had rather small tail base circumferences, indicating that if fattening was responsible for their large body mass, was not yet apparent in the tails where mouse lemurs usually store fat (Tables 7 and 8). Unfortunately progesterone levels (not shown) extracted from fecal samples of both females were much lower than expected for pregnant individuals (Chapter IV, this dissertation). Thus, the reproductive condition of these particular individuals captured late in the season could not be conclusively ascertained.

Climate records showed that total annual rainfall was much higher during 2007 and 2008 compared to 2004 and 2005. Moreover, rainfall was more regularly distributed among months, and similar amounts of rain fell between December and March (rainy season) and April to November (dry season) (Figure 6). No particular pattern could be linked between time of estrus and climate. The sequence of females who underwent estrus earlier in 2005 (e.g., K earlier than C, I and J) was reverted the following year, indicating that estrous onset did not follow, at least at the population level, any discernable environmental cue. In fact, the original sequence seemed to repeat itself every other year (Figure 1).

Discussion

This study presents the first long term record of estrous onset in a wild population of mouse lemur females. Inter-annual estrous intervals show consistent periodicities of ~365 days, as it would be expected in animals displaying circannual rhythms entrained by photoperiod. These field data corroborate that estrous onset does not correlate with climatic variation, body mass or age but instead shows an individual basis. Although physiological studies are necessary to unveil the mechanisms of reproduction, it is fair to speculate that estrous onset in brown mouse lemur females at Ranomafana has an internal (i.e., genetically or hormonally regulated) trigger and is not significantly influenced by additional environmental factors.

Reproductive observations showed that the majority of females were “successful” in that they underwent full time pregnancies and showed signs of lactation. This indirect assessment, however, may overestimate female reproductive success as offspring survival could not be directly determined. In at least one case, female A was observed with two

20-g babies around a nest location in early January, but with only one offspring later that month.

Observations during the late rainy season were extremely limited due to low capture success at Ranomafana during this period. Hence I was unable to demonstrate that a second litter is a common occurrence at this study site, although this remains a distinct possibility. Atsalis (2008) had reported lactating females in the month of April in her study of mouse lemurs also at Ranomafana conducted in the late 1990s. This would also support the notion that polyestry occurs in this forest, at least in part of the female population.

Even under captive conditions, regular polyestry is not common. Post partum estrus has been usually associated with early abortions or death of offspring (i.e., rebound polyestry) and estimated to occur between 2 to 20 days after reproductive failure in gray mouse lemur females (Glatston, 1979, Perret, 1986). Wrogemann and Zimmermann (2001) however, reported one captive brown mouse lemur female who successfully gave birth to two consecutive litters and experienced a postpartum estrus surprisingly ~ 5 days after first parturition. Yet, other females who only gave birth to a single litter experienced postpartum estrus between 4 and 39 days after parturition. If follicular phase is ~10-15 days (Perret, 1986) for gray mouse lemur females, renewed estrus can occur as soon as 15-20 days after parturition and earlier if offspring does not survive.

Comparative studies of wild mouse lemurs have reported variation in the number of litters that mouse lemur females have within a season. Génin (2008) reported that *M. griseorufus* from the southeast at Berenty had one litter per year but that females would undergo a second estrus if offspring was lost (e.g. rebound polyestry). Two litters were

observed in gray mouse lemurs at Ampijoroa, western Madagascar (Schmelting et al., 2000) and Lahann et al. (2006) reported that gray mouse lemurs from the eastern, less seasonal littoral forest of Mandena, had 2 and possibly 3 litters per season compared to the same species at Kirindy, a western dry forest. Schmelting et al. (2000) observed the first litter born between November 21 and December 5, and the second litter born around February 16 until March 1. Lahann et al. (2006) observed the first lactating females (first litter) in November and again later in February (second litter) at Mandena. Based on published gestation lengths for gray mouse lemurs (~62 days, Wrogemann et al., 2001), postpartum estrus occurred between 12 and 38 days, with a mean ~25 days, after parturition of surviving offspring in both populations of gray mouse lemur females. Observations from this study of several females with some evidence of reproductive activity (slightly swollen vaginas) between ~20 and 47 days after their first estimated parturition are indeed within the range of possibility of regular polyestry. More direct data, however, are necessary to confirm its occurrence at Ranomafana.

Variation in the number of litters within or among the widely distributed populations of *M. murinus* has been explained by effects of habitat variation and mortality rates. For example at Kirindy, with a dry season extending almost 8 months a year, gray mouse lemur females were observed to have only 1 litter per year (Eberle and Kappeler, 2004b). Lahann et al. (2006) suggested that a trade-off between reproduction and life span exists in gray mouse lemur populations, i.e., mouse lemurs at Mandena have more offspring per season but die younger as shown by the low recapture rates at this study site, of 13% compared to annual recapture rates of ~30-40% at Kirindy (but see Eberle and Kappeler, 2004b and quote from Kappeler 2000 in Schülke and Ostner, 2007

wherein predation pressure on Kirindy *M. murinus* is also reported to be intense resulting in a population turnover rate of 50%, one of the highest found in any primate).

Provisional recapture rates at Ranomafana are a higher than both sites. However, when considering recapture rates as a proxy for life spans, caution should be used. For instance, annual recapture rates are susceptible to changes depending on sampling time, because trapping success in mouse lemurs varies within a season. For instance, if the 12-month period starts and ends in February, when captures are low, recapture rates may be underestimated. Moreover, recapture rates have been shown to vary within forests.

Ganzhorn and Schmid (1998) showed higher recapture rates in the primary vs. secondary forests at Kirindy, possibly due to higher predation rates or reduced food availability in secondary forests. Finally, predation may differentially affect recapture success rates in forests with high and low predation, because predators tend to target young adults (members of the first year cohort), thereby strongly affecting the population profile. This has been demonstrated for gray mouse lemurs at Ampijoroa (Schmelting et al., 2007).

Although more data are necessary to document the expression of polyestry at Ranomafana, I do not expect brown mouse lemur females to have three surviving litters per year. Some females may have two litters, some will have only one, and a few individuals, possibly the first year cohort, may not be able to sustain successful pregnancy or raise offspring during their first reproductive season.

Conclusions

Onset of estrus was successfully determined for several females over multiple reproductive seasons at Ranomafana. This study has provided supporting evidence, in agreement with published captive data, that reproductive activation in mouse lemur

females is most likely endogenously regulated (as expressed by individual female variation within a ~4 week period) and entrained by photoperiod (as demonstrated by the regularity of interannual estrous intervals). If this is the case, females will be mating despite unfavorable conditions which can jeopardize reproductive success. Most females showed signs of successful pregnancies and early lactation, however, very low trapping success late in the season did not allow for confirmation of regular polyestry in brown mouse lemur females at Ranomafana. Indirect evidence, however, did suggest the expression of polyestry for at least part of the female population at this study site.

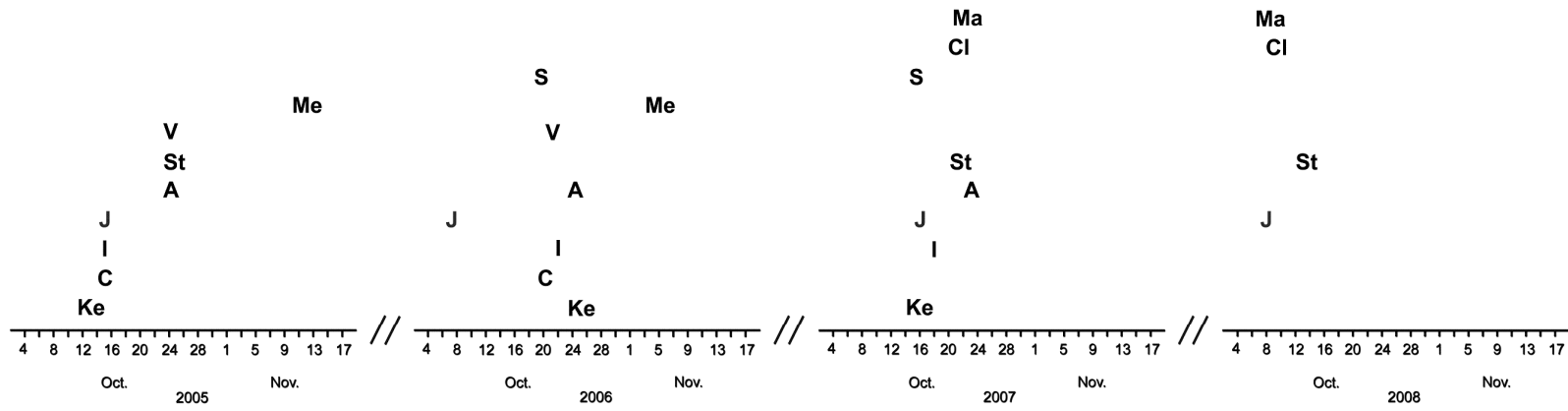


Figure II.1. Inter-annual variation in the time of estrus of frequently captured mouse lemur females

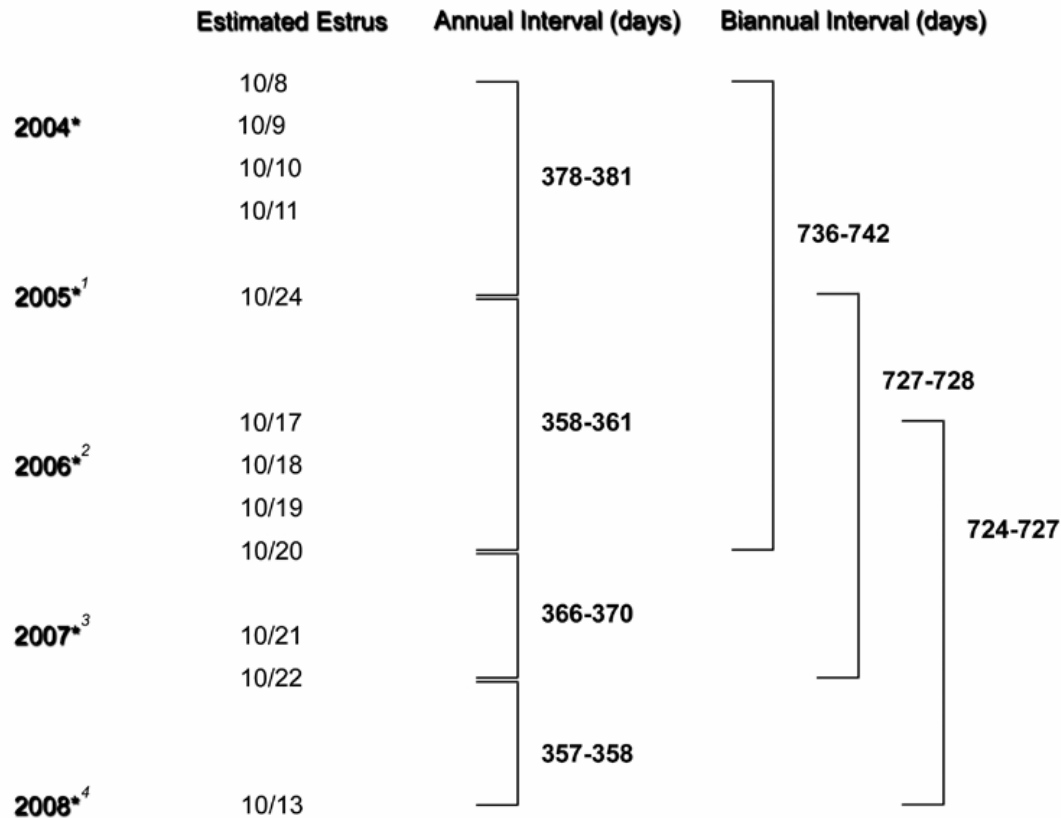
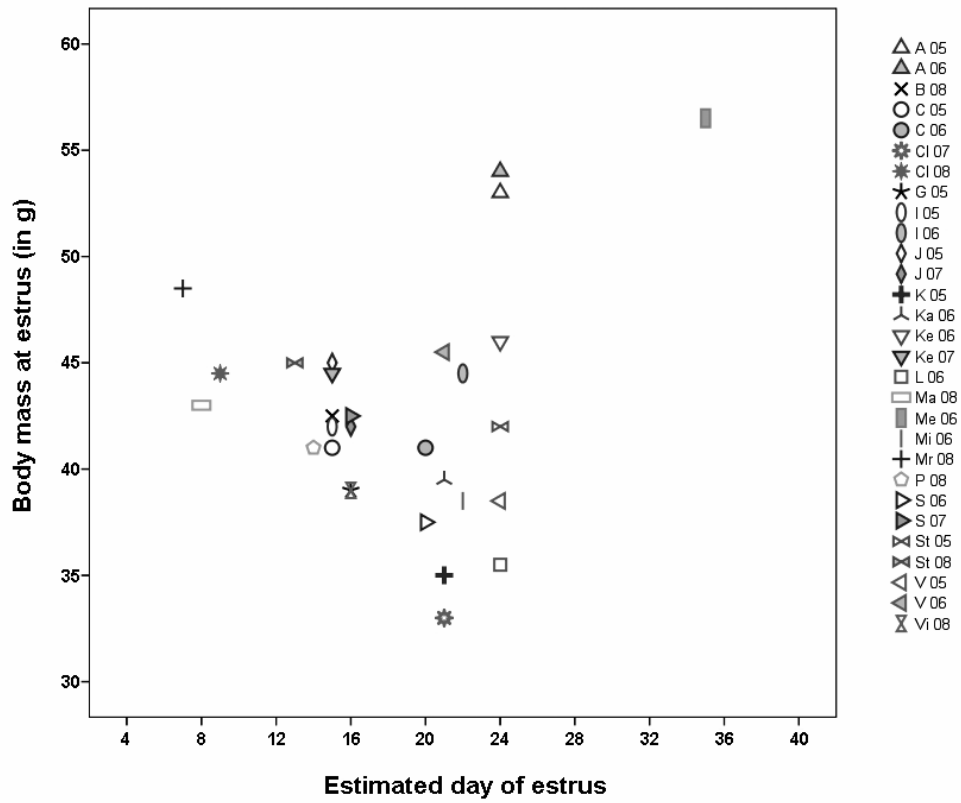


Figure II.2. Estimated onset of estrus in 5 consecutive reproductive seasons for a brown mouse lemur female.

*12/3/04 pregnant (body mass: 67.5g); 12/7 afternoon, already gave birth (51g); estimated dates of parturition: 12/4-12/7; possible estrus dates estimated on 57 days of gestation; ¹10/25/05 vaginal opening, early metestrus based on vaginal smears; ²10/16/06 swollen vagina; ³10/23/07 vaginal opening, possibly metestrus based on vaginal morphology; ⁴vaginal opening, estrus based on vaginal smears



Correlations are not significant, results shown in the text.

Figure II.3. Scatter plot of body mass (g) and day of estrus for captured brown mouse lemur females over multiple years. Correlations are not significant, results shown in text.

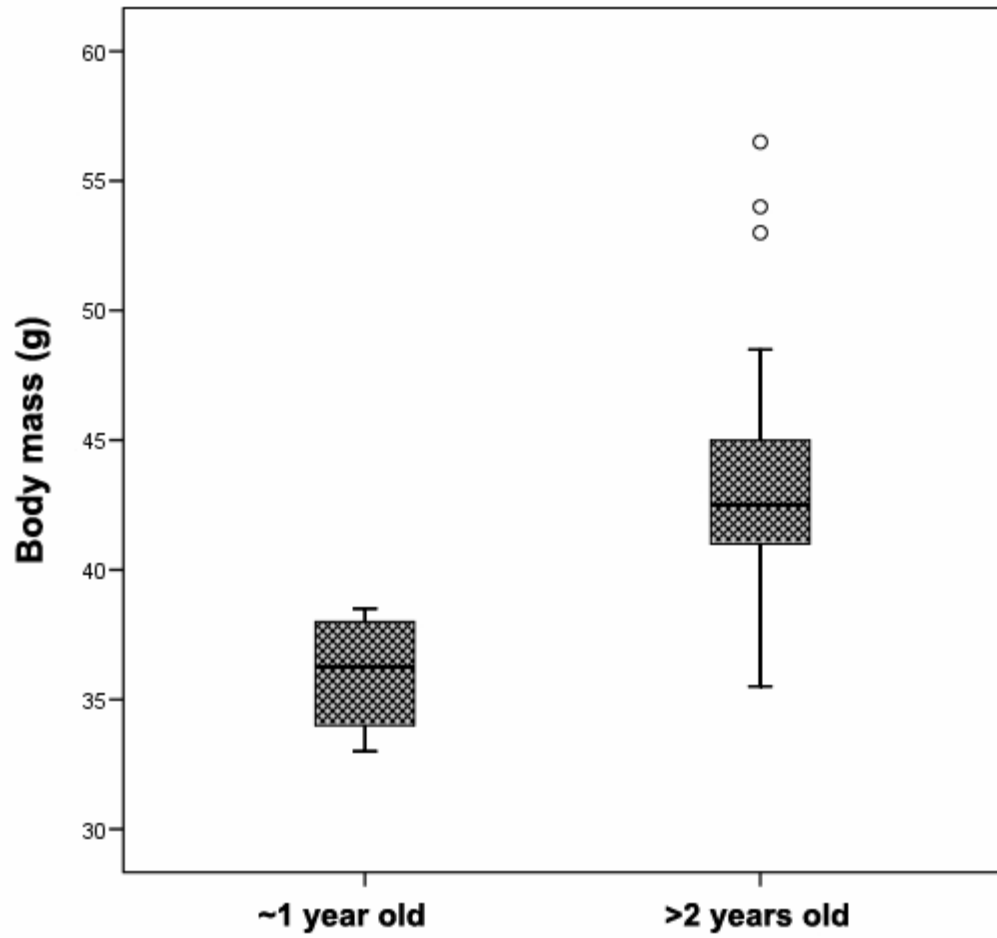


Figure II.4. Body mass (g) at day of estrus between ~1 year old and older brown mouse lemur females

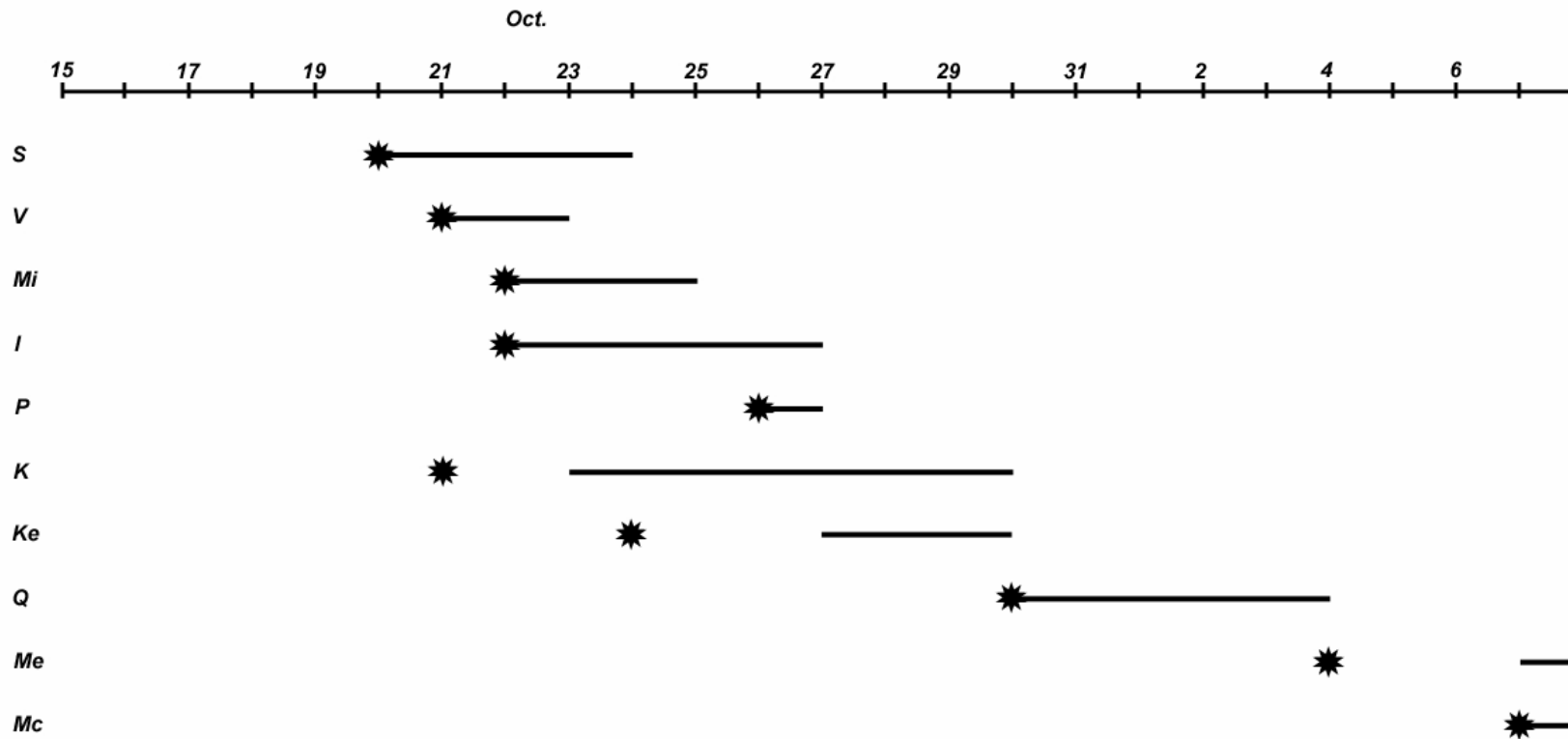


Figure II.5. Day of estrus and vaginal openings observed during first seasonal estrous cycle in 2006.

Horizontal lines represent the period between first and last observation of vaginal openings, and stars indicate the day of estrus as determined by inspection of vaginal smears

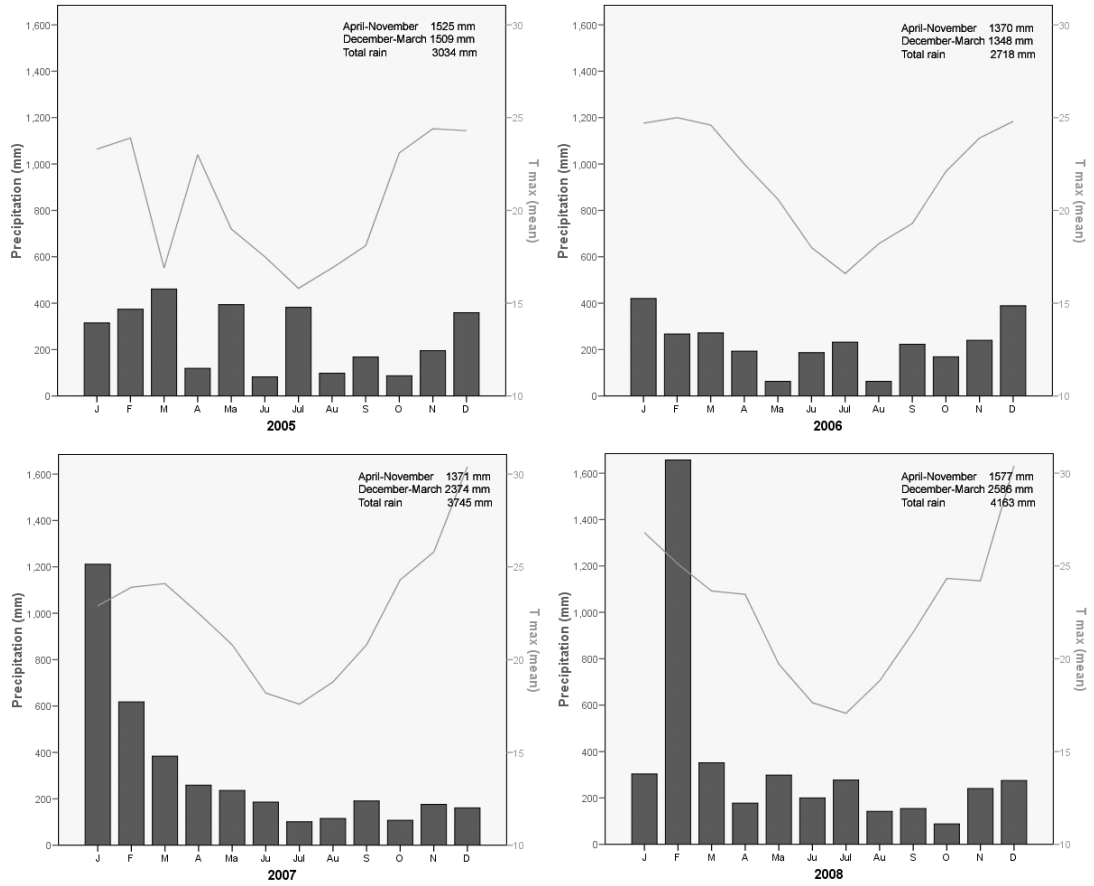


Figure II.6. Total rainfall (mm) and Temperature maxima (°C) recorded at Ranomafana National Park during years of trapping

Table II.1. Mouse lemur captures during study period, described by month and sex.

Year	Month	Trapping Nights	All captures	Female captures	Male captures	Females	Males	Females 1st capture	Males 1st capture
2004	October	17	104	27	77	9	13	9	13
2004	November	16	95	42	53	11	11	3	2
2004	December	6	35	24	11	12	8	3	0
2005	October	26	182	87	95	19	15	12	10
2005	November	23	118	66	52	21	18	5	4
2005	December	17	84	60	24	19	11	3	2
2006	January	3	19	12	7	11	6	0	1
2006	October	27	244	90	154	23	29	8	17
2006	November	11	77	41	36	20	16	2	0
2006	December	16	60	36	24	9	10	1	1
2007	January	14	56	43	13	12	4	0	0
2007	October	20	229	95	134	21	27	7	8
2007	December	5	20	9	11	5	8	4	0
2008	January	12	3	2	1	2	1	0	0
2008	February	8	4	1	3	1	2	0	1
2008	September	2	18	6	12	5	8	2	1
2008	October	13	126	50	76	16	22	7	8
	Totals	236	1474	691	783	216	209	66	68

Table II.2. Estimated timing of estrus for females captured during the 2005-2008 reproductive seasons at Ranomafana National Park.

Female	2005		2006		2007		2008	
	Est. estrus	V.S.?	Est. estrus	V.S.?	Est. estrus	V.S.?	Est. estrus	V.S.?
J	15-Oct	Y	7-Oct	Y	16-Oct	Y	8-Oct	Y
I	15-Oct	Y	22-Oct	N ¹	18-Oct	Y		
Ke	12-Oct	Y	24-Oct	Y	15-Oct	Y		
St	24-Oct	Y			21/22-Oct	N ²	13-Oct	Y
A	24-Oct	Y	24/25-Oct	Y	23-Oct	Y		
C	15-Oct	Y	20-Oct	Y				
Me	11/12-Nov	Y	4-Nov	Y				
V	24-Oct	Y	21-Oct	Y				
G	16-Oct	Y						
K	21-Oct	Y						
Je	18-Nov	Y						
Sh	16-Oct	N ³						
So	15-Oct	Y						
S			20-Oct	Y	16-Oct	Y		
P			26-Oct	Y			14-Oct	Y
Ka			21-Oct	Y				
L			24-Oct	Y				
Mc			7-Nov	Y				
Mi			22-Oct	Y				
Q			30-Oct	Y				
Cl					21-Oct	Y	9-Oct	Y
Ma					22-Oct	N ⁴	8-Oct	Y
An					20-Oct	Y		
Ae					16-Oct	Y		
Mar					14-Oct	Y		
T					21-Oct	Y		
Mr							7-Oct	Y
B							15-Oct	Y
Vi							16-Oct	Y

V.S.? = vaginal smears?, Y = yes, N = no; ¹vaginal plug, ²vaginal opening, possibly metestrus based on external morphology, ³vaginal plug, ⁴vaginal opening, possibly metestrus based on external morphology

Table II.3. Annual and bi-annual estrous intervals for frequently captured females at Ranomafana National Park

	2005-2006	2006-2007	2007-2008	2005-2007	2006-2008
A	365-366	363-364		728-730	
C	370				
Cl			354		
I	372	361		733	
J	357	374	358	731	732
Ke	377	356		733	
Ma			352		
Me	357-358				
St			358		
S		361			
V	362				

Table II.4. Female body mass (g) prior to estrus (6-15 days, PEBM) or at estrus (EBM) collected over four reproductive seasons (2005-2008). Second column indicates number of days between October 5th and time of estrus (RDE). Bolded numbers refer to ~1 year old females

ID	Year	RDE	PEBM	EBM
A	2005	19	55	53
A	2006	19-20	54	54
Br	2008	10	39.33	42.5
C	2005	10	42.5	41
C	2006	15	44.5	41
Cl	2007	16	31.5	33
Cl	2008	4	44	44.5
G	2005	11	38.3	39
I	2005	10	40.5	42
I	2006	17	43	44.5
J	2005	10	43	45
J	2007	11	45.5	42
Je	2005	13	41.5	45.5
K	2005	16	35.6	35
Ka	2006	16	40.5	39.5
Ke	2006	19	46.5	46
Ke	2007	10	40.5	44.5
L	2006	19	35	35.5
Ma	2008	3	47	43
Mr	2008	2	48.75	48.5
Me	2006	30	56	56.5
Mi	2006	17	35.5	38.5
P	2008	9	43.67	41
S	2006	15	36.8	37.5
S	2007	11	40	42.5
St	2005	19	49	42
St	2008	8	44	45
V	2005	19	39	38.5
V	2006	16	43.8	45.5
Vi	2008	11	41.75	39

Table II.5. Estimations of reproductive success based on observations from frequently captured individuals during the reproductive season

ID	Year	NE	FTP	SOL	RS	Obs
Ag	2006				N	S.V.
An	2005				Y/N	L.W.G.
An	2006				Y	W.O.
An	2007	N				B.V.
A	2005	Y		Y	Y	
C	2005	Y	Y	Y	Y	
Cl	2007	Y			Y/N	L.W.G.; S.V.
G	2005	Y		Y	Y	
I	2005	Y	Y	Y	Y	
I	2006	Y	Y	Y	Y	
I	2007	Y	Y			
J	2005	Y	Y	Y	Y	
J	2006	Y	Y	Y	Y	
J	2007	Y	Y			
Je	2005				N	L.W.G.; S.V.
K	2005	Y			N	L.W.G.; S.V.
Ke	2005	Y	Y	Y	Y	
Ke	2006	Y		Y	Y	
Mc	2006	Y		Y	Y	
Mir	2006			Y	Y	
R	2005			N	N	L.W.G.; B.V.
Q	2006	Y		Y	Y	
S	2006	Y		Y	Y	
Sh	2004			Y	Y	
Sh	2005	Y	Y	Y	Y	
St	2004			Y	Y	
St	2005	Y	Y			
St	2007	Y			Y/N	L.W.G.
V	2005	Y	Y			
V	2006	Y		Y	Y	

NE: normal estrus, FTP: full-term pregnancy, SOL: signs of lactation, RS: reproductive success, Obs: observations, Y: yes, N: no, S.V.: swollen vagina, L.W.G.: low weight gain during possible pregnancy, W.O.: seen with offspring, B.V.: bleeding vagina

Table II.6. Possible expression of polyestry based on evidence of vaginal activity in females frequently captured after estimated day of parturition

Female/Year	Est. Parturition	Vaginal Activity after Est. Parturition		Observations
		Within 15 days	Within 35 days	
I 05	12/11	NO	N/A	
G 05	12/12	NO	N/A	
A 06	12/9-12/19	NO	YES	NSV-W day 26-36
I 06	12/18	NO	YES	SSV-W day 31
J 06	12/3	NO	NO	SSV-W day 47
Ke 06	12/20	N/A	YES	SSV-W day 30
Me 06	12/31	N/A	YES	SSV-W day 19
Mc 06	1/3	NO	N/A	
Mir 06	12/21	N/A	NO	
S 06	12/15	NO	NO	
V 06	12/15	NO	NO	
C 07	12/17	N/A	YES	SV day 31

N/A= not available; NSV-W=not swollen, whitish vagina; SSV-W=slightly swollen and whitish vagina; SV=swollen vagina

Table II.7. Body mass (BM) and tail base circumference (TC) of mouse lemur females captured at the beginning of the reproductive season (around time of estrus or early undetectable pregnancies), mid-reproductive season (advanced pregnancies) and late in the season (two females).

		BM (g)	TC (mm)
	n	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Early reprod. season (Oct.)	14	45.57 ± 5.41	16.32 ± 1.04
Pregnant females (Dec.)	3	70.17 ± 2.93	17.32 ± 0.91
female "Ps" (mid-Jan.)	1	70.5	20.7
female "Q" (mid-Feb.)	1	71	16.78

Table II.8. Mann-Whitney U tests of significant differences for body mass (BM) and tail base circumference (TBC) from females captured early in the reproductive season, during advanced pregnancy, and late in the season

	Adv. Pregnancy		Late (females Ps and Q)	
	BM	TC	BM	TC
Early	*	NS	*	NS
Adv. pregnancy			NS	NS

* $p < 0.05$; NS = non-significant

CHAPTER III

TESTES VOLUME VARIATION IN MALE BROWN MOUSE LEMURS AT RANOMAFANA NATIONAL PARK

Implications for the duration of the reproductive season and expression of female polyestry

Introduction

Mouse lemurs are seasonal breeders, and reproductive activation in both sexes has been reported to be photoperiod-dependent (Perret and Aujard, 2001). Both sexes undergo a period of reproductive quiescence when males experience testis regression (and no spermatogenesis occurs) and females undergo diestrus, characterized by sealed vaginas and no ovulation (Wrogemann and Zimmermann, 2001; Wrogemann et al., 2001).

In captive gray mouse lemurs (*Microcebus murinus*), exposure to short days causes male testicular regression; however, individuals become refractory to the effects of short days and they develop testes before the transition to long days (Perret and Aujard, 2001; Génin et al., 2005). This “early” activation would prepare males to mate with females who generally undergo estrus about 3-4 weeks after testicular growth has begun, because spermatogenesis takes longer than ovulation does (Prendergast, 2005). In other small-bodied long day breeders, like Siberian and Djungarian hamsters, males also become refractory to the effects of short days and begin gonadal recrudescence ~4.5 months after exposure to short photoperiod treatment (Prendergast, 2005; Schlatt et al., 1995). Unlike Djungarian hamster males, whose testes reach only a third of maximum weight at 25 weeks after the beginning of gonadal regrowth, Siberian hamster males complete testis recrudescence only after 1-2 months. Yet, both species show great individual variability in the timing of photorefractoriness (i.e., gonadal recrudescence)

(Prendergast, 2005; Schlatt et al., 1995). Experiments conducted on Siberian hamsters showed substantial heritability ($0.61 < h^2 < 0.71$) between parent-offspring in the timing of testis recrudescence, but intra-population variance was significant and no two animals began testis regrowth at exactly the same time (Prendergast et al., 2004).

Similar to Siberian hamsters, mouse lemur testes are largest about 1 or 2 months after recrudescence and regression begins soon afterwards until testes achieve a non-reproductive condition (Lebec, 1984). In an early study, also conducted in captivity, Glatston (1979) noticed that testicular growth began earlier and lasted longer in gray mouse lemur males with relatively large testes and that, in fact, the period of testicular development in males appeared to be related to the final testicular size. Furthermore, Lebec (1984) showed that a high percentage of males who started testicular recrudescence earlier was socially dominant, though no correlation was found between social status and testes size or testosterone levels.

Variation in testes size between mouse lemur species has been reported under experimental conditions. Brown mouse lemur males (*M. rufus*)² maintain developed testes for 6 months, a month less than do gray mouse lemurs under simulated natural photoperiodic conditions (Wrogemann et al., 2001). However, Wrogemann et al. (2001) considered 1000 mm³ of testicular volume as the threshold for “reproductive capability,” based on the testicular volume of males who sired offspring inside the colony. This may underestimate the number of reproductively active males, if, as pointed out by Glatston

² Although described by Wrogemann et al., 2001 as *Microcebus rufus*, some of the individuals in the study colony (originally captured in the Mantadia region, eastern Madagascar) may belong to the species *M. lehilahytsara*, discovered recently by Kappeler

(1979), males with smaller testes are not necessarily infertile, even though they may not sire any offspring in captivity. Records of testis volume throughout the year show great individual variation (e.g. large standard deviations), particularly for gray mouse lemur males (Wrogemann et al., 2001).

In addition to photoperiod, other cues may modulate the reproductive output in mouse lemur males. For instance, serum testosterone levels and testes size in captive males were modified after urine exposure from proestrous/estrous females or dominant males (Perret, 1992; Perret and Schilling, 1995). Although the degree and direction of the effect (e.g., increase or decrease of testosterone values in each study group) depended on the particular experimental conditions, these studies demonstrated that social cues, e.g. communicated through urinary chemosignals, may affect male reproductive condition. Testosterone levels, for instance, were correlated with hierarchical status under experimental conditions (Schilling et al., 1984). Contrary to earlier findings by Perret (1992) and Andrès et al. (2001), however, Radespiel et al. (2002) found no correlation between dominance and reproductive success in gray mouse lemurs. Moreover, despite the fact that subordinate males mated fewer times than dominant males, they sired about half of the infants in the colony.

Earlier work by Martin (1973) discussed the possible co-existence of two types of males in wild populations of mouse lemurs. He suggested that larger, dominant gray mouse lemur males were located inside “population nuclei”, where they presumably had been able to establish territories to monitor estrous females, and that lighter males were

et al., 2005. I decided to keep original assignment to avoid confusion, and because it does not affect general statements made in this chapter.

relegated to peripheral areas where they had limited access to females. Later studies however, failed to identify these two classes of males (e.g. Eberle and Kappeler, 2002). A different idea put forward by Schmid and Kappeler (1998) and later reformulated by Génin (2007) suggested sex-biased mating strategies linked to fluctuating body mass (sexual dimorphism) in gray mouse lemurs. It had been observed that females more often than males undergo fattening at the beginning of the dry season, which incidentally allow them to sustain seasonal torpor (maybe reducing predation rates among other possible benefits), while most males remain active throughout the dry season and increase their body masses prior to the reproductive season (also Fietz, 1999a). This so-called “pre-mating fattening” of some males was described as being beneficial in that larger males would have an advantage in competition to access better territories and/or estrous females (Génin, 2007).

Variation in the period, amplitude and onset of testicular growth in males at the beginning of the reproductive season has been documented not only within populations but also among mouse lemur species in the wild (e.g., Atsalis, 2008; Randrianambinina et al., 2003; Schwab, 2000; Schmelting et al., 2000). For example, Schwab (2000) found that pygmy mouse lemurs (*M. myoxinus*) had relatively larger testes than sympatric gray mouse lemurs (*M. murinus*) at Kirindy, a dry deciduous forest in western Madagascar, and suggested that this would explain the longer reproductive season in the smaller species. Testes in gray mouse lemurs were largest in October but they showed complete regression by January, unlike testes of pygmy mouse lemurs, which were largest in November and still developed in January. Schwab (2000) also stated that testes size in both species decreased after their respective maximum values but yet slightly increased

again during the second month. However, this conclusion was based on the visual examination of testis mass from different males and there is no certainty that those monthly differences are significant. Schmelting et al. (2000) also suggested that gray mouse lemurs at Ampijoroa underwent a second increase in testes size prior to a breeding period in November, about two months after the presence of the first estrous females. However, as previously suggested by Atsalis (1999a), longitudinal measurements are necessary to truly determine whether or not monthly variation in testis mass is a population trend rather than the result of individual fluctuations.

Although there is a plethora of papers investigating social and mating systems in mouse lemurs (e.g., Andrès et al., 2003; Dammhahn and Kappeler, 2005; Fietz, 1999a; Schwab, 2000; Weidt et al., 2004), none has focused on how the biology of reproduction may pose constraints on or favor the expression of diverse social behaviors under different environmental conditions. Current knowledge of mouse lemur reproduction in captivity is limited to two species, the gray and brown mouse lemurs. While captive studies have illuminated the photoperiodic nature of mouse lemur reproduction –males becoming refractory to the stimulating cue of long days– (Perret and Aujard, 2001, Wrogemann et al., 2001), reproductive data collected on captive animals should be interpreted with caution, as artificial conditions may alter reproduction (Glatston, 1979). Whereas field observations are limited and conditions cannot be easily manipulated, I would argue that detailed observations of reproductive parameters across multiple seasons in the wild can provide a valuable source of biological data, and that particular ecological settings may elucidate questions regarding mouse lemur evolution and adaptation in a manner that no captive data can.

It is possible that part of the documented variation is an artifact of small sample sizes or a result of using different measuring standards; however underlying biological variation exists and should not be overlooked. In fact, there may be ecological benefits of a certain degree of flexibility. As suggested for Siberian hamsters, for example, variation in the timing of testis recrudescence (and regression) may be beneficial. In changing environments where favorable conditions for reproduction may differ between years, males attaining reproductive competence earlier or later may have differential success; if that is the case, natural selection would be maintaining a balanced polymorphism (Prendergast, 2005).

Under unpredictable environmental conditions, such as those existing in Madagascar (Chapter II, this dissertation), male reproductive variation in the timing and period of testicular development may be favorable. For instance, in the western forests of Madagascar, characterized by high seasonality (unpredictability mostly determined by alternations between dry and wet years) male mouse lemurs may display “narrower” variation and relatively shorter reproductive seasons (e.g. shorter period of testis development), because food sources decrease significantly during the dry season, posing a constraint to the energetics of reproduction. Eastern habitats, though still unpredictable, tend to be less seasonal and reproduction could be “extended”. If habitat variation indeed plays a role in the duration of the reproductive season, testicular development should vary among widely distributed populations of the same mouse lemur species.

Prior studies at Ranomafana National Park, an eastern montane rainforest, showed that testes size in brown mouse lemurs peaked in September, began to decrease during the following month and was reduced to pre-breeding levels by December (Atsalis, 2008).

However, low capture rates during the rainy months prevented a clear assessment of the timing of testicular regression at the population level.

During my field work at Ranomafana National Park, southeastern Madagascar, I collected reproductive observations in female and male brown mouse lemurs. In this Chapter, I aim to report male reproductive condition through measurements of testes size during multiple reproductive seasons.

I expected that variation in testes size should be more significant at the end of the reproductive season, during the period of testicular regression (I did not capture mouse lemurs at the beginning of the reproductive season when males experience gonadal recrudescence), particularly if, as Glatston (1979) pointed out for captivity, larger testes lasted longer. Overall, the extended reproductive competence of the male population may actually determine the duration of the reproductive season, as mouse lemur females will be undergoing more than one estrous cycle, i.e., polyestry.

My specific goals are to assess variation in testes size among individual males within a single reproductive season and in the same males across multiple years. Some of the questions I am interested in are: Is there variation in the timing of testis regression at Ranomafana? Are males with larger testes early in the season undergoing later testicular regression? Do frequently captured males have similar testis size across multiple reproductive seasons? In other words, do males with relatively large testes show the same trend in multiple years? On the basis of these data and published observations, I will explore the relationship between the duration of testes development and the timing of estrus in female mouse lemurs. Do males at Ranomafana show developed testes late in the reproductive season when females may be undergoing later estrous cycles? Based on

data collected from males captured prior to the presence of estrous females, I will compare body mass and tail circumference between sexes to test whether or not males show evidence of pre-mating fattening. Finally, I will address the question of reproductive flexibility in eastern mouse lemurs. This represents the first long term report of testis size in frequently captured males over consecutive reproductive seasons in wild mouse lemurs.

Methods

Mouse lemurs were trapped using intensive capture/mark/recapture techniques during the reproductive seasons of 2004-2008 at Ranomafana National Park, a southeastern montane rainforest (21°15'50"S, 47°25'08" E, 1000 m). I restricted the trapping area to ~9 ha within the Talatakely Trail System which includes ~5 km² of mapped trails. My local assistants and I trapped mouse lemurs during 43 nights between September and December in 2004, 69 nights between October 2005 and January 2006, 68 nights between October 2006 and January 2007, 20 nights in October 2007, 25 nights between December 2007 and February 2008 and 15 nights in September-October 2008.

A maximum of 50 Sherman traps were set up daily in pairs at ~17:00 each baited with a small piece of banana along selected trails and separated by 25-50 meters. Traps were checked 2-3 hours later and mouse lemurs were brought back to the Centre ValBio Research Station. First-time captured mouse lemurs were temporarily immobilized with Telazol and marked with Avid microchips. All mouse lemurs were weighed and examined to document their reproductive condition. For males, length and width of right and left testis were measured with calipers. In 2008, I also measured circumference of the

base of the tail in both sexes. After processing, all individuals were released at the site of capture around midnight on the same day.

Testes volume was calculated based on the equation for an ellipsoid (each testicular side was treated individually), described in Dammhahn and Kappeler (2005) and frequently used in the literature. Data on testes volume were pooled into half-month categories and means were used to compare across months and years. ANOVAs, T-tests and Mann-Whitney U tests were performed in SPSS 15.0.

Results

A total of 68 males were captured during the reproductive seasons of 2004-2008. A subset of males was captured during two consecutive seasons only: 3 in 2004-2005, 3 in 2005-2006, 4 in 2006-2007 and 3 in 2007-2008, and three consecutive seasons: 2 between 2004-2006, 4 in 2005-2007 and 9 in 2006-2008. Three males were captured four years in a row (1 in 2004-2007, 2 in 2005-2008). Males were captured more frequently than females in October, the sex ratio was similar in November and generally fewer males were trapped in December (Table 1). Mouse lemur trapping success (both sexes) is high in October and November, which coincides with the peak of the reproductive season, when most females undergo their first estrus (Chapter II, this dissertation).

For estimation of testes size I only include data from 53 individuals captured during the reproductive seasons of 2006, 2007 and 2008 because testicular measurements were only taken on the left testis in the first two years of captures and size estimations differed significantly between 2004-2005 and 2006-2008. Testes volumes are highest in October (but no data are available for earlier months) and show decreasing values in all months thereafter (Figures 1 and 2). Some individuals still exhibit somewhat developed

testes in December whereas others had already regressed all testicular tissue. By January, 67% of captured males (4 out of 6) showed completely regressed testes and the 2 males captured in February also showed complete regression (Figure 1). I ran an ANOVA with Tukey's post hoc tests of honestly significant differences (HSD) to compare individual variation in testes size among half-months for the 2006 reproductive season. Significant differences were found between testes size in October compared to all later months (Table 2).

I found a significant correlation between testes size in Early October and Early November in 2006, (i.e. males with relatively large testes in early October had also relatively large testes in early November and vice versa) but not between Early October and Early December (Figure 3A and B); however this last comparison excluded males with the largest testes who were not trapped in December.

Testes volume varied not only among individuals within a single season but also in the same individuals across years. In 6 males for which I calculated testes volume in early October of 2006, 2007 and 2008, I found significant differences in testes size between years (Paired samples T-tests: 06-07, $t=2.8$, $df=5$, $p=0.04$; 07-08, $t=-3.6$, $df=5$, $p=0.015$; 06-08, $t=-1.01$, $df=5$, $p=0.36$) (Figure 4).

As expected, there was a correlation between testes size and body mass, but this reflects the fact that testes weight contributes to overall body mass. These variables could not be measured independently; however when only males captured in more than one reproductive season were included, I did not find a consistent pattern between testes size and body mass (Figure 5). Some individuals were heavier but had smaller testes in 2008 compared to 2006 (e.g. C and H), others had similar body mass in 2007 and 2008 but

showed larger testes in the last year (e.g. E, D) and some males were heavier and had larger testes in 2008 compared to 2007 (e.g. P and M) (Figure 5). This is in agreement with results shown in Figure 4, where individuals captured at the same time in three consecutive years showed overall smaller testes in 2007 compared to 2006 and 2008, irrespective of body mass.

For comparative purposes, I estimated dates of second and third estrous cycles for mouse lemur females, provided regular polyestry exists at Ranomafana, on the basis of reproductive observations collected in 2006 –that established the onset of estrus between early October and early November– and gestation lengths previously calculated for brown mouse lemurs at Ranomafana (Blanco, 2008) (Table 3). It appears that by the time females may be undergoing a third regular estrous cycle, males will have undergone complete testicular regression.

I compared body mass and tail base circumference in males and females captured between September 29th and October 3rd in 2008, prior to trapping the first female in estrus (October 6th). I found significant differences in body mass ($p < 0.01$) but not in tail circumference between sexes (Table 4).

Discussion

I document for the first time inter- and intra-individual variation in testes size for mouse lemurs across multiple reproductive seasons at Ranomafana. Due to low trapping success during the rainy season (e.g. December-February) I could not thoroughly address the question of whether males with larger testes early in the reproductive season were the last individuals to display testis regression as Glatston (1979) had observed in captivity. Larger testes in October somewhat predicted larger testes in early November but the

correlation was lost between early October and early December, when I was able to trap fewer males.

A similar trend of testes size variation for 6 males captured in October of 2006, 2007 and 2008 (decrease from 2006-2007 and increase from 2007-2008) suggests that factors other than age affect testes volume. It is unknown whether males captured in 2007 initiated testes development earlier that year, but it is noteworthy that those individuals had similar or even greater testes size in December 2007 compared to 2006 despite having relatively smaller testes in October (Figure 3b, lower right corner). In other words, if testes were already smaller in early October 2007 than they were in 2006, they should have appeared more regressed in December of 2007, provided testicular regression occurs irreversibly and at a uniform rate. These seemingly conflicting results call for a more complex explanation for variation in testes size.

Atsalis (2008) observed that testes in brown mouse lemur males at Ranomafana were largest around the time of mating (September-October) and that they progressively reduce in size to achieve a non-reproductive condition a few months later, in December. Results from this study are consistent with her observations although no data prior to the end of September were available. Field observations from December and January, i.e. ~2-3 months after the beginning of the mating season showed that testes size is greatly reduced in the majority of captured males but that some of them completed testicular regression earlier than others. Contrary to Atsalis's findings a few individuals still showed some developed testes after mid-December, suggesting that several males could potentially mate with females undergoing second estrous cycles after first successful breedings (i.e., regular polyestry). I would argue, however, that three litters at

Ranomafana would be highly unlikely, if, as observed on a small sample size, males have completed testis regression by February.

Data from this study did not support the “pre-fattening” hypothesis. Males were significantly bigger than females but larger body masses were consistent with enlarged testicular tissue, which is already apparent a month prior to the beginning of the mating season (e.g., September) until late November at Ranomafana. For example, I dissected a male found dead in the forest of Ranomafana on November 1, 2006 (well after the peak of testicular development) with a body mass of ~54 g and testes that weighed ~3.7g, representing ~7% of the total body mass. These results partly agree with Atsalis (1999a) who found no significant differences in body mass and tail-base circumference between males and females prior to mating season in brown mouse lemurs at Ranomafana. Eberle and Kappeler (2004) had reported that heavier males had higher reproductive success, yet most litters had mixed paternities in gray mouse lemurs at Kirindy. Also at this site, Rasoazanabary (2006) did not find significant sex-differences in body mass prior to the mating season (although males were heavier than females) and larger males did not have an advantage in monopolizing better nest holes. Although Schmid and Kappeler (1998) originally acknowledged the fact that testicular tissue could contribute to a large proportion of the weight gain prior to the mating season, they did not control for testes mass. When circumference at the base of the tail was included in the analysis as proxy for fattening, because fat in mouse lemurs is commonly deposited in the tail (Génin et al., 2005), no significant differences were found between males and females prior to the mating season (Schmid, 1999). It is then unclear whether larger males would be dominant

because of larger body mass, their larger testes, or whether larger males would be dominant at all.

The relatively long reproductive season in brown mouse lemur males (although no data are available prior to late September, some males show testis development after December) is concordant with the idea that less seasonal habitats may accommodate a longer reproductive period. A comparison of western and eastern mouse lemurs might serve to demonstrate how environmental and internal factors might interact. For instance, eastern rainforests in Madagascar are relatively less seasonal than western dry deciduous forests (although both habitats are highly unpredictable, see Chapter II), so resource availability (e.g. preferred and keystone mouse lemur foods) can be expected to differ from east to west. On the other hand, captive studies have shown that gray mouse lemurs (west) under similar photoperiodic conditions have longer reproductive periods (i.e. testes were developed ~1 month longer) than do brown mouse lemurs (east), suggesting that species may differ in their internal mechanisms of reproduction. It is unknown whether biological differences accommodate habitat seasonality.

A correlation between environmental and reproductive factors has been partly supported by field data: grey mouse lemur males at Kirindy, a highly seasonal dry forest, showed highest testis size in October, a month later than gray mouse lemurs at Ampijoroa a dry but less seasonal forest in northwestern Madagascar. Lahann et al. (2006) also reported that gray mouse lemurs had longer reproductive periods at Mandena, a southeastern littoral forest, where females had multiple litters per year, though no data on testes mass were provided (Schmelting et al., 2000; Schwab, 2000). However, two studies of golden brown mouse lemurs (*M. ravelobensis*) which were trapped in nearby areas at

Ampijoroa, differed in their results: Schmelting et al. (2000) reported males with largest testis in August whereas Randrianambinina et al. (2003) captured males with largest testis in October. More studies are needed to unveil sources of reproductive variation, not only across species and habitats but also across reproductive seasons. In fact, this Chapter has shown intra-individual variation in testis mass across years.

The size and timing of testicular development may be also related to non-reproductive factors, such as the demands for a fully competent immune system. In fact, it has been suggested that a trade off may exist between reproductive success and immune function to maximize fitness (Lewis et al., 2008). For example, high levels of testosterone (produced in the testes) reduce immunocompetence in some mammalian species (e.g. Folstad and Karter, 1992). Moreover, recent experiments in a tropical but seasonal squirrel species subjected to different photoperiodic conditions showed that immunity was enhanced under short days (which are inhibitory for the reproductive system), and decreased during long days suggesting that improved immune response may be necessary to compensate for environmental stresses, such as low ambient temperature and reduced food availability that these mammals experience in the wild (Ahmad and Haldar, 2008). Given the fact that testosterone levels in mouse lemurs during the reproductive season are among the highest of any mammal studied so far (Petter-Rousseaux and Picon, 1981), it would be promising to study variation in gonadal hormones, testicular size and regression in mouse lemurs considering not only the apparent high costs of spermatogenesis per se (Wedell et al., 2002) but also the consequences of a compromised immune system during the season of scarce resources as these conditions would vary significantly in the highly unpredictable Madagascan habitats.

The seemingly overwhelming variation that appears to characterize testis size in mouse lemurs, and that ultimately determines the duration of their reproductive season should not discourage discussions about reproductive strategies in this opportunistic lemur group. I would argue that models of male mating strategies should not be restricted to the beginning of the reproductive season, as estrous females may be available later in the season. When mouse lemur females are undergoing their first estrous cycles, all males, despite testicular size variation, have the potential to mate (at least from a physiological point of view, without considering social constraints). As the reproductive season progresses and testicular regression begins, testes size will vary more dramatically among individuals. Some of them will display advanced regression when a portion of the female population may be undergoing second and third estrous cycles (i.e. regular polyestry) (Table 3). If, in fact, males with larger testes at the beginning of the season (or males with lower rates of testicular regression, if regression rate is a factor) are the last ones to completely regress their testes, they may be able to increase their reproductive success by mating with females when other males cannot. However, this implies a counterintuitive strategy as males should, in principle, delay complete testis regression until probabilities of mating are highly reduced. It is not clear, whether energetic, physiological or health constraints could override the increase of reproductive success by later matings. Variation in testicular regression may actually be maintained by natural selection in changing environments where resource availability vary between years and so do reproductive fitness of males who continue to allocate energy to reproduction later in the season.

Conclusions

Mouse lemurs are small-bodied nocturnal seasonal breeders. Variation in testis size has been documented both in the wild and captivity, within and between species, suggesting that biological and environmental components may be responsible for those differences.

This study is unique in that it shows testis size variation in frequently captured males over multiple reproductive seasons. Testicular regression begins earlier in some males and testis size also varies in the same individuals during different years.

Although more studies, both in the wild and captivity, are necessary to fully unveil the physiological underpinnings of reproduction and to document biological variation in the field, a picture of factors that likely influence reproductive variation within and across species is beginning to materialize. I hypothesize that variation in testis regression may be favorable in unpredictable environments, particularly in less seasonal forests, and that males who regress their testes later may be at an advantage to mate with females undergoing second or third estrous cycles. By monitoring testes development at a population level through multiple reproductive seasons, by making standard focal observations with records of mating behavior, and by conducting paternity analyses, a more complete picture of reproductive biology will emerge.

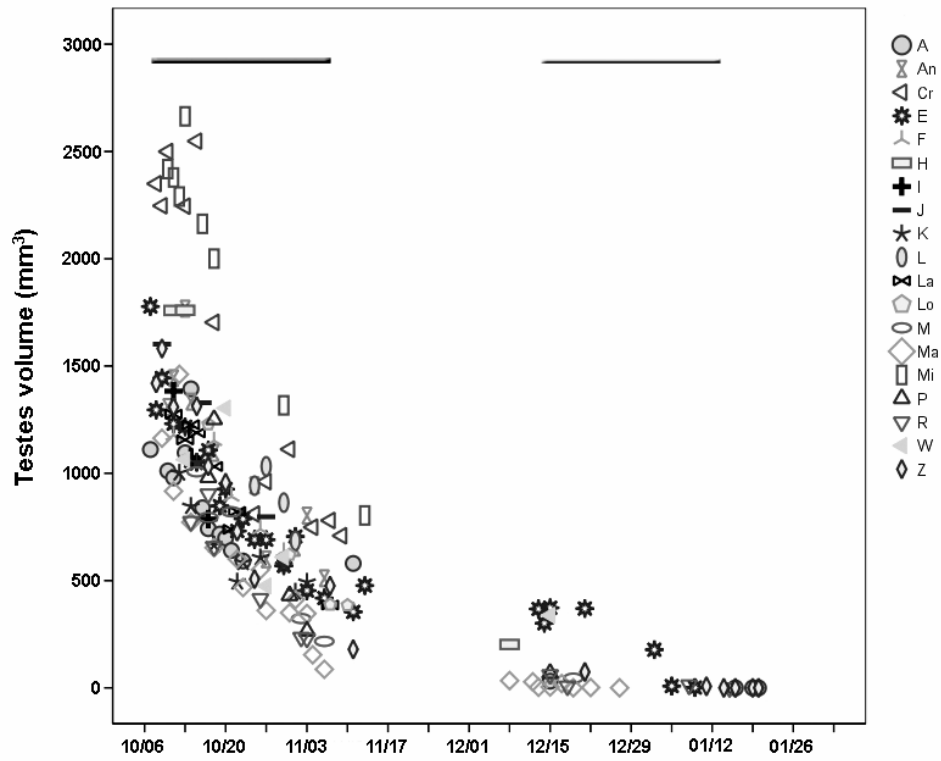


Figure III.2. Testes size estimated from frequently captured males during the 2006 reproductive season.

Upper line on the left indicates the period when estrous females were captured, upper thinner line on the right indicates the period when females would be expected to undergo a second estrus after successful gestation and parturition

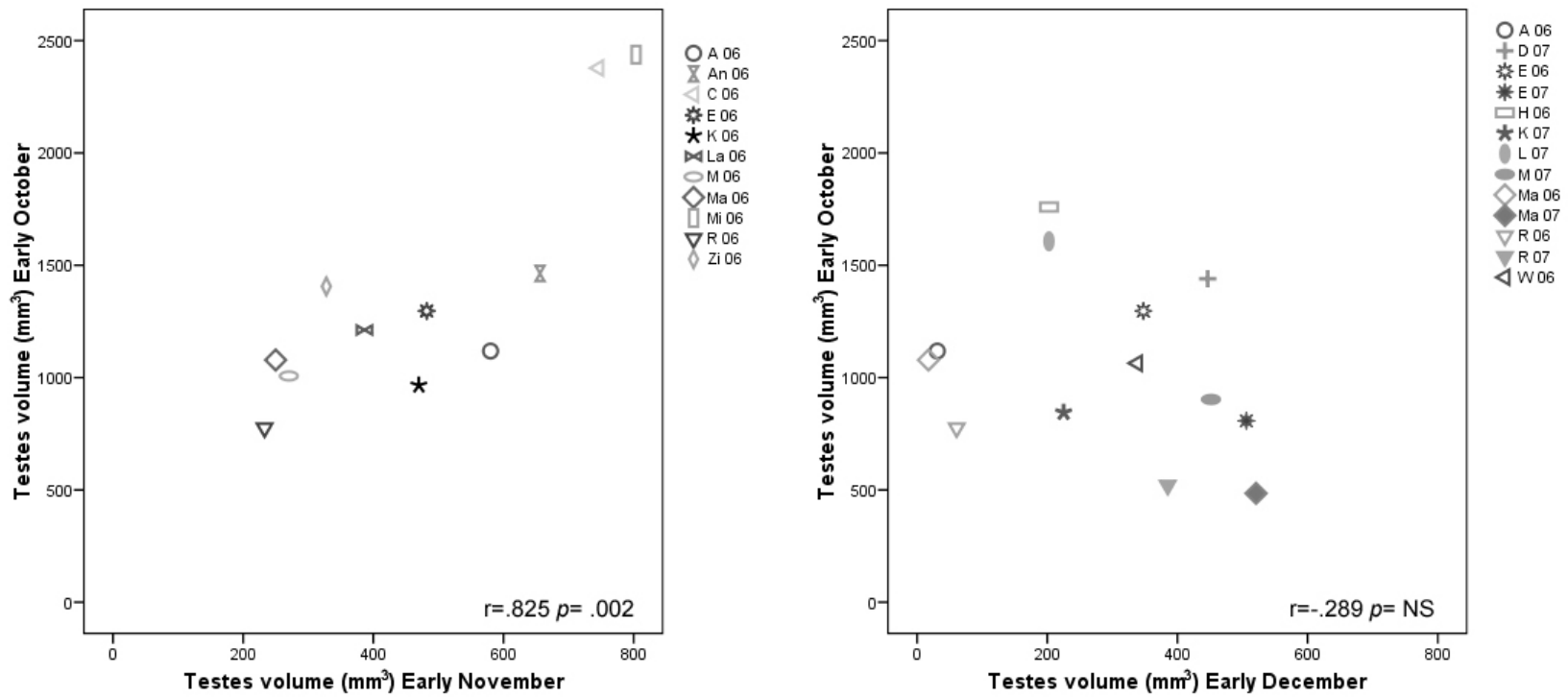


Figure III.3. Scatter plots of testes measurements between early October and early November (left) and early October and early December (right)

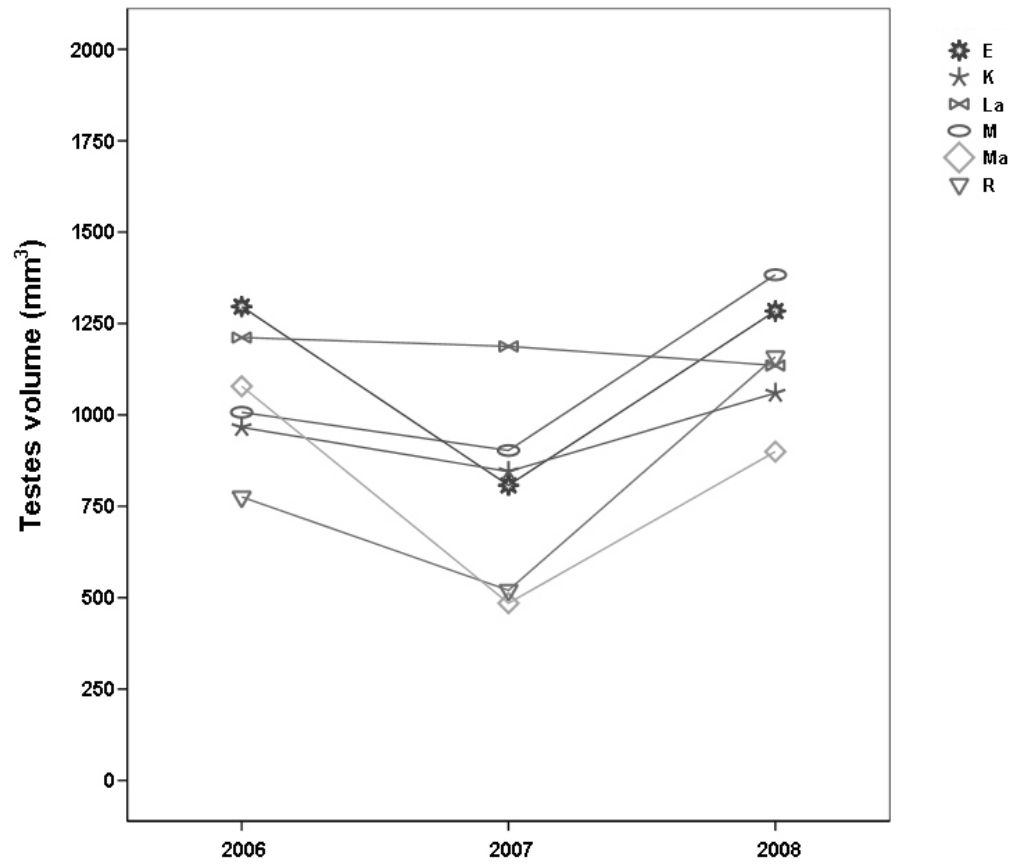


Figure III.4. Testis size (means) from 6 males captured in early October of 2006, 2007 and 2008

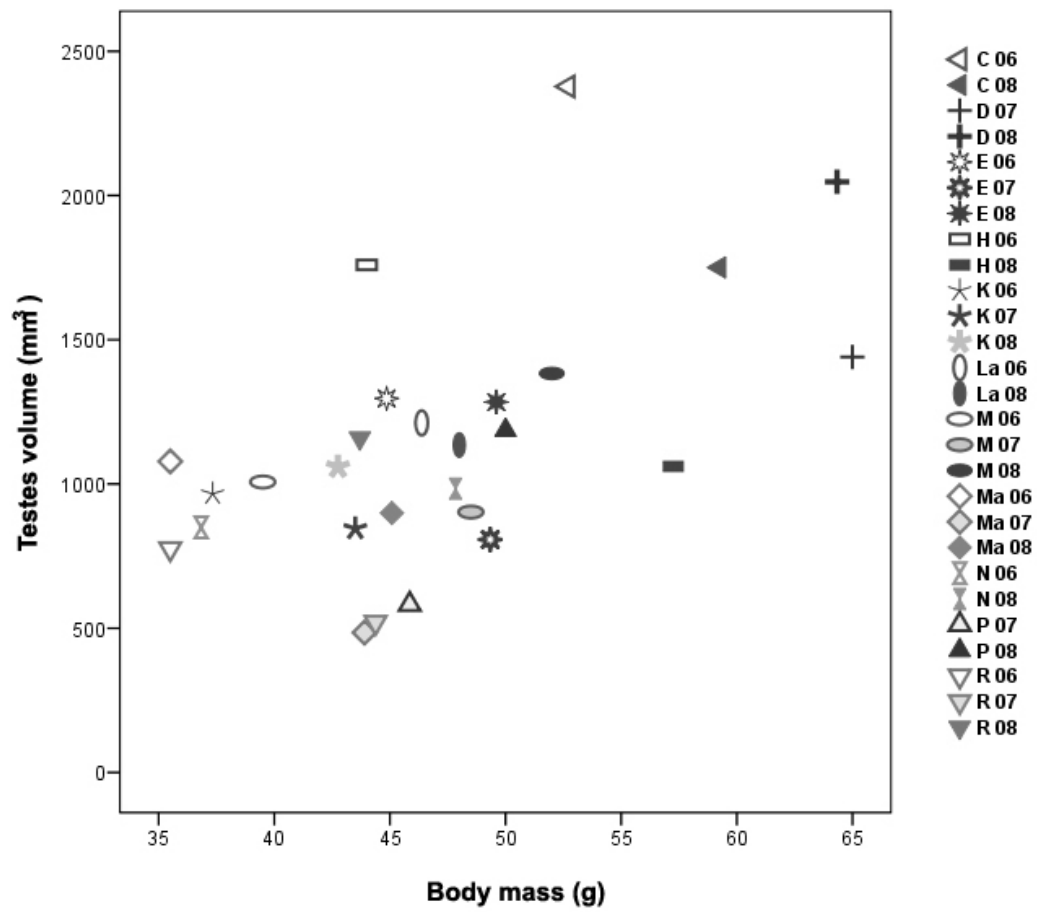


Figure III.5. Body mass (g) against testis volume in males captured early in October over multiple reproductive seasons

Table III.1. Summary of trapping schedule and monthly captures success by sex

month/ year	Nights trapped	# traps	# mouse lemurs	% capt.	# ♂	# ♀	% ♂	% ♀
Oct. 05	26	750	182	24.27	95	87	52.20	47.80
Nov. 05	23	512	118	23.05	52	66	44.10	55.90
Dec. 05-Jan. 06	20	682	103	15.10	31	72	30.10	69.90
Oct. 06	27	742	244	32.88	154	90	63.11	36.89
Nov. 06	11	242	77	31.82	36	41	46.75	53.25
Dec. 06	16	584	60	10.27	24	36	40	60
Jan. 07	14	478	56	11.72	13	43	23.21	76.79
Oct. 07	20	555	229	41.26	134	95	58.52	41.48
Dec. 07-Feb. 08	25	986	27	2.78	15	12	55.55	44.45
Sep.-Oct. 08	15	399	144	36.10	88	56	61.11	38.89

Table III.2. ANOVAs of testes volume during part of the 2006 reproductive season with Tukey's post hoc tests of significance of differences between half-months

2006	E Oct.	L Oct.	E Nov.	E Dec.	L Dec.	E Jan.
E Oct.						
L Oct.	***					
E Nov.	***	*				
E Dec.	***	***	NS			
L Dec.	***	***	NS	NS		
E Jan.	***	**	NS	NS	NS	
L Jan.	***	**	NS	NS	NS	NS

ANOVA: $F = 27.15$; $df = 6, 71$; $p < 0.001$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; NS = non-significant. Early months include days 1 to 15, late months 16 to 31.

Table III.3. Earliest and latest estrous dates for a single reproductive season estimated from field observations and known reports of gestation length and post-partum estrus from the literature

2006	Earliest estrus	Est. parturition ¹	Latest estrus	Est. parturition
First [observed]	7-Oct	3-Dec	7-Nov	3-Jan
Second [estimated] ²	13-Dec	8-Feb	13-Jan	11-Mar
Third [estimated]	18-Feb	16-Apr	21-Mar	17-May

¹Parturition dates are estimated by adding gestation length (57 days) to the day of estrus; ²Estimated estrous dates are based on a 10-day interval between estimated parturition and the following conception.

Table III.4. Descriptive statistics and Mann-Whitney U tests of significant differences for body mass (BM) and tail circumference (TC) of mouse lemurs captured before the first observed estrous female of the season

	B.M. (g)	T.C. (mm)
	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Females, n=11	44.6 ± 5.3	16.51 ± 1.61
Males, n=13	50.38 ± 4.75	16.94 ± 2.8
<i>p</i>	**	NS

** $p < 0.01$; NS = non-significant

CHAPTER IV

COMPARATIVE OBSERVATIONS OF MOUSE LEMURS FROM TSINJOARIVO FOREST, CENTRAL EASTERN MADAGASCAR

Preliminary data on morphology, reproduction and diet from forest fragment and continuous forest mouse lemurs

Introduction

The small-bodied nocturnal mouse lemurs occupy a wide variety of habitats in Madagascar, including very disturbed and fragmented environments. The taxonomy of the group has dramatically changed over the last decade due in part to extensive sampling of mouse lemurs throughout Madagascar. There were 3 recognized species in the mid-1990s and that number has risen to over 16 (Mittermeier et al., 2008); even more species are being described on the basis of previously unknown genetic variation (Louis, et al., 2008). Along with our knowledge of mouse lemur species diversity, knowledge of variation in metabolic, physiological and social responses among individuals and populations has also increased. Mouse lemurs exhibit a degree of variation in these parameters unknown in any other lemur group.

Due to their abundance and omnipresence in Madagascan forests, including secondary and highly disturbed habitats, mouse lemurs have been listed as the least threatened of all lemurs. However, questions remain as to whether forest fragmentation and habitat disturbance have a lasting negative effect on mouse lemur reproductive biology, population dynamics and ultimately their viability. Hints of probable negative effects of habitat disturbance were mentioned by Ganzhorn and Schmid (1998) who reported lower recapture rates, lower densities and less expression of seasonal torpor in mouse lemurs inhabiting secondary forests compared to primary forests at Kirindy, a

western dry forest. Variation in food resources, ambient temperature and predation rates were rated among the factors that could account for those differences. More recently, Schmid and Ganzhorn (2009) reported expression of true hibernation in mouse lemurs at a southern littoral forest in Madagascar. Only one of the two studied females underwent daily torpor whereas the other, inhabiting the more disturbed forest fragment, expressed hibernation (or seasonal torpor). The use of torpor and hibernation may be linked to individual survival in the short term but also it may affect aspects of life history as hibernation appears to be correlated with longevity (Chapter V, this dissertation).

Tsinjoarivo forest in central-eastern Madagascar is a particularly interesting place to study effects of disturbance, because it includes blocks of continuous and rather pristine forest and several forest fragments of varying sizes. Weather conditions were reported to differ between continuous and fragmented forests (Irwin, 2006). Daily temperature fluctuation was higher in at least one of the fragments in the Mahatsinjo area; in other words, the differences between temperature maxima and minima are usually greater in the fragments than in continuous forest. However, annual rainfall was greater in the continuous forest (Irwin, 2006). A great deal of environmental, botanical and biological information, including long term focal observations of the large-bodied sifakas, *Propithecus diadema*, has been collected since 2001 by Irwin and colleagues (Irwin, 2006; 2008). Results from botanical surveys and follow-up animal observations showed variation in dietary and behavioral patterns between sifaka groups inhabiting the continuous forest and those found in two of the fragmented forests (Irwin, 2006).

Mouse lemurs had been sighted, but not trapped, at Tsinjoarivo. An earlier biological inventory by Goodman et al. (2000) ascribed mouse lemurs in this forest to

Microcebus rufus, the brown mouse lemur. At the time of its publication, this was the only known and described eastern mouse lemur species. Since then, several eastern species have been discovered but taxonomic attribution in the field is complicated by the fact that some of the most recent discovered species, e.g., Goodman's mouse lemurs (*M. lehilahytsara*), cannot be easily distinguished from brown mouse lemurs due to similarities in their morphometrics and fur coloration (Kappeler et al., 2005). I will refer to individuals included in this study provisionally as *M. rufus*, with the caveat that species attribution may be changed after genetic analyses are eventually carried out.

The main goal of this study was to employ capture techniques at two sites with different levels of disturbance in the Tsinjoarivo area, i.e., within continuous forest and in one of the forest fragments to: 1- document the presence of mouse lemur populations and 2- determine whether morphological and size differences exist between locations. I also aimed to collect additional information on diet and reproductive condition. These data would then allow me to address possible effects of forest fragmentation on mouse lemur diet and reproduction. Are differences in recapture rates and reproductive parameters between sites in accord with published literature indicating negative effects of fragmentation in lemur population dynamics and viability? Are there clear differences in dietary preferences? Are there reproductive differences between the females at both study sites? Are reproductive observations consistent with those collected at Ranomafana? Is there evidence of reproductive failure, and if so is it more frequent in the fragment? Is polyestry (i.e. multiple estrous cycles per season) expressed at Tsinjoarivo and if so, where?

Methods

I captured mouse lemurs at two sites within the Tsinjoarivo forest, central-eastern Madagascar, that were originally established by Irwin and colleagues in 2001 (Irwin, 2006; 2008). The first study site, FRAG, was located at Andasivodihazo (19°41'15"S, 47°46'25" E, 1660 m), a 228-ha forest fragment in the Mahatsinjo area, and the second campsite, CONT, was set up within continuous forest at Vatateza (19°43'15"S, 47°51'25" E, 1396 m), ~10 km to the south-east. Trapping was conducted for 9 trapping nights in November 2006 (5 nights in CONT, 4 nights in FRAG), 20 trapping nights in November 2007 (11 in CONT, 9 in FRAG), 9 trapping nights in December 2007 (in CONT) and 5 trapping nights in January 2008 (in FRAG). Additional observations were recorded in April 2009 when mouse lemurs entered Tomahawk traps set up for larger-bodied dwarf lemurs (*Cheirogaleus*). A maximum of 50 Sherman traps were set up in pairs separated by ~25 meters in selected trails. Traps were baited with fresh banana around 17:00 and checked at ~4:00 the following morning. All mouse lemur captures were brought back to the campsite and all non-primate captures were identified and released immediately. Individual mouse lemurs were identified or marked with Avid microchips, weighed and measured. Reproductive condition was determined for females by inspecting vaginal morphology (i.e., sealed or swollen condition, or vaginal opening, which occurs only at estrus and parturition), nipple development and careful palpation to determine later stages of pregnancy or lactation; in males, testes were measured with calipers and testes volume was calculated based on the equation for an ellipsoid (each testicular side was treated individually), described in Dammhahn and Kappeler (2005) and frequently used in the literature.

If feces were available inside the traps, they were inspected for presence of seeds or food particles and a subset of female fecal samples were collected and dried in a Coleman oven for further reproductive hormonal analysis. For a description of the protocol for hormone analysis that I employed, see Blanco and Meyer (2009). Four botanical plots of 20x20m, two in the fragment and two in the continuous forest were chosen to monitor the presence of flowers and fruits at the beginning and at the end of trapping period.

Independent sample T-tests and Mann-Whitney U tests were used for morphometric and body mass comparisons between sites. All analyses were performed in SPSS 15.0.

Results

A total of 47 mouse lemurs were trapped at Tsinjoarivo, 35 of them in the forest fragment (FRAG) and 12 within continuous forest (CONT) out of a total of 1613 trapping opportunities. A few mouse lemurs were recaptured the second year, more often in the fragment than the continuous forest (Table 1). Five of the 11 mouse lemurs trapped in April 2009 inside dwarf lemur traps were recaptures from 2007. Trapping success was relatively higher in FRAG in November: 14.48% compared to 4.11% in CONT; in December 6.94% in the FRAG and 3.93% in January in CONT. However, trapping success was unfortunately much higher for rats (including a minimum of three species of endemic tufted-tail rats, genus *Eliurus*) at both sites during this study: in the FRAG, 23.30% in November, 35.28% in December; in CONT, 29.86% in November, 13.48% in January. Due to high percentage of rats entering the Sherman traps, trapping locations had to be frequently changed at both sites.

No distinguishable differences in fur coloration between mouse lemur populations were observed and no significant morphometric differences were found between CONT and FRAG mouse lemurs, except for head width (HEW) (Table 2). Body mass, however, differed between the sites (Mann-Whitney U test, years pooled together, $p=0.039$). Individuals captured in FRAG were heavier than those trapped in CONT (Table 3).

A total of 44 fecal samples were inspected for plant or animal parts. Out of 74 seed types (i.e., a type refers to presence but not number of a particular seed in a fecal sample), 59 were identified as belonging to 8 plant groups and 15 unidentified seeds were classified in 3 unknown types based on morphology. Insect parts were found in only one fecal sample and hair was found in two samples from the same individual female. Seventy-four percent of seeds present in fecal samples corresponded to four known genera (Table 4). The total number of fecal samples was insufficient to conduct inter-site comparisons. However, the three most frequent seeds found in feces were the same at both locations.

Botanical plot observations spanned a very short period within the reproductive season and they certainly do not represent the floristic diversity of each study site throughout a year. Although both plots at each site showed relatively more fruits (mostly unripe) during January compared to observations recorded a month earlier, generally the presence of fruits available was very low at both locations. The epiphytic hemiparasites, *Bakerella* spp. (vernacular names, “tongoalahy” and “hazomiavona”) and the hemiepiphyte liana *Medinilla* (“kalamasimbarika”) were present at both sites, and their fruits, mostly unripe, were available in mid-late November and January. This was consistent with the presence of seeds of these plants in mouse lemur fecal samples during

the same period. In April, 2009, observations of the same botanical plots in the FRAG showed no fruits available for *Bakerella* spp. and *Medinilla* and only three tree species bore fruits (“angavodiana”, genus *Agauria*; “rebosa”, genus *Melicope*; “nahaleonify”, unknown genus); however, it is yet unknown whether the latter fruits were consumed by mouse lemurs.

Reproductive observations from females trapped in mid-November, December and early January indicated that ~60% of individuals were gestating or lactating, while the rest of the females showed either signs of reproductive failure or no signs of reproductive activity at all (Table 5), combining both study sites. In the continuous forest, I confirmed the expression of polyestry in at least one female (Ni), who aborted early in the season (Nov. 18) and was captured pregnant around a month later (Jan. 10). A vaginal swelling was observed at the end of November in female F, who had displayed a bleeding vagina four days earlier, possibly as a result of early abortion, and also in female Pr, who was pregnant in mid-November, had a swollen vagina in early January, and developed nipples indicating recent lactation. Finally, female An showed vaginal opening in mid-November (when she weighed only 29.5g), and female Zo showed vaginal opening in early January, but no evidence of lactation (e.g., undeveloped nipples). In the fragment female Sa showed late unusual vaginal opening in mid-November, without prior vaginal swelling and females Na and Ai displayed vaginal swellings at the end of December in conjunction with significant nipple development (Table 5).

Fecal estradiol (E₂) and progesterone (P) levels were extracted from 18 samples of 7 females and those values agree overall with reproductive observations despite high intra- and inter-individual variation. For example, fecal P levels dropped both in female

St at the end of November as would be expected after an abortion, and in female An³ in January, after a presumably early parturition and possibly loss of offspring (Table 6).

For males captured both in the FRAG and CONT, average testicular size decreased from November to December, although no significant differences were found between months, possibly due to the small sample size (Table 7). Measurements could not be taken, due to reduced testis size from two individuals trapped at the end of December and the testes of the only two males captured in April in the fragment had already regressed.

Six out of 11 individuals captured in the fragment in April showed tail fattening. Two of the five adult females who showed fattening also showed signs of recent lactation. However, one adult female –a recapture from 2007, showed signs of recent lactation but no fattening. One the two adult males displayed fattening while the other did not. (Tables 8 and 9) (Figure 1).

Discussion

Mouse lemurs were captured within continuous forest and in one of the forest fragments at Tsinjoarivo. Although individuals captured in the forest fragment were morphologically similar to those trapped in the continuous forest, fragment mouse lemurs had significantly higher body masses. These results contradict those obtained at the same study sites for the larger-bodied sifaka (*P. diadema*). Irwin (2008) reported that male and immature sifakas captured in the fragment were lighter than individuals from the

³ Preliminary results from fecal endoparasite analysis, showed that female An had a significant number of unidentified parasitic larva present in her feces (over 90), compared to ~20 larvae from feces of another female trapped in the same area, and less than 5 and

continuous forest. Yet, dietary habits and social behavior differ between the larger-bodied sifakas and small-bodied mouse lemurs. Preliminary data from this study also differ from results obtained for a related mouse lemur species (*M. murinus*), inhabiting a different habitat. For gray mouse lemurs at Kirindy, a dry deciduous western forest, Ganzhorn and Schmid (1998) observed that individuals captured in secondary forest had lower body masses than mouse lemurs captured within primary forest. Recapture rates were also lower in the secondary forest. Limited food availability and less expression of torpor of mouse lemurs at this latter location were suggested as possible factors (but see Schmid and Ganzhorn, 2009). More research is needed at Tsinjoarivo, but the fact that fragment mouse lemurs were heavier and more frequently recaptured show that the dynamics within continuous and forest fragments may not be as simple as previously thought.

Mouse lemurs trapped at the beginning of the dry season provided valuable information. A number of mouse lemurs trapped in April 2009 exhibited fattening. Evidence of opportunistic fattening has also been reported for brown mouse lemurs at Ranomafana (Atsalis, 2008) and other mouse lemur species. It is unknown why certain adult individuals would undergo fattening while others would not. Lactating females, who have two or three litters, may not be expected to fatten to the same degree during the end of the rainy season than non-recently reproducing females given the energetic demands of lactation, but this hypothesis requires further testing. Scarcity of resources during this time of the year may render fattening unsustainable for the totality of the adult population.

none present in feces from three other females at CONT. Female An was possibly around 1 year old, the smallest “young adult” captured mouse lemur at Tsinjoarivo.

Food availability at Tsinjoarivo could only be assessed for a brief period within the reproductive season; hence, broad conclusions cannot be drawn from this preliminary survey. However, it appears that certain plants, such as the mistletoes (genus *Bakerella*) and a hemiparasitic liana (genus *Medinilla*) –highly abundant in the fragment– were preferred foods for mouse lemurs. In fact, Irwin (2008) argued that mistletoes are indispensable in the diet of the more folivorous sifakas, particularly in the fragments where they were utilized as staple food throughout the year compared to their use as fallback foods by the sifakas inhabiting the continuous forest. Both known cheirogaleid species present at Tsinjoarivo, the mouse and dwarf lemurs (*Cheirogaleus*) fed largely on *Bakerella* spp. and *Medinilla* during the reproductive season (pers. obs.). Indeed, cheirogaleids are most likely the main dispersers, as feces preserve intact seeds and evidence of germination (Figure 2) whereas sifakas are seed predators (Dew and Wright, 1998; Hemingway, 1996, cited in Atsalis, 1999).

This study could not identify dietary differences between the fragment and continuous forest, due to small number of fecal samples. However, the fact that the same few plants were identified at both locations and that they appear to be important food sources for a number of lemur species brings into question the degree of inter-specific competition, particularly in forest fragments during the dry season. Lower canopies due to fewer large trees, and overall reduced forest cover characteristic of fragments may not allow for vertical compartmentalization of resources, as Lahann (2007, 2008) reported for three sympatric cheirogaleid species whose diets highly overlap at Mandena, a southeastern littoral forest. Indirect evidence of competition at Tsinjoarivo was observed in April, when several mouse lemurs were trapped in dwarf lemur traps set up much

higher in tree branches than Sherman traps. It is also noteworthy that the only dwarf lemurs captured in April (i.e., not yet fully hibernating) had to significantly increase travel distances from their sleeping sites to find food sources, namely patches of mistletoes (pers. obs.). The importance of these hemiparasitic epiphytes not only resides in their unusual extended phenology, which allows for fruit availability throughout the reproductive season (Irwin, 2008), but also on their nutritional value. Atsalis (1999) performed chemical analysis on a variety of fruits consumed by mouse lemur at Ranomafana National Park, including a few species of *Bakerella* and reported an unusually high content of fat in mistletoes compared to all the other available fruits. Mistletoes were described as a keystone resource at Ranomafana, based on their dominant presence in the diets of mouse lemurs all year round (Atsalis, 1999). *Bakerella clavata*, a species also found at Tsinjoarivo and heavily consumed, had the highest fat content, so the implications of mistletoes for the survival of cheirogaleids in fragmented environments where these hemiparasitic epiphytes are abundant should not be underestimated. The viability of mouse lemur populations, however, is certainly determined by multiple factors that need to be studied in the long term.

Reproductive observations at Tsinjoarivo were not available at the beginning of the reproductive season, because mouse lemur trapping was conducted in late November until January. A study population of brown mouse lemur females at Ranomafana entered the first estrus of the season in October-early November (Blanco, 2008; Chapter II, this dissertation). Several females at Tsinjoarivo, both in the fragment and the continuous forest appeared to be gestating or lactating in late November, December and early January. These observations are consistent with estimated dates of parturition around the

third or last week of December at Ranomafana. However, a large percentage of females at both sites in Tsinjoarivo showed either no reproductive activity during this period or “unusual” vaginal swellings or vaginal openings, some of which apparently occurred as a result of earlier abortions. The presence of rebound polyestry (Blanco, 2008), i.e., renewed estrus after early abortion or loss of offspring, was demonstrated in at least one female (Ni) inhabiting the continuous forest. At least two other females (F, CONT and Sa, FRAG) displayed vaginal swellings as a result of presumed early abortions. Rebound polyestry has been reported in captive and wild populations of mouse lemurs (e.g. captive *M. murinus*, Glatston, 1979; Perret, 1982; wild *M. griseorufus*, Génin, 2008). Regular polyestry, i.e., renewed estrus after litter survival, has also been documented in captivity (e.g. *M. rufus*, Wrogemann and Zimmermann, 2001) and reported for wild *M. murinus* at Ampijoroa (Schmelting et al., 2000) and Mandena (Lahann et al., 2006). Although multiple litters within a single reproductive season has been reported in other mouse lemur species (possibly *M. ravelobensis* at Ampijoroa, Schmelting et al., 2000), the data are not conclusive, as individual females were not followed or frequently captured during the reproductive season to determine their timing of estrus, the status of their offspring (e.g. surviving or dead), and the presence of postpartum estrus.

Due to low trapping success later in the rainy season, I was only able to report *indirect* evidence of rebound and regular polyestry in brown mouse lemur females at Ranomafana (Blanco, 2008; Chapter II this dissertation). Due to the fact that only scarce data are available for Tsinjoarivo, I cannot conclude with certainty that regular polyestry occurs at this forest; however, the presence of vaginal swellings and well developed nipples indicating current/recent lactation in females Ai, Na at the end of December in the

forest fragment and in female Pr in January captured in the continuous forest is at least consistent with this possibility.

The fact that ~40% of females at Tsinjoarivo did not seem to be successfully reproducing at the time of capture is of interest in itself. Research conducted at Ranomafana, showed that most females, between 70 and 80%, trapped between October and December experienced normal estrus, full-time gestations and/or showed evidence of lactation. Interestingly, one year old females were more prone to reproductive failure (e.g. abortion, perinatal death of offspring) than older females (Chapter V, this dissertation). Identification of the first cohort of female mouse lemurs at Tsinjoarivo was not possible, as trapping was conducted in mid-November or later, when morphometrics and body mass are indistinguishable among age classes. However, in at least in one case, a female from the fragment (St) first captured in 2006 showed signs of abortion in mid-November of 2007, when she must have been at least two years old.

Conclusions

Mouse lemurs were captured both within continuous forest and in one of the forest fragments at Tsinjoarivo. Individuals could not be differentiated based on fur coloration and morphometrics so they are presumed to be the same species. External morphology is consistent with that of brown mouse lemurs, *M. rufus*, but genetic analyses are needed to confirm species attribution.

Mouse lemurs inhabiting the fragment were significantly heavier than those captured in the continuous forest. Although the number of recaptured mouse lemurs was low, partly due to small overall sample sizes, more mouse lemurs were recaptured in the

fragment. High unwanted trapping success of endemic rats was more significant in the continuous forest.

The three main dietary items identified in their fecal samples were two kinds of mistletoes and a liana, whose presence was established in both study sites but were particularly abundant in the fragment.

A higher percentage of females trapped at Tsinjoarivo did not appear to be reproductively active or they showed signs of reproductive failure (e.g., signs of abortion) compared to reproductive observations from females captured at Ranomafana, another eastern rainforest; however larger samples sizes and long term trapping are necessary to confirm these observations. Lack of data prevented inter-site comparisons. Evidence of polyestry (e.g. rebound polyestry at a minimum) was reported in a female trapped in the continuous forest and expression of opportunistic fattening was observed for at least some of the mouse lemurs captured in the fragmented forest.



Figure IV.1. Mouse lemur captured in April 09 showing fattening at the base of the tail



Figure IV.2. *Bakerella* seeds (tongoalahy) found in cheirogaleid feces (possibly from *Cheirogaleus*) showing germination

Table IV.1. Number of mouse lemurs captured at Tsinjoarivo in 2006 and 2007

Tsinjoarivo		N traps	First capture		Recapture	
Continuous	Year		Males	Females	Males	Females
	2006	170	4	2		
	2007	641	2	5	0	1
Fragment	2006	140	5	4		
	2007	662	12	12	2	2
	2009	N/A	3	8	1	4

Table IV.2. Morphometric comparisons and Mann-Whitney U tests of significant differences between mouse lemurs from the fragment and the continuous forests trapped in November

Variable	Site	n	$\bar{x} \pm SD$	<i>p</i>
C-TB (mm)	Continuous	13	86.43 ± 4.43	NS
	Fragment	19	87.49 ± 4.75	
TL (mm)	Continuous	13	111.84 ± 4.31	NS
	Fragment	22	113.7 ± 5.23	
HL (mm)	Continuous	13	79.57 ± 2.91	NS
	Fragment	19	79.46 ± 3.33	
HFL (mm)	Continuous	13	28.09 ± 1.56	NS
	Fragment	19	29.23 ± 1.46	
HEL (mm)	Continuous	13	33.03 ± 0.9	NS
	Fragment	22	33.67 ± 1.12	
HWL (mm)	Continuous	13	18.86 ± 0.68	**
	Fragment	22	19.77 ± 0.74	
SNL (mm)	Continuous	13	9.13 ± 1	NS
	Fragment	22	9.66 ± 0.74	
IPD (mm)	Continuous	13	13.23 ± 1.06	NS
	Fragment	22	13.37 ± 0.85	
EL (mm)	Continuous	13	17.05 ± 1.85	NS
	Fragment	22	17.69 ± 1.02	
EW (mm)	Continuous	13	8.78 ± 1.05	NS
	Fragment	22	9.08 ± 1.04	

Mann-Whitney U tests: ** $p \leq 0.01$; NS=non-significant. Measurements from top to bottom: taken with flexible tape, Crown to Tail base, dorsally, from the forehead to the cranial margin of the first caudal vertebra; Tail length, ventrally, from the base of the anus to the distal end of the last caudal vertebra; Hind-limb length, from the femoral head to the distal end of the 4th digit, measured with the limb and foot extended; taken with calipers, Hindfoot length, from the calcaneal heel to the distal end of the 4th digit, measured with foot extended; Head length, maximum length of the head from the most projecting margin of the rhinarium to the back of the braincase; Head width, maximum bizygomatic breadth; Snout length, maximum length from the most projecting margin of the rhinarium to a virtual line projected transversely across the base of the orbits; Interpupillary distance; Ear length, maximum length between the base of the tragus and the tip of the pinna; Ear Width, maximum distance between base of tragus and border of pinna measured perpendicular to ear length. Pregnant females are excluded.

Table IV.3. Body mass comparisons between mouse lemurs from the fragment and the continuous forest trapped in November

<i>Microcebus</i>		2006		2007	
		n	BM (g)	n	BM (g)
Fragment	females*	3	42.33	11	43.36
	males	5	45.70	14	41.55
Continuous	females*	2	33.88	3	36.88
	males	4	42.38	2	36.25

*Pregnant females are excluded

Table IV.4. Identified seeds found in mouse lemur fecal samples

Vernacular name	Family	Form	Scientific name	N of feces
Kalamasimbarika	Melastomataceae	LI	<i>Medinilla</i> spp.	27
Tongoalahy	Loranthaceae	HPR	<i>Bakerella</i> sp.	17
Hazomiavona	Viscaceae	HPR	<i>Viscum</i> sp.	9
Voananananala	Rubiaceae	TU	<i>Pauridiantha</i> ?	2
Dendemilahy	Loganiaceae	TC	<i>Anthocleista</i> sp.	1
Kitonda	Ericaceae	TU	<i>Vaccinium</i> sp.	1
Takaloparihy	Myrsinaceae	LI	<i>Embelia</i> sp.	1
Voamangalela	?	TU?	?	1

LI: liana; HPR: hemiparasite; TU: understory tree; TC: canopy tree

Table IV.5. Summary of female reproductive observations at Tsinjoarivo

Female	Reprod. Obs.	Date of SwV or VO	Probable Polyestry
Ai	PP, PL, SwV	21-Dec	Yes
E	NP, NL		
Ja	PL		
Ji	NP, NL		
Ka	PL		
Na	PP, PL, SwV	26-Dec	Yes
Ri	PL		
Ro	PP		
Sa	PP, VO	29-Nov	Yes
St	PP, A		
An	VO, PP, PPDO		
Chr	NP, NL		
F	A, SwV	25-Nov	Yes
Ni	P, A, P		Yes
Pr	PP, PL, SwV	11-Jan	Yes
Zo	NL, VO	6-Jan	

PP: possibly pregnant; PL: possibly lactating; SwV: vaginal swelling; NP: not pregnant; NL: not lactating; VO: vaginal opening; A: abortion; PPDO: possibly perinatal death of offspring; P: pregnant

Table IV.6. Hormone values for E₂ (pg/mg) and P (ng/mg) and reproductive observations for seven females captured at Tsinjoarivo

ID	Date	E ₂	P	Reprod. Obs.
Ni	9-Nov-07			SV, PP
Ni	13-Nov-07	20.68	2.13	PP
Ni	14-Nov-07	46.47	5.58	PP
Ni	16-Nov-07			SV, PP
Ni	18-Nov-07			RV, A
Ni	10-Jan-08	121.66	3.13	P
An	13-Nov-07	45.73	1.76	VO
An	14-Nov-07	35.81	2.42	VO
An	15-Nov-07	40.19	3.85	ASV
An	6-Jan-08	64.13	5.08	SV, DN, RB?
An	8-Jan-07	59.91	0.98	SV, DN, NL
E	21-Dec-07			SV, NP
E	29-Dec-07	13.84	2.35	NP
E	2-Jan-08			SV, NP/NL
Zo	6-Jan-08	18.94	2.20	VO, NL
St	21-Nov-07	373.85	8.38	SV, DN, P
St	27-Nov-07	247.60	1.69	RV, A
St	29-Nov-07			SV
Sa	21-Nov-07	375.86	0.30	SV, PP
Sa	27-Nov-07	108.25	2.42	SV
Sa	29-Nov-07	151.24	1.23	VO, DN
Sa	30-Nov-07	40.39	5.22	ASV
Na	24-Nov-07			SV, DN
Na	29-Nov-07			SV, DN, PP
Na	1-Dec-07	31.41	0.42	SV, PP
Na	26-Dec-07	335.12	5.17	SWV, DN

SV, sealed vagina; SWV, swollen vagina; RV, reddish vagina; VO, vaginal opening; ASV, almost sealed vagina; A, recent abortion; RB? possibly gave birth recently; P, pregnant; PP, possibly pregnant; NP, not pregnant; DN, developed nipples; NL, not lactating

Table IV.7. Testes volume (mm³) and Mann-Whitney U tests of significance, estimated from males captured in the forest fragment during the reproductive season

Month	n	$\bar{x} \pm SD$	<i>p</i>
Nov.	14	397.68 ± 103.04	
Dec.	7	308.67 ± 133.25	NS

Table IV.8. Body mass (BM), tail base circumference (TBC), evidence of fattening (FAT), recent lactation (RL) or visible testis development (VT) from mouse lemurs captured in the forest fragment in April at the beginning of the dry season

Sex	Age	BM (g)	TBC (mm)	FAT	RL	VT
Female	adult	90.5	34.16	Yes	Yes	
Female	adult	87	33.26	Yes	No	
Female	adult	77.5	29.24	Yes	No	
Female	adult	67	25.08	Yes	Yes	
Female	adult	66	30.38	Yes	No	
Female	adult	49	18.6	No	Yes	
Female	adult/juvenile	50.5	22.02	No	No	
Male	adult	73.5	32.44	Yes		Yes, but regressed
Male	adult	39	18.42	No		Yes, but regressed
Female?	juvenile	38	16.6	No	No	
male?	juvenile	42.5	16.02	No		No

Table IV.9. Body mass and tail base circumference from individuals captured early in the reproductive season, i.e., at their lowest annual values

	n	Min	Max	$\bar{x} \pm SD$
BM (g)	21	37.5	55	45.81 ± 5.56
TBC (mm)	16	13.5	20.7	16.41 ± 1.85

CHAPTER V

CHEIROGALEIDS IN THE WILD: DO FAST OR SLOW LIFE HISTORIES CHARACTERIZE EASTERN MOUSE AND DWARF LEMURS?

Introduction

Mammalian life histories are classically arranged along a fast-slow continuum. Individuals belonging to small-bodied species tend to live faster and die younger than larger species. Other factors, such as the demographics of mortality rates, covary with life history traits independently of body size (Promislow and Harvey, 1990). Simply stated, species in the fast lane compensate for high adult mortality rates by reproducing at an early age, having multiple, low-mass litters, short gestations, precocial neonates, and early weaning. On the slower end, species with lower intrinsic mortality rates can afford to delay reproduction and invest in fewer, larger-bodied offspring over longer life spans (Promislow and Harvey, 1990).

It is widely recognized that the fast-slow continuum does not adequately describe all variation in life histories. Many species, including for example some caviomorph rodents, express a mosaic of life history traits such as early age at maturation and precocial offspring (suggestive of fast life histories) but long gestations (suggestive of slow life histories) (Kraus et al., 2005). For this reason, some researchers prefer to use multivariate techniques to identify suites of traits, as opposed to single variables, that best characterize variation in mammalian life histories (Bielby et al., 2007). Nevertheless, the fast-slow continuum may be useful in broad comparative analyses. In a recent study, Dewar and Richard (2007) invoked the fast-slow continuum to explain some unusual life history characteristics of Malagasy lemurs and other mammals. They suggested that

Malagasy mammals display extremes of “fastness” and “slowness” unseen in their counterparts from other regions, and that this relates to unique environmental conditions in Madagascar –most importantly to highly variable (“unpredictable”) intra- and inter-annual patterns of rainfall. According to environmental stochasticity models, longer reproductive spans should be favored if fertility and juvenile survival are highly variable. This life history solution, called bet-hedging, is observed in large-bodied lemurs (such as sifakas, *Propithecus verreauxi*) and other Malagasy mammals. However, early age at reproduction should be selected if adult survival is tenuous, as observed in small-bodied lemurs. In fact, Dewar and Richard (2007) argue that gray mouse lemurs may have the fastest life history of any primate, with their early age at first reproduction, multiple offspring and multiple litters within single reproductive seasons. This is a favorable strategy for animals suffering high adult predation rates along with habitat unpredictability.

Indeed, the environmental unpredictability of Madagascar has also been invoked to explain a suite of unusual energy-saving lemur traits including hypometabolism and female dominance (Young et al., 1990; Wright, 1999). Daily torpor and hibernation are extreme energy-saving strategies employed by some members of the family Cheirogaleidae to cope with strong seasonality of resources (Schülke and Ostner, 2007). In general, as studies of non-primates have shown, hibernating species tend to have life spans that are longer than expected based on body mass alone (Wilkinson and South, 2002). Hibernation is usually characterized by an overall decrease in metabolic rate, resilience to environmental stress, and seasonal loss of body mass (e.g. caloric

restriction), all of which are positively correlated with longevity (Stuart and Brown, 2006).

This raises some interesting questions: Do mouse lemurs and other cheirogaleids really live their lives in the fast lane, or do they exhibit life history characteristics of “slow” species? Does the fast-slow continuum adequately describe their life histories? How does the variation in the metabolic characteristics of cheirogaleids (obligate hibernation only exists among species belonging to the genus *Cheirogaleus*) correlate with variation in life history traits? Although the use of torpor or hibernation in cheirogaleids has been described (for example, Fietz and Dausmann, 2006; Schülke and Ostner, 2007), the relationship between these metabolic strategies and life history traits in this group of small-bodied lemurs has not been directly investigated. This is the goal of this study.

Laboratory work conducted in the early 1980s featured western gray mouse lemurs (*M. murinus*) and fat-tailed dwarf lemurs (*C. medius*). Comparisons between these species showed apparent similarities in a number of life history parameters such as gestation length (*M. murinus*: ~62 days and *C. medius*: ~62 days) and age at sexual maturation (around 1 year old for both species) (Foerg, 1982). Remarkably also, these values differed from those manifested in captive bushbabies of comparable body sizes (gestation length in the mouse lemur-sized *Galagoides demidoffi*: ~112 days, and in the somewhat larger, and more similar in mass to *C. medius*, *G. moholi*: ~122 days, Zimmermann, 1989). These differences appeared to support the inference of exceptionally fast life histories for cheirogaleids. To date, however, little has been documented about life history parameters of wild mouse and dwarf lemurs. Indeed, the

life history similarities and differences among cheirogaleids and other small-bodied prosimians that have been observed under captivity may not hold in the wild, where different environmental conditions would impose specific limitations to the biological potential of the species.

Within the context of mammalian life history theory alone, both mouse and dwarf lemurs would be expected to display “fast” life histories because (1) they live in highly unpredictable climates, (2) they are small-bodied animals and body mass is positively correlated with life span, and (3) small-bodied species tend to show higher adult mortality rates than larger species. However, the use of torpor and hibernation, unusual metabolic strategies for a primate, may affect overall growth and development and “slow down” their life histories, consequently increasing their life spans.

To further explore how the life histories of cheirogaleids are affected by torpor or hibernation, I collected data on growth, dental development and reproduction from wild populations of eastern mouse and dwarf lemurs. In particular, I investigated similarities and differences in their growth schedules, and ask whether their life history parameters vary according to expectations based on their different activity patterns and energy saving strategies. I also examined dental and growth comparative data on cheirogaleids and similarly-sized prosimians collected from museum specimens and captive data.

Methods

Study sites

I trapped mouse and dwarf lemurs during the reproductive season at two eastern rainforests. *Microcebus rufus* was captured in the Talatakely trail system at Ranomafana National Park, a montane rainforest in southeast Madagascar (21°15'50"S, 47°25'08" E,

1000 m) in October-December 2005, October 2006-January 2007 and October 2007-February 2008 and October 2008. *Cheirogaleus* spp. were captured at two study sites in the high-altitude forests of Tsinjoarivo, central-eastern Madagascar in November-December 2006, November 2007-January 2008, October-November 2008 and March-April 2009. There are two sympatric species of dwarf lemurs at Tsinjoarivo that have been described elsewhere (Blanco et al., 2009; Groeneveld et al., 2010). *C. cf. sibreei* was captured at Andasivodihazo, one of the fragmented forests (~228 ha) within the Mahatsinjo area (19°41'15"S, 47°46'25" E, 1660 m) and *C. crossleyi* was captured in the same fragment and exclusively in the second study site, Vatateza, which was established ~10 km to the southeast within continuous forest (19°43'15"S, 47°51'25" E, 1396 m). For more details about Tsinjoarivo forest, see Irwin (2006). Because there are significant differences between the Tsinjoarivo dwarf lemur species in morphometrics and body mass, I analyzed the two separately.

At Ranomafana National Park, a maximum of 50 Sherman traps were set daily at ~17:00 at heights of 1.50 m along pre-established trails and separated by a minimum of 25 m. Traps were checked at ~20:00 and all captured individual mouse lemurs were brought back to the Centre ValBio research station. After routine procedures, all individuals were released at their sites of capture around midnight on the same day. At Tsinjoarivo, a maximum of 25 Tomahawk traps were set at ~17:00 at heights of between 4-10 m and separated by a minimum of 50 m along preexisting trails. Traps were checked at ~4:00 the following morning and all individual dwarf lemurs were brought back to the campsite. All captured animals were released at dusk later the same day. Both mouse and dwarf lemurs were marked with Avid microchips, weighed and measured. Molds of the

lower right teeth of dwarf lemurs were taken. Reproductive condition was assessed for all females based on vaginal and nipple morphology. The vagina remains sealed in both cheirogaleids except for estrous cycles and parturition. If vaginal openings were observed, vaginal smears were taken to determine proestrous, estrous or metestrous conditions following published protocols (Blanco, 2008; Wrogemann and Zimmermann, 2001). Later stages of pregnancy could be detected by careful palpation and by assessing patterns of weight gain.

Samples

I include in the analysis data from 45 *Cheirogaleus*, 18 of which (4 juveniles, 2 subadults, 12 adults) were *C. crossleyi* captured in the continuous forest, 8 of which (5 infants, 3 adults) were *C. crossleyi* captured in the forest fragment, and 19 of which (4 juveniles, 2 subadults, 13 adults) were *C. sibreei* captured in the forest fragment. No *C. sibreei* was captured in the fragment in late March-April and no trapping was conducted in the continuous forest at that time. To compare infants, juveniles, subadults, and adults of the same species, *C. crossleyi* infants captured in the fragment in April 2009, prior to hibernation, were pooled with juveniles, subadults and adults of the same species captured in the continuous forest during previous years. Because two different populations of *C. crossleyi* are combined in the analysis, I first compared the three adult *C. crossleyi* individuals from the fragment (captured late in October 2008) to the 13 adult *C. crossleyi* from the continuous forest, to check for interpopulation differences.

Age determination in dwarf lemurs was possible for infants (~3-4 months old), juveniles (~1 year-old) and subadults (~2 years-old). Infants had deciduous dentition (at least deciduous third and fourth premolars –dp3s and dp4s) and were trapped in March-

April, prior to their first hibernation period. Juveniles were recognized because they retained deciduous dentition (dp3s, dp4s or both) when they were captured at the beginning of the reproductive season (October-November), after they came out of hibernation. However, they showed complete dental replacement before undergoing their second hibernation period beginning the following March-April. Subadult individuals could be accurately classified as such when they had been trapped during the previous year as juveniles. These individuals showed little dental wear and no sign of sexual activity (lack of nipple development in females, testes development in males). Therefore, I considered as subadults all females who showed no sign of prior pregnancy, sexual activity, or dental wear, but who displayed permanent dentition. For body mass comparisons, only individuals captured around the same time of year (Oct-Nov) were analyzed unless otherwise indicated.

More than 130 individual mouse lemurs were captured at Ranomafana over four reproductive seasons, but I only considered a subset of trapped individuals in this analysis, 13 for morphometric tests (6 juveniles and 7 adults) and 13 for body mass comparisons (5 juveniles and 8 adults). My rationale for the selection was to choose only individuals whose measurements were collected by me (to eliminate inter-individual measurement error) and females for which captures were made around the time of estrus (early October). This enabled me to compare body masses in a consistent manner.

For mouse lemurs, age determination was complicated by the fact that all individuals captured at the beginning of the reproductive season had their permanent dentition and were sexually mature (females underwent estrus and males had visible testes). However, 1-year-old females could be identified on the basis of their lower body

mass at the time of estrus and the date of their first appearance in the trapping area⁴. Museum data confirmed that year old mouse lemurs are not fully grown (nor are all of their postcranial epiphyses fully fused). Mouse lemur individuals were classified as adults if they were ~2 years old or older (which could be verified if they had been captured in at least two different years). The ages of the individuals included in this study have been confirmed by independent methods for aging mouse lemurs through dental wear analysis tested in an extensive sample at Ranomafana (Zohdy et al., 2009). Independent sample T-tests and Mann-Whitney U tests were used for morphometric and body mass comparisons of age categories. All analyses were performed in SPSS 15.0.

Comparative samples

Museum specimens and captive data were added to this study to compare dental and growth parameters between cheirogaleids and similarly-sized prosimians. Dental developmental schedules of cheirogaleids and bushbabies were recorded by Laurie Godfrey and others after inspection of live-trapped specimens in museum collections (Blanco et al., in prep.). Early growth records for captive *C. medius* and *G. moholi* were also available from the Duke Lemur Center. Table 1 shows the specimens selected for this analysis. For wild immature cheirogaleids, ages could be inferred under the assumption of reproductive synchrony, i.e., by calculating the differences between field capture dates and birth dates predicted on the basis of field observations for each species (see Godfrey et al., 2001). For bushbabies, ages were inferred by plotting body masses

⁴ The same trapping locations have been used since 2004 at Ranomafana to maximize recaptures. Female mouse lemurs are trapped in the same areas over the years (unlike some males who may emigrate or immigrate). After a few months of intense trapping

from museum records onto growth curves published in the literature or generated for Duke Lemur Center individuals using Origin software (Blanco et al., in prep.).

Results

Patterns and timing of growth in *Cheirogaleus*, Tsinjoarivo fragment vs. continuous forest

The three adult *C. crossleyi* individuals captured in the fragment were slightly bigger than the 13 adult *C. crossleyi* trapped in the continuous forest in most of the morphometric variables, although significant differences were found only for tail length (TL) and head width (HEW) (Table 2). Infants from the fragment had significantly wider heads and higher means for several morphometric variables than did juveniles from the continuous forest (Tables 3-6). These differences may derive from interpopulation variation; however, they may also derive from the fact that infants were measured shortly before the hibernation period, when they are most fat, and juveniles were measured just after hibernation, i.e., after the prolonged period of weight loss.

Unsurprisingly for *C. crossleyi* as well as *C. sibreei*, juveniles were significantly smaller than adults for most of the morphometric variables. They had attained, on average, ~90-91% of the adult body size by ~9-10 months of age. No significant differences were found between subadult and adult dwarf lemurs (Tables 3-6, Figures 1 and 2). With regard to body mass, infant dwarf lemurs were significantly heavier than juveniles, as would be expected for individuals captured soon before and after their first hibernation period (Tables 7-10, Figure 3). Weighing on average 70% of adult weight early in the reproductive season (~1-2 months after hibernation), juveniles of both dwarf

every year, all animals are marked, so we assume that “new” females captured at the

lemur species were significantly lighter than adults. Subadults did not differ in body mass from adults (Tables 7-10).

Growth in *Microcebus* at Ranomafana

A similar but relatively faster growth profile was found in mouse lemurs. Juvenile individuals were significantly smaller than adults but, on average, they reached ~94% of adult size at around 1 year of age (Table 11). Body mass, as measured in young females at the time of estrus, averaged ~83% that of adults (Table 12). Two-year old females were indistinguishable from older females but heavier than year-old females in their first reproductive season (Table 13).

The timing of reproduction in wild eastern cheirogaleids

Age at first reproduction differs in eastern rain forest mouse and dwarf lemurs. Juveniles (~1 year old) and subadults (~2 years old) *Cheirogaleus* showed no signs of sexual activity with two exceptions: one juvenile and one subadult female who displayed slightly swollen vaginas, but did not become pregnant. Juvenile and subadult males showed no visible testicular development, except for an individual, possibly a subadult, with very small testes.

Unlike dwarf lemurs, year-old mouse lemur females undergo normal estrus (as shown by vaginal smears) and generally do become pregnant during their first reproductive season. However, they experience more abnormal pregnancies (with low weight gain profiles) and abortions or perinatal death of the offspring than older females, as evidenced by observations of trapped females around the estimated time of parturition

beginning of the reproductive season represent the cohort born during the prior season.

(Table 14). Thus, even for mouse lemurs, reproduction is precarious for year-old individuals.

Comparisons to bushbabies (*Galago*)

Dental eruption schedules and early growth rates differ between cheirogaleids and bushbabies. Museum data reveal that infant dwarf lemurs erupt their deciduous dentition earlier than similarly-sized bushbabies (Figure 4) – i.e., within the first few weeks of life. Within cheirogaleids, however, eruption schedules begin to differ after ~ 3 months: whereas mouse lemurs display complete permanent dentition by 4 months, dwarf lemurs retain their deciduous premolars (e.g., dp4s and/or dp3s) throughout and slightly beyond their first hibernation period (Figure 4). Museum data were corroborated by field data collected in this study (Figure 5), as all young *Cheirogaleus* captured shortly after they emerged from hibernation (at ~10-12 months old) were dentally immature. In contrast, all captured mouse lemurs at Ranomafana, including those that were apparent members of the first year cohort (and captured at ~1 year of age), possessed their full permanent dentitions.

Growth curves calculated on the basis of body mass records for two captive individuals indicate faster rates in *C. medius* compared to a similarly-sized bushbaby (*G. moholi*)⁵ for the first 100 days of life. At 60 days, the fat-tailed dwarf lemur weighs almost 200 g while the bushbaby weighs half that. This amounts to more than two thirds of the adult weight in *C. medius*, and half the adult weight in *G. moholi*. At 100 days, *C. medius* surpasses normal (wild) adult body weight, while same-aged *G. moholi* barely

exceeds 60% of adult mass (Figure 6). No growth data are available for wild bushbabies; however, much younger dwarf lemurs that I captured in the Tsinjoarivo forest fragment in April (at ~4 months old) weighed on average 258g, which is about 90% of average adult body mass of individuals captured after hibernation (Table 7). This suggests more rapid early growth and development in cheirogaleids than in like-sized bushbabies.

Discussion

Field data on growth, development and reproduction in cheirogaleids, including this study, deviate from those collected under captive conditions. Foerg (1982) reported extended high growth rates for juvenile dwarf lemurs who reached adult body mass at ~ 4 months old. Müller, in her study of *C. medius* at Ampijoroa (1999a, b), and Fietz, in her study of the same species at Kirindy (1999b,c), noted differences in the growth profiles of wild and captive dwarf lemurs. *Cheirogaleus* under laboratory conditions were reported to be ~100% heavier than wild individuals of comparable age, and infants in captivity reached adult mass within the first 15 weeks after birth, i.e., much earlier than wild *Cheirogaleus* at Tsinjoarivo; as we have seen, the latter do not achieve adult size until they are 2 or 3 years old, despite their rapid infant growth rates. This difference in the growth trajectories of captive and wild *Cheirogaleus* is not surprising as captive individuals do not undergo natural hibernation, which would markedly reduce metabolic rates (Dausmann et al., 2000, 2009) compromising overall growth and development.

Differences in growth were also observed between wild and captive mouse lemurs. Perret (1992) showed that gray mouse lemur infants grew from 5-6 g at birth to

⁵ Adult female body mass for *C. medius* at Ampijoroa: 141-220g (Müller, 1999), in captivity about 100g heavier (Foerg, 1982); adult female body mass for *G. moholi* in

~30-40 g in less than 2 months in captivity. By 3 months, they showed full permanent dentition and adult body size. Radespiel and Zimmermann (2003) reported that 4-5 months old gray mouse lemurs could not be clearly distinguished from adult individuals. Wild-caught individuals from Ranomafana reveal a different pattern: ~1 year-old individuals are smaller than adults in body mass and linear measurements. However, they have progressed further along their growth trajectories to adult body size than have year-old dwarf lemurs.

Reproductive schedules also differ between wild and captive individuals, particularly belonging to the genus *Cheirogaleus*. Müller (1999a) speculated that the unusually fast dwarf lemur growth rates seen in the laboratory (i.e., individuals attaining adult body size during the first year of life) may be correlated with early sexual maturation (~ 1 year) of *Cheirogaleus* in captivity. According to Müller (1999a), juvenile *Cheirogaleus* at Ampijoroa were smaller than adults and individuals showed no signs of sexual activity until they were 2 years old. Indeed, some females delayed reproduction until they were ~3 years old (Müller, 1999a). My data for eastern dwarf lemurs at Tsingoarivo show a similar pattern. Juvenile and subadult individuals showed no signs of pregnancy or lactation. Lack of testicular development in juvenile males and presence of non-gestating juvenile females is suggestive of reproductive retardation that may occur as a result of hibernation. A similar phenomenon has been described in golden-mantled ground squirrels which restrict gonadal growth to periods of arousals and normothermic states (Barnes et al., 1986). Studies have also showed that reproduction may not occur until a critical mass is attained, as it was observed in Columbian ground squirrel females

captivity: 150-252g (Zimmermann, 1989).

whose body condition was one of the main factors in determining early age at reproduction (Neuhaus et al., 2004).

The expression of polyestry (multiple estrous cycles within a season) also seems to vary between the wild and captivity. In the laboratory, female dwarf lemurs show post-partum estrus and up to four estrous cycles in any given reproductive season (Foerg, 1982). Although data are lacking for wild *Cheirogaleus*, the narrow window for mating, gestation, lactation and fattening prior to hibernation (~5 months) may render polyestry a very unlikely phenomenon. Later than normal gestations may increase the risk of offspring mortality in years when food resources may be scarce at the time of highest energetic demands for infant growth and development. In fact, Müller and Thalmann (2002) reported that smaller-bodied infants remain usually active several weeks after adults have entered the period of inactivity possibly in order to gain sufficient body reserves. Results from this study support this inference as the majority of dwarf lemurs (5 out of 6) captured during the month of April at Tsinjoarivo were infants.

Unlike *Cheirogaleus*, both captive and wild mouse lemurs are sexually mature during their first breeding season. Year-old mouse lemurs captured at Ranomafana were sexually mature, although the relatively lower body mass of young females at Ranomafana may affect their ability to carry out successful pregnancies and/or to support lactation (Chapter II, this dissertation). More data are necessary to test this hypothesis. In addition to having an early age at first reproduction, mouse lemurs do express polyestry in the wild. This assertion is supported by indirect evidence at Ranomafana (Blanco, 2008; Chapter II this dissertation) and direct evidence at other study sites (Chapter IV, this dissertation; Lahann et al., 2006; Schmelting et al., 2000). Mouse lemurs occupy a

variety of habitats throughout Madagascar, including dry deciduous, spiny and rain forests. The number of litters per season appears to be correlated with the degree of seasonality (Lahann et al., 2006) and other environmental factors such as predictability of rainfall, which in turn may affect the expression of torpor or hibernation (Chapter II, this dissertation).

Data on cheirogaleids and similarly-sized bushbabies, who do not experience torpor or hibernation, show early ontogenetic differences in dental schedules and growth trajectories. Young dwarf lemurs begin to erupt the deciduous toothcomb, lower second premolars and upper canines during their first week of life (Eaglen, 1985). Infants had their first molar erupting by day 26, whereas similarly-sized bushbabies experience gingival eruption of the upper deciduous incisors by the second week and gingival eruption of the first molars at 36 days (Holly Smith et al., 1994). By two months of age, however, bushbabies (e.g. *G. senegalensis*) have caught up with dental schedules and in fact, they look more “advanced” than similarly-sized dwarf lemurs (*C. crossleyi*) (Figure 4). Dental analysis presented in this study is consistent with the argument that hibernation affects dental development in *Cheirogaleus*. Retardation of dental crown formation and the age at eruption of incisors has been demonstrated to occur in hibernating thirteen-lined ground squirrels (Sarnat and Hook, 1942).

Growth rates are higher in cheirogaleids than bushbabies during the first month of life. Although captive conditions can certainly alter the pattern of growth and development, I expect early rates to represent true biological differences, as infants suckle for at least one month prior to weaning.

Comparisons between cheirogaleids, particularly *Cheirogaleus* and bushbabies have shown differences in those life history parameters that would both maximize early infant independence (fast early growth rates, early dental development) and minimize the duration of the reproductive season (e.g. short gestation lengths). These strategies are consistent with the fact that dwarf lemurs are constrained by hibernation whereas bushbabies are not.

According to life history theory, cheirogaleids, a group of small-bodied primates, are expected to show relatively fast growth and development and to produce multiple litters of precocial infants. In some respects, mouse and dwarf lemurs fit this pattern. They have the shortest gestation lengths of any lemur (~60-70 days) and infants grow relatively faster than any similarly-sized prosimian. By 3-4 months of age both mouse and dwarf lemurs have all permanent molars. However, whereas 1-year-old mouse lemurs are sexually mature and have nearly attained full adult body size, dwarf lemurs cannot sustain these rapid growth rates due to the effects of hibernation on growth and development.

Indeed, it may be that the early rapid growth and development displayed by *Cheirogaleus* and *Microcebus* does not signal adaptations to life in the fast lane at all, but rather is part of the expected growth and developmental complex associated with torpor or hibernation, and ultimately with a relatively slow life history profile. Under this interpretation, rapid *early* growth and development (including a shortened gestation period) is necessitated by the need for infants to prepare for an extended season of scarce resources. If adult survival is variable (i.e. increase in adult mortality rates) in cheirogaleids, a classic life history strategy would favor early reproduction with multiple offspring per litters and multiple litters per season (polyestry). Whereas mouse lemurs can

rely on fallback and keystone food sources during the season of scarce resources and may indeed exhibit polyestry, dwarf lemurs are highly frugivorous and polyestry is not feasible.

These patterns of growth and reproduction have been found in other mammals. In a comparative study, hibernating female squirrels showed shorter gestation and lactation periods, and their infants developed and grew faster than their counterparts in non-hibernating species (Waterman, 1996). Despite early growth rates, juveniles were smaller and had lower body masses than adults, as they needed to switch from structural growth to fattening prior to their first hibernation period. This strategy has been documented in several mammalian species such as common hamsters (Lebl and Millesi, 2008) and European ground squirrels (Millesi et al., 1999) among others.

The counterpart of undergoing long periods of inactivity is the extension of the life span. Since the classic experiment on Turkish hamsters by Lyman et al. (1981) reporting that individuals who spent more time hibernating lived longer than controls, other examples have documented the correlation between hibernation and longevity (Wilkinson and South, 2002). Dwarf lemurs may exhibit prolonged life spans compared to similarly-sized species, but unfortunately no ages are available for wild populations and reports from captivity vary dramatically in range, with the oldest age for a fat-tailed dwarf lemur recorded at 22 years (John Allman, pers. comm.). Although the mechanisms responsible for longevity in animals that hibernate or employ torpor are only beginning to be understood, cheirogaleids make excellent subjects to explore the consequences that different energy-saving strategies may indeed have in the variation of primate life history parameters. Particularly intriguing is the fact that mouse lemurs can experience different

degrees of torpor or hibernation between species, populations of the same species and even sexes or individuals within the same populations. Although data are lacking, it can be hypothesized that patterns of growth and development and age-related changes over the life span in mouse lemurs should not differ to the same extent as dwarf lemurs do and several questions remain as to whether individual variation in energy saving strategies is translated into individual variation in longevity. Further studies are also necessary to link torpor/hibernation and polyestry and their relationship with life history parameters such as life span.

Unique lemur traits, including torpor and hibernation, have been linked to environmental unpredictability in Madagascar. Small-bodied cheirogaleids can be expected to live fast and die young in such environments, as it has been suggested for gray mouse lemurs (Dewar and Richard, 2007). However, as this study has showed, a more complex, biphasic pattern of growth and development, e.g., fast infant growth rates and dental eruption schedules, but delayed age at first reproduction, appears to characterize some cheirogaleids (e.g. genus *Cheirogaleus*). If habitat unpredictability favors the use of metabolic strategies such as hibernation to cope with harsh conditions and seasonality of resources, then growth, development and reproduction should be constrained by those same environmental limitations. These constraints favor the “speeding up” of early growth and development. On the other hand, the delayed age at first reproduction and prolonged life spans resulting from hibernation in dwarf lemurs are more characteristic of a primate living its life on the slow lane. Despite their small-sized bodies, cheirogaleids depart from the typical pattern “live fast, die young.” This variation cannot be accommodated within the framework of the classic fast-slow life history

continuum, and requires the consideration of additional factors, independent of body mass. Sibly and Brown (2007) found two “life history” axes of variation in a comparison of production rates (i.e. mass of neonate tissue produced per female per year) in a variety of mammalian species. This analysis would indicate that within size classes, certain groups live slower and die older (and have lower production rates) than expected based on their body masses (first axis). The second axis is represented by variation in “lifestyles”, i.e., suites of ecological, physiological and behavioral traits that directly affect birth and/or death rates. Whether energy saving strategies would represent a particular lifestyle or be correlated with an additional axis of life history variation requires further investigation; however, it can be hypothesized that life histories in small-bodied cheirogaleids are slowed down by the use of torpor and/or hibernation, unique metabolic strategies for a primate.

Conclusions

Mouse and dwarf lemurs are small-bodied nocturnal primates living in highly unpredictable environments. Under the classic life history model, cheirogaleids (as small-bodied species with high adult mortality due to predation) would be expected to live fast and die young. However, studies of non-primate mammals have shown that the use of energy-saving strategies such as torpor and hibernation, unusual for primates but manifested in the Cheirogaleidae, can affect a suite of life history traits including gestation length and age at first reproduction. In this chapter, I sought to explore the relationship between metabolism and life history traits in cheirogaleids. My working hypothesis was that animals experiencing torpor and hibernation are constrained in the degree to which they can experience life in the fast lane, and indeed may be expected to

display a biphasic life history strategy, with rapid early development interrupted by slow development (correlated with the onset of the hibernation). If this is the case, I would predict that those cheirogaleids with the most extended seasons of hibernation would deviate the most from the classic pattern of variation in life history, influenced by variation in adult mortality. Whereas the comparative data presented here are limited, they do present supporting evidence for the working hypothesis.

Life history in cheirogaleids, particularly in dwarf lemurs, is neither fast nor slow but a combination of both. On the one hand, female dwarf lemurs have short gestation and lactation periods and infants experience rapid early growth and dental development to wean early and achieve independence prior to the season of hibernation. On the other hand, dwarf lemurs delay their age at first reproduction and slow down juvenile and subadult growth to minimize energy expenditure during hibernation. Furthermore, dwarf lemurs appear to have longer life spans than similarly-sized, non-hibernating prosimians. This conforms to expectations, as, among non-primates, hibernation has been shown to correlate positively with longevity.

Microcebus, in turn, experience varying degrees of daily and/or seasonal torpor affecting species and sometimes sexes differently. It has been recently documented that mouse lemurs can even experience true hibernation like *Cheirogaleus* do, although they are not obligate hibernators and individuals within the same population can employ different metabolic strategies (Kobbe and Dausmann, 2009; Smith and Ganzhorn, 2009). The extent to which this variation affects life history characteristics of mouse lemurs, as compared to *Cheirogaleus*, is not known and requires further investigation.

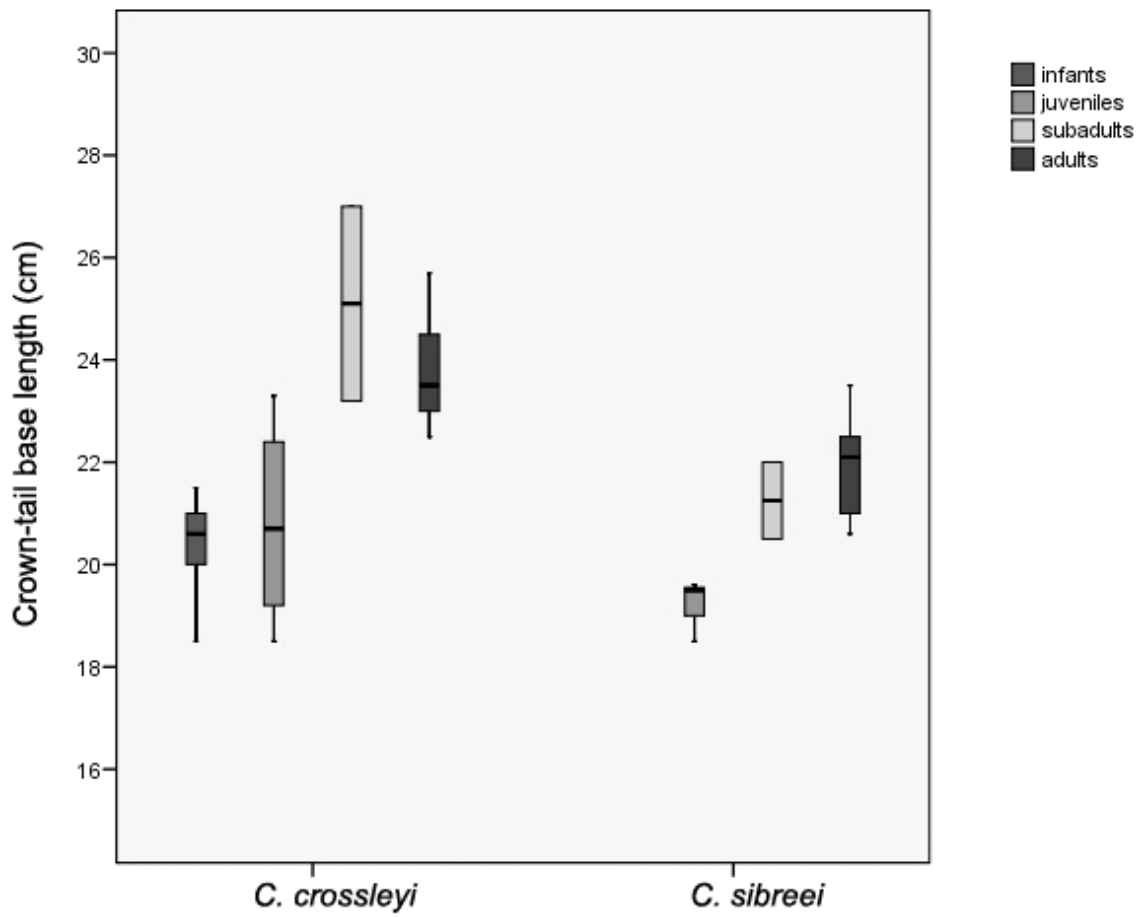


Figure V.1. Morphometric differences in crown to tail base length across age categories in two dwarf lemur species

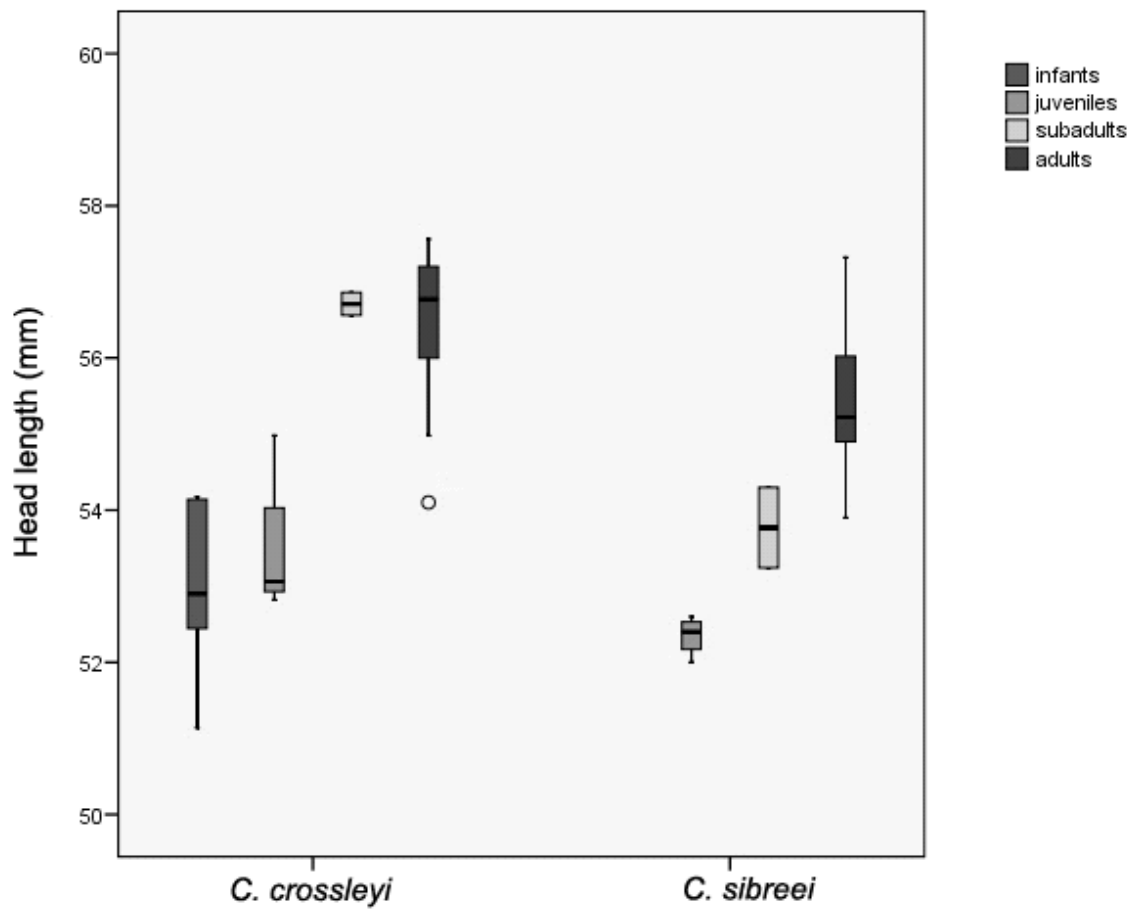


Figure V.2. Morphometric differences in head length across age categories in two dwarf lemur species

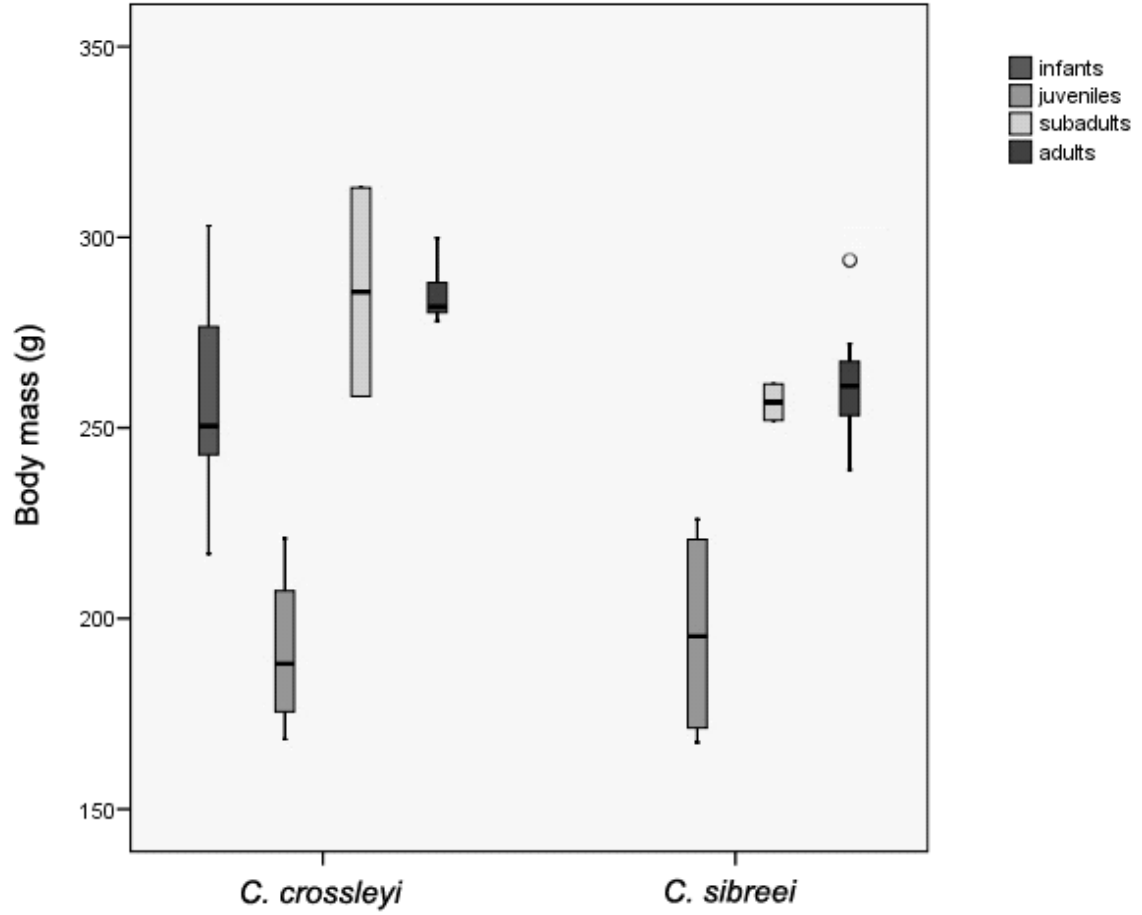


Figure V.3. Body mass differences across age categories in two dwarf lemur species

<i>M. griseorufus</i>				$\frac{I^1 I^2 C^1 P^2 P^3 P^4 M^1 M^2 M^3}{I_1 I_2 C_1 P_2 dp_3 P_4 M_1 M_2 M_3}$		
<i>M. rufus</i>			$\frac{I^1 I^2 dc^1 dp^2 dp^3 dp^4 M^1 M^2}{I_1 I_2 C_1 dp_2 dp_3 dp_4 M_1 M_2}$		$\frac{I^1 I^2 C^1 P^2 P^3 P^4 M^1 M^2 M^3}{I_1 I_2 C_1 P_2 P_3 P_4 M_1 M_2 M_3}$	
<i>C. crossleyi</i>				$\frac{I^1 I^2 C^1 P^2 dp^3 dp^4 M^1 M^2 M^3}{I_1 I_2 C_1 P_2 dp_3 dp_4 M_1 M_2 M_3}$		$\frac{I^1 I^2 C^1 P^2 dp^3 P^4 M^1 M^2 M^3}{I_1 I_2 C_1 P_2 dp_3 P_4 M_1 M_2 M_3}$
<i>C. medius</i>	$\frac{di^1 di^2 dc^1 dp^2 dp^3 dp^4}{di_1 di_2 dc_1 dp_2 dp_3 dp_4}$					
<i>G. moholi</i>	di^1					
<i>G. senegalensis</i>		$\frac{di^1 di^2 dc^1 dp^2 dp^3 dp^4 M^1 M^2}{I_1 I_2 C_1 dp_2 dp_3 dp_4 M_1}$	$\frac{I^1 I^2 C^1 P^2 dp^3 dp^4 M^1 M^2 M^3}{I_1 I_2 C_1 P_2 dp_3 dp_4 M_1 M_2 M_3}$			
	0.3-0.5 months	1.4 months	2 months	3 months	4 months	11 months

Figure V.4. Dental schedules of cheirogaleids (*Microcebus* and *Cheirogaleus*) and bushbabies (*Galago*) who do not experience torpor or hibernation. Data are based on observations from museum specimens

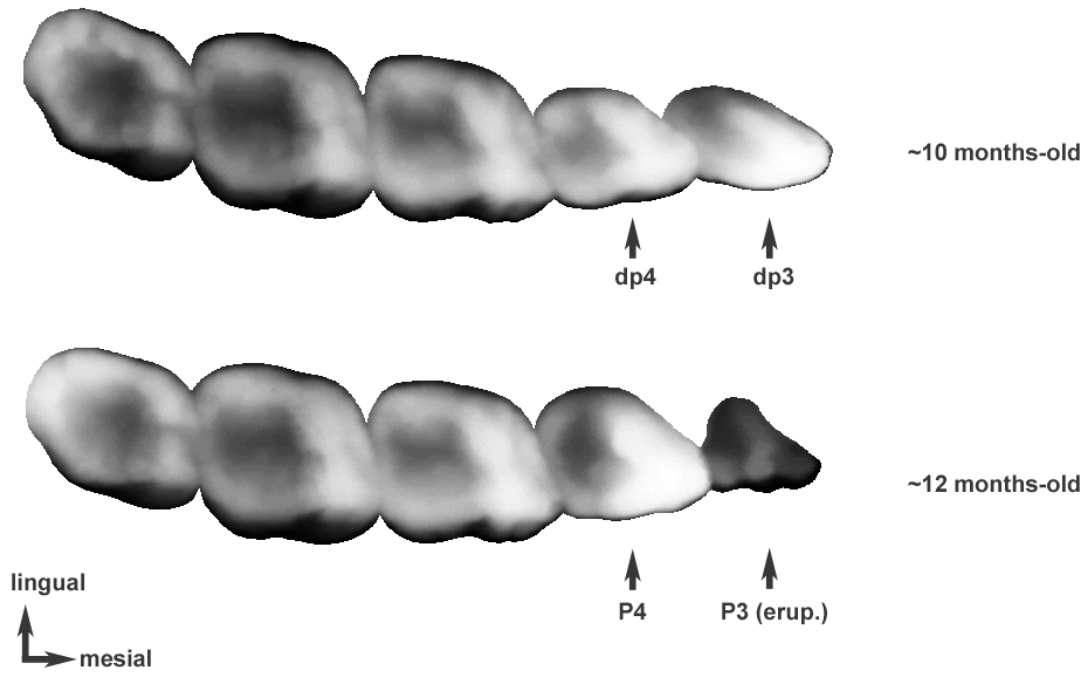


Figure V.5. Scans of lower right dentitions of a young *Cheirogaleus* trapped in early November and early January, showing replacement of deciduous premolars

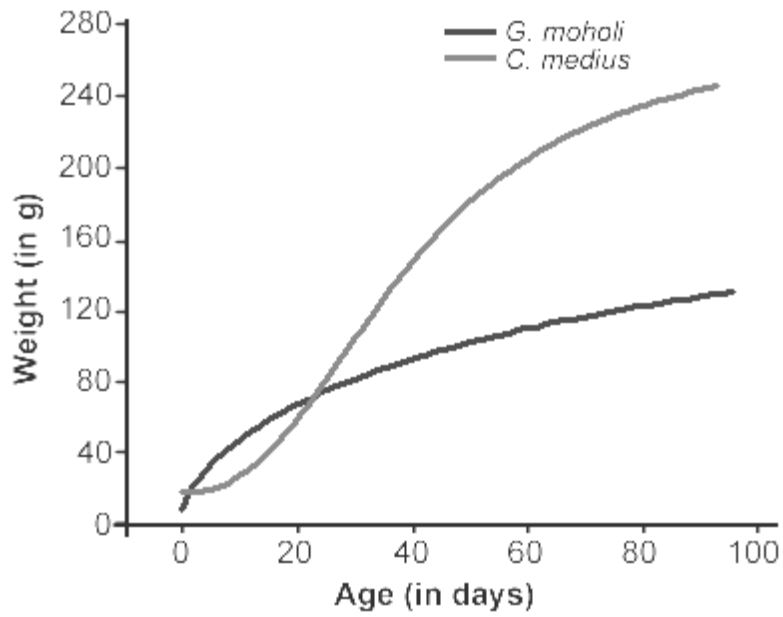


Figure V.6. Early growth rates calculated for a captive dwarf lemur and a similarly-sized bushbaby

Table V.1. Museum samples of immature cheirogaleids and bushbabies included in dental comparative analysis

Catalog N	Species	Locality	Sex	Capture date	Age	Notes
MCZ44847	<i>Microcebus rufus</i>	Antsihanaka	female	April, 1928	4 months	assuming birth synchrony
ZMB71427	<i>Microcebus rufus</i>	unknown	unknown	March 2, 1881	2 months	assuming birth synchrony
FMNH1567a,b	<i>Microcebus griseorufus</i>	Beza Mahafaly	unknown	Feb. 20, 1991	<3 months	assuming birth synchrony
FMNH 5656	<i>Cheirogaleus crossleyi</i>	Fandriana, Fianarantsoa Prov.	male	March 6, 1896	~3 months	assuming birth synchrony
MNHN 1967.1653	<i>Cheirogaleus crossleyi</i>	Andasibe	male	Nov. 27, 1962	~11 months	assuming birth synchrony
UMAPC 189	<i>Cheirogaleus medius</i>	captive	male		16 days	known age
SUNY-DPC3176M	<i>Galago moholi</i>	captive	unknown		10 days	known age
USNM37805	<i>Galago senegalensis</i>	Casamanca, Senegal	male	Feb. 4, 1966	~63 days	according to growth curves
USNM376224	<i>Galago senegalensis</i>	Sinesaloum, Senegal	male	Nov. 22, 1965	~41 days	according to growth curves

Table V.2. Morphometric comparisons and Mann-Whitney U tests of significant differences between adult *C. crossleyi* individuals from the continuous forest (CF) and forest fragment (FF)

	C-TB	TL	HL	HFL	FL	FFL	HEL	HEW	SNL	IPD	EL
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
CF n=13	23.8 ± 1	25.3 ± 1	15.6 ± 0.4	49.2 ± 1.7	10.2 ± 0.6	33.1 ± 1.8	56.5 ± 1	33.5 ± 1	17 ± 0.9	22.5 ± 0.7	22 ± 1.5
FF n=3	24 ± 1.3	27.2 ± 0.2	15.3 ± 0.8	50.3 ± 2.2	10.2 ± 0.6	34 ± 0.3	57.4 ± 1.3	36.1 ± 0.8	16.7 ± 0.4	23.5 ± 1.7	22.9 ± 0.9
p	NS	**	NS	NS	NS	NS	NS	**	NS	NS	NS

Mann-Whitney U tests: ** $p \leq 0.01$; NS=non-significant. Measurements from left to right: taken with flexible tape, Crown to Tail base (cm), dorsally, from the forehead to the cranial margin of the first caudal vertebra; Tail length (cm), ventrally, from the base of the anus to the distal end of the last caudal vertebra; Hind-limb length (cm), from the femoral head to the distal end of the 4th digit, measured with the limb and foot extended; taken with calipers, Hindfoot length (mm), from the calcaneal heel to the distal end of the 4th digit, measured with foot extended, Forelimb length (cm), from the humeral head to the distal end of the 4th digit measured with limb and hand extended, Forefoot length (mm), from distal end of palmar pad to 4th digit measured with hand extended, Head length (mm), maximum length of the head from the most projecting margin of the rhinarium to the back of the braincase; Head width (mm), maximum bizygomatic breadth; Snout length (mm), maximum length from the most projecting margin of the rhinarium to a virtual line projected transversely across the base of the orbits; Interpupillary distance (mm); Ear length (mm), maximum length between the base of the tragus and the tip of the pinna.

Table V.3. Morphometric comparisons between infant, juvenile, subadult and adult dwarf lemurs of *C. crossleyi* (*C.c.*) at Tsinjoarivo. Same variables as Table V-2

<i>C. c.</i>	C-TB	TL	HL	HFL	FL	FFL	HEL	HEW	SNL	IPD	EL
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Inf. n=5	20.3 ± 1.2	22.8 ± 1.1	16 ± 1.1	48.5 ± 2.1	9.4 ± 0.6	32.9 ± 0.9	53 ± 1.3	32.7 ± 0.8	17 ± 0.7	22.8 ± 1	22 ± 1.4
Juv. n=4	20.8 ± 2.1	22.5 ± 1.1	14.2 ± 0.6	46.7 ± 4	9.1 ± 0.6	31 ± 2.6	53.5 ± 1	30.7 ± 1	15.3 ± 1.4	20.7 ± 1	19.6 ± 1.9
Sub. n=2	25.1 ± 2.7	26.2 ± 1.1	15.5 ± 0.7	52.1 ± 2.1	10.3 ± 0.7	33 ± 0.2	56.7 ± 0.2	33.2 ± 0.9	17.6 ± 2.2	22.1 ± 0.3	21.6 ± 1.3
Ad. n=12	23.8 ± 1	25.2 ± 1	15.7 ± 0.3	49.2 ± 1.7	10.2 ± 0.6	33.3 ± 1.7	56.5 ± 1	33.3 ± 0.9	17.1 ± 0.9	22.5 ± 0.7	21.9 ± 1.4
% ¹	87.5	89.1	90.7	94.9	88.9	93.0	94.7	92.3	90	92.2	89.6

¹percentage of adult size attained by juveniles at ~10-12 months old

Table V.4. Morphometric comparisons between juvenile, subadult and adult dwarf lemurs of *C. sibreei* (*C.s.*) at Tsinjoarivo. Same variables as Table V-2

<i>C. s.</i>	C-TB	TL	HL	HFL	FL	FFL	HEL	HEW	SNL	IPD	EL
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Juv. n=4	19.3 ± 0.5	20.5 ± 1.3	13.6 ± 0.3	45.4 ± 1.8	8.9 ± 0.6	31.7 ± 1.2	52.3 ± 0.3	30.4 ± 0.8	14.4 ± 1.6	21.4 ± 0.2	20.4 ± 1.3
Sub. n=2	21.2 ± 1.1	24.1 ± 0.8	15.5 ± 1.4	50.1 ± 2.5	8.8 ± 0.1	32 ± 0.3	53.8 ± 0.7	33 ± 0.3	14.4 ± 0.9	20 ± 2.4	19.7 ± 0.9
Ad. n=13	22 ± 0.9	23.7 ± 1.2	15.1 ± 1.2	46.9 ± 1.4	9.4 ± 0.6	32.2 ± 1	55.4 ± 1	33.6 ± 1.8	15.6 ± 1	22.3 ± 0.9	23.3 ± 0.9
% ¹	87.8	86.8	89.8	96.8	94.8	98.6	94.5	90.4	92.5	95.9	87.7

¹percentage of adult size attained by juveniles at ~10-12 months old

Table V.5. Mann-Whitney U tests of significant differences between age categories in *C. crossleyi* individuals. Morphometric variables described in Table V-2

<i>C. crossleyi</i>	I - J	I - S	I - A	J - S	J - A	S - A
C-TB	NS	NS	**	NS	*	NS
TL	NS	NS	**	NS	**	NS
HL	*	NS	*	NS	**	NS
HFL	NS	NS	NS	NS	NS	NS
FL	NS	NS	*	NS	**	NS
FFL	NS	NS	NS	NS	NS	NS
HEL	NS	NS	**	NS	**	NS
HEW	*	NS	NS	NS	**	NS
SNL	*	NS	NS	NS	NS	NS
IPD	*	NS	NS	NS	*	NS
EL	NS	NS	NS	NS	*	NS

I=infants, J=juveniles, S=subadults, A=adults. * $p \leq 0.05$, ** $p \leq 0.01$; NS=non-significant

Table V.6. Mann-Whitney U tests of significant differences between age categories in *C. sibreei* individuals. Morphometric variables described in Table V-2

<i>C. sibreei</i>	J - S	J - A	S - A
C-TB	NS	**	NS
TL	NS	**	NS
HL	NS	*	NS
HFL	NS	NS	NS
FL	NS	NS	NS
FFL	NS	NS	NS
HEL	NS	**	NS
HEW	NS	**	NS
SNL	NS	NS	NS
IPD	NS	*	NS
EL	NS	*	*

J=juveniles, S=subadults, A=adults. * $p \leq 0.05$, ** $p \leq 0.01$; NS=non-significant

Table V.7. Body mass (g) comparisons between infants (before first hibernation at 3-4 months old) and juvenile, subadult and adult individuals (soon after hibernation) of *C. crossleyi*

<i>C. crossleyi</i>	$\bar{x} \pm SD$
Infants (n=5)	258 ± 32.9
Juveniles (n=4)	191.4 ± 22.27
Subadults (n=2) ¹	285.63 ± 38.71
Adults (n=7)	285.2 ± 8.1
% ²	67.11

¹one of the subadults was captured in early January 08 and was ~50g heavier than the one captured mid-November 07; ²percentage of adult size attained by juveniles at ~10-12 months old

Table V.8. Body mass (g) comparisons between juvenile, subadult and adult individuals (soon after hibernation) of *C. sibreei*

<i>C. sibreei</i>	$\bar{x} \pm SD$
Juveniles (n=4)	196.04 ± 29.02
Subadults (n=2) ¹	256.75 ± 6.72
Adults (n=7)	262.18 ± 17.53
% ²	74.77

¹one of the subadults was captured in early January 08 and was ~10g heavier than the other one captured late in October 08; ²percentage of adult size attained by juveniles at ~10-12 months old

Table V.9. Mann-Whitney U tests of significant differences between age categories in *C. crossleyi*

<i>C. crossleyi</i>	Juveniles	Subadults	Adults
Infants	*	NS	NS
Juveniles		NS	**
Subadults			NS

* $p \leq 0.05$, ** $p \leq 0.01$; NS= non-significant

Table V.10. Mann-Whitney U tests of significant differences between age categories in *C. sibreei*

<i>C. sibreei</i>	Subadults	Adults
Juveniles	NS	**
Subadults		NS

** $p \leq 0.01$; NS= non-significant

Table V.11. Morphometric comparisons between juvenile (~1 year old) and adult (± 2 years old) mouse lemurs. For descriptions of measurements see Table V-2.

<i>M. rufus</i>	TL (cm)	HL (cm)	HEL (mm)	HEW (mm)	SNL (mm)
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Juveniles (n=6)	109.9 \pm 4.9	79.8 \pm 6.4	33.7 \pm 1	20 \pm 1.1	9.1 \pm 1.5
Adults (n=7)	118.2 \pm 8	83.4 \pm 3.6	35.3 \pm 0.8	21.9 \pm 1.1	9.6 \pm 0.7
<i>p</i>	*	NS	**	*	NS
% ¹	92.9	95.7	95.4	91.2	94.2

Mann-Whitney U tests: * $p \leq 0.05$, ** $p \leq 0.01$; NS= non-significant; ¹percentage of adult size attained by juveniles at ~1 year old

Table V.12. Body mass (g) of juvenile females (~1 year old) and adult mouse lemurs captured at Ranomafana

<i>M. rufus</i>	$\bar{x} \pm SD$
Juveniles (n=5)	35.8 \pm 2.2
Adults (n=8)	43.31 \pm 4.8
<i>p</i>	**
% ¹	82.66

Mann-Whitney U tests: ** $p \leq 0.01$; ¹percentage of adult size attained by juveniles at ~1 year old

Table V.13. Female body mass (g) around the time of estrus over 4 reproductive seasons.

	2005	2006	2007	2008
“A”	53	54		
“C”	41	41		
“CP”			33	44.5
“I”	42	44.5		
“J”	45	41.5	42	50.5
“Ke”		46	44.5	
“P”		35	42.67	41
“S”		37.5	42.5	
“V”	38.5	45.5		

Bold numbers represent body mass of females estimated to be 1 year old at the time of capture

Table V.14. Reproductive success of juvenile (~1 year old) and adult female mouse lemurs estimated on the basis of body mass profiles and vaginal and nipple morphology during the reproductive season

	Reproductive observations		
	Failure*	Success	Total
Juvenile	4	4	8
Adult	0	11	11
Total	4	15	19

Pearson Chi-Square: 6.97; df=1; $p=0.008$

*Unsuccessful reproduction was characterized by low weight gain during pregnancy, signs of abortion or perinatal death of offspring and/or renewed vaginal swelling

CHAPTER VI

CONCLUSIONS

This dissertation provides detailed observations of the reproductive biology of brown mouse lemurs females. A simple question – i.e., whether factors other than photoperiod affect the timing of estrus (or whether females in the wild begin reproduction around the same time each year) – motivated my initial data collection. Most of previous studies on mouse lemur reproduction in the field had primarily focused on the beginning of the mating period (i.e. the first “reproductive wave”) and little was known about later reproductive events or whether females expressed polyestry. My broader goal was to determine how environmental variation affects reproduction in mouse lemurs – a question that cannot be answered in the laboratory. It turned out that this is a complex question that can be addressed only minimally by studying mouse lemurs in a small number of field sites.

In Chapter II, I addressed questions about female reproduction in wild mouse lemurs and reported intra-population variation in the time of estrus at Ranomafana; yet, data collected over multiple reproductive seasons showed that individual females underwent the first seasonal estrus with a periodicity of ~365 days, which was consistent with an endogenous regulation and entrainment by photoperiod. Although the number of litters per season – monoestry or polyestry – was difficult to document at Ranomafana, I inferred, based on indirect evidence, that some females would likely experience a second estrus ~30 days after successful gestation (i.e. around weaning), or sooner, ~5 days after reproductive failure, such as a late abortion or perinatal death of offspring. Chapter III focused on male reproduction and variation in testis size. I showed that some males

regress testicular tissue earlier than others and that, as a consequence, reproductive success among males may vary greatly late in the season. I hypothesized that variation in testis regression may be actually favored in unpredictable environments, where a trade-off may exist between the number of potential estrous females that could be found late in the season and the energetic constraints of maintaining reproductive capability at the beginning of the season of scarce resources. Non-reproductive factors, such as the need to acquire a fully competent immune system may be also related to the timing of testicular regression, but data are lacking to support this hypothesis. In Chapter IV, I presented data from mouse lemurs inhabiting fragmented and continuous forests at Tsinjoarivo, another eastern rainforest. I hypothesized that Tsinjoarivo may be a “marginal” or “extreme” habitat for mouse lemurs in that it is high altitude, or in that forest fragmentation is proceeding at a rapid rate. I also hypothesized that, within Tsinjoarivo, the fragmentary forests would have fewer mouse lemurs than the continuous forest. However, this is to my knowledge the first field study of mouse lemurs at high altitude forests, so it was impossible to know *a priori* to what extent mouse lemurs might find the environment challenging. Furthermore, high population density may not signal a healthy population, if individuals are simply squeezed into a space that cannot in the long term accommodate them. Higher percentages of female reproductive failure were found at Tsinjoarivo compared to Ranomafana and I documented the expression of polyestry (rebound polyestry at a minimum) in at least one female in the continuous forest site and opportunistic fattening in >60% (6 out of 9) of adult mouse lemurs trapped in the forest fragment at the beginning of the dry season. Assessment of long term viability of mouse lemur populations in highly degraded habitats (e.g. small forest fragments) would require

further monitoring, as these preliminary results showed that mouse lemurs in the surveyed fragment were heavier and that they were recaptured more often than those trapped in the continuous forest site. These results were contrary to expectations, given previously published reports for gray mouse lemurs in secondary western forests. Finally, in Chapter V, I broadened the analysis to include the other member of the family Cheirogaleidae which employs hibernation, the dwarf lemur (genus *Cheirogaleus*). I compared patterns of growth, dental development and reproduction in mouse and dwarf lemurs to other similarly-sized prosimians. I discussed how the use of metabolic strategies unusual for a primate may have shaped unique life histories in cheirogaleids, which do not simply fit the classic pattern of living fast–dying young that generally characterizes other small-bodied mammals.

Despite the primary role that endogenous regulation appears to play in cheirogaleid reproduction (at least shown in this dissertation for mouse lemurs), a deep understanding of the reproductive biology and flexibility of the group demands a consideration of habitat and climate conditions where cheirogaleids live and most importantly, a documentation of the use of metabolic strategies. This approach has not been fully explored in the literature. Oftentimes, the socioecological model has been applied to characterize cheirogaleid social and mating systems: in very simplistic terms, this model states that due to their differences in reproductive investment, sexes arrange differently in space: females group themselves according to the distribution of high quality food sources and predation risks (effectively competing for access to food), whereas males, because they have higher reproductive potential (and greater reproductive variance than females) will compete for access to females. Male mating strategies will

therefore depend on the females' distribution (Eberle and Kappeler, 2002). Within this context, it has been proposed that mouse lemur males who are the most active at the end of the dry season will be better able to find estrous females (Eberle and Kappeler, 2002). It has also been proposed that the lack of complete estrous synchrony that is found in mouse lemurs and some other cheirogaleids effectively allows for potential male monopolization (Génin, 2008) and that emerging early from seasonal torpor to forage allows male dwarf lemurs to establish territories before the reproductive season begins (Müller, 1999a) among others behaviors.

It can be argued that metabolic and reproductive strategies should be tightly connected as aspects of reproduction such as lactation are energetically-expensive and reproduction must rely on either previously stored or readily available resources. This should be particularly true of organisms living under seasonal and unpredictable environmental conditions. It is essential for an organism to be able to monitor internal and external energy availability as well as to be able to modulate behaviors (e.g., by increasing or decreasing time spent foraging; by engaging in or suppressing reproduction) to ensure survival (Schneider, 2004). Yet, studies on energy-saving strategies in mouse lemurs are usually linked to social systems (Génin, 2007), and usually fail to assess possible constraints imposed by the physiology of reproduction.

The connection between reproductive physiology and metabolic strategies may be particularly relevant in cheirogaleids, which are the only primates to use heterothermy (as expressed by use of torpor or hibernation) to cope with Madagascar's environmental unpredictability. Perhaps socioecological models borrowed from other, more socially complex primates may not have high explanatory power when applied to small-bodied

lemurs. This is not to disregard the importance of social behaviors; for example, it appears that parental care from both the male and female is necessary for survival of dwarf lemur infants (Müller and Thalmann, 2002). Also, social complexity appears to benefit infant survival despite reducing the number of offspring per capita per year in other small mammals like sciurids (Blumstein and Armitage, 1998).

When metabolic and reproductive strategies are considered, then alternative explanations can be given to the same behaviors mentioned above: mouse lemur males may have to remain active at the end of the dry season not because they are competing for access to the best females, but because they did not store enough fat to sustain seasonal torpor; mouse lemur females will differ in the day of estrus because there is individual variation in the regulation of circannual rhythms (perhaps with a relatively high heritability component); and dwarf lemur males may have to come out of hibernation earlier than females simply because spermatogenesis takes longer than ovulation does and it cannot begin during hibernation.

My field work on cheirogaleids in Madagascar has opened up a vast number of intriguing new questions. For instance, is there a relationship between habitat unpredictability, use of torpor and polyestry in cheirogaleids? Habitat seasonality and unpredictability have been linked to the duration of mouse lemur reproductive seasons and to the differential use by mouse lemurs of metabolic strategies such as torpor (Génin, 2008). Génin (2008) described a suite of features of reddish-gray mouse lemurs (*M. griseorufus*) at Berenty, which he considered to be the most unpredictable habitat in Madagascar. These included high population turnover from year to year, opportunistic fattening, the use of daily torpor during any season, and the use of fallback foods such as

gums. He also reported at least one female with renewed estrus after loss of offspring (i.e. rebound polyestry). Seasonal torpor appears to be favored in habitats with relatively low temperatures (greater differences between temperature maxima and minima will increase energy savings from torpor) and it is correlated with opportunistic fattening during the early dry season (torpor as preparation for hibernation hypothesis). In other words, those individuals who are able to fatten at the beginning of the dry season will be more likely to undergo seasonal torpor (Schülke and Ostner, 2007), simply because hibernation cannot occur without prior accumulation of fat deposits. Obligate hibernation has only been documented in the related genus *Cheirogaleus*, the dwarf lemurs, which remain inactive between ~6-8 months a year. True hibernation, generally defined as extended bouts of torpor (more than 24hs), with highly reduced metabolic rates and body temperature maintained within few degrees above ambient temperatures (Carey et al., 2003), has been recently documented in two species of mouse lemurs: for *M. murinus* in a two-individual study at Mandena, and for *M. griseorufus* in a longer study conducted at Tsimanampesotse, southwest Madagascar. Its expression, however, appears to be opportunistic, as only one of the two gray mouse lemurs and 3 of 16 reddish-gray mouse lemurs underwent hibernation for several weeks, by passively tracking daily variations in ambient temperature (Kobbe and Dausmann, 2009; Schmid and Ganzhorn, 2009). These reports not only highlight the opportunistic expression of hibernation in mouse lemurs, but they also suggest the possibility that this extreme energy saving strategy may be more widespread than previously considered. Indirect data from trapping and focal observations from several mouse lemur populations such as *M. rufus* at Ranomafana (Atsalis, 1999; 2008), *M. griseorufus* at Berenty (Génin, 2008), *M. murinus* at Kirindy (Schmid, 2000)

also showed opportunistic fattening (i.e., only some individuals within a population significantly increase body mass prior to the dry season) and seasonal inactivity for several weeks at a time. Other mouse lemurs such as *M. berthae* at Kirindy (Dammhahn and Kappeler, 2005; Schmid et al., 2000) and *M. ravelobensis* and *M. murinus* at Ampijoroa, however, underwent daily torpor but showed no evidence of seasonal torpor or hibernation (Schülke and Ostner, 2007).

Some researchers have suggested a relationship between reproduction (e.g. number of litters), environment (e.g. seasonality) and life span (Lahann et al., 2006; Génin, 2008). I propose a model encompassing use of torpor/hibernation, reproduction, habitat conditions with implications for life history parameters.

A simple model including a combination of environmental conditions (e.g. degree of seasonality of food and water resources) and biological constraints (e.g. dietary specialization determined by the ability of individuals belonging to a species to exploit certain resources) can be constructed to predict reproductive and metabolic strategies in predictable environments (Figure 1). At one extreme (with loose external and internal constraints) species might be expected to maximize total reproductive output. The opposite (“constrained” conditions – i.e., strong seasonality, strong dependence on seasonally constrained resources) might favor bet-hedging (i.e., fewer offspring but longer life spans to compensate for bad years). Dwarf lemurs (genus *Cheirogaleus*), appear to exemplify one end of the spectrum (e.g. extreme “tight” in Figure 1): adult females have one offspring per year and experience obligate hibernation for most of the year. They feed mainly on highly seasonal fruits and nectar, with high sugar content. Although dwarf lemurs live most of their lives sleeping, they probably have longer life

spans than other cheirogaleids as there is a positive correlation between hibernation and longevity (Chapter V, this dissertation).

This model, however, is unrealistic. The fact is that environmental unpredictability is characteristic of all of Madagascar's habitats, and thus no single combination of reproductive and metabolic strategies will always succeed (Figure 2). Some researchers have argued that extreme unpredictability favors extreme responses (very fast or very slow life histories) (Dewar and Richard, 2007). Indeed, heterothermy and the ability to experience torpor and hibernation likely developed in cheirogaleids as a strategy to cope with unusually harsh habitat conditions and fluctuations in resource abundance and availability. But the manner in which torpor and hibernation is used in cheirogaleid species has proven to be surprisingly variable, sometimes even within species.

Life history strategies of cheirogaleids, particularly in mouse lemurs are difficult to characterize as very fast or very slow, and "opportunistic" may be a more accurate depiction. The "opportunistic" model presented in Figure 2 posits that similar metabolic and reproductive responses can work under different environmental conditions (and vice versa, i.e., that different responses can work under similar environmental conditions). Thus, polyestry could be expressed not only in less seasonal environments but also in habitats with high seasonality (and maybe lower predictability) if females for instance, can fulfill dietary requirements with available fallbacks resources. In those habitats, females might even increase reproductive investment, undergoing rebound and/or regular polyestry, particularly if long term survival of adults in those environments is compromised (e.g. high mortality rates).

The use of seasonal torpor/hibernation could occur under a variety of conditions, for example in populations inhabiting both western dry or eastern rain forests. Hibernation requires fat stores and fattening is dependent, among other factors, of food, water availability and very importantly ambient temperature variation. In habitats with relatively low temperature minima, metabolic savings obtained by using of torpor are higher than those in warmer environments (Schülke and Ostner, 2007). If this is true, one would expect adaptive responses of individuals belonging to any particular species to be variable or flexible, and environmental correlates of life history variation to be weak. Individual “choices” may be shaped by individual experiences and favored under multiple circumstances (Table 1). For instance, rules of social dominance may prevent certain individuals to access to high quality food sources (fruit patches), and thus they may be unable to lay enough fat stores to sustain hibernation at the beginning of the dry season. Females may or may not express polyestry or fattening in a given population (e.g. Chapter IV, this dissertation). A possible explanation may be that females have only a limited number of reproductive “opportunities” that can be lost if they experience early resorption, abortion or perinatal death of offspring more than once in a season, i.e., they may be “eligible” to undergo fattening early during the dry season if food sources are available. Another possibility is that individual females “shut down” reproduction late in the season when energy saving mechanisms are turned on cued by internal (e.g. energy balance) or environmental factors (e.g. lower ambient temperatures).

Cheirogaleids, as a group, appear to have unique life histories, and they do not seem to fit the classic model of small-bodied mammals which live fast and die young. Their unusual use of energy saving strategies such as torpor and hibernation both appear

to constrain behaviors (e.g. young dwarf lemurs do not reproduce) and allow great flexibility of opportunistic responses particularly evident in some mouse lemur populations. Mouse lemurs are risk takers and opportunistic and occupy every habitat in Madagascar, whereas the more conservative and constrained dwarf lemurs may be at higher risk of extinction because of their reliance on fruits to be able to sustain obligate hibernation. Even if dwarf lemurs may be on the slow lane and live longer, mouse lemurs may be able to adjust more quickly to changing environmental conditions, which is the reality of Madagascar today.

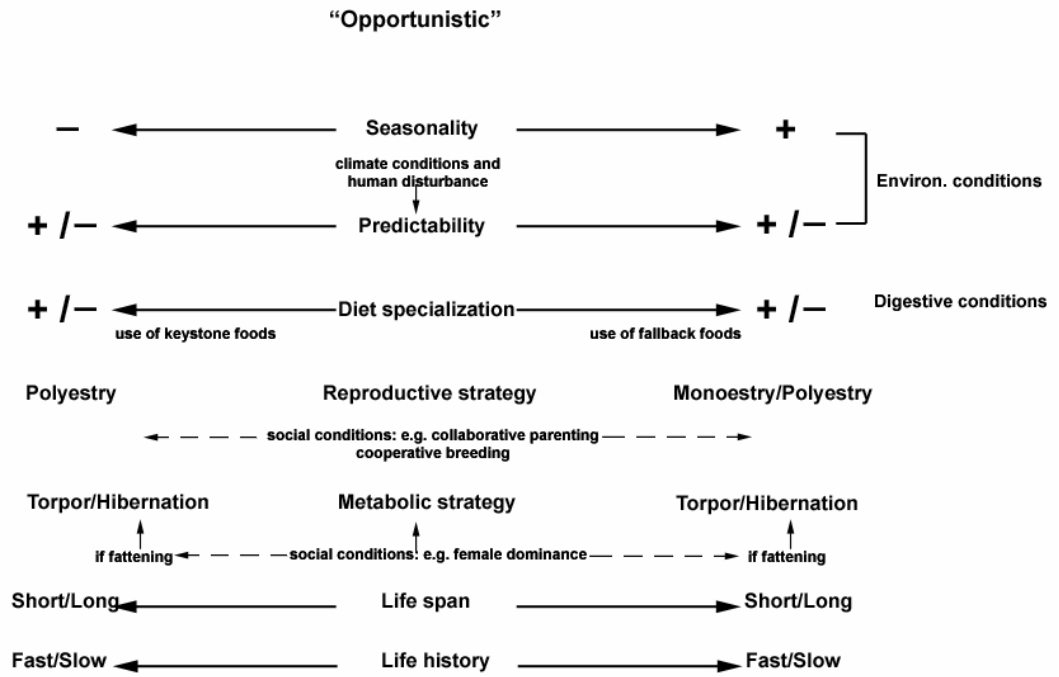


Figure VI.2. Descriptive model including a variety of possible metabolic and reproductive strategies expressed under different environmental and dietary conditions

Table VI.1. Individual variation in degree of fattening, use of torpor or hibernation and expression of polyestry, reported or expected to occur within and between mouse lemur populations.

Microcebus sp. cf. *M. rufus* at Tsinjoarivo, pers. obs., and *M. griseorufus* at Tsimanampesotse, southwest Madagascar, Kobbe and Dausmann, 2009

Seasonal.	Predict.	Diet Sp.	Species/Sex	Fattening	Torp./Hiber.	Polyestry
-/+	+/-	-	<i>M.cf. rufus</i> ♀	Y	H?	Rb/Rg?
			<i>M.cf. rufus</i> ♀	N	T	Rb/Rg?
			<i>M.cf. rufus</i> ♂	Y	H?	N/A
			<i>M.cf. rufus</i> ♂	N	T	N/A
+	-	+/-	<i>M.griseorufus</i> ♀	Y	H	Rb?
			<i>M.griseorufus</i> ♀	N?	T	Rb?
			<i>M.griseorufus</i> ♂	Y	H	N/A
			<i>M.griseorufus</i> ♂	N?	T	N/A

Y=yes, N=no, H=hibernation, T=torpor, Rb=rebound polyestry, Rg=regular polyestry, N/A=not applicable

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