Senescence Ecology: Aging in a Population of Wild Brown Mouse Lemurs (*Microcebus rufus*)

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ACADEMIC DISSERTATION

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Sometimes you can tell a large story with a tiny subject.

-Eliot Porter
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List of original publications

I. Sarah Zohdy, Marina Blanco, Brian Gerber, Stacey Tecot, Patricia C. Wright and Jukka Jernvall. 2011. **Mouse lemurs in the wild survive past the captive age of senescence.** (submitted)

II. Sarah Zohdy, Addison Kemp, Lance Durden, Patricia C. Wright, and Jukka Jernvall. 2012. **Mapping the Social Network: Tracking lice in a wild primate population (Microcebus rufus) to infer social contacts and vector potential.** BMC Ecology. (in press)

III. Sarah Zohdy, Stacey Tecot, Patricia C. Wright and Jukka Jernvall. 2011. **Testing the Immunocompetence handicap hypothesis (ICHH) in both sexes of wild brown mouse lemurs.** (manuscript)

IV. Sarah Zohdy, Stacey Tecot, Patricia C. Wright and Jukka Jernvall. 2011. **Age-related changes in Testosterone, Cortisol, and DHT in wild mouse lemurs.** (manuscript)


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**Abbreviations**

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<tr>
<td>T</td>
<td>Testosterone</td>
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<tr>
<td>DHT</td>
<td>Dihydrotestosterone</td>
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<tr>
<td>C</td>
<td>Cortisol</td>
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<tr>
<td>ICHH</td>
<td>Immunocompetence handicap hypothesis</td>
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<tr>
<td>DHEA-S</td>
<td>5α-dehydroepiandrosterone</td>
</tr>
<tr>
<td>IGF</td>
<td>Insulin-like growth factor</td>
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<tr>
<td>RNP</td>
<td>Ranomafana National Park</td>
</tr>
<tr>
<td>CVB</td>
<td>Centre Val Bio</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>NK</td>
<td>Natural killer</td>
</tr>
<tr>
<td>APC</td>
<td>Antigen presenting cells</td>
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<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
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<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
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Summary

Mouse lemurs (Microcebus spp.) are the world’s smallest member of the order Primates, and a model species for ancestral primates that lived 55 million years ago. In captive conditions, mouse lemurs live over six times longer than similarly sized mice and have been found to exhibit many symptoms of human senescence, including Alzheimer’s-like neurodegeneration. These traits make captive mouse lemurs an exemplary model for the study of aging. Despite this, to date no study has examined the aging process in wild mouse lemurs.

In this thesis I use mark-recapture data and a combination of field and laboratory techniques to examine the aging process in wild brown mouse lemurs (Microcebus rufus). A technique was developed to estimate ages of individual wild mouse lemurs from dental wear. I found that in their natural habitat these tiny primates live up to 8 years of age, well past the captive age of senescence (5 yrs). Among old individuals, both males and females are represented, and age-dependent survival rates do not differ between sexes.

With the ages of wild mouse lemurs identified, other age-related factors such as hormone levels and parasite loads were subsequently examined. Contrary to findings in captivity no observable symptoms of senescence were found in older wild mouse lemurs, i.e. over the age of five. Further, new findings highlight mouse lemurs as an exception to many assumptions of mammalian physiology. In this study, testosterone levels were not found to differ between sexes, potentially providing an explanation for the lack of difference in age-dependent survival/mortality rates between sexes. Similarly, the presence of testosterone and DHEA-S, two hormones typically found to decrease with age, did not differ between young and old lemurs. Cortisol did decrease with age in male mouse lemurs, but not in females.

Differences in immunity with age were examined indirectly via parasite dynamics. Specifically, I described the ectoparasites found on brown mouse lemurs, and created and implemented a novel method which allows the tracking of parasite-host interactions in the wild. This method revealed that parasite movement between lemurs of a single population suggests a much more complex social network than would be indicated by trapping events, and that a few individual lemurs are responsible for widespread parasite dispersal. However, age did not appear to play a role in whether or not a lemur was more of a parasite „donor“ or „receiver“. In addition to shedding light on the social behavior of mouse lemurs and the natural movement of parasites in a wild population, I used the presence of ecto- and endoparasites as an indicator of immune health in young and old individuals, exposing a decline in endoparasites with age, but not in ectoparasites.
Hormone measures and parasite loads were also used to test the immunocompetence handicap hypothesis (ICHH) (which implicates testosterone as a cause for immunosuppression and hence higher parasite intensities) in both sexes. No correlation between testosterone and parasite intensities was found; however, when cortisol and testosterone positively co-varied higher parasite loads were observed in both sexes.

In conclusion, for this thesis I examine physiological (dental, endocrinological, and parasitological) shifts in age in a wild population of brown mouse lemurs. This thesis details the development and implementation of novel methods, results that challenge several assumptions of mammalian biology, and new conclusions that further our understanding of the natural world.

**Summary (Malagasy)**

Famintinana

Anisan’ny primaty kely indrindra eran-tany izao ny tsiditsidy ary fantatra fa misy fitoviana amin’ny vianakaviambane’ny gidro izay niaina 55 tapitrisa taona lasa izay. Raha ompian’ny olombelona amin’ny toerana voatokana ho amin’izany ny tsiditsidy dia hita fa avo enina-eny mihoatra amin’ny totozy mitovy habe aminy ny taom-pahavelomany ary fantatra koa fa mampiseho famba- ran’aretina mitovy amin’ny olombelona eo amin’ny lafiny fahanterana, anisan’izany ny fahasimbana ny sela ao amin’ny atidohy toy ny Alzheimer. Noho ireo toetrany ireo dia anisan’ny bibi fanaovana fikarohana mikasiaka ny fahanterana ny tsiditsidy. Na izany aza anefà dia mbola tsy nisy ny fikarohana momba ny fivoaran’ny fahanteran’ny tsiditsidy dia.

Ny fikarohana nataoko dia nampiasa ireo vokatra azo tamin’ny famandriahana sy fametrahana marika ny bipy anaty ala ary ny tekniaka anaty labôratoara hana- rahana ny fivoaran’ny fahanteran’ny tsiditsidy (*Microcebus rufus*). Nisy fomba vaovao noforonina hahafantarana ny taon’ny tsiditsidy izay mifototra amin’ny fahasimban’ny nifiny. Ny valin’ny fikaroana nataoko dia maneho fa ny tsiditsidy any amin’ny toerana voajahary dia miaina mihoatra ny valo taona, izany hoe manana fahavelomana lava lavitra raha ampitahaina amin’ireo tsiditsidy karakarain’ny olombelona (mahatrastrata 5 taona eo eo). Tsy nisy fahasamihafana loatra ny isan’ny lahy sy vavy raha ny bipy efa antitra no jerena izay midika fa tsy miainkina loatra amin’ny maha-lahy na maha-vavy ny taham-pahavelomany’ny tsiditsidy.

Ny fahalalana ny taonan’ny tsiditsidy dia nahafahana namantatra ireo toetra hafa izay mifandraika amin’ny fitomboan’ny taona tahaka ny habetsaky ny hormanina sy ny parazita ao aminy. Tsy hahitana fambara ivelany ny fahanteran’ny tsiditsidy dia, vokatra izay mifanohitra amin’ny azo tamin’ny bipy izay tsy miaina

Nojerena manokana ny tsy fiovan’ny “immunity” (hery fiarovana anaty hahafahana miady amin’ny aretina) rehefa miha antitra ny bibly amin’ny alalan’ny fivoarana ny parazita any amin’ny lahy sy ny vavy. Nohalaliniko koa hoe inona avy ireo “ectoparasites” (parazita ivelan’ny vantana) hita ao amin’ny tsiditsidy, ary namorona fomba vaovao aho hahafahana manaraka ny fivoarana ny fifandraisian’ny tsiditsidy sy ny parazita any amin’ny toerana voajanahary. Ireo fomba vaovao ireo dia nanambara ny hasarotan’ny fipondran’ny parasite ao amin’ny vondrana tsiditsidy iray. Manarak’izany, andiana tsiditsidy vitsivitsy ihany no nanaparitaka ny parazita. Na izany aza, tsy mifandraika amin’ny taoan’ny bibly ny maha mpanaparitaka na mpandray hao ny tsiditsidy. Ankoatrizay fahalalana azo momba ny fifandraisam-piara-hamonina eo amin’ny tsiditsidy izay, nahafahana nampitaha ny fahatanjahan’ny hery fiarovana amin’ny aretina eo amin’ny bibly antitra sy tanora ny fisian’ny “ectoparasites” sy “endoparasites” (parazita miaina ao anatin’ny bibly). Hita fa mihena ny “endoparasites” rehefa miha antitra raha tsy miova kosa ny hamaron’ny “ectoparasites”.

Ny fandrefesana ny tahan’ny hîrmônina sy ny habetsaky ny parazita dia natao hanamarinana ny “Immunocompetence handicap hypothesis” (ICHH) (izay milaza fa ny tahan’ny testosterono no antony mampihena ny hery fiarovana anaty ka manamora ny fitomboan’ny parazita) eo amin’ny lahy sy ny vavy. Tsy misy fifandraisian’any anefa ny tahan’ny testosterônina sy ny hamaroan’ny parazita. Rehefa miara mitombo kosa anefa ny “cortisol” sy ny testosterônina dia mitombo ny isan’ny parazita amin’ny tsiditsidy.

Raha fintinina ny fikarohana nataoko dia nijery ny fiovan’ny fahasimban’ny nify, ny tahan’ny hîrmônina vokarina sy ny isan’ny parazita ateraky ny fahanteran’ny tsiditsidy dia. Namorona sy namelabelatra fomba vaovao izay mame-traka fanamby ho an’ny fikarohana mikasika ny bibly mampinono aho ary inoanako fa hampitombo ny fahalalana ny zava-boary ny vokatra azo.
1. Review of literature

1.1 Aging and senescence

"All diseases run into one, old age."

Ralph Waldo Emerson

Everything ages. In inanimate objects like wine and cheese this process is often one of refinement. However, in the biological world organisms experience physiological declines with time eventually leading to death, and this process is known as senescence. Senescence is an intrinsic biological phenomenon that limits an organism’s maximum potential lifespan, even in the absence of extrinsic sources of mortality such as predation, disease, and environmental hazards. The fascination behind human mortality dates back to the earliest civilizations. The fundamental question of why some individuals die faster than others led to the search for a single underlying mechanism of aging. However, with time and research it has been shown that the process of becoming old and the time frame in which this occurs varies greatly by taxa. Some organisms are semelparous and only survive through one breeding cycle (Cole, 1954, Braithwaite & Lee, 1979), while others live hundreds of years (George et al., 1999, Thomson et al., 1995). Moreover, some plants live thousands of years (Lanner & Connor, 2001, Larson, 2001, Flanary & Kletetschka, 2005), and some organisms are even found to have characteristics that render them immortal (Piraino et al., 1996). Thus, there is no clear formula for survival as all organisms vary in their longevity; however certain traits have been found to contribute significantly to longevity. These traits include increased body mass and metabolic rate, the ability to escape extrinsic mortality factors through flight, gliding, arboreality or other methods of evading predators, and finally the life history traits unique to the Order: Primates.

It is widely thought (Kirkwood & Austad, 2000) that animals in captivity survive longer than those in the wild. This is typically attributed to organisms and their susceptibility to extrinsic and intrinsic mortality factors. Extrinsic mortality factors are those found heavily in a natural setting and include things such as: disease, predation, environmental stressors, starvation, and other threats to mortality in the wild that captive animals are typically safe from. Intrinsic mortality factors are things that affect both wild and captive animals and contribute to mortality from the outside in. These factors include: cellular senescence, cancer, immunocomprirminisation, oxidative damage, free radical build up, etc. It is thought that captive animals survive longer than animals in the wild and experience symptoms of senescence because they are safe from extrinsic mortality factors. However, evidence from wild animals; (Reznick et al., 2005, Reznick et al., 2004, Cartar, 1992, Charmantier et al., 2006, Promislow, 1991) suggests
that senescence may occur in the wild, even though individuals are exposed to extrinsic risks.

### 1.1.1 Aging in the Natural World

While captive data is critical and allows for more intense studies, these data can only tell us so much about the aging process. Animals in captivity are safe from environmental stressors, predators and often from parasites. These are all factors that may contribute to the aging and survival of wild populations, therefore lifespan in captivity may be dramatically different from that in the wild. For example captive bank voles can live up to 40 months when safe from extrinsic mortality factors (Godfrey, 1958) in a captive environment, whereas wild bank voles are known to live up to only 18 months of age (Stoddart, 1971). Therefore, to gain a more accurate depiction of the true aging process of an organism one must also understand what the process looks like in a natural habitat. There are great difficulties in studying aging in the wild; however, advancements in modern technology and long term data sets have allowed such studies to be possible. In fact, several studies have provided evidence that senescence in the wild does indeed occur, in the form of age-related increases in mortality rates or decreases in reproductive success (Holmes & Austad, 1994, Promislow, 1991, Reznick et al., 2005). However, the physiological mechanisms behind the deterioration of these systems are still widely unexplored and presents the potential for a burgeoning field of research.

### 1.1.2 Patterns in longevity

The fact that larger animals live longer than smaller animals was noted as early as 350 BC by Aristotle. In 1908 Rubner tested this hypothesis in domestic animals and showed that the rate of metabolism increased as a function of body size, and that the larger domestic animals (cows, pigs, horse, man) were also lived longer (Rubner, 1908). However, in some cases, smaller individuals with higher rates of metabolism, such as birds, live longer than their slower, larger conspecifics (Pearl, 1928).

In the wild, volant animals such as birds and bats experience lower rates of extrinsic mortality (Pomeroy, 1990) and greater longevity (Austad & Fischer, 1991, Comfort, 1979, Holmes & Austad, 1994, Jürgens & Prothero, 1987, Podlutsky et al., 2005, Wilkinson & South, 2002) than their nonvolant relatives, presumably because of decreased predation pressures.
Review of literature

Figure 1. Mammals of similar body mass that occupy different ecological niches differ in longevity. It is thought that the behaviors associated with arboreality, gliding, flight and burrowing can prolong mammalian lifespan by protecting the animals from extrinsic mortality factors such as disease, predation, and environmental hazards. In this figure (from bottom to top) subterranean, terrestrial, arboreal, gliding and volant mammals are represented. The direction of the arrow represents an increase in lifespan with the direction of the arrow, such that terrestrial mammals have the shortest lifespans, followed by arboreal mammals, followed by gliding and volant mammals. Due to their burrowing nature, similar to volant mammals, subterranean mammals have prolonged lifespans when compared to terrestrial, arboreal, and gliding mammals of similar size.
Although other factors such as hibernation and reproductive rate have been shown to play a role in bat longevity (Wilkinson & South, 2002) with bats surviving up to 41 years in the wild, these factors are accordant with the evolutionary theory of aging, and it is perhaps safe to conclude that the exceptional longevity of Chiroptera as a whole is the result of flight. In addition to flying birds and mammals, gliding mammals are longer-lived than nonvolant, nongliding mammals (Austad & Fischer, 1991, Holmes & Austad, 1994). As with flight and gliding behavior, arboreality is thought to lower extrinsic mortality rates and increase longevity by providing a relatively protected environment with reduced exposure to predation, disease, and environmental hazards (Shattuck & Williams, 2010). Subterranean animals, protected from predators and extreme climates have increased longevity for their body mass when compared to terrestrial, arboreal and gliding mammals of similar body mass. One example of this can be seen in the longest lived rodent, the naked mole rat which lives up to 28 years of age (Buffenstein, 2008). Indeed, comprehensive analyses of living mammalian species suggest that hiding and predator avoidance behaviors such as torpor or use of burrows lower extinction risk (Liow et al., 2009). The concept of predator avoidance extending lifespan is not a new one. Darwin himself in *The Descent of Man* (Darwin, 1871) identified an association between arboreality and extrinsic mortality, and noted the “power of quickly climbing trees, so as to escape from enemies.” Furthermore, several researchers have suggested that primates are long-lived among mammals at least in part because they are largely arboreal (Austad & Fischer, 1991, Kaplan & Robson, 2002). Taken together, these data suggest that mammals that can fly, climb, glide, hide or otherwise elude heavy predation pressures experience extended longevity when compared to terrestrial mammals of similar body sizes (Figure 1).

### 1.1.3 Laboratory models of aging

Aging research in laboratory model organisms from yeast to mice has demonstrated that some mechanisms of aging are evolutionarily conserved, and that common processes occur (Johnson et al., 1999, Guarente & Kenyon, 2000, Longo & Finch, 2003, Partridge & Gems, 2002, Nyström & Osiewacz, 2004, Johnson, 2008, Gems & Partridge, 2008). These mechanisms are often based on genetic factors. For example, in the nematode *Caenorhabditis elegans*, extensive research has been conducted on the *daf*-2 locus. Loss of function mutations at this locus have been found to extend the life span of *C. elegans* under laboratory conditions (Walker & Lithgow, 2003, Henderson et al., 2006). This has provided information on the comparative biology of the *daf*-2 locus, which can be found in many organisms, including mammals. This locus contains a gene encoding a receptor for the IGF (Insulin-like Growth Factor) pathway that regulates many aspects of cellular metabolism and development in a range of organisms, including mammals (Holzenberger et al., 2003, Longo & Finch, 2003, Carter et al., 2006).
Another gene that has received attention in laboratory aging research is \textit{p53}, a tumor-suppressor gene involved in the recognition of cell damage and initiation of programmed cell death or apoptosis (Derry \textit{et al.}, 2001, García-Cao \textit{et al.}, 2006, Gatza \textit{et al.}, 2006, Matheu \textit{et al.}, 2007). Increased expression of this gene has been shown to reduce tumor formation, but also to accelerate the aging process (Pinkston \textit{et al.}, 2006). In spite of the strong effects of certain gene loci on life span (Bartke \textit{et al.}, 2001) the mechanisms by which these genes influence aging can be extremely complex.

Interpreting genetic effects on rate of aging and life span requires an understanding of the evolutionary and environmental context of the experimental systems (Austad, 1993, Austad & Podlutsky, 2006). Studies of the effects of individual gene loci on aging and life span have been possible only in laboratory populations of animals with well studied and understood genetics, and genomes that are fully sequenced. Typically, these organisms, such as yeast, \textit{C. elegans}, \textit{Drosophila melanogaster}, and laboratory mice, have well established feeding, housing, and reproductive protocols, short life spans and high fecundity. These traits have made them readily studied organisms. At the same time, however the life histories of these model organisms differ greatly from birds, humans and other large mammals. Lifespan evolves in the context of the life history of the organism. This then beckons the question of whether \textit{D. melanogaster} and \textit{C. elegans}, for example, are suitable models for understanding human aging. Although these species share conserved longevity mechanisms with mice (McElwee \textit{et al.}, 2007), uncertainties about the genes responsible for differences in longevity between these species and differences in the life histories of model organisms make it difficult to draw broader organismal conclusions.

Humans, birds, as well as many large mammals are characterized by repeated reproduction over years, determinate growth, lack of resting stages, and continued proliferation of cells in the course of tissue function, maintenance, and repair. However, other organisms can have fundamentally different life histories, and therefore different contexts under which lifespan evolves. For example, plants exhibit continuous meristematic growth, in which case distinctions between the soma and the germ line are blurred and age-related damage to proliferating cells can be sorted by clonal selection. Similarly this can be done in single cell organisms like yeast (Petit & Hampe, 2006). Due to this type of growth, it is perhaps not surprising that that some plants reach ages of thousands of years (Lanner & Connor, 2001, Larson, 2001, Flanary & Kletetschka, 2005). Other plants and many insects, however, have annual life cycles in which individuals suffers an inevitable death at the end of the growing season, which allows for the evolution of a programmed senescence in which resources are preferentially allocated to reproductive investment rather than continued life (Gan, 2007). Another strategy is found in water fleas (Cladocera), which alternate
phases of parthenogenetic (asexual or clonal) and sexual reproduction (Dudycha, 2001).

Many vertebrates, including fish and turtles, have indeterminate growth and thus increase in size with age and also remain reproductive. As a result of their increasing fecundity with age and size, selection to postpone senescence remains strong late into life (Baudisch, 2005) and many long-lived species show little evidence of age-related declines in reproductive success (Congdon et al., 2001, Coulson & Fairweather, 2001, Nisbet et al., 2002).

The roundworm *C. elegans*, which is one of the main model species of aging research, is unusual in having a completely post-mitotic adult life. It has a resting, or dauer, stage in which cell metabolism is reduced and the individual enters a state of „suspended life“ (Kenyon, 1988, Riddle, 1988). Even the more typical mammals used in aging research, such as laboratory mice and rats, are unusual, from a human point of view, in having been selected for high fecundity, rapid development and short generation times (Miller et al., 2002). In addition, laboratory strains of mice typically are highly inbred and genetically uniform, which is advantageous for genetic analysis and experimentation, but likely presents a highly atypical background for lifespan manipulation.

Currently it is not clear that life span can be extended much beyond that in natural populations. (Van Voorhies et al., 2005) showed that *C. elegans* tends to prefer laboratory agar to natural soil. In laboratory settings, daf -2 mutants on agar live about twice as long as wild-type individuals on agar. When the mutants are on soil, however, the lifespan of daf -2 mutants was not extended but instead reduced. Therefore, the influence of daf -2 on aging depends dramatically on the environment and further understanding of aging in natural habitats is necessary.

### 1.1.4 Primate aging

Primates stand out as an exception in terms of aging and life history research. Primates, humans included, mature later, live longer, and have lower fertility than most other mammals (Leigh, 2001, Walker et al., 2006, Shattuck & Williams, 2010, Ernest, 2003, Austad & Fischer, 1991). Humans are the product of millions of years of primate evolution, and studying aging in non-human primates helps provide insight into the evolutionary impetus of human aging.

Primate life histories lie at the slow end of the fast-slow continuum, which is generalized to all mammals (Charnov & Berrigan, 1993, Harvey & Clutton-Brock, 1985, Read & Harvey, 1989, Ross, 1998). More specifically, primates have longer gestation periods, smaller litter sizes, larger neonates, slower postnatal growth rates, a later age at first reproduction, and a longer life span than
do most other mammals of comparable body masses (Charnov, 1991, Charnov & Berrigan, 1993, Lee, 1991, Martin & MacLarnon, 1985, Ross, 1998). As a consequence, primates experience an extended infancy and juvenile periods, and have lower reproductive rates in comparison to other mammals of the same size (Charnov, 1993, Charnov & Berrigan, 1993). Many hypotheses have been derived attempting to explain why primates are so unique. These hypotheses broadly discuss three possibilities. 1) The evolutionary relationship between life history traits and brain size. Studies of allometry have found that large-brained primates generally have long gestation periods, slow, prolonged growth periods, late sexual maturation, and long lives (Harvey et al., 1987, Allman et al., 1993, Charnov & Berrigan, 1993, Barton, 1999a, Ross, 2003, Ross & Jones, 1999). 2) Mortality rates that are environmentally imposed (Promislow & Harvey, 1990, Charnov, 1991, Charnov, 1993, Stearns, 1992, Janson et al., 1993, Ross & Jones, 1999). It has been suggested that primate mortality rates are either the result of high juvenile mortality that favors the evolution of prolonged juvenile periods (Janson et al., 1993, Ross & Jones, 1999), or of low rates of adult mortality which are linked to late age of first reproduction and hence delayed maturation (Charnov, 1993, Promislow & Harvey, 1990, Stearns, 1992). 3) The importance of ecological factors such as diet and predation on age at first reproduction and hence the speed of life (Eisenberg, 1981, Ross, 1988, Ross, 1992, Rowell & Richards, 1979).

Primate life span is notoriously difficult to measure, since either the age of captive animals is often well known but may not necessarily reflect the life span that is typically achieved under natural conditions. Because of their long life histories, studying primate lifespan in natural conditions requires long-term research commitment to individual populations. As some primates can live up to 50 years or longer, this commitment has the possibility of spanning the entire life-time of a researcher.

Lifespan data available from non-human primates range from about 9 years in the fat-tailed lemurs (Cheirogaleus medius) (Harvey et al., 1987) up to about 60 years in chimpanzees (Pan troglodytes) (Hakeem et al., 1996). When compared to other mammals, primates have longer lives for their body size (Charnov & Berrigan, 1993, Jones & MacLarnon, 2001). Allometric analyses show that life span within the primate lineage is positively correlated with body mass and brain size (Allman et al., 1993, Austad & Fischer, 1991, Deaner et al., 2003, Harvey et al., 1987). In fact, it is thought that brain size is more influential than body size, because the partial correlation between lifespan and brain size is positive after the effects of body size have been removed (Sacher, 1959, Allman et al., 1993, Deaner et al., 2003, Harvey et al., 1987). In addition, maturation age correlates strongly with lifespan, and it is thought that life span and brain size may linked to age at maturity and may be only secondarily correlated with each other via the intermediate variable of maturation age (Harvey et al., 1987).
Brain size and brain growth pattern are strongly correlated with almost all life history traits. Large brained primates usually have long gestation periods, high neonatal body mass, and give birth to few offspring that have delayed maturation and long lives (Allman & Hasenstaub, 1999, Allman et al., 1993, Austad & Fischer, 1991, Charnov & Berrigan, 1993, Harvey et al., 1987, Purvis & Harvey, 1995). These relationships occur independently of body size and have been functionally linked either to the high energetic costs of maintaining large brains (Allman et al., 1993, Foley et al., 1991, Martin, 1996, Ross & Jones, 1999, Sacher, 1959) or to developmental processes like learning during the juvenile period that require large brains (Barton, 1999b, Dunbar, 2003, Harvey et al., 1987, Joffe, 1997).

In terms of life history, there is one notable exception among primates: humans. When compared to other great apes, human infants are quite large at birth, have slow growth, and an extended childhood (post weaning, but dependant on others) period (Bogin, 1997). Furthermore, humans reach reproductive age much later, yet have short interbirth intervals, and increased longevity compared to other apes (Leigh & Park, 1998, Kaplan et al., 2000, Mace, 2000). Maximum life span in humans is reported to range between 90 and 122 years (Weiss, 1981). This is about twice as long as chimpanzees (Kaplan & Robson, 2002). However, a large proportion of this time (around 30-40%) is postreproductive (menopause) in women, which suggests that total life span and reproductive life spans are disconnected in humans. While the fertile lifespan of humans is similar to other great apes (Alvarez, 2000), the prolonged post reproductive period is in stark contrast to other primates, who have typically short, if any, postreproductive periods (Hawkes et al., 1998, Johnson & Kapsalis, 1998, Pavelka & Fedigan, 1999). Women, however, can have a much higher fecundity than other nonhuman apes due to much shorter interbirth intervals (Hawkes, 2003).

In addition, human brains are three to four times larger than in chimpanzees and gorillas (Kaplan & Robson, 2002), which is much higher than expected for a primate, when adjusted to body size (Pagel & Harvey, 1989). The human brain grows faster and continues to grow longer after birth (Bogin, 1999, Kaplan & Robson, 2002). Furthermore, humans show broad intraspecies variations in growth rate, age of maturation, birth rate, age-specific mortality, and senescence, which not only depend on ecological factors but also on food technology, medical care, and culture (Mace, 2000, Coulson & Fairweather, 2001, García-Cao et al., 2006, Kaplan, 2002). In developed countries during the last 150 years, the average age at menarche among girls has decreased by about 4 years; birth rate in women has changed from six to about two children per family, and the percentage of people reaching more 100+ years of age has increased (Kaplan, 2002).
There is no general consensus on how to explain these unusual human life history patterns, however, there is one widely accepted hypothesis explaining selective pressures shaping the evolution of the peculiarly long lifespan in humans with the emergence of menopause in women. This is the „grandmother hypothesis” (Hawkes, 2003, Hawkes et al., 1998, Alvarez, 2000, Lahdenpera et al., 2004). This hypothesis is based on (Charnov, 1993) and assumes that the long post reproductive period evolves when post reproductive females gain greater fitness by increasing the success of their offspring instead of continuing to breed themselves. Support for this hypothesis comes from some historic and contemporary data sets that showed that grandparents may assist grandchildren by transferring knowledge and by participating in household tasks and child care. This help may increase offspring breeding probability, the nutrition of the grandchildren, and survival. One study of fitness benefits in post reproductive women in multigenerational farming communities in Finland and Canada during the eighteenth and nineteenth centuries (Lahdenpera et al., 2004) found that post reproductive women living in the same house or near their offspring had more grandchildren and greater fitness, because they enhanced the lifetime reproductive success of their offspring by allowing them to breed earlier, more frequently and more successfully. Fitness benefits, however, disappeared as the reproductive output of the offspring declined; and the rates of female mortality accelerated as their offspring ceased reproduction.

Taken in combination, the life history traits of primates (both human and non human) demonstrate long-lived, slowly maturing mammals with large brains, low reproductive output and in the case of humans, long post reproductive periods.

1.2 Madagascar

The island of Madagascar is thought to be one of the oldest islands in the world. It separated from Africa about 165 mya and remained attached to India. The island then separated from India about 90 mya settling in its current location, while India moved north into Asia, creating the Himalayas. Because the island has been isolated for such a long period of time the organisms on the island have diversified into their own unique biota. Over 90% of the animals and 80% of the plants on the island of Madagascar exist nowhere else in the world. This makes the island one of the most unique places for biologists to conduct research. Of the unique organisms on the island perhaps the most recognizable and diverse mammalian taxa are the lemurs.
1.2.1 Lemurs

Taxonomically, lemurs are a family of primates in the sub order Strepsirrhine, a sub order that also includes the lorises of Asia, and the galagos of mainland Africa. Strepsirrhine primates are called so because they retain the primitive “wet nose” that is found in non-primate animals. These primates have also retained a few traits reminiscent of their small mammalian past, such as nocturnality (with the exception of some cathemeral and diurnal lemurs) and a thin membranous reflective layer behind the retina that allows for visibility in low light or dark conditions called a tapetum lucitum.

Lemurs are the most distantly related primate to humans. It is thought that a small, relatively basal primate (that possibly resembled something like the extant mouse lemurs) arrived to the island of Madagascar about 65 mya, and from there diversified into the over 101 species of lemur found on the island today. A principle driving factor behind Madagascar’s high species diversity and local endemism is its topography. The island is a ring of coastal lowlands encircling a high plateau. The plateau is highest in the east (giving rise to mountain chains and the eastern corridor running north-south) and slopes gradually towards the sea in the west. This topographical pattern, which creates two major floral zones the humid eastern and the dry western, is closely tied to the speciation and present distribution of the island’s lemurs.

The phylogenies and distribution of nocturnal lemur genera are complicated and our understanding of them is constantly evolving through integrative studies that combine morphological, molecular and geographical data (Yoder, 2000).

1.2.2 Mouse lemurs

Mouse lemurs (*Microcebus* spp.) are a species-rich strictly nocturnal genus comprised of the world’s smallest primates. Prior to the 1970’s only one species of *Microcebus* was recognized, *M. murinus* (Napier, 1967, Schwarz, 1931). To date, 19 species have been proposed and their precise distribution cannot be easily deduced from the data available (Yoder *et al.*, 2000, Olivieri *et al.*, 2007, Radespiel *et al.*, 2008, Radespiel *et al.*, 2011).

Members of this genus exhibit great flexibility in adapting to different environments characteristic of Madagascar (Rasoloarison, 2003, Atsalis, 1999a). They occur in all forest habitats on the island, in nearly all the remaining forests, from very humid evergreen habitats to the dry spiny forests in Southern Madagascar (Rasoloarison, 2003, Radespiel *et al.*, 2006). *Microcebus* are even found in secondary forests affected by human activity and in plantations, and they seem to do well along forest edges (Radespiel et al., 2006, Lehman *et al.*, 2006).
Mouse lemurs generally move quadrupedally on branches, including running and leaping short distances, and they sometimes come to the ground to hunt for insects or to cross short open areas (Martin, 1973b, Pages-Feuillade, 1988). *Microcebus* uses all heights of the forest (Radespiel et al., 2006, Atsalis, 2007), but seasonal differences have been observed in preferred forest strata; at the end of the dry season in the forest reserve of Ampijoroa located in Ankarafantsika National Park in Northwestern Madagascar, when little plant food was available, some animals spent 70% of their time searching for insects below 3 m (Pages-Feuillade, 1988).

1.2.2.1 Torpor

To reduce energetic expenditures, mouse lemurs can enter states of hypothermia, either through daily torpor, or through deep and prolonged hibernation during the dry season. Even though these primates are tropical or subtropical they are able to save energy by becoming completely inactive and lowering their body temperatures when food resources are low and the climate is unpredictable (Perret, 1992, Schmid, 1996, Ortmann et al., 1997).

The external trigger for seasonal torpor is photoperiod, a predictable environmental cue, and indeed, several researchers have shown that even in captivity changes in photoperiod can be used to trigger torpor in mouse lemurs (Perret, 1992, Perret et al., 1998) with the amplitude of change in body temperature dependent on ambient temperature (Aujard et al., 1998). In past studies of *Microcebus*, individuals were observed to be active throughout the year in the forest (Hladik, 1980, Martin, 1973b), however, it has since been discovered that there is a great deal of variation in mouse lemur torpor patterns.

In addition to seasonal torpor, mouse lemurs engage in bouts of daily torpor during which time their body temperatures decrease and they become lethargic while sleeping. Evidence of daily torpor has been reported for *M.murinus* (Schmid et al., 2000, Schmid, 2001), *M.rufus* (Atsalis pers comm.; this thesis), *M.ravelobensis* (Randrianambinina et al., 2003) and *M.griseorufus* (Genin, 2008), all species studied thus far. Whereas seasonal hibernation is part of a photoperiod associated seasonal regime for surviving in unfavorable environmental conditions, daily torpor is a much more flexible way to adjust to energy requirements.

Only populations of two species, the dry forest dwelling *M.murinus* and the rainforest dwelling *M.rufus* have been found to enter seasonal torpor. *Microcebus ravelobensis*, *M. berthae*, *M. griseorufus* inhabitants of dry forests, were not observed hibernating seasonally (Schmid et al., 2000, Schmid, 1996, Ortmann et al., 1997, Genin, 2008). Even within a species, some populations may hibernate while others do not; at Ampijoroa *M.murinus* sympatric with *M. ravelobensis*
did not exhibit seasonal fattening and hibernation (Schmelting, 2000, Lutermann, 2001). Moreover, even within the same population some individuals may hibernate some years while others do not (Schmid & Kappeler, 1998, Atsalis, 1999a). This variety in seasonal torpor patterns is unlike what happens in the fat-tailed dwarf lemur, *Cheirogaleus spp.* where no individuals are sighted in the forest in the dry season.

In captive studies, mouse lemurs underwent daily bouts of torpor when food was in short supply even under high ambient temperatures, independent of photoperiod (Genin & Perret, 2003). Food-restricted animals experienced lowest body temperatures. In other words, given food freely, animals did not become torpid even during short photoperiod. These observations suggest that genetic variability and differences in local habitats, including climatic conditions, may drive the variation reported in seasonal torpor patterns. Schmid (1999) speculated that females in the wild entered the seasonal period of inactivity once they had reached a critical mass of at least 50 g, allowing them to remain in hibernation for several months.

When inactive, either as part of their daily cycle or when in seasonal hibernation, mouse lemurs sleep in tree holes, leaf nests, or in a variety of other sleeping options (Radespiel et al., 1998, Radespiel et al., 2003, Thorén et al., 2010, Atsalis, 2008) and there are differences in how mouse lemurs use their nests. In Ankarafantsika National Park *M.murinus* females selected tree holes that averaged 4 m above the ground, significantly higher than the location of nests chosen by males, which averaged only 0.77 m (Radespiel et al., 1998). Males often chose dead tree hollows and leaf nests, changing these poor quality sleeping sites frequently (Radespiel et al., 1998). In contrast, females selected well insulated tree holes that offered better protection from predators and the elements (Radespiel et al., 1998). Furthermore, compared to the gray mouse lemur, sympatric *Microcebus ravelobensis* used a broader variety of sites, including collections of branches (Radespiel et al., 2003). But, golden brown mouse lemur females like gray mouse lemur females were also concerned with safety: although they used tree holes only in 46% of cases (some located at the base of trees) compared to 86% for coexisting *M.murinus* (Radespiel et al., 2003), females chose to construct leaf nests that were higher in trees during the rearing season when offspring would need protection from predators (Thorén et al., 2010).

Male-biased trap sex ratios have been reported in both west and east coast populations of mouse lemur in studies that took place during the dry season (Harcourt, 1987, Fietz, 2000, Schmid & Kappeler, 1998, Atsalis, 1999b, Dammhahn & Kappeler, 2009, Rasozanabary, 2006, Atsalis, 2000). However, Atsalis (2008) discovered that the sex ratio of trapped animals was approximately 1:1 between January and April, but began to favor males in May and became
highly biased in favor of males from May through September which may indicate that females are in torpor and therefore do not enter traps.

1.2.2.2 Diet

Dietary data on mouse lemur have been collected through direct observation of animals feeding in the forest, examination of the fecal matter of live trapped animals, and, in the past, through analysis of stomach contents (Martin, 1972, Hladik et al., 1980, Atsalis, 1999b, Atsalis, 2008, Dammhahn & Kappeler, 2009, Lahann, 2007). The data suggest that although mouse lemurs are the smallest primates and expected to be highly insectivorous, like some galagos, (Charles-Dominique & Martin, 1972, Harcourt, 1986, Harcourt & Bearder, 1989, Harcourt & Nash, 1986) less than half of their diet is made up of insects. Mouse lemurs consume a broad range of dietary items that change seasonally and whose specific composition depends on species’ geographical distribution (Radespiel et al., 2006, Atsalis, 2008). A recent publication (Crowley et al., 2011) examining geographic variation in dietary isotope analysis of lemur species from all ecosystems found in Madagascar revealed that trophic differences among mouse lemur species were small; however, nitrogen isotope values in Microcebus and plants covaried, which suggest that nitrogen values could be a good predictor of isotopic differences among habitats. Higher nitrogen values are indicative of a diet rich in insects, therefore these nitrogen differences may be due to differing insect abundance and hence consumption in different habitat types.

In general, gums and the secretions of homopteran larvae have been found to be of special importance in the seasonal diet of mouse lemurs studied in dry forests. As with M. griseorufus Génin (2001) found that M. murinus fed on gums at Kirindy and at Berenty in which 75–80% of feeding episodes involved gums though gum trees visited were different. Génin concludes that gum trees are a keystone resource for mouse lemurs at least during the dry season.

It has been confirmed from other studies that the secretions of homopteran larvae were an important food resource in the dry season for M. microcebus, M. ravelobensis and M. berthae (Corbin & Schmid, 1995, Dammhahn & Kappeler, 2005, Rademeyer et al., 2006, Reimann & Zimmermann, 2002). These secretions are produced by colonies of larvae and they drip onto branches below, drying into a crystalline form that mouse lemurs lick (Corbin & Schmid, 1995). Being high in sugar but low in protein, insect secretions are supplemented by animal matter (Dammhahn & Kappeler, 2005, Rademeyer et al., 2006).

We have seen examples of the potential importance of mouse lemurs as pollinators and seed dispersers. *M.murinus* enjoys floral nectar (Hladik et al., 1980, Martin, 1972, Martin, 1973a), and very likely serves as a pollinator to some plant species (Nilsson et al., 1993, Hladik et al., 1980).

Seed dispersal may be among the most important components of plant–animal interactions (Reid, 1989). In Madagascar the diversity of birds and other mammals that eat fruits is low anyway (Goodman et al., 1997, Eliezer et al., 1997, Hawkins & Goodman, 2003), leaving lemurs to be primary seed dispersers (Smith & Ganzhorn, 1996, Dew & Wright, 1998). At Ranomafana National Park (RNP) the brown mouse lemur has been confirmed to disperse mistletoe seeds; the characteristic sticky appearance of mouse lemur feces containing intact mistletoe seeds was seen on forest substrates, such as on the trunks of trees (Atsalis, 2008). In the New and Old World, mistletoe fruits are considered by some to be almost exclusively consumed by birds and therefore essential to their seed dispersal (Godschalk, 1985, Reid et al., 1995). Because mammals are rarely dispersers of mistletoes (Amico & Aizen, 2000), the discovery of a close relationship between mistletoes and brown mouse lemurs is significant. Epiphytes play an important role in forest nutrient cycling (Nadkarni, 1983). Thus, mouse lemurs may play an important role in the ecological dynamics of forests in Madagascar.

The diet of *M. rufus* (the brown mouse lemur) in relationship to resource availability was studied intensively by Atsalis (1999, 2008) in RNP. As in other similar studies, plants were checked monthly for flower and fruit production, and insect abundance was monitored through various collection methods. To determine diet, fecal samples from live captured mouse lemurs were examined and radiocollared individuals were followed in the forest. Even in this rainforest with annual precipitation of over 4000 mm, the dry season is characterized by relative scarcity in fruit and insect abundance (Atsalis, 2008, Hemingway, 1995, Overdorff, 1991). Atsalis found that *M. rufus* relied heavily on fruit, eating possibly as many as 75 species and they increased the quantity and diversity eaten during rainy season when fruit availability was high. This period also corresponded with seasonal fattening in preparation for the depletion of resources that occurred during the dry season, and with the time when the young began to feed independently. In contrast to fruit consumption, insect feeding did not increase during the rainy season when insect abundance was at its highest, but as in *M.murinus*, beetles were a regular part of the diet. At the same location (RNP), the brown mouse lemur relied heavily on the fruits of several varieties of the mistletoe *Bakerella*, which occurred in small patches but was widely distributed in the forest (Atsalis, 1999b, Atsalis, 2008). Mistletoes are considered to be highly nutritious fruits that are dispersed by specialized frugivores (Godschalk, 1983, Howe & Estabrook, 1977, McKey, 1975). The small berries of *Bakerella* were found to be high in fat content and fat has twice the energy content of the
carbohydrates typical of fruits. Based on this, Atsalis (1999) proposed that *Ba-kerella* served as a keystone and staple resource to this population of mouse lemurs because they ate it year round as well as during the dry season when resources were low in abundance.

Figure 2. Testicular volume increase in males and estrus opening in female mouse lemurs associated with the onset of the breeding season. The image on the left demonstrates the testicular expansion in male mouse lemurs that occurs prior to the mating season. The image on the right is of an open vulva in a female mouse lemur. As mouse lemurs are seasonal breeders, the females typically only have open vulvas, and hence are receptive to mating one night of the year.

1.2.2.3 Reproduction

Mouse lemurs typically become sexually active within the first year of their lives (Andriantsiferana et al., 1974, Martin, 1972, Radespiel et al., 2001, Glaston, 1979). Reproduction, like the seasonal changes in body mass and activity levels that occur in mouse lemurs, appears to be triggered by photoperiod changes. When day length begins to increase (and night shortens) at the end of the dry season, the vulval area in females begins to swell and male testes undergo a considerable enlargement (Figure 2) (Petter-Rousseaux, 1980, Perret, 1990, Schmid & Kappeler, 1998, Schwab, 2000, Dammhahn & Kappeler, 2005, Atsalis, 2008, Randrianambinina et al., 2003). A photoperiodic trigger of estrous in females and an increase in testicular volume in males was observed in
three species of mouse lemurs (*Microcebus murinus*, *M. rufus*, *M. ravelobensis*). Estrous was prolonged in *M. ravelobensis*, indicating the evolution of species-specific controls of female reproduction (Schmelting *et al.*, 2000, Randrianambinina *et al.*, 2003). A similar example of reproductive isolation in sympatry has been observed for *M. murinus* and *M. griseorufus* (Yoder *et al.*, 2002). Changes in morphology related to reproduction typically begin in September both in the east and west coast of Madagascar (Radespiel, 2000, Atsalis, 2008). In the west coast at the Ankarafantsika reserve, estrus took place between mid-September and early to mid-October and no second estus occurred (Radespiel, 2000, Radespiel *et al.*, 2006). As the majority of females captured in late October were gestating, mating must have occurred in September (Radespiel, 2000). Similarly, in the eastern rain forests at Ranomafana National Park *M. rufus* females were found to be in estrus between mid-September and mid-October, and a marked increase in male testicular volume began in August and continued throughout the mating season (Blanco, 2008, Atsalis, 2008).

During estrus in mouse lemurs, the female’s vulva is open for only 1 day of the year and copulation must occur during that period (Atsalis, 2008, Blanco, 2008, Wrogemann *et al.*, 2001, Eberle & Kappeler, 2003). Recent evidence in wild *M. rufus* has suggested that if a female loses her offspring it is possible for her to enter a second estrus, an example of “polyestry” (more than one estrus in a year) (Wrogemann & Zimmermann, 2001, Blanco, 2008, Radespiel *et al.*, 2001).

Female mouse lemurs usually have more than one infant per birth, producing 1-3 offspring in *M. rufus* and 1-4 in *M. murinus*, though the average litter size for both species is two infants (Radespiel *et al.*, 2002, Foerg, 1982, Martin, 1972, Wrogemann & Zimmermann, 2001). With these life history traits, the potential for rapid population growth is considerable. Goodman (1992) estimated that within a base population of 2100-2300 mouse lemurs at Beza Mahafaly reserve, if two infants are successfully weaned per female per year, the population would increase to 4200-4600 by the following year. Thus, even a loss of 580 lemurs due to predation per year would not confer population decline.

Mouse lemurs have a weaning period of about forty days (Colas, 1999, Glaston, 1979). Recent studies have also elucidated the fact that some mouse lemurs have rare breeding patterns, such as cases of multiple paternity within litters in *M. murinus*. Using genetic paternity analysis on known individuals in both the wild and captivity, Radespiel (2002) found that infants born in the same litter are sometimes found to be fathered by two or more different males suggesting that successful multiple mating occurs (Andres *et al.*, 2003, Radespiel *et al.*, 2002).
1.2.3 Aging mouse lemurs in captivity

In 1953, a grey mouse lemur (Microcebus murinus) breeding colony was established in the Museum National d’Histoire Naturelle in Brunoy, France by A. Petter and J.J. Petter. The colony descends from a stock of mouse lemurs captured in the southwestern coastal areas of Madagascar (Perret & Aujard, 2001). While these mouse lemurs were originally brought into captivity to study reproduction and thermoregulation, the discovery of human like symptoms of senescence in these small primates occurred. In captivity, grey mouse lemurs are found to live up to 12-15 years of age (Perret & Aujard, 2005), more than 6 times longer than the similarly sized mouse. In addition to their extended longevity, in captivity grey mouse lemurs also develop age-related pathologies and symptoms of senescence that are comparable to those found in human aging. Some of these changes include neurodegenerative plaques and tangles synonymous with human Alzheimer's diseases, whitening of the hair, ocular degeneration and cataract development, declines in sensory perception, locomotor stagnation, and age related changes in hormones. These symptoms begin to occur as early as 3 years in captivity, and past the age of 5 over 20% of mouse lemurs exhibit neurodegeneration. Because most of the symptoms of senescence occur between 4-5 years of age, animals that are 5 years of age in captivity are considered elderly.

Early research on this captive population (Perret, 1997) demonstrated the importance of photoperiod on mouse lemur lifespan and reproduction. The daily light cycle can be modified such that the annual breeding cycle can be shortened to 8 months, exhibiting a way to not only “accelerate time” in captive mouse lemur colonies, but also to stimulate multiple breeding cycles in these animals that typically only mate once or twice a year. Additionally, Perret and colleagues found that by altering photoperiod, captive grey mouse lemurs could be made to age prematurely. These animals exhibit the age-related brain and morphological pathologies that are seen in aged individuals in normal light cycles. However, altering the light cycles in the reverse manner does not appear to extend the lifespan of these lemurs.

1.2.3.1 Brain Pathologies

Aged mouse lemurs in captivity exhibit cerebral pathologies that are comparable to those found in elderly humans (Bons et al., 1995). Dhenian and colleagues (1997) have demonstrated by using magnetic resonance imaging (MRI) that aged mouse lemurs experience brain atrophy and cerebral iron deposits and amyloid plaques, as well as morphological changes in the cortex, corpus callosum, thalamus, hypothalamus, brainstem and ganglion with age (Bons et al., 1991). Additionally, amyloid deposits are seen in the arterioles and capillaries in the brain of 60% of adult animals. The cholinergic system shows the most
obvious age related degeneration among neurotransmitter systems studied in the mouse lemur brain. The number of cholinergic neurons declines dramatically with age, and the neurons that remain often exhibit cytological damage (Mestre & Bons, 1993, Jallageas et al., 1998). The number of lesioned neurons also increases with age, and in mouse lemurs aged 8 and older, Tau protein densities in the brain are comparable to those seen in human patients with Alzheimer’s disease (Giannakopoulos et al., 1997).

Approximately 20% of aged mouse lemurs experience severe changes in behavior including aggressiveness, loss of social contact, and the inability to perform cognitive tasks in a test setting. These animals develop an age-associated neurodegeneration, comparable to human Alzheimer’s disease, which is called „Microcebus age-associated neurodegeneration” or MAAN (Bons et al., 2000, Jallageas et al., 1998).

1.2.3.2 Hormonal changes

In humans, plasma concentration of the androgen precursor dehydroepiandrosterone sulfate (DHEA-S) declines with age and is considered as a potential predictor of longevity in males (Labrie et al., 1997, Mazat et al., 2001). In captive grey mouse lemurs aged from 8 months to 11 years, plasma DHEA-S levels were measured, and similar to the trends found in humans, these DHEA-S values showed significant changes with age. The age-related decline of DHEA-S levels occurred after the age of 3 years and accelerated after the age of 6 years. DHEA-S levels were 30–40% of their adult values past 6 years of age. This pattern closely resembles the pattern seen in human males, perhaps further suggesting DHEA-S as an aging biomarker. Using that data to extrapolate the DHEA-S trend, Perret and Aujard find the intercept to be at 13.2 years of age, an age which is close to the maximum lifespan seen in captive mouse lemurs (Perret & Aujard, 2005). Similarly, plasma testosterone levels in males were found to show significant changes with age, increasing from the first year of life to the 5th year, after which testosterone levels plummet to 30-40% their adult values. The ages at which these hormonal shifts occur heavily coincide with the onset of captive senescence, suggesting the age of 5 to be the turning point in captive mouse lemurs at which point a plethora of senescent physiological changes occur.

1.2.3.3 Morphological and behavioral changes

In addition to brain-related changes in aged mouse lemurs, changes in the body appearance are quite readily seen in mouse lemurs from the age of 4 years onwards (Bons et al., 1991). For example, aging mouse lemurs often experience graying and whitening of the hair. This usually begins at the head and then runs down the abdomen. Old lemurs often also experience an increase in body
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weight and decrease in mobility (Bons et al., 1991, Perret & Aujard, 2001). This decrease in mobility is often so extreme that the lemurs rarely even jump (Bons et al., 1991).

Another change that is seen in aged mouse lemurs in captivity is a decrease in visual acuity and olfaction. The visual decline has been attributed to retinal degeneration and bilateral cataracts, which begin as early as 3 years of age (Eberle & Kappeler, 2003, Bons et al., 2000). And the decrease in olfaction abilities, or anosmia, has been attributed to the structural degeneration of olfactory brain structures with age (Mestre et al., 1992, Bons et al., 2000). Captive mouse lemurs also experience a significant loss of hearing from the age of 7 years (Chabert & Bons, 1998, Niaussat & Petter, 1980).

In some mouse lemurs an age related behavior shift is seen. This subset of lemurs demonstrates a behavior indicative of ‘pathological aging’. These lemurs remain secluded in the corners of the case, and often become very aggressive towards other lemurs, requiring them to be placed in some sort of isolation (Bons et al., 1991).

These studies have shown mouse lemurs to be a promising model to understand human aging. Given their small size and phylogenetic proximity to humans they may also provide an interesting biological model organism for other human pathologies in the future.

1.3. Studying factors related to aging in the wild

Historically, it has been quite difficult to study the aging process in wild organisms, primarily due to their unknown histories. However, there are several morphological and physiological changes that occur with age that may be used to better understand aging in a natural context. These include things like dental wear, declines in immune function, and the age-related shifts in hormone levels.

1.3.1 Age estimation using dental wear

One major obstacle in examining the aging process in wild animals is the difficulty in determining the ages of animals without a known birth date. One method that has been used to overcome this obstacle is through dental wear assessments. Dental wear primarily occurs on the occlusal surface of the tooth through tooth-tooth and food-tooth contact. Prolonged tooth wear occurs with chronological age and can therefore be used as a biomarker to predict the ages of wild mammals for which birth dates are unknown.
1.3.1.1 Teeth

“And there is nothing that an evolving animal worries about more than how his teeth are getting along.”
Roy Lewis: The Evolution Man

Mammalian teeth are hard structures anchored into the jaws of the mouth that prepare food for digestion. The part of the tooth that is exposed in the mouth is known as the tooth crown, and tooth crown surfaces are variable in their working surfaces. Teeth are typically comprised of two primary layers of tissue: dentine, and enamel, a hard shiny layer of tissue that coats the dentine (Figure 3). There are four types of teeth in mammals: incisors, canines, premolars and molars (or cheek teeth). Teeth are durable structures and often the only remaining features found in fossil record due enamel hardness, therefore understanding associations between dietary habits and tooth morphology, tooth wear, and physical tooth properties can provide insight into reconstructing past environments and understanding the ecology of extant populations.

Teeth have different shapes, and the occlusal tooth surface morphology of an animal can often provide rich insight into dietary habits. This is because certain food sources are more easily digestable than others and hence require less fracturing through mastication. For example, herbivorous animals feed on plant parts, which can be difficult to digest and hence require extensive breakdown by the teeth prior to entering the gut. Because of this, herbivores tend to have complex teeth with many cusps, crenulations and shearing crests that have adapted to break down tough plant parts (Evans et al., 2007). One unique example among herbivores are the bamboo feeding mammals. Unlike their grass eating counterparts, bamboo feeders have low crowned teeth; however, these teeth have become very complex, presumably to chew on the fibrous bamboo plant tissue. Most carnivorans on the other hand have relatively simple teeth (less complex) because animal matter is easier to digest than plant matter and requires less physical breakdown in the mouth.

1.3.1.2 Using teeth to estimate age

While mark-recapture techniques in wild populations provide minimum estimates of ages, additional methods are required to obtain accurate age tabulations for all individuals. One way to determine the ages of known and previously unknown individuals is through dental wear.
Figure 3. The anatomy of a tooth. In this schematic cross section of a tooth, the layers of dental tissue are shown. The crown is the part of the tooth that is exposed in the mouth and is covered by enamel. Underneath the enamel is the dentine layer. The gum (gingiva) forms a roof on top of the periodontal ligament, which is the soft tissue of the tooth socket. The ligament is full of collagen fibers anchored in the cement of the tooth and the bone of socket wall. The tooth is sustained by blood vessels that enter through holes in the tooth roots. This blood supply feeds the cells that form the dentine layer. Nerves that supply the periodontal ligament also pass through the tooth.

The upper and lower teeth of non-mammalian vertebrates rarely ever make contact, and they predominantly wear through abrasion when they come into contact with food. Mammalian teeth, however, require upper and lower teeth to come into contact in order to break apart food particles. When this occurs over time, the teeth wear down, and the tooth crown features can be obliterated, decreasing the efficiency of nutrient intake (King et al., 2005, Lanyon & Sanson, 1986, Logan & Sanson, 2002). The effects of long term tooth wear have been shown in the ringtail possum (Gipps & Sanson, 1984), and in koalas where the decrease in digestive efficiency also impairs the chance of males finding a mate.
(Lanyon & Sanson, 1986, Logan & Sanson, 2002). This decline in dental functionality has been coined *dental senscence* (King et al., 2005) and may jeopardize an animal’s health or reproductive success.

King et al. (2005) showed in sifakas (*Propithecus edwardsi*) that while tooth wear causes overall decreases in tooth crown relief, three dimensional shearing crests lengths are maintained until extreme stage of wear. Furthermore, aspects of crown features, most notably exposed dentine area, show a linear change with age (Radespiel et al., 2006, King et al., 2005). Because the area of exposed dentine increased linearly with age, this measurement could be used to predict the ages of individuals in this wild population.

Additional studies have used changes in tooth crown height and/or in exposed dentine to predict the ages of a many different wild taxa (Phillips-Conroy *et al.*, 2000, Dennis *et al.*, 2004, King *et al.*, 2005, Cuozzo & Sauther, 2006, Galbany *et al.*, 2011, Skogland, 1988, Hewison *et al.*, 1999). While most of these studies were conducted in relatively large mammals ranging from koalas, wolves, and reindeer to baboons, howler monkeys and chimpanzees, the potential for use in smaller trappable animals has yet to be explored.

Indeed, there is a long history of using tooth wear to estimate age in mammals in their natural habitats. The combination of repeat captures and dental impressions has allowed for the calculation of dental wear rates in different mammals, providing a relatively accurate estimator of age in the wild.

**1.3.2 Parasites, immunity, and aging**

“According to one estimate, parasites may outnumber free-living species four to one. In other words, the study of life is, for the most part, parasitology”

*Carl Zimmer*

In natural habitats, parasites can often be considered to be the top predators of wild animals. Almost all organisms are parasitized in one way or another. Sometimes the parasites can be extremely pathogenic causing illness and death, while others are asymptomatic, causing little or no harm to the host, or in some cases even benefitting the host’s fitness. Parasitology often refers to the study of helminths (flatworms and roundworms), protozoans, and ectoparasites, leaving the fungal, bacterial, and viral work to the field of Microbiology.

Parasites and their hosts often co-evolve together very intimately, sometimes even leading to morphological adaptations specific to one another (Poinar Jr & Poinar, 1998, Barker, 1994). In some cases the parasite even alters the behavior or morphology of the host to pass on to its definitive host (Webster, 2007, Van Den Abbeele *et al.*, 2010, Levri, 1999).
Susceptibility to parasitic infection increases with age through the process of immunosenescence, an age-specific deterioration in the efficiency of the immune system (Tarazona et al. 2002). While this subject has been addressed in humans and in laboratory models (Gruver et al., 2007), only a few studies in natural populations have breached the topic. In these studies on wild populations of birds and mammals parasite loads, used as an indirect measure of immunity, were found to increase with age (Hayward et al., 2009a, Palacios et al., 2007b, Cichon et al., 2003, Pelletier, 2005) providing evidence of immunosenescence in natural populations.

These interactions and specializations make parasite ecology a crucial study when trying to understand an animal in its natural habitat. Often ecologists examine the world from the host’s perspective taking into account interactions with parasites and parasite prevalence. However, it is also important to conduct parasite studies from the parasite’s perspective with the host as a skipping stone on the way to a parasites definitive host or reproductive stage of its life cycle.

1.3.2.1 Endoparasites

Endoparasites typically refer to parasites that live within the body of the host. These are primarily protozoans or helminths that feed off of and gain nutrition from living inside the host. Some of the most lethal human diseases are caused by protozoan endoparasites such as malaria, schistosomiasis, African sleeping sickness, and many more (Zhu et al., 2011, Steverding, 2008, Liu et al., 2010, Ford, 2007, Ekpo et al., 2010) Helminths on the other hand tend to cause less pathogenic diseases and can live in their hosts for up to 70 years while remaining asymptomatic (Figueiredo et al., 2010).

1.3.2.2 Ectoparasites

Ectoparasites are parasites that reside on the outside of the host’s body and obtain nourishment from the outer surface or from penetrating bites into the host. While not all ectoparasites are blood feeders, a few are obligate blood feeders that feed solely on the host’s blood. In humans, these include arthropods like sucking lice, bed bugs, South American kissing bugs (assasin bugs), and fleas. These obligate hemathophages typically can not reproduce or survive without feeding on host blood. Some other ectoparasites are optional blood feeders, only feeding on blood occasionally, and these include insects like mosquitoes and ticks. Due to regular contact with host blood and regular host switching, many hematophagous ectoparasites are also vectors of medical or veterinary significance. Their ability to carry, transfer, and sometimes even support the development and growth of blood parasites can make them lethal to their hosts.
Ectoparasites loads have been found to directly affect fitness and survival in multiple taxa, including fish, lizards, birds, and mammals (VanVuren, 1996, Tschirren et al., 2007, Lehmann, 1992, Owen et al., 2009, Cheney & Cote, 2003b, Cheney & Cote, 2003a, Uller & Olsson, 2003). The cost of maintaining heavy ectoparasite loads often results in increased juvenile mortality, decreases in body mass and growth rates, and smaller clutch/litter sizes (VanVuren, 1996, Tschirren et al., 2007, Lehmann, 1992, Owen et al., 2009, Cheney & Cote, 2003b, Cheney & Cote, 2003a, Uller & Olsson, 2003). While most of the fitness costs imposed by parasites affect juveniles, the costs seen in adults include reduced reproduction, decreased litter/clutch size, lower survival rates during hibernation periods (Møller & Erritzøe, 2000, Atkinson, 1991, Lehmann, 1992, VanVuren, 1996). The results thus far providing evidence that higher mortality of juveniles compared with adults due to parasitism may be the common rule in nature. It is these results that suggest that age-related changes in ectoparasite loads may contribute significantly to the survival rates and maximal lifespan of wild animals.

1.3.2.2.1 Sucking Lice

Lice can be divided into two taxonomic groups, sucking lice (Anoplura) and chewing lice (Mallophaga). These insects are small, wingless, and dorso-ventrally flattened with sclerotized plates on the abdomen that provide rigidity when the abdomen becomes distended during feeding bouts. As hemimetabolous insects, lice have an egg stage and three nymphal instar stages, the last of which molts into an adult. Sucking lice are obligate, hematophagous ectoparasites of placental mammals and are known to parasitize 12 of the 29 mammalian orders (Light et al., 2010, Durden & Musser, 1994). Whereas chewing lice can spend time off of their hosts and can transfer to new hosts phoretically (via intermediate organisms such as flying insects) or fomitically (via inanimate objects) sucking lice have evolved a morphology that is highly specialized for life on their hosts (Durden & Musser, 1994), including features that permit secure attachment to host hair and feeding directly upon host peripheral vasculature (Figure 4). This specialization confers considerable advantages to sucking lice while they are on the host; however, it also restricts the amount of time lice can spend off a host to just a few hours (Balashov, 2009). This limitation necessitates that louse transfers between hosts occur when two or more host individuals come into direct contact.

As arthropods that feed on blood, sucking lice come into contact with blood borne pathogens, and have been found to act as vectors of various pathogens.
Figure 4. Morphological features of sucking lice (Anoplura) A. Ventral view of an adult female sucking louse B. The feeding tubes (stylets) protruding from the mouth (haustellum) pierce the host’s blood vessels to begin imbibing blood C. The tibio-tarsal claw morphology is specialized for secure attachment to host hair.

For example, sucking lice can transmit tapeworm eggs to dogs, filarial nematodes to waterfowl, and various bacterial pathogens to livestock (Bartlett & Anderson, 1987, Hornok et al., 2010). Historically, sucking lice have been responsible for widespread epidemics (trench fever, epidemic typhus, epidemic relapsing fever) in human populations (Roux & Raoult, 1999). Nowadays with improved sanitation these outbreaks occur less frequently in developed countries. However, with the occurrence of global population increase and overcrowding in developing countries, sucking lice present a potential source of public health concern.

The role of sucking lice in wildlife is understudied, and to date, only one study has examined the movement of lice in a wild population (Durden 1983) and none have assessed the role of these arthropods as vectors of pathogens.
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These data suggest that the features unique to sucking lice make them an organism of significant medical and veterinary relevance that can provide information about the epidemiology and ecology of human and wildlife populations.

1.3.2.3 Sex differences in immunity


1.3.2.3.1 Innate immunity

Innate immunity represents the first line of defense against parasites. This type of immunity is found in almost all multicellular organisms. Because these responses do not require prior exposure or sensitization, they can be initiated immediately following exposure to a novel parasite. Males and females differ in their innate immune responses, suggesting that some sex differences may be germline encoded. Studies of both humans and rodents illustrate that inflammatory immune responses are generally higher in females than males and may explain why women are more likely to develop inflammatory rheumatic diseases, such as rheumatoid arthritis and systemic lupus erythematosus, than men (Da Silva, 1995). Female mice also exhibit stronger delayed-type hypersensitivity reactions to Schistosoma mansoni than males (Boissier et al., 2003). The number and activity of cells associated with innate immunity differ between the sexes. Phagocytic cells, including macrophages and neutrophils, can kill parasites by generating reactive oxygen metabolites and nitric oxide, as well as by secreting enzymes. Among humans and lizards, the phagocytic activity of neutrophils and macrophages is higher in females than males (Spitzer, 1999, Mondal & Rai, 1999). Natural killer (NK) cells also represent a critical first line of defense against parasites. Women with regular menstrual cycles as well as women tested during the luteal phase of their menstrual cycle were found to have lower NK cell activity than men (Yovel et al., 2001, Souza et al., 2001). Studies of mice have shown that estradiol can reduce both the number and activity of NK cells (Hanna & Schneider, 1983). Antigen-presenting cells (APC) from females are more efficient at presenting peptides than are APC from males (Weinstein et al., 1984). Following infection of the central nervous system, MHC class II expression on astrocytes, endothelial cells, and microglia is enhanced in female compared with male mice (Barna et al., 1996).
Taken together, these data suggest that the activation of innate immunity differs between males and females. Whether the sexes differ in their reliance on innate immunity to defer parasitic infections has not been well documented and represents an important area for future research.

### 1.3.2.3.2 Adaptive immunity

The innate and adaptive immune systems work together, and the functionality of one is often dependent on the other (Luster, 2002). Adaptive immunity relies heavily on T and B cells, however without the chemokine signaling that links the activation of the innate immune cells with the delivery of an adaptive immune response, T and B cells would not be able to function properly. Similarly, the innate immune system would likely suffer and be unable to allocate tasks without the adaptive immune system in vertebrates (Hoebe et al., 2004). Therefore these two systems are reliant on one another and work in tandem to handle the pathogens of vertebrates.

Primary humoral immune responses (e.g. antibody production by B-cells) are typically greater in females than males (Gomez-Guerrero et al., 1993, Falter et al., 1991). In mice infected with the parasite *Giardia muris*, females have lower infection rates and produce more antibodies than males, suggesting a functional advantage for increased humoral immunity in females (Daniels & Belosevic, 1994). Cell-mediated immune responses also differ between males and females. T-cells, in particular CD4+ helper T-cells (Th cells), are functionally and phenotypically heterogeneous and can be differentiated based on the cytokines they release. Reliance on subsets of Th cells (i.e. Th1 or Th2 cells) to overcome infection differs between males and females, with females reportedly exhibiting higher Th2 responses (i.e. higher IL-4, IL-5, IL-6, and IL-10 production) than males (Roberts et al., 2001, Bijlsma et al., 1999). Female rodents also have higher mitogen-stimulated lymphocyte proliferation, faster wound healing, and increased immunological intolerance to foreign substances than males (Krzych et al., 1981, Graff et al., 1969, Blankenhorn et al., 2003). Sex differences in Th cell responses may mediate sex differences in response to infection. After experimental inoculation with coxsackievirus, male mice primarily generate Th1 phenotypic responses and females exhibit predominantly Th2 phenotypic responses (Huber & Pfaeffle, 1994). Treatment of males with estradiol and females with testosterone prior to coxsackievirus infection reverses the Th responses (Huber & Pfaeffle, 1994). In humans, however, women generate a stronger delayed-type hypersensitivity response (i.e. Th1-related), whereas men have higher IgE concentrations (i.e. Th2-related) following infected with *L. mexicana* (Lynch et al., 1982). Taken together, these data illustrate that sex differences in cytokine responses to infection play a critical role in determining susceptibility to parasites.
1.3.2.4 Immunosenescence

Humans are living longer than they ever have before, and experiencing ages not
seen in previous centuries. Along with old age, the elderly are experiencing a
plethora of diseases and immune deficiencies never seen before and an in-
creased risk of infection, autoimmune disease and cancer, resulting in high mor-
bidity and mortality rates among the elderly (Pawelec et al., 2002, Miller, 1996,
Effros, 2003). The deterioration of immune function with age is a phenomenon
known as immunosenescence. Most knowledge about immunosenescence
comes from studies of humans or mammalian laboratory models. The findings
from these suggest that the aging immune system occurs when many compo-
nents of the immune system begin to demonstrate altered activity (Pawelec et
Often, immunosenescence affects adaptive immunity (Weksler & Szabo, 2000,
Aspinall, 2003), although certain components of the innate immune system
(such as functioning phagocytes) may also decline with age (Pawelec et al.,
1998). On the other hand, there is evidence that other components (such as in-
flammation) may actually increase in functionality with age (Franceschi et al.,
2000).

In recent years the concept of immunosenescence has been expanded to studies
in both vertebrates (Ujvari & Madsen, 2006, Saino et al., 2003, Palacios et al.,
2007a, Lozano & Lank, 2003, Hayward et al., 2009b, Haussmann et al., 2005,
Cichoń et al., 2003) and invertebrates (Kurtz, 2002, Adamo et al., 2001) in
their natural habitats. While these studies have found that immunosenescence
does appear to occur and may even be a common phenomenon in free-living
organisms, the actual mechanisms behind this decline in functionality are still
widely unknown.

There are many potential explanations for immunosenescence, these include
hormonal changes with age, the effect of oxidative stress on the immune sys-
tem, and telomere shortening in immune cells (Panda et al., 2009). However,
one of the most widely accepted hypothesis implicates the involvment of the
thymus gland or thymic atrophy in old age, and hence the decline in production
of T cells, as the mechanism behind this decline in immune function and in-
creased susceptibility to infection (Pawelec et al., 1995). This is believed to be
an example of antagonistic pleiotropy, a concept which suggests that natural
selection has favoured genes conferring short-term benefits at the cost of dete-
rioration in later life. If this is the case, the immune system is selected to serve
individuals only until reproduction. After that, biochemical processes proceed
freely without past selective pressure to improve the life of an individual.
Thymic involution in late age supports this hypothesis; however, the true
mechanisms behind the functional decline in immunity with age are still widely
unknown. Incorporating studies on various taxa in both captive and wild conditions may better elucidate our understanding of this phenomenon.

1.3.3 Hormones and aging

Hormones vary in function from actively playing a role in sexual maturation and reproduction to mediating stress. Regardless of their roles, hormones function primarily in the body’s homeostasis, or maintenance of equilibrium or stability. Their role is to adjust metabolic imbalances and restore them to homeostatic levels. However, hormones may also have negative effects. Too much or too little of specific hormones can be deadly, and slight imbalances can lead to chronic illnesses like diabetes or severe immune dysfunction. Because of their role in development and sexual maturation, changes in hormone levels are often seen with age, and the effects of these age-related changes in hormones can dramatically influence the overall health of an individual.

1.3.3.1 Androgens

Androgens are steroid hormones responsible for the growth and development of male sex organs, masculine characteristics, and male reproduction by promoting spermatogenesis. Androgens however are present and active in both male and female organisms. The most commonly known androgen is the hormone testosterone (T), which is produced in the gonads. In females T is aromatized into estrogens (female sex hormones) and hence is important in both sexes. Only in recent years have researchers begun to explore the functional roles of androgens in females. It is commonly assumed that T levels are higher in males than in females, however recent research in female dominant mammals such as the rock hyrax, the Milne Edward’s sifaka, and mouse lemurs in this thesis have exposed that the males of a species do not always have higher T than females (Tecot, 2010, Koren et al., 2006).

Another androgen, which is synthesized in the adrenal glands from cholesterol, is dehydroepiandrosterone (DHEA). In humans, this is the most abundant androgen in the body, as it is a precursor to androstenedione, T, and the estrogens (Shealy, 1995). The sulfated version of this hormone (DHEA-S) is catalyzed in the liver and adrenal glands. Human studies of DHEA-S, have found it to increase exponentially during sexual maturation at adrenarche, and past the age of 30, declines in a predictable rate in men. Because of this age related decline, DHEA-S has been proposed to be a predictor of mortality in human males. Finally, the androgen dihydrotestosterone (DHT), produced in the adrenal cortex, is a metabolite of T which is more responsible for masculinization than T. DHT binds to androgen receptors with a higher affinity than T does, and is the hormone responsible for male secondary sexual characteristics. Unlike T, DHT
does not aromatize into estrogens and has therefore been used in studies examining male secondary sexual characteristic development.

1.3.3.2 Glucocorticoids

Glucocorticoids, such as the hormone cortisol (C), are steroid hormones secreted in parallel with the „fight-or-flight” stress response, and are commonly measured indicators of stress in vertebrates (Wingfield & Romero, 2001). These hormones are involved in the sustained response of the body to a stressor (Kudielka & Kirschbaum, 2005), and they control the expression of up to 1% of genes in the human genome (Janeway, 2001). In mammals, glucocorticoids decrease glucose transport and metabolism in peripheral tissues, reduce the permeability of the blood–brain barrier to glucose, initiate gluconeogenesis, increase protein catabolism and serum glucose levels, and are involved in immunosuppression and reduced inflammation (Janeway, 2001, De Leon et al., 1997, McEwen & Seeman, 1999). Glucocorticoids divert energy from long-term storage to cope with immediate crises (Sapolsky et al., 2000), and elevated glucocorticoid levels have been observed in animals facing both natural challenges (Tecot, 2007, Goymann et al., 2001, Foley et al., 2001, Alberts et al., 1992) and anthropogenic environmental disturbances (Wingfield & Romero, 2001, Wasser et al., 1997, Creel et al., 1997). Because high glucocorticoid levels suggest the presence of environmental threats, and chronically high glucocorticoid levels are themselves associated with health risks (Dhabhar & McEwen, 2001), it is believed that high glucocorticoid levels indicate lower individual fitness or population viability (Pride, 2005). Since glucocorticoid levels fluctuate in response to environmental challenges, they provide a spatio-temporally fine-grained measure of the stress landscape animals’ experience. If glucocorticoid differences indicate individual differences in survival, then glucocorticoid patterns could reveal even short-term or subtle selection pressures that would otherwise be difficult to detect. Because they can be measured in feces, glucocorticoids provide a powerful non-invasive tool for evolutionary ecologists (Hofer & East, 1998).

1.3.3.3 Hormonal changes with age

Hormones influence physiological processes such as growth, immune, and reproductive functions of the body. In many cases, the endocrine physiology of infancy significantly differs from that of adolescence and old age. Life history changes in the secretion of hormones can potentially result in significant modifications to both physiology and behavior, impacting the individual’s ability to survive and reproduce. Although a part of the natural ontogenetic process, such alterations in neuroendocrine functioning may be especially felt by wild animals because changing demands for adaptation may more constantly challenge them. The relative ease or difficulty with which animals achieve the physiological and
behavioral modifications necessary for social and ecological adjustment may vary according to age, thus contributing to unequal risks of morbidity and mortality. Developmental stages involving increased androgen secretion mark the maturation of both the hypothalamic–pituitary–adrenal (HPA) and –gonadal (HPG) systems. Both androgens and glucocorticoids have been linked to the psychophysiological correlates of aging. For example, the loss of visuospatial ability, processing speed, learning, memory, and other cognitive functions are associated with declining T in aging men (Martin et al., 2007). In their examination of men in their mid 30s to men in their 80s, Martin et al., (2007) found advancing age to be associated with reductions in total and biologically available levels of T and its hypothalamic–pituitary precursors. Despite a further association with impoverished learning, executive, and memory functions, Martin et al., (2007) additionally found losses in total and free T among men of middle and old age to be connected with enhanced processing speed.

1.3.4 Hormonal mediation of parasite infection

Aside from maintaining homeostasis, certain hormones play crucial roles in immunity. In most animals, sex differences in parasite infection are found and these differences are often attributed to sex differences in hormones, or social behaviors that are influenced by hormones. Some reasons behind these sex differences, and the specific interactions between certain hormones on the immune system are discussed further below.

1.3.4.1 Sex differences

Among human and non-human animals, the prevalence and intensity of parasitic infections is higher in males than females (Zuk & McKean, 1996, Roberts et al., 2001, Klein, 2000). Sex differences in exposure as well as susceptibility to parasites probably contribute to sex-based differences in the intensity and prevalence of parasites. Several field and laboratory studies link sex differences in immune function with circulating steroid hormones (Zuk & McKean, 1996, Roberts et al., 2001, Klein, 2000, Raouf et al., 2006, Pedersen & Greives, 2008, Evans et al., 2000, Muehlenbein, 2006). Increased susceptibility to infection is thought to be one of the leading causes of increased death rates among elderly men as compared with women (Owens, 2002). Studies of vertebrates in natural habitats illustrate that the prevalence and intensity of parasitic infections are often higher in males than females. The prevalence and intensity of infection with Leishmania, Plasmodium, Entamoeba, Necator, and Schistosoma parasites, for example, is higher among human males when compared to females (Klein, 2004). Although clinical studies of humans and field studies of non-human animals are suggestive, there are many factors, such as exposure rates, social behavior, habitat, and diet, which cannot be held constant and could contribute to the observed sex differences in parasite infection. Studies of rodents in a controlled laboratory
setting, however, revealed that sex differences may be mediated by immune-endocrine interactions.

Although males are more susceptible to a majority of the studied parasite infections, exceptions are noted (Morales-Montor et al., 2004). The prevailing hypothesis for the immunological and parasitological differences between the sexes is that sex hormones, in particular, T, estradiol and progesterone, influence the immune system and alter immune competence. Immune cells, including lymphocytes, macrophages, granulocytes, and mast cells all have sex hormone receptors. This illustrates that there are direct connections between the endocrine and immune systems and that endocrine factors can, theoretically directly modulate the expression of target genes in immune cells. A considerable amount of research has aimed to explain sex steroid hormone actions in the immune system and how these influence sex differences in healthy and diseased conditions (Klein, 2000). Although sex differences in the synthesis and release of sex steroids are hypothesized to underlie discrepancies in responses to infection, it remains unclear whether it is the sex steroids alone that explain all sexual variation in responses to infection.

1.3.4.2 Mediation of parasitic infection by androgens

Sex differences in parasitic infection are partly mediated by the effects of androgens, including dihydrotestosterone (DHT) and T, on the immune system (Roberts et al., 2001, Olsen & Kovacs, 1996). Androgen receptors have been identified in many different lymphoid tissues, including the thymus, bone marrow, and spleen as well as in immune cells (Wunderlich et al., 2002, Roberts et al., 2001, Cutolo et al., 1996). In laboratory experiments, exposure to T reduces natural killer (NK) cell activity in mice (Hou & Zheng, 1988). Similarly, when macrophages in mice are stimulated with T, the synthesis of pro-inflammatory products are reduced (D’Agostino et al., 1999). In contrast, T increases synthesis of anti-inflammatory cytokines, such as IL-10 (D’Agostino et al., 1999). The immunosuppressive effects of T may demonstrate the inhibitory effects of androgen receptor signalling mechanisms on the transcriptional factors that mediate the production of pro-inflammatory and anti-parasitic cytokines (McKay & Cidlowski, 1999). Alternatively, androgens may suppress different immune responses by increasing the expression and translation of heat shock proteins and factors that induce apoptosis (Jones et al., 2000, Hofmann-Lehmann et al., 1998, Vegeto et al., 1999). In another study of gonadectomized male rats (Rattus norvegicus) a reduced number of Angiostrongylus malayensis helminths were found, as well as increased numbers of circulating white blood cells, and heavier thymic mass than gonadectomized males that were injected with a synthetic T propionate (Kamis et al., 1992). Studies show that gonadectomized male mice demonstrate greater resistance to several protozoan parasites, including Leishmania major, Plasmodium berghei, and P. chabaudi when compared
to mice with intact gonads (Zhang et al., 2000, Mock & Nacy, 1988, Benten et al., 1992). In one study, the infection of Indian soft-furred rats (Millardia mel-tada) with the parasite Nippostrongylus brasiliensis resulted in a higher worm burden in gonadally intact males than in females or in castrated male rats (Tiuria et al., 1994, Klein, 2004). Similarly, gonad intact male reindeer (Rangifer t. tarandus) show higher incidence of warble fly (Hypoderma tarandi) infestation than both females and castrated males (Folstad et al., 1989).

**1.3.4.3 Mediation of parasitic infection by glucocorticoids**

Glucocorticoids, such as corticosterone in small mammals and cortisol in pri-mates, have the potential to influence sex differences in immune function. Glu-cocorticoid receptors have been identified throughout the immune system and on circulating T-cells, B-cells, and macrophages (Webster et al., 2002, Elenkov & Chrousos, 1999). High circulating concentrations of corticosterone suppress innate, cell-mediated, and humoral immune responses in laboratory mice and rats (Webster et al., 2002, Elenkov & Chrousos, 1999). Generally, glucocortico-ids suppress the synthesis of pro-inflammatory cytokines, including various in-terleukins (IL-1, IL-2, IL-6, IL-8, IL-11, IL-12), and enhance the production of anti-inflammatory cytokines, such as IL-4 and IL-10 (Webster et al., 2002, Elenkov & Chrousos, 1999). The immunosuppressive effects of glucocorticoids are mediated by the antagonistic effects on NFkB-mediated responses (McKay & Cidlowski, 1999). Glucocorticoids also cause lymphocyte apoptosis and the redistribution of lymphocytes from blood to organs such as lymph nodes, skin, and bone marrow (Klein, 2004, Dhabhar & McEwen, 1999, Brunetti et al., 1995). Laboratory studies in rodents have shown that basal corticosterone con-centrations are higher among females and rise more rapidly in females than males following exposure to stressors (Brunetti et al., 1995; Dhabhar and McEwen, 1999).

Males and females often differ in the amount of stress they experience and the types of stressors they encounter. Presumably, if infection disrupts homeostasis differentially between males and females, then the effects of glucocorticoids on immune responses may differ between the sexes. The role of glucocorticoids as mediators of sex differences in susceptibility to parasitic infection has only re-cently been explored and presents an important area of future research.

Androgens and estrogens have been the primary focus of studies examining sex differences in infection; however, several other steroid hormones, including glucocorticoids, growth hormone and prolactin, exhibit sexually dimorphic pat-terns of expression and may additionally influence the sex differences that are seen in infection. Because peptide hormones are directly affected by steroid hormone concentrations via feedback mechanisms, it is equally likely that sex differences in infection represent the interplay between peptide and steroid hor-
mones (Morales-Montor et al., 2004, Klein, 2004). Therefore, the endocrine system may mediate sex differences in infection through a variety of different mechanistic pathways.

1.3.4.4 Parasite mediation of host hormones

Not only can host hormones affect responses to infection, but parasites can have enormous effects on hormone signaling within the host. For example, male mice are more susceptible to two malaria species, *Plasmodium berghei* and *P. chabaudi*, than females (Wunderlich et al., 1991, Klein, 2004). Although several studies illustrate that host sex steroids influence the course of *Plasmodium* infection, additional studies suggest that in fact these protozoan parasites can alter hormone concentrations in their hosts. Infection of female mice with *P. berghei* increases concentrations of estradiol and progesterone and disrupts estrus cycles (Aina et al., 1990). Similarly, infection with *P. chabaudi* suppresses T concentrations in males (Barthelemy et al., 2004). Whether these hormonal changes following infection are mediated by the parasite or the host remains unknown. However, host castration by parasites is reported in both vertebrate and invertebrate hosts and is hypothesized to increase the availability of host resources for parasite growth and development (Baudoin, 1975). *Schistosoma mansoni* infection, for example, suppresses T production in male mice (Isseroff et al., 1986). Because elevated estrogen and suppressed androgen concentrations can facilitate immune responses against *Plasmodium* infection, these hormonal changes may be adaptive host responses against infection as opposed to examples of parasite-mediated castration.

In contrast, female mice are more susceptible to infection with the tapeworms *Taenia crassiceps* and *T. taeniaeformis* than males because estradiol is utilized by the worms to enhance parasite reproduction (Morales et al., 1996, Larralde et al., 1995). Interestingly, when male rodents are infected with *T. crassiceps* or *T. taeniaeformis* both serum and testicular testosterone concentrations are reduced, estradiol concentrations are increased, and mating behavior is inhibited (Morales et al., 1996, Larralde et al., 1995, Klein, 2004). *Taenia crassiceps* parasites also produce steroid hormones, including T (Romano et al., 2003). Because T can be aromatized into estradiol in the host, T production by the parasite may further facilitate growth and reproduction of the parasite and inhibit host responses to infection (Romano et al., 2003).

Taken together, these studies illustrate that the effects of parasites on host hormones may contribute to the observed (and often unexplained) variability in the expression of sex differences in response to different parasite species. To date, however, very few studies have examined the mediation of host hormones by parasite; however, current available technologies make this an attractive potential future field of research.
1.4 Study site: Ranomafana National Park

Ranomafana National Park (RNP) is located in the Fianarantsoa province in southeastern Madagascar approximately 43,500 hectares of continuous rainforest (Figure 5) at altitudes varying between 500 and 1500m, with the majority of the park between 900 and 1200m. The rainforest is montane and contains relatively undisturbed lowland rainforest, cloud forests and high plateau forests (Grenfell, 1995). The park is surrounded by a 3km wide buffer zone. The park was established in 1991 by Dr. Patricia Wright, after the discovery of a new species of lemur (Hapalemur aureus) and the rediscovery of a lemur previously thought to be extinct (Prolemur simus) in 1986 (Meier et al., 1987, Wright et al., 1987).

The establishment of the Centre ValBio research station in 2003 expanded research capabilities by providing electricity and laboratory equipment in a rainforest setting. In addition to acting as a research station Centre ValBio staff are actively involved in Conservation Education, Health and Hygiene, and Research programs. Besides visiting villages surrounding the park and teaching about conservation, these teams also educate about health and hygiene practices, sustainable agriculture techniques, and the importance of ecohealth in maintaining human health. CVB also has full time experienced field technicians that are experts in local flora and fauna that work closely with international researchers. These field technicians are primarily from the villages nearest to the research station. Their expertise not only provides invaluable advice and assistance in the field but also provides local communities with a sustainable income.

RNP and the other national parks around the island are crucial for conservation efforts. Madagascar is one of the places that has lost the most terrestrial vertebrates since 1500 AD (Brooks et al., 2002), and due to rapid deforestation in recent years there is a particularly high proportion of threatened species across multiple taxa (Baillie et al., 2004). The high species richness and endemism make the island of Madagascar a top priority for conservation and a biodiversity hotspot (Brooks et al., 2006). In recent years the growing population and increases in deforestation and tavy (a form of slash-and-burn agriculture) had become a serious threat to the endemic species. It is speculated that less than 5% of the original forests on the island remain, therefore conservation efforts and the preservation of Malagasy national parks such as RNP are crucial to the survival of these unique species.
Figure 5. Ranomafana National Park (RNP). Ranomafana National park is nestled within the southeastern rainforests of Madagascar. The figure on the top is a schematic of Madagascar showing where on the island the park is. RNP is comprised of 42,500 hectares of continuous rainforest (left). Surrounding the borders of the park (right) is the buffer zone (dotted region). Situated close to the road, the Talatakely region is visited regularly by tourists and is one of the most heavily researched sites in the park. The second study site used in this thesis called ‘Camp-site’ is immediately across the road from CVB.
2. Aims of study

1. To determine whether or not mouse lemurs live to the captive age of senescence (5yrs) in the wild

2. To determine whether or not male mouse lemurs are more vulnerable to mortality in the wild due to parasites, if so can this be attributed to their high testosterone levels

3. To determine whether or not males suffer from higher parasite loads than females, and if so, is this due to high T levels in males?

4. To determine whether or not male and females are equally susceptible to parasites in the wild

5. To determine whether or not there are age-related changes in ecto- and endoparasite loads in wild mouse lemurs

6. To determine whether age-related changes in hormones T, DHT, Cortisol and DHEA-S can be seen in wild brown mouse lemurs
3. Materials and methods

3.1 Methods used in this thesis

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Laboratory Methods

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Before addressing the research aims preparatory work, such as method development, was necessary. In addition to addressing the research questions, several methods were established or modified in this thesis.

3.2 Methods developed and implemented in this thesis

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4. Results and discussion

Summary of results

I. Wild mouse lemurs live up to 8 years (possibly more) in the wild. Survival rates do not differ between the sexes and neither do testosterone levels, perhaps explaining the lack of differences in survival/mortality rates.

II. By tracking lice we were able to construct a mouse lemur social network while simultaneously gaining information about the natural movement of parasites in a wild population. We were also able to identify which individual lemurs would be responsible for a population wide louse-borne epidemic.

III. Contrary to the ICHH, T does not appear to be responsible for male biased parasitism; however, there does appear to be an effect of C (alone and when combined with T) on parasite loads in both sexes. We also found that old mouse lemurs have more endoparasites than younger lemurs, and no effect of age on ectoparasite loads.

IV. We found no age related changes in T or DHEA-S in either sex; however, C decreases with age in male mouse lemurs.

V. We found that mouse lemurs are parasitized by three species of ticks and one species of lice. We describe the male and third instar of the louse species (*Lemurpediculus verruculosus*) and found that the ears are the most heavily parasitized part of the body.

VI. We found that mouse lemur dental wear does not differ between relatively undisturbed and disturbed habitats.
4.1 (I) Mouse lemurs in the wild survive past the captive age of senescence

In contrast to what we know from captive populations, our results suggest that, mouse lemurs in the wild are capable of survival past the captive age at which symptoms of senescence begin to manifest. In polygymous vertebrates, males typically have much higher mortality rates than females. One widely accepted explanation is that high T levels in males and the costly effects of the androgen result in higher mortality. We found that age-dependent survival in brown mouse lemurs is the same in both sexes. This is highly unusual because mouse lemurs are polygamous primates and comparable survival of the sexes is very unusual in a non-monogamous vertebrate. One potential explanation for this finding may be the discovery that comparable T levels are seen in both sexes of these lemurs.

While it was surprising that wild mouse lemurs survive past the age of 3 or 4, long life span in the wild is not a foreign concept. Linnen and colleagues in 2001 (Linnen et al., 2001) examined the age-specific patterns of mortality in wild and laboratory strains of *D. melanogaster*. Wild *Drosophila* brought into the laboratory showed a typical exponential increase in mortality rate with age. Experimental strains kept in culture for hundreds of generations showed the same pattern, only with elevated mortality at each age compared to the wild flies. That is, the laboratory strains had shorter life spans. However, while selection on life span in the laboratory strains registered impressive gains in the length of life, the age-specific mortality rates merely decreased to the levels observed in wild-caught flies, and no further. Thus, selection can improve life span, but might only be effective in removing genetic factors incorporated in laboratory strains selected for short development time and rapid early reproduction.

In this manuscript, for the first time, we combine mark-recapture techniques with dental molding techniques in a small mammal and use these to estimate the ages of the individuals in this population. These data were collected from 2007-2010, over four mouse lemur breeding seasons. During that period 10,536 traps were set over 444 trap nights for a total of 1,091 mouse lemur captures.

The lack of sex difference in age-dependent survival and lack of sex differences in T present a unique and interesting finding. Other studies investigating sex differences in survival in polygamous animals have found that males survival rates are much lower than female survival rates, and higher levels of T are typically proposed as the reason for these sex differences. To our knowledge this is the first study that examines both survival and T in a wild population of small mammals. When examining T values in a larger-bodied, diurnal, sympatric le-
mur from RNP we found the same results: both males and females experience comparable T levels (Tecot, 2010). These T findings have only been seen in one other animal, the rock hyrax (Koren et al., 2006) another female dominant mammal. However ring tailed lemurs and spotted hyenas, two other female dominant mammals do have male biased sex differences in T. Further behavioral and endocrinological research may reveal interesting patterns of elevate T in some female dominant species and not in others. It would be interesting to further examine the other species of lemur that live in RNP, a location that has monogamous lemurs, sexually dimorphic lemurs, and polygymous lemurs.

The T findings suggest that perhaps the stereotypically elevated T seen in males does contribute to the higher mortality often seen in non-monogamous male vertebrates. However, this is often attributed to the hypothesis that elevated T compromises the immune system and makes males more vulnerable to infection, parasitism, and eventually predation. Our findings in this population of mouse lemurs (IV) suggest that not T but rather Cortisol appears to be responsible for elevated parasite loads in male mouse lemurs. Similarly, elevated parasite loads are seen in tandem with high C in females as well.

There are many potential reasons that may explain why wild mouse lemurs survive past the captive age of senescence. One potential reason may be that mouse lemurs, as large brained primates, are capable of predator avoidance strategies that non-primate arboreal mammals may not have. Nocturnality and arboreality have been proposed as mechanisms of predator avoidance, and mouse lemurs are both nocturnal and arboreal, allowing them to avoid diurnal and terrestrial predators. Another potential mechanism behind long lifespan in wild mouse lemurs may be their ability to hibernate, a trait unique among primates and shared only with a few other Cheirogaleids. Recent work has shown that the ability to torpor/hibernate increases survival in mammals, and has been linked with slow life histories (Turbill et al., 2011, Lyman et al., 1981). While this may provide a potential explanation for long lifespan in wild mouse lemurs, we would have expected dental wear rates to reveal these patterns of sex specific hibernation if it occurs in this population, with females experiencing half the annual wear of males. We did not find any sex differences in dental wear; however, we did find a few individuals with predicted ages less than minimum possible ages (I) that may reflect hibernation in those individuals. Nonetheless, there was no sex biased pattern and only very few individuals had these slow wear rates. This suggests that perhaps there is no sex bias in hibernation in brown mouse lemurs, but that only individuals that can attain a large enough body mass can undergo the process of torpor/hibernation.

Another potential explanation for the presence of old mouse lemurs in the wild may be natural selection. There is the possibility that the „aged“ individuals we documented in the wild may just represent the subset of aged individuals that
survive in captivity without experiencing any symptoms of senescence. However, there are no published statistics on the percentage of elderly mouse lemurs in captivity that are free from age-related pathologies, so whether or not this explains the number of ‘aged’ individuals in the wild is still yet to be seen. Further information in the matter may provide an explanation for the number of aged individuals found in the wild.

**4.2 (II) Mapping the Social Network: Tracking lice in a wild primate population (*Microcebus rufus*) to infer social contacts and vector potential.**

In this manuscript we introduce and implement a simple, cost effective method that exploits the dependence of sucking lice on direct host-host interactions. We report louse transfer patterns, vector potential, and reconstructed social interactions in the louse’s primate host, the wild brown mouse lemur (*Microcebus rufus*), of Ranomafana, Madagascar. We found that louse transfers occur exclusively between males, and dramatically increase in frequency at the onset of the short host breeding season. While testosterone levels may be causing this increase, we do not have hormone values to incorporate with this louse exchange data. Our findings from I and III, however, show that testosterone is unlikely to be the culprit responsible for the high parasite loads seen in males. Other sex-dependent non-hormonal characteristics, including morphological, physiological, behavioral, dietary, and life history traits may contribute to this pattern of heavily parasitized males.

Louse transfer data from this study indicate that mouse lemurs exchange lice over longer distances than suggested by trapping data, and that the social contact revealed by louse exchange patterns are distinct from those that predicted by trapping data alone. We also found that just a few parasitized animals could be responsible for a population wide louse-borne epidemic.

(Beldomenico & Begon, 2010) proposed that animals with poor body conditions are more susceptible to parasitic/pathogenic infections, which in turn would perpetuate their poor body conditions. They refer to this as a „vicious circle”, which results in heavily infested animals that become „superspreaders”, who disproportionately contribute to the dispersal of parasites in a population. Our technique of following the movement of lice through a wild population revealed the presence of lemurs that disproportionately contributed lice to the rest of the population, confirming the presence of „superspreaders”. In addition, however, the mouse lemurs most heavily parasitized in this study were also the ones that collected the most lice from others and hence not superspreaders, but rather what we call „supercollectors”.
These results demonstrate that our method reveals mouse lemur social contacts unique to those predicted from trapping data alone, and much broader ranging behavior of mouse lemurs (and lice) than previously anticipated. This also indicates the rapidity with which blood-borne pathogens could spread throughout this wild population. And our finding of “superspreader” mouse lemurs indicates that just a few lemurs could be responsible for population-wide infestation.

We believe that our method and results from its application will be of interest to a wide array of integratively-minded biologists. To further supplement this research, we could examine blood borne pathogens in mouse lemurs and genetically examine whether or not the *Lemurpediculus verruculosus* sucking lice are vectors of these potentially fatal pathogens. Those wishing to study behavior or parasite-host interactions and infection patterns in trappable species may employ this method to obtain otherwise inaccessible data. Disease ecologists may be interested in using this approach to reveal the potential that individuals have to transmit blood-borne diseases through a population. Conservation biologists can use this to inform how to plan animal reintroductions, transfers, or how to determine causes of sudden population decline. These perspectives are diverse but interconnected, and have potential to inform one another and give rise to new integrative studies. This method has the potential to be widely applicable to any trappable species, and may provide insight into the poorly understood field of pathogen infection patterns in the wild.

Our study suggests a potential change in the way that diseases are thought to be spread. Superspreaders are likely to have poor body conditions, and should thus be highly susceptible to predation. The presence of superspreaders alone as the primary distributers of parasites (as suggested in Beldomenico and Begon 2010) poses the problem of potential parasite eradication. However, the probable presence of superspreaders and supercollectors provides a potential explanation for ‘ecostasis’, the fine balance between parasites and their hosts that led to their intimate coevolution.

**4.3 (III) Testing the immunocompetence handicap hypothesis (ICHH) in both sexes of wild brown mouse lemurs.**

Polygamous vertebrate populations typically have increased mortality rates in males when compared to females. This phenomenon has often been explained through T mediated immunosuppression in males (Roberts *et al.*, 2004, Mougeot *et al.*, 2004, Hillgarth & Wingfield, 1997). The notion that maintaining high levels of T is metabolically costly and defers resources from the immune system has led to several hypotheses implicating high T levels in males as
the mechanism behind weakened immunity, increased susceptibility to parasites, and higher mortality rates. In previous work, we found that that male and female brown mouse lemurs have comparable survival rates and T levels in the wild. To further test whether or not sex differences in parasite loads are seen, and whether or not T plays a role in immunosuppression we examined the effects of T, C, secondary sexual characteristics (testicular volume in males), and age on ecto and endoparasite loads in both sexes. We exploited the unique situation of elevated T in female mouse lemurs, and found that although T levels are comparable in males and females, males are still more heavily parasitized by ectoparasites than females. We found that while T alone does not explain this sex difference, the high levels of C found in males might.

Ectoparasites have been shown to decrease host fitness, reproductive success, and survival (Van Vuren, 1996; Cheney and Cote 2003; Uller and Olssen 2003). By combining this study with (I, II) we find that although mouse lemurs are ectoparasitized, we do not find evidence decreased survival rates, or decreased body mass in the most heavily ectoparasitized individuals of the population (I and unpublished data). Perhaps by examining the effects ectoparasites on prolonged torpor, juvenile mortality, and reproductive success the negative relationship between ectoparasites and host fitness will be revealed.

We found C to have more of an effect on parasite loads than T (perhaps not surprising due to C’s immunosuppressive properties). This effect was seen in both sexes, however, in females an increase in C led to an increase in endoparasites, while an increase in C in males led to an increase in ectoparasites. When T and C were combined we found an interesting result. Rather than T alone being responsible for immunosuppression, we found that when controlling for C, T levels can explain the ectoparasite loads. This could perhaps explain why so many studies testing the ICHH find conflicting results. Many of those studies use T implants to test the hypothesis and these implants presumably cause stress to the study subject, increasing C levels. Perhaps if future studies addressing the ICHH take both C and T into consideration these conflicting results will be resolved.

We also separated male and female mouse lemurs into young (0-4) and old (5+) groups to assess whether or not an age-related decline in immunity, as examined through indirect measures (e.g.-parasite loads), is seen in wild brown mouse lemurs. In humans, soay sheep, albatross and bees (Palacios et al., 2007a, Hayward et al., 2009b, Amdam et al., 2005), immunosenescence, or the age related decline in immune function, has been shown to occur. Here we compare aged and young individuals and found that although there is no significant difference in ectoparasite loads between young and old individuals, we did find a significant difference between young and old fecal nematode counts. This difference can be seen in both sexes, suggesting that if there is a decline in immunity with age in wild mouse lemurs it does not experience a sex bias as well. The
prevalence of nematode larvae is 97% in old individuals, and 76% in young individuals. These findings suggest the possibility that some sort of immune decline does appear to occur in old age in wild mouse lemurs. Similar to the findings in other studies of immunosenescence, this decline in functionality appears to affect the adaptive, humoral immune response rather than the innate immune response.

4.4 (IV) Age-related changes in testosterone, cortisol, and DHEA-S in wild mouse lemurs.

Glucocorticoids appear to increase with age in humans and several other species such as chimpanzees, ring-tailed lemurs, and humans (Robbins & Czekala, 1997, Pride, 2005). However, some other studies have shown no change with age. The increase in glucocorticoids has been heavily attributed to an age-related increase in dysregulation of the hypothalamic–pituitary axis (HPA), which leads to elevated levels of circulating corticosterone (Montaron et al., 2006). Corticosterone dysregulation has also been linked to Alzheimer’s disease in humans and brain pathologies in other animals that experience increases in C with age, or in the case of semelparous animals, before death (Braithwaite, 1974, Bradley, 2003, Bradley et al., 1980, Bradley et al., 1975, Bradley, 1987, Barker et al., 1978). It is thought that brain tangles occur in these animals as circulating corticosterone increases and slowly becomes neurotoxic to neurons as it inhibits glucose uptake and glucose metabolism. In doing so it causes selective dendritic brain damage (McEwen & Seeman, 1999, Heininger, 2000, De Leon et al., 1997).

In this study, rather than seeing an increase in C with age in wild brown mouse lemurs, we see significant decline with age in males. In (III) we show that males typically have higher C than females and that high C is associated with an increase in ectoparasite loads. Further, this manuscript shows that ectoparasite loads do not significantly increase with age in male mouse lemurs, and that (I) males survive as long as females in the wild. The findings in (III) that indicated that males were parasitized more often than females were surprising, as male survival is comparable to females; however, this study showing a decrease in C with age in males may provide a potential mechanism behind the lack of decline in male survival. If high C levels in males lead to high parasite loads, perhaps if C did increase with age we would see an increase in parasites and a decrease in survival among male mouse lemurs.

Androgens are hormones crucial during development and sexual maturation and their continued secretion throughout the body through life allows for the maintenance of reproductive organs and spermatogenesis. However, findings in humans and other animals suggest that the abundance of certain hormones changes
with age. For example, in human males a gradual decline in T is seen with age. It is estimated that 30% of men after the age of 60 (Harman et al., 2001) have low T. This decline in T often accompanies symptoms such as lowered muscle and bone mass. In some studies lowered T has also been seen to increase the chances of Alzheimer’s disease (Moffat et al., 2002, Hak et al., 2002).

In our study the finding that T, DHT, and DHEA-S do not change in either sex with age was intriguing but perhaps not surprising. It is possible that mouse lemurs in the wild survive well past the maximum age at which we captured them (8 yrs, I), and perhaps wild mouse lemurs even experience ages comparable to maximum lifespan in captivity. If this is the case, there is the possibility that our aged animals have yet to experience senescent symptoms and declines in androgens. With more long term data a decline in androgens may be revealed.

4.5 (V) Lice and ticks of the eastern rufous mouse lemur, Microcebus rufus, with descriptions of the male and third instar nymph of Lemurpediculus verruculosus.

Very little is known about ectoparasites in lemurs, or in malagasy mammals in general, and in this manuscript, we describe the male, and third instar of the sucking louse Lemurpediculus verruculosus, a louse previously documented to parasitize “mouse lemur”. We identify for the first time, Microcebus rufus as the host of this louse. Additionally we identify the three species of ticks that parasitize the brown mouse lemur. Brown mouse lemurs were assessed for ectoparasites during the 2008 and 2009 field seasons, and after a literature review it was revealed that no parasites had previously been described for this species of mouse lemur.

We found that male lemurs were more heavily parasitized by ectoparasites than females, and lice were primarily found on the ears and testicles. These are two areas with thin skin and sparse hair, presumably facilitating the ease of access to a blood meal. Interestingly, no lice were ever found on the dorsum of the lemurs, perhaps because sucking lice typically have tarsal claw adaptations to specific hair sizes, and the hairs on the ears and testicles are fine relative to those on the dorsum.

Sucking lice are typically species-specific parasites, and some in cases are even specific to a location on the body of a mammalian species. For example, squirrels are parasitized by 16 species of sucking louse, each specific to a different part of the body. Humans are also parasitized by 3 species of sucking lice that are region specific: one parasitizes the hairs on the head, one on the body, and one in the pubic region, although pubic lice have also been found to parasitize
eyebrow hairs and mustaches and beards, as those hairs have thicknesses and morphologies more similar to pubic hair than to body or head hair.

Since sucking lice are obligate blood feeders they have the potential to disperse blood borne pathogens, the finding that more male lemurs had lice than females may suggest some sort of immunosuppression, or immune vulnerability in males mouse lemurs. However, as we found in (IV) it is unlikely that this immunosuppression is due to T, but rather C likely plays a role in the sex difference seen.

One interesting finding was that all three species of ticks found to parasitize the mouse lemurs of this population were nymphs. Over four trapping seasons, and 1300 captures, we never found an adult tick. This suggests that mouse lemurs are not the definitive host of these three tick species, but rather an intermediate host. This begs the question of what the definitive host(s) are. There is the potential that the definitive host is a predator of mouse lemurs, or an animal that shares a treehole with mouse lemurs, such as the larger bodied sympatric fat tailed dwarf lemur *Cheirogaleus major*, or the tree dwelling sportive lemur *Lepilemur microdon*.

4.6 (VI) Lemur habitat and dental senescence in Ranomafana National Park, Madagascar.

In this article, we combined dental wear data from two areas of differing levels of habitat disturbance in two lemur taxa that represent the largest and smallest lemurs in Ranomafana National Park. The two sites that mouse lemurs were captured in differ in their level of human disturbance. One is comprised of rainforest that was selectively logged in the 1980s but has grown back substantially since then, the other was a rice paddy clear cut 15 years ago, but has been growing back since then, although it is exposed to daily human disturbance. Due to the different habitats and hence resource availability we expected to see differences in dental wear rates between the two sites. Similarly dental wear in two habitats of differing levels of disturbance were assessed in the larger bodied, diurnal Milne Edward’s sifaka *Propithecus edwardsi*. We found that although the animals were living in differing levels of disturbance, there was no significant difference in dental wear rates between sites. Furthermore, we examined C14 and N15 isotopes from hair samples collected from both sites to see if dietary differences exist. Carbon values are indicative of vegetation based diets, and nitrogen values reflect the amount of insect matter consumed. Using 2008 data from hair samples collected that season, we found no significant difference in C14 or N15, suggesting comparable dietary habits in the two sites (unpublished data). However, when examining isotope data from the 2009 field season, we found that animals in the less disturbed site (Tala) had significantly higher N
values than the more disturbed site (Camp). These higher N values suggest a diet more heavily comprised of insects. However, dental wear rates between the two sites do not differ, even with this 2009 difference in dietary isotopes. Insects do comprise a large portion of the annual diet of brown mouse lemurs (Atsalis, 2008), so perhaps higher N values in one site during one season would not significantly impact the rate at which mouse lemur teeth wear annually. Additionally, dental wear does occur from food-tooth contact, but also occurs from tooth-tooth contact, so a slight difference in diet one season might not change the rate at which the upper and lower dentition come into contact with one another, perhaps explaining why no difference in dental wear was seen. To further investigate this, one could experiment with different dietary supplementation to captive mouse lemurs and regularly examine how tooth wear rates change when provisioning a diet rich in insects (not including soft grubs). This would provide interesting insight into the dental effects of feeding on insects.

Another beneficial experiment would be to examine the age-related dental wear in captive populations of eastern and western mouse lemurs where the ages are known and the diets remain constant. If those findings suggest constant mouse lemur dental wear among congeners, even with slight variation, our method of age estimation (I) would have the potential to be applicable to all mouse lemurs in Madagascar and would allow for the rapid age estimation of one time captures outside of RNP.

Further research into the functional importance of dental wear on feeding on insects may reveal that individuals with heavily worn teeth are unable to feed on insects as efficiently as those with pristine cusps. With additional long term data collection in both captive and wild conditions we may be able to better understand the effects of dietary fluctuations on overall age-related dental wear.
## Age-related changes in wild and captive mouse lemurs

<table>
<thead>
<tr>
<th>Captive</th>
<th>Page</th>
<th>Wild</th>
<th>Article</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lifespan 12.3 yrs</td>
<td>32</td>
<td>Lifespan minimally 8 yrs</td>
<td>MS I</td>
</tr>
<tr>
<td>unknown</td>
<td></td>
<td>14% individuals live past 5 yrs.</td>
<td>MS I</td>
</tr>
<tr>
<td>unknown</td>
<td></td>
<td>Survival rates same in both sexes</td>
<td>MS I</td>
</tr>
<tr>
<td><strong>Morphological symptoms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cataracts</td>
<td>35</td>
<td>No evidence of cataracts</td>
<td>MS I</td>
</tr>
<tr>
<td>Gray hair</td>
<td>35</td>
<td>No evidence of graying hair</td>
<td>MS I</td>
</tr>
<tr>
<td>Obesity</td>
<td>35</td>
<td>No evidence of obesity</td>
<td>(unpub. data)</td>
</tr>
<tr>
<td>Diminished large scale movements</td>
<td>35</td>
<td>No diminished large scale movement</td>
<td>MS II</td>
</tr>
<tr>
<td>Anosmia</td>
<td>35</td>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td><strong>Brain pathologies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain atrophy</td>
<td>33</td>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td>Decline cholinergic neurons</td>
<td>33</td>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td>Neurodegeneration (MAAN)</td>
<td>34</td>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td><strong>Parasites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>unknown</td>
<td></td>
<td>Increase in endoparasites</td>
<td>MS III</td>
</tr>
<tr>
<td>unknown</td>
<td></td>
<td>No change in ectoparasites</td>
<td>MS II, MS III</td>
</tr>
<tr>
<td><strong>Hormones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decline in T</td>
<td>34</td>
<td>No decline in T with age</td>
<td>MS I, MS IV</td>
</tr>
<tr>
<td>Decline in DHEA-S</td>
<td>34</td>
<td>No decline in DHEA-S with age</td>
<td>MS IV</td>
</tr>
<tr>
<td>unknown</td>
<td></td>
<td>Decline in C with age in males</td>
<td>MS IV</td>
</tr>
<tr>
<td><strong>Effects of Hormones on Parasites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>unknown</td>
<td></td>
<td>T alone not responsible for increased parasite loads</td>
<td>MS III</td>
</tr>
<tr>
<td>unknown</td>
<td></td>
<td>Increase in parasite loads when T and C positively co-vary in both sexes</td>
<td>MS III</td>
</tr>
</tbody>
</table>
5. Concluding remarks

In this thesis I addressed multidisciplinary questions relating to mouse lemur aging and life history. Some of the findings were unexpected and do not conform to the trends found in other studies and typically seen in other organisms. First, comparable T levels were found in both sexes of mouse lemurs, a female-dominant primate species. Very few studies address T in females; therefore, given the results of this study the assumption that males have significantly higher T levels needs to be questioned, particularly in female dominant taxa. Another finding was that tracking lice can be used to predict social networks and ranging data in small trappable mammals. Additionally, the wide scale movement of lemurs and louse exchange dynamics provide insight into the way diseases are spread in wild populations. Another unanticipated finding was the presence of “old” mouse lemurs in the wild and the lack of male-biased mortality rates. In polygamous vertebrates males typically have much lower survival than conspecific females, whereas monogamous vertebrates tend to have comparable survival rates. In this case, once again wild brown mouse lemurs are an exception, begging the question of whether or not this trend will be seen in other female dominant vertebrates.

Throughout this research I also developed original, minimally invasive methods which are widely applicable to studies of other organisms. These include the tracking of individual parasites in order to uncover previously unknown information about social interactions and parasite exchange in a host organism; using dental molds to precisely determine ages in small trappable mammals; the isolation and culturing of nematode larvae from fecal samples to rapidly quantify the number of nematodes from a fecal sample; and mouse lemur-specific fecal hormone assays that were created for this study.

In the search for a better understanding of the aging process in wild brown mouse lemurs I examined multifaceted physiological transformations (parasitological, endocrinological, and dental wear). This research has produced novel, replicable methodologies and findings with wide-reaching implications that extend beyond aging and challenge some of the previously-held assumptions of mammalian physiology.
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