Doctoral Dissertation Improvement: Feeding on phytoestrogens – Implications for red colobus monkey physiological ecology

Research Objectives

For millennia, plant species containing chemicals that alter the vertebrate endocrine system have been consumed by humans for nutritional or medicinal purposes (Ososki and Kennelly 2003). However, the importance of such plant chemicals (e.g., phytoestrogens) to humans remains unclear. Because more than 99% of human history has relied on a hunter-gatherer subsistence strategy (Milton 2000), investigating the prevalence of phytoestrogens in wild plant foods fed on by free-ranging, non-human primates is necessary to improve understanding of human origins. The nutritional environment in which human ancestors evolved can be best reconstructed by studying living non-human primates in a natural setting (Milton 1999). As phytoestrogens likely had a significant influence on the evolution of both human and non-human primate physiology and behavior (Wynne-Edwards 2001), it is important to take a deeper look at the relationship between these compounds and the primates ingesting them. Therefore, the overall objective of the proposed dissertation research is to examine the role of phytoestrogens as endocrine disruptors in the physiological ecology of the Uganda red colobus monkey (Procolobus rufomitratus tephrosceles) in Kibale National Park, Uganda. More specifically, the proposed research aims to address three main questions:

1. How prevalent are phytoestrogens in the tropical forest tree community?

2. Does the presence of phytoestrogens influence diet choice in red colobus monkeys?

3. Does the ingestion of phytoestrogens act as a stressor (i.e., increase fecal cortisol levels) and/or impact sex steroid levels (i.e., fecal estrogen, progesterone, and testosterone levels) in red colobus individuals?

These questions will be addressed by screening various plant species for estrogenic activity and quantifying fecal steroid hormone levels from 28 individually recognized red colobus adults over a 12-month study. This will be one of the first field studies to collect these data concurrently.

Project Significance

Utilizing a novel approach to examine the relationship between primates and the plants they depend on will provide important knowledge thus far neglected in studies of primate biology. Phytoestrogens are plant compounds that may significantly affect both wild non-human primate and human biology at numerous levels: physiologically, behaviorally, and evolutionarily. From an ecological standpoint, discussions of primate ecology may be neglecting a critical factor influencing health, fertility, diet choice, and abundance. For example, many studies cite the importance of the availability of high protein, low fiber foods to primates (Chapman et al. 2002); however, it may be that fiber and phytoestrogen content are correlated,
and that the causal agent is actually phytoestrogen content. From an anthropological perspective, since non-human primates are the closest living relatives of *Homo sapiens sapiens* and live in an environment more similar to that of pre-agricultural human ancestors, data on the primate-phytoestrogen relationship will provide an improved evolutionary and ecological context for human origins. It is likely that these plant compounds have had a significant influence on primate evolutionary development (i.e., increased plasma steroid hormone levels to reduce impacts of ingestion of plant steroid hormone mimics) (Wynne-Edwards 2001). Nonetheless, this potential influence will remain hidden unless anthropological research seeks data only obtainable from studies of current primate-plant interactions.

**Previous Studies on Primate-Phytoestrogen Relationship**

Identifying nutritional factors that significantly influence primate biology is a main concern within the field of physical anthropology. Research on leaf-eating primates has shown that the availability of high protein, low fiber leaves is critical to their diet choice and biomass (Chapman et al. 2002; Milton 1979; Oates et al. 1990). It is less clear how plant secondary metabolites impact the physiology, feeding patterns, and biomass of these primates. Because plants defend themselves from herbivory by producing certain types of chemicals (e.g., tannins, alkaloids), it is hypothesized that primates must also deal with minimizing consumption of these defensive compounds when selecting their foods (Coley and Barone 1996). However, numerous mammalian herbivores can deal with toxins either behaviorally or physiologically (Freeland and Janzen 1974). This is especially true for ruminants and other foregut fermenters (e.g., colobines), since they have specialized alkaloid stomachs with microbes that can detoxify many of these defensive compounds (Smith 1992). Thus, it would be extremely beneficial for a plant to produce a compound that could not be detoxified by the consumer.

Milton (1998) discusses two types of plant defensive compounds that primates are faced with: 1) those that are toxic to the feeder or the feeder’s gut microbes and 2) those that inhibit digestion or absorption. An overlooked third type exists, and it can be defined by its ability to significantly alter long-term internal processes of the feeder, such as endocrine functioning or reproductive potential. Research on human and domesticated livestock foods has revealed an important class of plant chemicals exhibiting characteristics of this third type: the phytoestrogens.

Phytoestrogens are naturally occurring plant compounds structurally or functionally similar to vertebrate estrogens and their active metabolites (Whitten and Patisaul 2001). It has been proposed that plants produce these compounds to defend themselves from herbivory, as their presence would be an effective strategy to reduce local populations of herbivores (i.e., by suppressing fertility) (Hughes 1988; Wynne-Edwards 2001). Further, steroid production is a cheap, low-risk defense mechanism for plants since it requires only minor modifications to existing biosynthetic pathways (Wynne-Edwards 2001). Plant estrogen mimics are found throughout human plant-based foods, being especially prevalent in soybeans, flaxseed, and alfalfa sprouts, and occurring in smaller amounts in cereal brans, vegetables, and fruits (Kurzer and Xu 1997). The physiological impact of phytoestrogens was first documented in western Australia, when it was discovered that they could have a dramatic negative impact on vertebrate populations. Formononetin, an estrogen mimic found in a species of clover that domesticated sheep were feeding on, caused an epidemic of infertility that led to great economic loss to
farmers (Bennets and Underwood 1951). Most studies to date have been concerned primarily with the economic costs or health effects of phytoestrogen consumption for humans. As a result, almost all investigations into the impact of phytoestrogen consumption have been conducted on domesticated and captive mammals or have analyzed cultivated plants for phytoestrogen content. The role of these compounds in vertebrate herbivory in the wild has thus far been neglected (Wynne-Edwards 2001). Furthermore, there is a growing need to place phytoestrogen research into an ecological and evolutionary perspective.

As non-human primates are a well-studied group with much known about their feeding strategies, as well as modern human’s closest-living relatives, examining the importance of phytoestrogens to wild primates offers an excellent opportunity to further understanding of the ecological and evolutionary role of these plant compounds for both human and non-human primates. Although data on the relationship between primates and phytoestrogens in the wild are scarce, the potential importance of such compounds to primate ecology has been hypothesized (Huffman 1997; Nash and Whitten 1989). Additionally, the importance of phytoestrogens to the human evolutionary experience has been theoretically addressed (Jackson 1991). Studying the impact of these plant compounds on primate behavior and physiology in a natural setting will allow for the testing of these hypotheses.

**Study Site & Species**

Uganda is a center of high biodiversity within Africa, with the larger closed-canopy forests of western Uganda having the greatest biodiversity importance (Howard et al. 2000). Kibale National Park (795 km²), a mid-altitude, moist-evergreen forest in western Uganda (0 13' - 0 41' N and 30 19' - 30 32' E) near the foothills of the Rwenzori Mountains, is home to the highest recorded biomass of primates in the world with 11 species represented (Chapman et al. 1997; Chapman and Lambert 2000; Struhsaker 1997). This high level of biodiversity and complex community of primates, along with the rapid loss of forest outside the park (Howard et al. 2000), make understanding ecological relationships within this forest a critical endeavor.

The Uganda red colobus monkey (*Procolobus rufomitratus tephrosceles*) is a 9 kg folivorous African primate that usually feeds heavily upon *Celtis durandii* (Ulmaceae) young leaves, but also consumes a wide variety of other plant foods (Chapman et al. 2002). This primate lives in multimale-multifemale groups with an average group size of 30 individuals (Chapman et al. 2002); however, much larger groups of 70 to 100 individuals do exist (*personal observation*). *P. r. tephrosceles* is considered vulnerable, with the only likely viable population living in Kibale National Park (Struhsaker 2005).

The Uganda red colobus is an ideal study species for the proposed research, as substantial information on its feeding patterns and food preferences exists. Moreover, it is highly folivorous, with foregut fermentation. Therefore, phytoestrogen defense would likely be an appropriate strategy for its food plants because these compounds appear to be active after microbial metabolism and can even be converted into more biologically active forms in the gut (Gultekin and Yildiz 2006). Furthermore, collared red colobus groups with individual recognition (i.e., each individual has a unique collar color/tag shape combination) are available for study in Kibale.
Research Hypotheses & Methodology

The proposed dissertation research will examine the prevalence and variance of phytoestrogens in a tropical forest and determine whether their ingestion has a significant behavioral and physiological impact on a wild primate. Three main hypotheses will be tested:

1) Phytoestrogens are prevalent in the tropical forest tree community, especially in leguminous plant species, and there is a significant degree of spatial and temporal intraspecific variation in their quantity:

Little is known about how common phytoestrogens are in the leaves of tropical forest tree species. Leguminous plants are high in estrogenic compounds (Gultekin and Yildiz 2006), but data on other tropical tree families are lacking. Because phytoestrogens would likely be an effective, cheap defense strategy for plants to deal with vertebrate herbivory (Wynne-Edwards 2001), it is predicted that estrogenic compounds occur in a number of plant species found in Kibale, including the leguminous plants found there (e.g., Albizia grandibracteata). In addition, Chapman et al. (2003) showed that a number of plant nutritional (e.g., protein) and chemical (e.g., saponins) characteristics varied significantly between individuals of the same species and for the same individual across time. Therefore, it is also predicted that the amount of phytoestrogens in a given tree species will vary significantly according to these dimensions.

To address this hypothesis, mature and young leaves of the 20 most abundant and 20 most commonly eaten tree species by red colobus will be screened for estrogenic activity using cell culture bioassays in the laboratory of Dr. Len Bjeldanes [Dept. of Nutritional Sciences & Toxicology, University of California-Berkeley (UCB)]. Additionally, all parts (e.g., leaves, seeds) of common leguminous tree species at this site will be screened. This will be done for three individuals of each species. Those species showing estrogenic activity in the bioassays will be analyzed for isoflavonoid content using reversed phase high performance liquid chromatography with ultraviolet detection in the laboratory of Dr. Isao Kubo [Dept. of Environmental Science, Policy, and Management (ESPM), UCB]. Seasonal variation in phytoestrogen content for a particular species will be examined across two wet and two dry seasons. Further, variation in the same individual tree over time and variation between different individual trees of the same species at the same time will also be examined. Variation between species and across wet and dry seasons will be statistically analyzed using ANOVA models.

Plant Collections

Long-term data compiled by Dr. Colin Chapman (Dept. of Anthropology, McGill University) will be used to generate the list of most abundant and most commonly eaten tree species within Kibale. Plants will be identified in the field with the help of local field assistants and a plant identification guide (Katende et al. 1995). Specimens will be collected using a tree-pruning pole or the skills of a trained tree-climber. Leaves will be collected fresh, taken back to the field station, and dried using a food dehydrator. Dried plant material will be stored in sealed plastic bags out of direct sunlight until transported back to UCB.

All foods that make up 95% of the wet weight intake of foods for a given month, based on behavioral observations of the focal red colobus group, will be collected that month. This
will likely result in 30 different foods collected each month across the 12 months of the field study. These samples will be collected from the exact individual tree that was fed on by the group. However, only those species showing estrogenic activity in bioassays will be analyzed for phytoestrogen content. These samples will be used to examine interspecific and intraspecific variation in phytoestrogen content.

2) The presence of phytoestrogens does influence diet choice for red colobus monkeys, but there is a tradeoff between benefits of protein intake and costs of phytoestrogen intake.

Although many medical studies focus on the benefits of a diet high in phytoestrogens for protecting against certain diseases (e.g., cancer) (Kurzer and Xu 1997), for a wild primate species, the costs of such compounds to fitness likely outweigh the possible benefits for increased survival. Therefore, it is predicted that red colobus will select against plants with high amounts of phytoestrogens. However, since legumes are known to be especially prolific in these compounds (Ososki and Kennelly 2003), but also tend to have high amounts of protein, it is predicted that there will be a tradeoff between increasing protein intake and keeping phytoestrogen consumption to a minimum. Such a finding could explain the lack of ability to predict colobine biomass using legume biomass, even though such a relationship is theoretically supported (Chapman et al. 2002).

To address this hypothesis, a focal group of red colobus in Kibale will be followed for one year, with behavioral data collected six days per week. This focal group has individually recognizable adults, based either on a unique collar color/tag shape combination or easily detectible scars and features (e.g., fur color pattern, bends in tail). Behavioral data on the feeding strategies of these adults (n = 28) collected for this project, detailed nutrient intake data from other current studies of red colobus, and published long-term data on red colobus feeding preferences will be used to examine the relationship between phytoestrogen content and diet selection. This will be done following methods outlined in Wasserman and Chapman (2003). In sum, to evaluate if phytoestrogen content influenced red colobus diet selection, behavioral data will be used to calculate the percent of foraging effort devoted to each particular plant species and part. This value will then be compared to each particular food’s phytoestrogen content using a simple regression model. However, since plant foods are not equally available in the tropical forest, with some species abundant and some rare, data on the biomass of various species in the tree community will be used to statistically adjust for the influence of plant availability. Finally, since single factor explanations for a species’ choice of foods is unlikely, the influence of nutrients (e.g., protein) and toxins (e.g., tannins) will be controlled for using published nutritional data and a multiple regression approach.

3) The ingestion of phytoestrogens acts as a stressor (i.e., impacts fecal cortisol levels) and alters sex steroid levels (i.e., fecal estradiol, progesterone, and testosterone levels) in red colobus individuals:

The hypothalamo-pituitary adrenal endocrine axis (HPA) plays a central role in allowing an animal to return to homeostasis in the face of external disturbances. Through the HPA axis, the steroid hormone cortisol is released in response to stressors (Sapolsky 2005). After
performing its role in the stress response, it is degraded and excreted from the body as various metabolites (Palme et al. 1996). Since steroid hormones are fairly stable, cortisol and its metabolites (as well as estradiol, testosterone, and progesterone) in the feces can be reliably measured if collected immediately after defecation in order to quantify the stress response (or reproductive state) in primates (Whitten et al. 1998). Gathering quantitative data on the stress response of primates via cortisol metabolite measurement is relevant to primate health and fitness because a chronically stressed animal suffers a series of detrimental effects that lowers its ability to survive and reproduce. The cumulative long-term effects of the physiological response to stress include cardiac, vascular, and metabolic problems, as well as the suppression of digestion, growth and development, reproduction, and the immune response (Sapolsky 2005). Such consequences likely lead to an overall reduced fitness. The sensitivity and activity of the HPA axis are affected by a number of factors, including maternal care (Francis et al. 1999), maternal diet (Cooney et al. 2002), social status in dominance hierarchy (Sapolsky 2005), food availability and predation (Cavigelli 1999), and the ingestion of phytoestrogens (Hartley et al. 2003). Furthermore, it has been shown that phytoestrogen consumption also affects the hypothalamic-pituitary gonadal axis (HPG) (Whitten and Patisaul 2001). However, the impact of phytoestrogen consumption on the HPA and HPG axes in wild primates has not been examined. Because of results from captive studies, it is predicted that phytoestrogen consumption by wild primates will impact their HPA and HPG axes (i.e., alter cortisol, estradiol, testosterone, and progesterone levels).

To address this hypothesis, fecal samples will be collected from 12 individually recognized adult males and 16 individually recognized adult females living in the focal red colobus group once per week, every week, for one year, to monitor the steroid hormone levels (i.e., cortisol, estradiol, testosterone, progesterone) of each individual. Since female reproductive state greatly influences steroid hormone levels (Weingrill et al. 2004), each female will be categorized as either pregnant, lactating, or cycling each week based on behavioral observations. For females that are cycling, at least two fecal samples will be collected each week. Furthermore, fecal samples will only be collected between 0830 and 1230 hrs to reduce the contribution of diurnal variation seen in the excretion patterns of fecal steroids (Sousa and Ziegler 1998). To determine the amount of hormones in a fecal sample, and to avoid having to transport frozen samples internationally, hormones will be solubilized in the field. These processed samples will subsequently be analyzed for steroid hormone content using immunoassays at an appropriate facility, such as the National Primate Research Center at the University of Wisconsin-Madison under the direction of Dr. Toni Ziegler. The hormone data thus produced will be analyzed with the feeding behavior data to assess the relationship between phytoestrogen consumption and red colobus physiology (e.g., by comparing hormone profiles from the month when most phytoestrogens were consumed to the month when the least were consumed).

Fecal Hormone Analyses

When a known adult defecates, the time, temperature, and humidity will be recorded, and the sample will be immediately collected, placed in a sterile vial, and stored on ice in a cooler. At the end of the day, all fecal samples will be transferred to a -20°C freezer and stored until the hormones are separated from the fecal sample via solubilization. To solubilize the steroid hormones in the field, a fecal sample will be removed from the freezer, thawed, and homogenized. Then, 0.5 g will be solubilized using a 5.0 pH citrate buffer / 95% ethanol
solution (10 ml, 1:1) that will be mixed for 24 hrs (Guillette [2003]; personal communication). After mixing, samples will be spun in a centrifuge for 20 min to separate the supernatant containing the hormones from the fecal pellet, and then 2 ml of the supernatant will be passed through an Alltech maxi-clean filter cartridge for storage and transport back to the U.S. (Palme 2005; Touma and Palme 2005; Ziegler and Wittwer 2005). In order to account for the effects of diet variability on hormone excretion in feces, dry matter values will be determined in the field by drying each fecal sample to constant weight and calculating the percent water by weight (Wasser et al. 1993). Thus, final steroid hormone quantification will be reported as nanograms of steroid hormone and metabolites (e.g., fecal cortisol) per gram of dry feces.

**Analysis of Factors Influencing Hormone Profile**

Spatial data on group movement will be recorded hourly using a handheld GPS unit (to estimate energy expenditure), unusual stressful events (e.g., fights, intergroup encounters, predation attempts) will be recorded ad libitum, and climate data will be recorded daily to account for other potential variables influencing hormone levels. Furthermore, data concurrently collected by Dr. Colin Chapman and Dr. Jessica Rothman (Dept. of Anthropology, McGill University) will provide detailed information on genetic relationships of individuals, gastrointestinal parasite loads, and dominance hierarchies to account for other non-nutritional stressors. All of this data, along with the detailed feeding behavior data, will be used to identify significant factors influencing the levels of each of the four steroid hormones (cortisol, estradiol, progesterone, testosterone). This will be accomplished using multiple regression, ANOVA, and time series analysis.

**Past Experience with Methodology**

As an undergraduate, the applicant began studying the nutritional ecology of colobines in the laboratory of Dr. Colin Chapman [then in the Dept. of Zoology, University of Florida (UF)]. For one and a half years, the applicant conducted a senior honors thesis project that examined the importance of energy availability to colobine diet choice and biomass. After this, the applicant continued working on colobine nutritional ecology in the Chapman laboratory as a research assistant for two years, analyzing plant foods for various nutrients and toxins (e.g., sugars, cyanogenic glycosides). Also during this time, the applicant learned to use noninvasive endocrinological techniques to examine stress hormone levels by developing a project that examined several red colobus populations living in a series of forest fragments adjacent to Kibale. This resulted in the applicant’s first field season at Kibale in 2003, during which time the applicant worked with field assistants to collect fecal samples and setup a laboratory at the field station for extracting steroid hormones from those samples. Since then, the applicant has spent a total of seven months conducting research in Kibale. The applicant has also spent six months conducting fieldwork at sites in Central America and the U.S.

In addition to these experiences with the nutritional chemistry of plants and field studies of primates, the applicant gained experience running radioimmunoassays for measuring fecal steroid hormones in the laboratory of Dr. Louis Guillette (Dept. of Zoology, UF). Additionally, the applicant has worked with Dr. Toni Ziegler since 2003 on validating and using immunoassay techniques to measure various steroid hormones. All of these research experiences have fine-
tuned the applicant’s acquisition of skills needed to execute a year-long primate-based field project, as well as conduct laboratory analyses to examine the phytoestrogen content of plants and quantify the steroid hormone levels of primate fecal samples. Furthermore, the applicant has taken extensive coursework in endocrinology, ecology, primatology, plant chemistry, and anthropology, which has provided a solid theoretical training relevant to the proposed project.

**Preliminary Research Completed**

Research relevant to the proposed study began in 2003, when the applicant set up a laboratory for extracting steroid hormones from fecal samples at Kibale. During this time, the applicant also began validating the technique for quantifying fecal cortisol levels, including an examination of diurnal variation in red colobus fecal cortisol levels. Results indicated that fecal cortisol levels increased 1.9% per hour over the course of the day (Fig. 1). This study also provided the first insight into individual variation in cortisol levels in this species (Table 1). Finally, during the field season of 2003, the applicant completed the first project using this noninvasive endocrinological technique on the Uganda red colobus monkey to examine the fecal cortisol profile of individuals living in a series of forest fragments adjacent to Kibale (Chapman et al. 2006).

During a 2006 field season, the applicant conducted a study showing that the fecal cortisol technique appropriately quantifies the red colobus stress response. The method was biologically validated by examining the effects of a known stressor, darting and capture, on fecal cortisol levels (Palme 2005). Two adult males were followed for five consecutive days, starting on the day of darting, and again 22 and 23 days after darting, and all fecal samples (n = 32) were collected opportunistically to quantify fecal cortisol levels (Figs. 2 & 3). The hormone data indicated that the two males had baseline fecal cortisol levels for the first two days of sampling (mean = 131 ng/g, n = 11), elevated levels for the next two days (mean = 235 ng/g, n = 7), and baseline levels for the remainder of the study (mean = 115 ng/g, n = 14). The elevated levels were significantly higher than the baseline levels (t = -4.21, P = 0.002, n = 32). These results indicate that this noninvasive technique does appropriately quantify the red colobus stress response.

During a recent field season from July to October 2007, the applicant began setting up the proposed long-term project by selecting the study group and individuals, training field assistants, and conducting an initial collection of plant (n = 170) and fecal samples (n = 300) to begin developing the laboratory analyses. Two full-time field assistants were hired and trained to continue with sample and data collection, while the applicant returned to UC-Berkeley to learn the laboratory methodology for measuring phytoestrogens. The applicant will return to Kibale in February 2008 to continue with the fieldwork for this project. All necessary permits for conducting this research and exporting plant and fecal samples from Uganda to California have been obtained.

**Broader Impacts**

By utilizing a unique approach that combines methodology from the fields of primatology, ecology, endocrinology, and nutritional chemistry, this project will provide
information that contributes to a deeper understanding of primate biology. Additional benefits for society include a better understanding of the conservation issue of human-induced endocrine disruption in wildlife and the public health issue of the effects of phytoestrogen consumption on human biology. Because the study species is considered vulnerable (Struhsaker 2005), a better understanding of red colobus endocrinological physiology and the effects of consuming endocrine disruptors is likely vital to its continued survival. As for the implications for public health, discussions of the costs and benefits of phytoestrogen consumption in human diet are currently very popular in the medical literature. Herbal treatments found in drugstores, along with soy and soy-based products, can contain high levels of these compounds. An accurate understanding of the potential impacts of phytoestrogen consumption on public health cannot be realized until a Darwinian medical approach is utilized: a better understanding of how much human biology has been exposed to these chemicals in the past is needed.

Additionally, by working with field assistants from villages surrounding Kibale National Park, graduate students from Makerere University in Kampala, and professionals from both Makerere and McGill University, this study will facilitate international research exchange among the U.S., Canada, and Uganda. Further, by continuing to develop an environmental endocrinology laboratory in Kibale, the applicant will disseminate important knowledge and scientific infrastructure to this developing nation. Working closely with local field assistants will also promote the participation of an underrepresented group in science.

The applicant will also advise and train an undergraduate female laboratory assistant in phytoestrogen analyses at UC-Berkeley to assist in the proposed research project and conduct a separate senior honors thesis project. These activities will promote the advancement of women in science. Her honors thesis project will analyze plant-based gorilla foods for phytoestrogens using the same methods outlined in this proposal. The applicant will help this student in every step of the research process (e.g., obtaining funding, lab work, data analysis), as well as in the publication process. This work is currently occurring and is scheduled to run through May 2009.

Finally, the applicant will continue to be involved with education and outreach throughout the tenure of this project. For example, the applicant served as co-president of the Berkeley Chapter of the Society for Conservation Biology for one year (2005-2006), and during this time coordinated a benefit concert which raised funds for a sister chapter located in Kenya. A scientific book and journal donation were also organized, with the donations then shipped to Kenya. To promote a better understanding of the scientific research process, primate biology, and Africa, the applicant has given presentations to kindergarten, elementary, high school, undergraduate, and graduate classes, and will continue to do so throughout this project. At Kibale National Park, the applicant will continue to help with an ongoing public health program located at the Kanyawara Research Station, as well as co-manage two community projects designed to promote environmental and public health awareness.

**Research Schedule**

The proposed project will consist of five phases of research over the next two and a half years. The first and second phases have recently been completed. The third phase, currently occurring, consists of a 12-month field study in Kibale National Park. The fourth phase consists of laboratory analysis of phytoestrogen content of plant samples and is also currently being conducted. It is for the fifth phase of research, the laboratory related costs of measuring steroid
hormone levels, that the applicant is requesting funds from the National Science Foundation. A detailed schedule for the five phases is listed below, with funding sources in parentheses:

**Phase 1 (NSF Graduate Research Fellowship & Center for African Studies, UC-Berkeley)**

June – July 2006: Biological validation of fecal cortisol methodology, examination of effects of darting and capture on red colobus adult male physiology and behavior

**Phase 2 (International Primatological Society & NSF Graduate Research Fellowship)**

July – Sept. 2007: Research design & method development, training of field assistants, behavioral data collection, plant & fecal sample collection

**Phase 3 (NSF Graduate Research Fellowship & The Leakey Foundation - pending)**


Feb. – Mar. 2008: Laboratory processing of plant & fecal samples by applicant at field station

Aug. – Dec. 2008: Conclusion of field portion of research, laboratory processing of plant & fecal samples by applicant

**Phase 4 (Advisor Funding, EPA STAR – pending, & Wenner-Gren – to be submitted)**

Apr. – July 2008: Phytoestrogen analysis of plant samples by applicant, training of undergraduate assistant

Aug. – Dec. 2008: Phytoestrogen analysis of plant samples by undergraduate assistant

Jan. – Dec. 2009: Phytoestrogen analysis of plant samples by applicant

*Phase 5 (Funding Requested from NSF)*


**Dissemination of Results**


Jan. – May 2011: Publication of results, presentations at professional meetings
**Tables**

Table 1. Summary of cortisol levels collected from different individual red colobus monkeys (*Procolobus rufomitratus tephrosceles*) in Kibale National Park, Uganda.

<table>
<thead>
<tr>
<th>Individual</th>
<th>Number of Samples</th>
<th>Mean Cortisol Level (ng/g) (SE)</th>
<th>Minimum Cortisol Level (ng/g)</th>
<th>Maximum Cortisol Level (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>158 (23.7)</td>
<td>127</td>
<td>197</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>189 (31.2)</td>
<td>145</td>
<td>225</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>418 (81.0)</td>
<td>301</td>
<td>510</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>223 (59.4)</td>
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<td>335</td>
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<td>5</td>
<td>6</td>
<td>171 (21.7)</td>
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<td>10</td>
<td>123 (24.4)</td>
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</table>
Fig. 1. Scatterplot of log-transformed fecal cortisol levels from eight red colobus adult individuals in Kibale National Park and time of defecation with fitted lines from a log-transformed parallel lines model (n = 59). The linear regression equation with individual 1 as the baseline:

\[
\ln(\text{cortisol level}) = 4.7817 + 0.1914*\text{ind2} + 0.9831*\text{ind3} + 0.3463*\text{ind4} + 0.1031*\text{ind5} - 0.2846*\text{ind6} - 0.0789*\text{ind7} - 0.2341*\text{ind8} + 0.0189* \text{time of defecation}
\]
Fig. 2. Fecal cortisol profile for individual 14 (red colobus adult male) darted at 7:51 AM on June 13, 2006, in Kibale National Park.
Fig. 3. Fecal cortisol profile for individual 40 (red colobus adult male) darted at 9:52 AM on June 19, 2006, in Kibale National Park.