

**PROTOCOL FOR ANIMAL USE AND CARE**

Email to: [campusvet@ucdavis.edu](mailto:campusvet@ucdavis.edu)

EH&S USE ONLY

**PROTOCOL: 10502**  
**EXPIRES: 4/24/04**

Investigator		Contact	
Last Name:		Last Name:	
First:		First:	
Middle:		Middle:	
email:		email:	
Department:		Department:	
Phone / Fax:		Phone:	
After hrs. #:		After hrs. #:	

Species (common names):	Number:	Source:
Gray-cheeked mangabeys	Approx. 40	Wild, free-ranging in Uganda

**Project Title** Ecology of female relationships in wild gray-cheeked mangabeys (*Lophocebus albigena*)

Overnight housing location::	<input type="checkbox"/> Free-ranging	Day use:	<input type="checkbox"/> N/A
Animals will be maintained by:	<input type="checkbox"/> Vivarium <input type="checkbox"/> Investigator (If investigator maintained, attach husbandry SOP's.)		

**Procedures:** Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

Our research requires being able to identify wild female mangabeys as individuals but their uniformly black pelage makes it impossible to identify them by natural markings alone. We will apply Clairol Nice 'n Easy 98 Natural Extra Light Blonde hair dye to their pelage, squirted from a syringe, to avoid handling them or causing them other unnecessary stress.

**Special Husbandry Requirements:** Describe any special requirements your animals have with respect to food, water, temperature, humidity, light cycles, caging type, bedding, or any other conditions of husbandry.

Natural food and water available; temperatures range from 40-80 degrees Farenheit; humidity ranges from 0-100%; light cycles roughly 12-12; no cages; bedding provided by trees.

Other instructions for animal care staff: (check applicable entries)

Sick Animals	Dead Animals	Pest Control
<input type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator
<input type="checkbox"/> Clinician to treat	<input checked="" type="checkbox"/> Save for Investigator	<input type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input type="checkbox"/> Necropsy	

**Hazardous Materials (only if in the animal room):**

Infectious Agents?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Radioisotopes?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Toxic Chemicals?  Yes  No

Agent(s):

Funding source:	NSF, Leakey, Wenner-Gren (potential sources)	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Previous protocol number (if any):	

What Veterinarian or veterinary clinic will provide care for your animals? (check one) None required

<input type="checkbox"/>	Lab Animal Health Clinic ( 2-0514 )	<input type="checkbox"/>	California Primate Research Center ( 2-0447 )
<input type="checkbox"/>	VMTH Large Animal Field Service ( 2-0292 )	<input type="checkbox"/>	Another Veterinarian

If you checked "Another Veterinarian", please provide:

Veterinarian:		Address:	
Day phone:			
Emergency phone:		Email:	

*If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pctillman@ucdavis.edu) for current information about training and record keeping requirements.*

### Summary of Procedures:

a) Briefly describe the **overall intent** of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

The overall intent of the 18-month field study is to investigate the ecological determinants of female social relationships in gray-cheeked mangabeys (*Lophocebus albigena*) in Kibale National Park, Uganda. Our main objectives are to

- 1) describe female dominance hierarchies in two groups of mangabeys in unlogged forest and two groups in logged forest, where food availability is poorer than in unlogged forest;
- 2) determine whether rank is "maternally inherited" using behavioral observations and supplementing them with DNA analyses of maternal relationships of adult females whose mothers are unknown;
- 3) assess the relative importance of various scales of spatial distribution and depletion time of foods in determining competitive relationships, and;
- 4) investigate the behavioral processes that exacerbate food competition and reduce female reproductive success in mangabeys living in logged areas, as a means of contributing to both theoretical and applied issues in primatology.

This study will contribute most significantly to primate behavioral ecology in four areas. First, it will help to fill a large gap in our knowledge of the social behavior of forest living cercopithecines. Second, it will address the issue of spatial scale of food in relation to competition among females. Third, it will increase the number of studies that compare the importance of spatial vs. temporal distributions in causing agonism between females. Finally, it will clarify the effects of food abundance, food distribution, and ranging behavior on female social relationships, with implications for understanding the causes of lower female reproductive success in poorer habitats.

### b) Procedures employed in this project:

Please check the appropriate boxes if any of these procedures will be employed in your project:

- |  |   |   |
|--|---|---|
| <input type="checkbox"/> Monoclonal Antibody Production **       | <input type="checkbox"/> Food or water restriction        | <input type="checkbox"/> Special diets; food or water treatment.              |
| <input type="checkbox"/> Polyclonal Antibody Production **       | <input type="checkbox"/> Non-recovery surgical procedures | <input type="checkbox"/> Induced illness, intoxication, or disease            |
| <input type="checkbox"/> LD 50 or ID50 studies.                  | <input type="checkbox"/> Survival surgical procedures     | <input type="checkbox"/> Death as an endpoint (see i below)                   |
| <input type="checkbox"/> catheters, blood collection, intubation | <input type="checkbox"/> Multiple survival surgery        | <input checked="" type="checkbox"/> Trapping, banding or marking wild animals |
| <input type="checkbox"/> Prolonged restraint. (8 hrs+)           | <input type="checkbox"/> Behavioral modification.         | <input type="checkbox"/>  |
| <input type="checkbox"/> Fasting prior to a procedure.           | <input type="checkbox"/> Aversive conditioning.           | <input type="checkbox"/>  |

\*\* If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.

c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

The pelage of females will be squirted with Clairol Nice 'n Easy 98 Natural Extra Light Blonde hair dye from a syringe when they come low and close to the observer. This method (with black dye) was successfully used to mark immature patas monkeys in Kenya in 1999 (see protocol #8485). It is feasible to use this method on mangabeys because they frequently come down to as low as three meters and sometimes to the ground, well within squirting range. Potential hazards include accidental contact with the eyes. To minimize this hazard, we will apply Clairol Nice 'n Easy 98 Natural Extra Light Blonde hair dye only when the animals' faces are turned away. No other health hazards can be imagined.

d) Study Groups and Numbers: Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; **you may add as many rows as you require**. The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

Group	Procedures / Drugs	Number of Animals	Category
4	Mark pelage with Clairol Nice 'n Easy 98 Natural Extra Light Blonde hair dye from a syringe	Est. 36-40	1

Categories of invasiveness

Category	Description
1	<p>Little or no discomfort or stress</p> <p><b>Examples:</b> domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral.</p>
2	<p>Minor stress or pain of short duration</p> <p><b>Examples:</b> cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress</p>
3	<p>Moderate to severe distress</p> <p><b>Examples:</b> major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation</p>
4	<p>Severe pain near, at or above the pain tolerance threshold</p> <p><b>Examples:</b> exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness.</p>

Further descriptions of these categories are included in the instructions following this document.

e) **Rationale for species and numbers:** How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

1) Female social relationships have not yet been described for gray-cheeked mangabeys.

2) We need to compare four groups of mangabeys, a large and small group in logged and a large and small group in unlogged forest, and all juvenile and adult females in each group will need to be part of the study.

f) **Surgery:** If the project involves survival surgery, where will the surgery be conducted?

Building:  Room:

Who will be the surgeon?

g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?

h) **Neuromuscular blocking agents** can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent?

What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

Under what circumstances will incremental doses of anesthetics-analgesics be administered?

**i) Adverse effects:**

Describe any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

The only potential adverse effects are 1) eye irritation from accidental contact with the hair dye and 2) ingestion of dye by licking the pelage. Previous research using black dye on patas monkeys resulted in no obvious changes in social interactions between dyed and undyed animals or between dyed animal.

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of post-operative analgesics or other means, explain why this is necessary.

We will minimize eye irritation by aiming the syringe at the animals' hindquarters and by applying the dye only when their heads are turned away from us. We cannot stop the animals from licking themselves but our experience with patas suggests that they will not react to the dye in this way.

*Note: if any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.*

Is death an endpoint in your experimental procedure?     Yes     No

*(Note: "Death as an endpoint" refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.*

**j) Literature search for alternatives and unnecessary duplication:**

*Federal law specifically requires this section. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this class/lab, or 2) there are alternative methodologies, but these are not appropriate for your particular class/lab. "Alternative methodologies" refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that this use of animals is not **unnecessarily** duplicative of other studies.*

*UC Davis provides on-line access to a number of databases that can be used to search for alternatives. Visit*

*[http://trc.ucdavis.edu/jawelsh/Databases\\_Med\\_Vet\\_Researchers.htm](http://trc.ucdavis.edu/jawelsh/Databases_Med_Vet_Researchers.htm) (email: [jawelsh@ucdavis.edu](mailto:jawelsh@ucdavis.edu))*

*or [http://www.vetmed.ucdavis.edu/Animal\\_Alternatives/main.htm](http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm) (email: [mwwood@ucdavis.edu](mailto:mwwood@ucdavis.edu))*

What was the date on which you conducted this search?

February 3,  
2003

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

Database Name	Years Covered	Keywords / Search Strategy
Anthropological Literature	1984-present	Marking techniques, darting, collaring
BIOSIS Previews	1969-present	Marking techniques, darting, collaring

Anthropological Index	1957-present	Marking techniques, darting, collaring

What were your findings with respect to alternative methodologies?

All alternative methods are more invasive and thus suboptimal. These include 1) darting and putting collars on animals, 2) darting and putting ear tags on animals, and; 3) darting and shaving sections of animal's pelage.

Has this study been previously conducted?

Yes

No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

**k) Disposition of animals:** At what point in the study, if any, will the animals be euthanized?

The animals will not be euthanized.

**l) Methods of euthanasia:** Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

Species	Method	Drug	Dose (mg/kg)	route

**m) Surplus animals:** What will you do with any animals not euthanized at the conclusion of the project?

They will continue living in their natural habitat in Uganda.



Assurances for the Humane Care and Use of Vertebrate Animals:

Principal Investigator's Statement:

I have read and agree to abide by the *UC Davis Policy and Procedure Manual* section 290-30 (Animal Use and Care). This project will be conducted in accordance with the *ILAR Guide for the Care and Use of Laboratory Animals*, and the *UC Davis Animal Welfare Assurance* on file with the US Public Health Service. (These documents are available from the Campus Veterinarian and at <http://ehs.ucdavis.edu/>). I will abide by all Federal, state and local laws and regulations dealing with the use of animals in research.

I will advise the Animal Use and Care Administrative Advisory Committee in writing of any significant changes in the procedures or personnel involved in this project.

	Associate Professor	13 February, 2003
<i>Principal Investigator</i>	<i>Rank / Title</i>	<i>Date</i>

Committee Use Only Below

<b>** Conditions necessary for Committee Approval:</b>
Final Disposition of this protocol: <input type="checkbox"/> Approved <input type="checkbox"/> Not Approved <input type="checkbox"/> Withdrawn by Investigator  Date of Action: ____/____/____

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

<i>Campus Veterinarian</i>	<i>Date</i>

Antibody Production Project Description

If your project involves only antibody production, either polyclonal or monoclonal, you may complete this page in lieu of section c), project description.

c) Will these animals be used for antibody production?  Yes  No

1. Polyclonal or Monoclonal antibodies?   
 If Monoclonal, will you be producing ascites tumors in the animals?  Yes  No

2. What type(s) of antigen will be used?   
 Will the antigens be sterile?

3. What adjuvant will be used for the initial injection?   
 What adjuvant will be used for subsequent injections?

4. What route will be used for injections?   
 What anatomical location will be injected?   
 How many injections at one time?   
 How frequently will injections be given?   
 What volume will be injected at each site?

5. Polyclonal Blood collection Procedures:  
 Who will collect the blood?   
 From what anatomical location?   
 How frequently will blood be collected?  Volume?   
 Will the animals be sedated?  Yes  No

6. Will monoclonal antibodies be produced in mice bearing ascites tumors?  Yes  No  
 How often will the animals be assessed for abdominal distention?   
 How often will they be tapped?   
 How many times will they be tapped?   
 Will the animals be sedated for tapping?

**Note:** If you are producing monoclonal antibodies using ascites tumors in mice, section j), alternatives, must explain why an *in-vitro* system is not suitable for your study.

7. Sedation / Anesthesia for blood or ascites collection: If the animals will be sedated for either injections or collections, please indicate the species, drug, dose and route:

Species	Drug	Route	Dose (mg / kg)
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

h) What criteria will be used to determine that the animals should be euthanized rather than continue to be used?

### Categories of Invasiveness in Animal Experiments

*Use these categories when completing item d), Study Groups and Numbers*

Each year, the US federal government requires a report from the campus in which animal projects are categorized as to degree of invasiveness. Please assist the IACUC in this determination by assigning the animal procedures in your project to one of the categories below. The *US Government Principles Regarding the Care and Use of Animals* state, "Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals."

#### 1. Experiments which cause little or no discomfort or stress.\*\*

**Examples:** domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral, but not intrathoracic or intracardiac (Category 2); acute non-survival studies in which the animals are completely anesthetized and do not regain consciousness; approved methods of euthanasia following rapid unconsciousness, such as anesthetic overdose or decapitation; short periods of food and/or water deprivation equivalent to periods of abstinence in nature.

#### 2. Experiments which cause minor stress or pain of short duration.

**Examples:** cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress; short periods of food and/or water deprivation which exceed periods of abstinence in nature; behavioral experiments on conscious animals that involve short-term, stressful restraint: short term exposure to noxious but non-lethal levels of drugs or chemicals. Such procedures should not cause significant changes in the animal's appearance, in physiological parameters such as respiratory or cardiac rate, or fecal or urinary output, or in social responses.

#### 3. Experiments which cause moderate to severe distress or discomfort

**Examples:** major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation, aggression, predator-prey interactions; procedures which cause severe, persistent or irreversible disruption of sensorimotor organization; the use of adjuvants which cause clinically evident swelling or abscesses.

Other examples include induction of anatomical and physiological abnormalities that will result in pain or distress: the exposure of an animal to noxious stimuli from which escape is impossible; the production of radiation sickness; exposure to drugs or chemicals at levels that impair physiological systems.

Note: procedures used in Category 3 studies should not cause prolonged or severe clinical distress as may be exhibited by a wide range of clinical signs, such as marked abnormalities in behavioral patterns or attitudes, the absence or grooming, dehydration, abnormal vocalization, prolonged anorexia, circulatory collapse, extreme lethargy or disinclination to move, and clinical signs of severe or advanced local or systemic infection, etc.

#### 4. Procedures which cause severe pain near, at, or above the pain tolerance threshold of unanesthetized conscious animals

**Examples:** exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs or chemicals at levels that (may) markedly impair physiological systems and which cause death, severe pain, or extreme distress: completely new biomedical experiments which have a high degree of invasiveness; behavioral studies about which the effects of the degree of distress are not known; use of muscle relaxants or paralytic drugs without anesthetics; burn or trauma infliction on unanesthetized animals; a euthanasia method not approved by the American Veterinary Medical Association; any procedures (e.g. the injection of noxious agents or the induction of severe stress or shock) that will result in pain which approaches the pain

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\*\* The text of these categories has been freely adapted from a document originally published by the Canadian Council on Animal Care (CCAC).

tolerance threshold and cannot be relieved by analgesia (e.g. when toxicity testing and experimentally-induced infectious disease studies have death as the endpoint).

