Prosimians in U.S. Ex-Situ Institutions: the Duke Lemur Center as an Example of Animal Welfare Science and its Contribution to Biodiversity Conservation.

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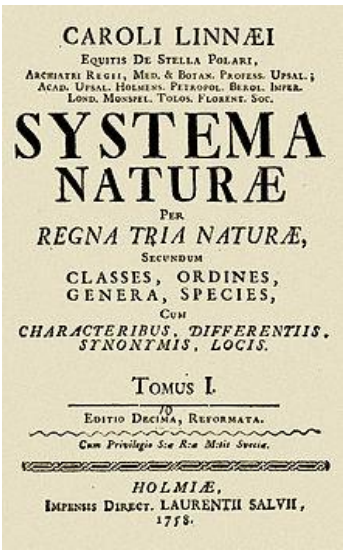
1.- Introduction

Sign at the entrance of the center, picture taken by Gloria Fernández Lázaro

The most well known and popular prosimians, the lemurs started to be known I the Western World at the beginning of the 17th century due to accounts mainly of French sailors and merchants. By the beginning of the 1th century some of them were brought to Europe, just in time for Carolus Linnaeus to include some illustrations in several editions of his *Systema Naturae* the starting moment for the classification of all species and which, for animals, is at the origins of the modern International Code of Zoological Nomenclature


CAROLI LINNÆI
EQUITIS DE STELLA POLARI,
ARCHIATRI REGII, MED. & BOTAN. PROFESS. UPSAL.;
ACAD. UPSAL. HOLMENS. PETROPOL. BEROL. IMPER.
LOND. MONSPEL. TOLOS. FLORENT. SOC.

SYSTEMA NATURÆ

PER
REGNA TRIA NATURÆ,
SECUNDUM
CLASSES, ORDINES,
GENERA, SPECIES,
CUM
CHARACTERIBUS, DIFFERENTIIS,
SYNONYMIS, LOCIS.

TOMUS I.

EDITIO DECIMA, REFORMATA.

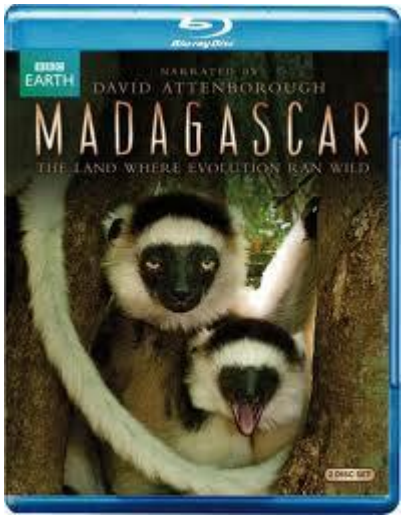
Cum Privilegio Sæ Rmæ Mtis Sueciæ.

HOLMIÆ,
IMPENSIS DIRECT. LAURENTII SALVII,
1758.

The Ring-tailed lemur (*Lemur catta*), one of the first lemurs to be classified by Linneus in 1758 (it appeared in *Systema Naturae´*s 10th edition) .The engraving (Edwards) is courtesy of Wikimedia commons: http://es.wikipedia.org/wiki/Archivo:Lemur_catta_-_George_Edwards.jpg

On site research started more or less by the last third of this same century during the peak of the Enlightment naturalists voyages organized to describe the natural world. During the 19th century, there was an explosion of new lemur descriptions and names, which later took decades to sort out and menageries, museums and cabinets started their systematic collections . On site search continued also during this century. However, the modern form of lemur taxonomic nomenclature was not really established until the 1920s and 1930s, being standardized in 1931, and it was not until the 1950s and 1960s that the on-site study of lemur behavior and ecology began to blossom.The name of "the Petters" (Jean-Jacques Petter and Arlette Petter-Rousseaux) became then bound to knowledge about lemurs when they toured Madagascar in 1956 and 1957, surveying many of its lemur species and making important observations about their social groupings and reproduction.

In 1960 Madagascar, since 1890 a French colony, obtained its independence. That same year a commercial film of David Attenborough introduced lemurs to the West.



Sir David Attenborough and his most recent (2011) collection of clips recorded since 1960 in his later numerous visits to the island, put together in a flamboyant BBC edition.
http://www.bbc.co.uk/nature/collections/p00db3n8

U.S. research started almost immediately afterwards, under the leadership of the famous physical anthropologist and controversial character –due to his fatal destiny years later, after he moved to New York University in 1973-, John Buettner-Janusch. Just after moving to Duke University, North Carolina, in 1965, he founded Duke´s Lemur Center the next year. He had sent the scientist Alison Bishop (later Alison Jolly, when she married) to Madagascar in 1962 to study the diet and social behavior of the ring-tailed lemur and Verreaux's sifaka at Berenty Private Reserve.

Other followed the Petters and Jolly but the political turmoil of the new Malgasy Republic during the mid-1970s stopped the explosion of interest and it was not until the 1980s that field studies resumed, thanks in part to the renewed involvement of the Duke Lemur Center under the direction of Elwyn Simons (from 1977 to 1991), and the conservation efforts of Patricia Wright during the eight years she spent at the Center.

Ex situ research in the Center is of course also of essence is also popular among researchers looking to answer questions that are difficult to test in the field. The Duke Lemur Center has the largest captive lemur population outside of Madagascar, which it maintains for captive breeding and non-invasive research such as those on vocalizations, basic locomotor research, bipedalism, the effects of social complexity transitive reasoning, and cognition studies involving a lemur's ability to organize and retrieve sequences from memory.

Duke LEMUR CENTER
Studying & Caring for Lemurs

Primate models in research are mainly focused in some species of Old and New World monkeys for practical and evolutionary concerns. Many guides record their psychological and biological needs for the wellbeing of the different species of primates in captivity but there is not so much information for prosimians. If it is going to happen, how are we are going to ensure their welfare? It has recently been proposed by the ILAR (Institute for Laboratory Animal Research) to develop "small non human primate models" for research which include prosimians, but is it an exception or a beginning of a new trend?

Nowadays there are not too many prosimians used in biomedical research but there are many used in fundamental research (both on site and in captivity) and in particular the available information about their maintenance in captivity is poor. For this reason this case study explores the knowledge and the applied welfare science for prosimians in the Duke Lemur Center (Duke University, North Carolina) which seems to be the unit which has the most comprehensive experience in the care of prosimians of any institutions in the United States and is the largest captive prosimian colony in the world. The reader is presented with some of the taxonomy, habitat, feeding, behavior, reproduction, and conservation information for the species needed to understand the peculiarities of this suborder of primates. Also the best practices for their maintenance in captivity are analyzed and the regulations and protocols applied in US for them as well as some of the efforts for prevent the threat of extinction of these beautiful animals.

Chapter I, "Prosimians: Who are they?" explains the characteristics of the suborder providing information about the taxonomy, habitat, feeding, behavior, reproduction and conservation status of the species.

Chapter II, "Welfare in captivity" describes the best practices for the maintenance of the species, analyzing the housing conditions, nutrition, group composition, enrichment program and veterinary care needed by these animals.

Chapter III, "Regulations and protocols in U.S" details federal laws and other regulations concerning prosimians in North American institutions and those applied in the Duke Lemur Center to achieve its mission.

I.- PROSIMIANS: WHO ARE THEY?

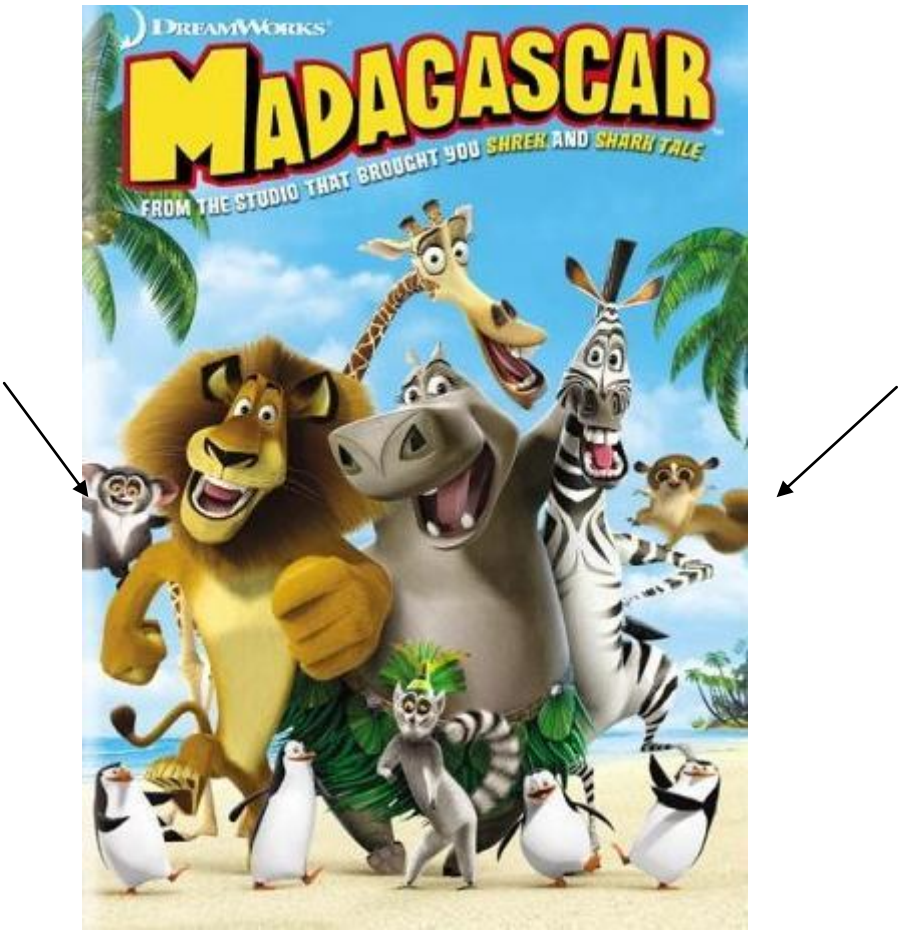
The order **primates** is commonly divided, since Simpson´s 1945 division (see Section on Works cited) into two major groups, or suborders, **Prosimii - prosimians** (lemurs, lorises, bushbabies, and tarsiers) and **Anthropoidea - anthropoids** (monkeys, apes and humans).

Although specialized in many aspects, living prosimians generally retain more primitive features than do anthropoids so it is no wonder that their name suggests this primitive nature: pro-simian (before apes).

The **simians** are better known and usually have attracted more interest since we humans are members of this order, in particular the **apes**: the tailess, with fingered and toenailed prehensile (grasping) hands and prehensile feet -all but humans- Catarrhines (*rhis* is "nose" in Greek, *cata* meaning "down," "against," "back," .. , thus Catarhines

mean with drooping or downward nosed, describing their narrow, downward pointing nostrils). And even much more attention, especially in recent times, has been grasped by the Great Apes: orangutans, gorillas, chimpanzees, bonobos and… humans [the rest of the apes being called the "lesser apes" or gibbons.]

Much less seduction has been triggered by the prosimians, at least until recently, although there is increasing interest in this order, even from children, the film industry not being alien to this phenomenon.



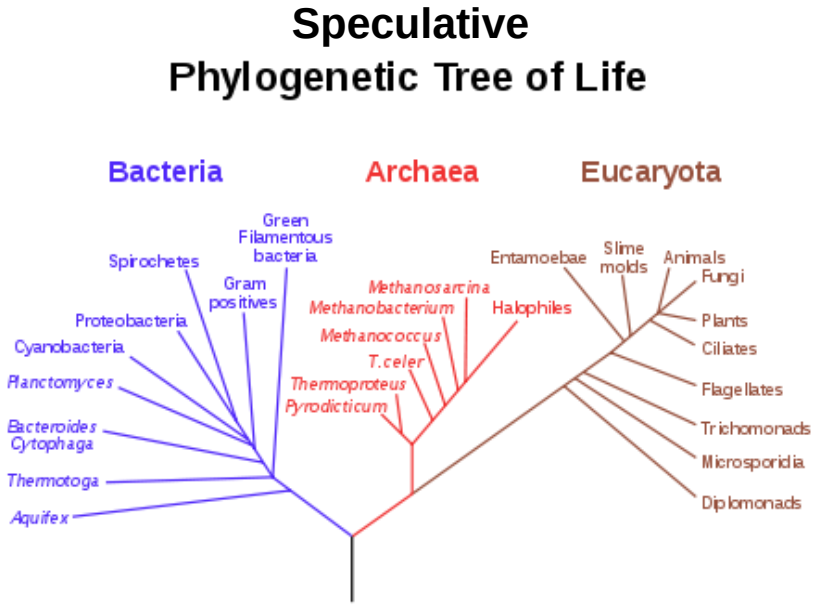
Simpson´s classification, included in 1945 another lesser family, the treeshrews Tupaiidae –tupaiids (from "tupai", the Malay word for treeshrew and also for squirrelto which the tupaiids superficially resemble), which nowadays in considered a separate order (Scandentia), which helps understanding how prosimians are the link between the insectivores and the monkeys.

Tupaia glis. Source: Wikimedia commons

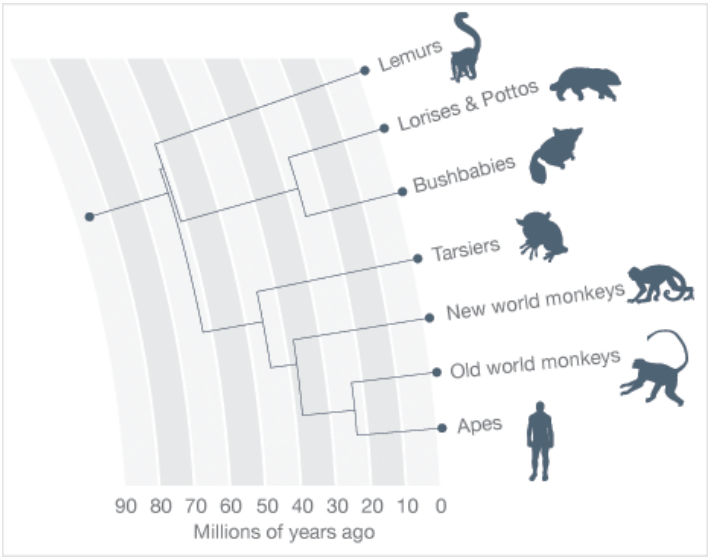
In any case the classification really does not provide any indication of which group of living prosimians may be closer to the origin of anthropoids, nor does it emphasize the derived characteristics that may be used to group prosimians [derived characteristics being, in taxonomic science, the less common characters shared by a given group, or, in other words, the traits shared by only the members of the group or, in evolutionary terms, the evolutionary novelties that are unique to the lineage being considered.]

It simply expresses the fact that prosimians are primitive primates that lack anthropoid features. But today these criteria have been reviewed and it is generally agreed,

a) that classification should reflect phylogeny -the history of organismal lineages as they change through time due to evolution; it implies that different species arise from previous forms via descent, and that all organisms, from the smallest microbe to the largest plants and vertebrates, are connected by the passage of genes along the branches of the phylogenetic tree that links all of Life (the “Tree of Life”); and



b) that the taxonomic groups should be monophyletic, that is, that they should have a single common ancestor that gave rise to all members of the group.



A family tree showing the evolution of the primates from a common ancestor some 85 million years ago (courtesy of BBC Nature Wildlife)

Under this approach, the more evolved within the prosimians at the opposite end of the tupaiids, the Tarsiers, are then grouped with anthropoids because they share a number of derived anatomical features with them such as lack of tapetum [the layer of tissue in the eye of many vertebrates that lies immediately behind or sometimes within the retina and that reflects visible light back through the retina, increasing the light available to the photoreceptors], partial postorbital enclosure [the enclosure of the eyes in a bony ring or a bony socket, supposedly (Szalay, 1976:349. Although that is been recently disputed; see Alfred L. Rosenberger et al, 1978) "…protecting the eyeballs and associated structures from the contraction of the temporalis", one of the muscles of mastication, that covers much of the temporal bone, although it is not as complete as it is in anthropoids], type of nose [see below], very large teeth for their body size and hemocorial placenta [which means that the fetal weave penetrates the endometrium until the point to be in contact with the maternal blood, as in anthropoids, including humans, although some rodents also have it].

Actually, the tarsiers are Haplorrhini (the Greek name means "simple-nosed") and thus considered "dry-nosed" primates, their upper lip, which has replaced the ancestral rhinarium, is not directly connected to the nose which allows a large range of facial expression; and not Strepsirrhini, who are characterized as "wet-nosed" because of the retention of the rhinarium [the moist, naked and hairless area of roughened skin around the nostrils of the nose at the tip of the snout in most mammals].

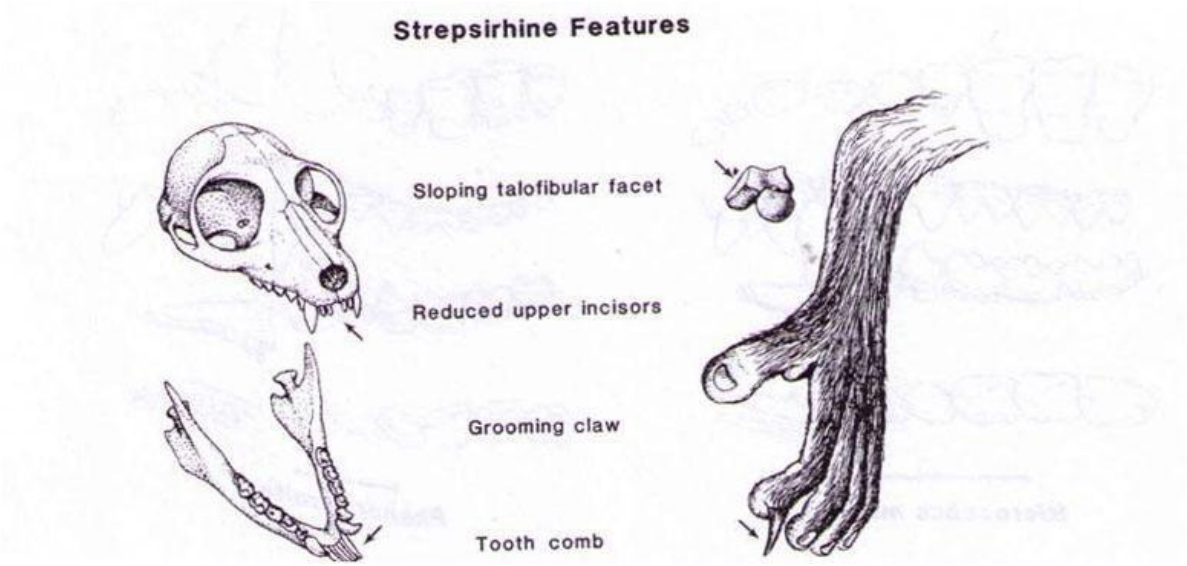
Picture: Wikimedia Commons

In fact the phylogenetic position of tarsiers within the order primates has been debated for much of the past century, and tarsiers have alternately been classified as prosimians or as the sister group of anthropoids (simians). Genetics are also non conclusive: analysis some DNA mutations, is argued to offer very persuasive evidence for the monophily of haplorhines while other lines of evidence, such as DNA sequencing remain ambiguous. Also, in common with simians, tarsiers have a mutation in a gene (the L-gulonolactone oxidase –GULO- gene) which confers need for vitamin C in the diet. Since the strepsirhines do not have this mutation and have retained the ability to make vitamin C, the genetic trait which confers the need for it in the diet would tend to place tarsiers with haplorhines. Thus, most systematists as well as geneticists argue the debate is conclusively settled in favor of their anthropoid characteristics.

Philippino Tarsiers. By the way, only very recently a team of researchers led by Marissa Ramsier of Humboldt State University in California, came upon new findings that put the Philippine Tarsier among the highest recorded (70 kHz) terrestrial mammals emitting a high-pitched ultrasound-range voice (not audible to humans). The tiny tarsiers, otherwise thought of to be "quiet" nocturnal animals, can actually be very loud and communicate through a secret high-pitched code that they use as a private-channel warning sound for fellow tarsiers when in danger, or for finding prey in pure ultrasound since they can emit up to 70 kHz sounds and hear even at 90 kHz. Picture: Courtesy of Roberto Verzo, Chocolate Hills of Bohol, Philippines.

So the prosimians are really strepsirhines and thus considered by primatologists to have more evolutionarily ancestral features and adaptations than their haplorhine ("dry-nose") cousins. The group is characterized by their “wet-noses” (because of the retention of the rhinarium) and the different families inhabit in Madagascar (where they are the only non-human primates), southeast Asia and Africa and who are united by at least three specialized features (see figure below, by Fleagle, 1999):

- their unusual dental tooth comb .with the exception of the aye-aye (and associated small upper incisors),
- the laterally flaring talus (ankle bone that contacts the tibia), and
- the grooming claw on the second digit of their feet



Their skull is characterized by the retention of primitive primate features such as simple postorbital bar (without postorbital closure), a relatively small braincase, and a primitive mammalian nasal region.

Many of the distinctive soft structures of the strepsirhine, such as the well-developed rhinarium, are primitive features found in many other groups of mammals. Since their moist nose is connected to the upper lip, which is connected to the gum, they have limited facial expressions capabilities.

It is true, though, that the reflecting tapetum, although it is a common feature in many mammalian groups, seems to involve different chemicals in the strepsirhines, suggesting that this characteristic may be a derived feature of that group (Martin, 1995).

The reproductive system of all strepsirhines is characterized by at least two pairs of nipples, a bicorne uterus, and an epitheliochorial type of placentation [A type of placenta in which the maternal epithelium and fetal epithelium are in contact due to the formation of a layer of fetal tissue merely pressed close against the uterus wall].

There are seven families of living strepsirhines.

Five of these are from the island of Madagascar: cheirogaleids, lemurids, indriids, and the two families that contain only a single living genus each, the lepilemurids (or megalapids) and the daubentoniids. They are the five families of primates typically called lemurs (infraorder Lemuriformes).

The other two families are split with the lorises, pottos and galagos (infraorder Lorisiformes). Lorises live in Africa and Asia and galagos in Africa (see map in next page).

Order Primates

Suborder Strepsirrhini: Non-tarsier prosimians

- Infraorder Lemuriformes
  - Family Cheirogaleidae: Dwarf and mouse lemurs (30 species)
  - Family Lemuridae: Lemurs (22 species)
  - Family Lepilemuridae: Sportive lemurs (25 species)
  - Family Indriidae: Woolly lemurs and allies (19 species)
  - Family Dauentoniidae: Aye-ayes (1 species)
- Infraorder Lorisiformes
  - Family Galagidae: Galagos (19 species)
  - Family Lorisidae: Lorises, pottos and allies (9 species)

Suborder Haplorrhini: Tarsiers, monkeys and apes


Dwarf & mouse lemurs


Aye-ayes


Lemurs


Sportive lemurs


Woolly lemurs

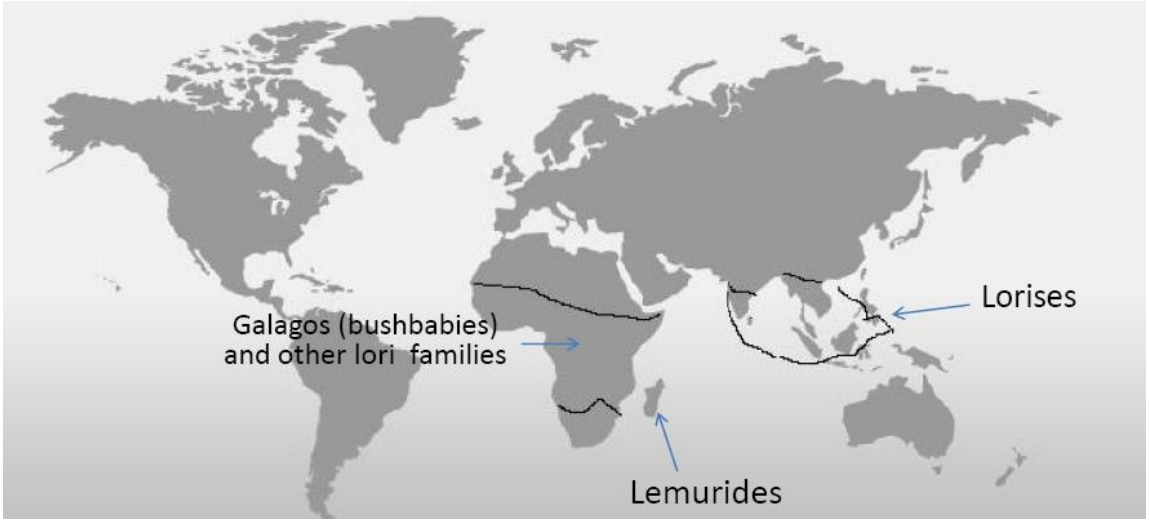

Lorises


Galagos

In brief, the greatest abundance and diversity of strepsirhines occur on the island of Madagascar, off the eastern coast of southern Africa. The world´s fourth largest island, Madagascar has an area approximately that of California and Oregon combined and lies totally in the southern hemisphere. There is tremendous regional diversity in the flora, with tropical rain forest along the east coast and in the northwest, mountain regions in the north, dry forest and spiny deserts in the west and south, and heavily cultivated central plateau that has been almost totally denuded of natural vegetation.

Madagascar has been separated from African mainland for over 100 million years, meaning that most of its plant and animal life has evolved in isolation. This has resulted in very high levels of endemism, both at species level and, more importantly, at higher

taxonomic levels. Recent information on endemic families and genera (Mittermeier et al., 2004) indicate that Madagascar is one of the world leaders in these categories as well, with many more unique evolutionary lineages than any other hotspot. The 100 % of the primates that live on the island are endemic; it has 5 families, 15 genera and 97 species found nowhere else.



PROSIMIANS OF MADAGASCAR

1.- DWARF AND MOUSE LEMURS (Family Cheirogaleidae)

The smallest strepsirhines with a mass of less than 500 g. They are all nocturnal and nest-building animals. All move quadrupedally and most have elongated bodies with short legs. Some of them can communicate using ultrasonic vocalizations (Zimmermann, 1995).

The family has five genera and 30 species: *Microcebus*, the mouse lemurs (18 species); *Allocebus*, the hairy-eared dwarf lemur (one species); *Mirza*, giant mouse lemurs (two species); *Cheirogaleus*, the dwarf lemurs (five species); and *Phaner*, the fork-marked lemurs (four species). No subspecies are recognized at the present time.

The smallest are the mouse lemurs (Picture 1). They seem to be subject of extraordinary predation pressure from a wide range of carnivores, snakes and birds.

Picture 1: Two *Microcebus murinus* or Gray Mouse Lemur (Courtesy of David Haring, Duke Lemur Center photographer).

The **mouse lemurs** are the most faunivorous in their diet, eating invertebrates as well as small vertebrates (tree frogs and chameleons). They also take fruits, flowers (nectar), buds and leaves. Giant mouse lemurs (Picture 2) feeds on fruits, flowers, gums, insects and are specialized on secretions produced by the larvae of colonial insects.

Dwarf lemurs are predominantly frugivorous and fork-marked lemurs (Picture 3) are specialized in gums, their gut is characterized by a large caecum [the pouch-like portion of the large intestine whose main function is to digest plant-based foods] in which the gums are chemically broken down.

Picture 2: Northern giant mouse lemur (*Mirza zaza*) photographed in captivity at the Duke Lemur Center (Courtesy of David Haring).

Picture 3: Fork- marked lemur (photo by R.A. Mittermeier).

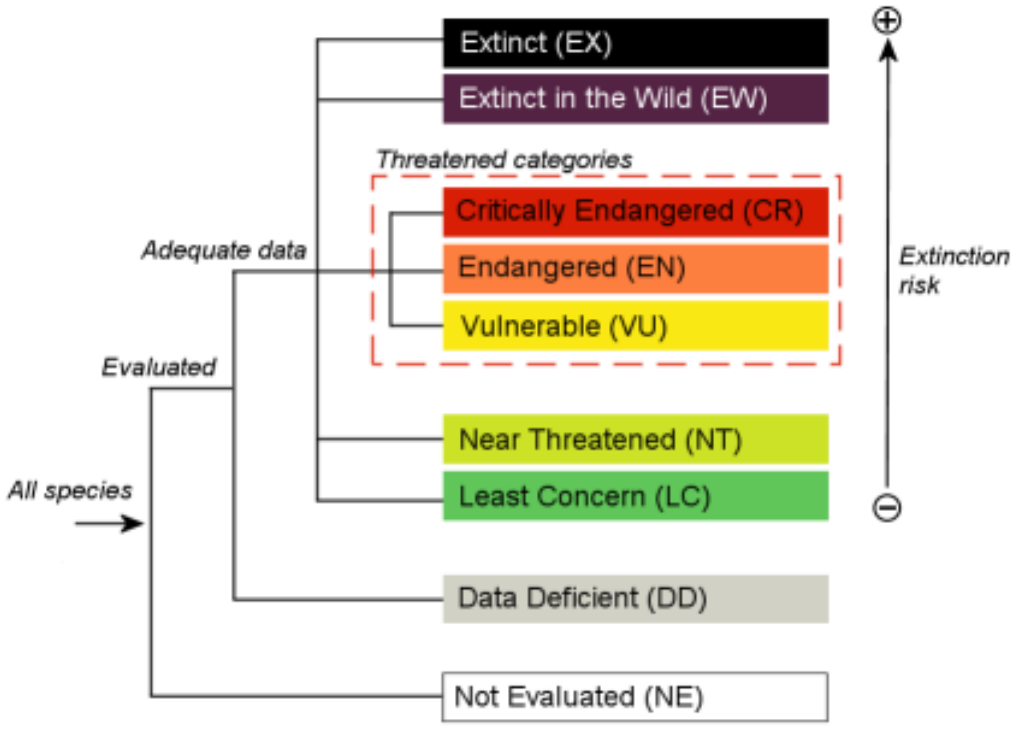
Cheirogaleids are usually solitary foragers with individuals foraging in separate overlapping home ranges but fork-marked lemurs seem to live in more or less permanent groups, many of which contain one male and one female.

Some of them undergo prolonged periods of seasonal torpor (temporary hibernation). Dwarf lemurs (Picture 4) adapt to the dry season of Madagascar by hibernating for six to eight months of each year. During this time, they metabolize the enormous fat reserves stored in their tails during the wet season.

Picture 4: Fat-tailed Dwarf lemur (*Cheirogaleus medius*) courtesy of David Haring, photographer at Duke Lemur Center.

They are seasonal breeders mating in the dry season (September-October) and their birth season coincides with the wet season (November- February). Litters usually are of two or three newborns.

Concerning their conservation status, the most recent (2011) assessment of the International Union for Conservation of Nature, the so-called IUCN Red List, which is the most comprehensive information source on the status of wild species whose overall aim is to convey the urgency and scale of conservation problems to the public and policy makers and to motivate the global community to work together to reduce species extinctions, classified 4 *Microcebus* as Endangered, one *Mirza* and two *Phaner* as Vulnerable, one *Mirza* as Near Threatened, two *Cheirogaleus*, three *Microcebus* and 2 *Phaner* as Least Concern.



Others like *Allocebus* (see picture 5) are classified as Data Deficient because there is inadequate information to make a direct, or indirect, assessment of its risk of extinction based on its distribution and/or population status.

Picture 5: Hairy-eared dwarf lemur (*Allocebus trichotis*), photo by E.E. Louis Jr.

2.- LEMURS (Family Lemuridae)

This family consist of two main subgroupings that include the best known and the most spread of the lemurs. The three genera *Lemur, Eulemur* and *Varecia* all are considered "true lemurs" and have diets that are largely made up of fruits and leaves. The bamboo lemurs, which include the genera *Hapalemur* and *Prolemur*, specialize in eating bamboo although they take other food items as well.

Members of the Lemuridae range in weight from 700g to 4.5 kg. With forelimbs slightly shorter than hindlimbs, they move largely quadrupedally along the branches and will leap across gaps in the forest. Typically they live in groups and are active during the day, but all except the ruffed lemurs (*Varecia*) and the ring-tailed lemurs (*Lemur*) are also active a certain amount of time during the night, exhibiting an activity pattern referred to as cathemeral (Tattersall, 1987), that is, "distributed approximately evenly throughout the 24 h of the daily cycle, or when significant amounts of activity, particularly feeding and/or traveling, occur within both, the light and dark portions of that cycle" (Ankel-Simons, 2007). The extent of this nocturnal activity remains poorly documented in many species, and represents a growing area of interest among primatologist (Curtis and Rasmussen, 2006; Donati *et al.*, 2007; Kappeler and Erkert, 2003).

The **genus lemur** only has one species, the ring tailed lemur or *Lemur catta* (Picture 6) who is the most widely recognized and best studied of all Madagascar strepsirhines. Is a relatively large, diurnal lemur, easily recognized by its black-and white ringed tail and it is by far the most terrestrial of the lemurs. Despite being well known, this lemur has an uncertain taxonomy and its exact position on the lemur branch of the primate family tree has been much debated. It shares some behavioral traits with the bamboo lemurs (scent communication) and also the many *Eulemur* species, which were originally classified in the same genus. They form particularly large and complex groups with strict social hierarchies, the typical group consisting of a dominant adult female and several males who will later disperse between various other groups when they reach three to four years of age.

Picture 6: Ring-tailed lemurs or *Lemur catta* photographed in captivity at Faunia (Madrid) by Gloria Fernández Lázaro.

The **genus *Eulemur*** has been subject of many changes in its taxonomic classification at the level of species but for the evidence at the moment we recognize 12 species overall. They present sexual dichromatism in the coat color (Picture 7) and the extreme is observed in *E.flavifrons*, in wich males are black and females blond. They tend to scent mark with anogenital region and their social structure is variable: some species tend to form pair bonded groups while others occur in large, multi-male/multi-female groups that can number close to twenty individuals (Overdorff *et al.*, 1999). Females typically carry their offspring on their abdomens and wean them at six to seven months of age (Overdorff and Johnson, 2003).

Picture 7: Male (at the right) and female (at the left) of *Eulemur fulvus albifrons* photographed in captivity at Faunia (Madrid) by Gloria Fernández Lázaro.

Ruffed lemurs of the **genus *Varecia*** are the largest and most frugivorous members of the family. They are clearly recognizable by their long faces and their raucous loud call. This genus has the shortest gestation period and produces the largest litters of the family (Vasey, 2007). Females commonly have litters of two or three infants that are kept in a nest for the first two weeks of life. This genus has many color variations within the same species and a lot of debates have been opened around their taxonomy. For the moment, as many authors (Groves, 2001; Mittermeier *et al.*, 2010), we recognize two species *Varecia variegata* (with three subspecies) and *Varecia rubra* (Picture 8).

Picture 8: *Varecia rubra* (at the right) photographed in Faunia (Madrid) and *Varecia variegata* (at the left) photographed at Duke Lemur Center by Gloria Fernández Lázaro.

Concerning their conservation status, according to the most recent IUCN Red List assessment (2011) four species of this family, *Varecia variegata, Eulemur flavifrons, Hapalemur alaotrensis* ,and *Prolemur simus,* are Critically Endangered which means that they are considered to be facing an extremely high risk of extinction in the wild. Four species are Endangered, nine are Vulnerable and three Near Threatened.

Of the "bamboo lemurs" members of the genus *Hapalemur* are best known for a diet dominated by bamboo (Rand, 1935); an unusual ecological specialization among primates. Different species prefer to eat different parts of the bamboo plant, which contains high levels of poisonous cyanide, so they have clearly developed a mechanism for withstanding extremely large amount of this deadly chemical. All of them have relative short faces and small, hairy ears. We currently recognize five species and seven taxa because in 2007, Rabarivola *et al.* described two new subspecies of *H. griseus* (see Picture 9 next page).

The other "bamboo lemurs" genus, *Prolemur,* contains only a single species, *Prolemur simus* (See Picture 9 next page) . It is much larger than any of the other species of bamboo lemurs (Albrecht *et al.*, 1990) and has at least nine craniodental features that are distinctive (Vuillaume-Randriamanantena *et al.*, 1985).

Picture 9: At the right two *Prolemur simus* (photo by Tony king) and at the left one *Hapalemur griseus* (courtesy of David Haring, Duke Lemur Center).

3.- SPORTIVE LEMURS (Family Lepimuridae)

Scientists used to place the sportive lemurs of the genus *Lepilemur* together with the extinct giant lemur, genus *Megaladapis* (in the Megaladapidae family) but, based on recent genetic analyses (Yoder *et al.*, 1999; Karanth *et al.*, 2005), other experts have decided that the relationship between these two lineages is not as close as originally thought

So nowadays we recognize this family as separate from the Megaladapidae, and containing a single genus, *Lepilemur* with 26 species, most of which have been described only in the past ten years (Picture 10).

All are medium-sized, largely folivorous, and nocturnal. Sportive lemurs generally weight less than one kilogram and are approximately half a meter in length, including the tail.

They are vertical clingers and leapers. And their face is covered with short hairs.

Leaves constitute the bulk of the diet, with flowers, buds and sometimes fruit taking on greater importance toward the end of the dry season. They have evolved a large caecum to extract the maximum nutrition from this diet. Some researchers have also reported observing them redigestering their feces, a behavior known as cecotrophy which allows further digestion of partially processed plant material. [Cecotropes, also called 'night feces' or 'soft feces,' are the material resulting from the fermentation of food in a part of the digestive system called the caecum. They are nutrient-rich and are passed out of the body, like feces, but are reingested by the animal so the nutrients can be absorbed. Cecotropes have twice the protein, and half of the fiber of the typical hard fecal pellet.

They also contain high levels of vitamin K and the B vitamins. They are typical of rabbits and other rodents.]

Their social and mating system is poorly understood but some species are thought to lead entirely solitary lives, and others seem to live in dispersed pairs with a male and a female sharing a tree hole during the day and separating at night to forage.

Picture 10: *Lepilemur leucopus* (photo: Wildlife Madagascar).

There is insufficient information to determine the conservation status of most of the species of this family. The latest IUCN Red List assessment (2011) classified 23 of them as Data Deficient.

Where there is information, only on three species, their status has been classified as follows: *Lepilemur septentrionalis*: Critically Endangered; *Lepilemur ankaranensi:* Endangered; and *Lepilemur edwardsi*: Vulnerable.

4.- WOOLLY LEMURS AND ALLIES (Family Indriidae)

This family contains three genera: *Avahi*, *Propithecus*, and *Indri*. The first one is small (roughly 1 kg) and nocturnal, the latter two are large (3-9 kg) and diurnal, and include the largest of all living lemurs, the indri (*Indri indri*).

All three genera are "vertical clingers and leapers" an unusual postural and locomotor complex among primates. This is characterized by resting postures in which the trunk of the body is held vertically on upright supports and locomotor behavior that involves leaps between vertically supports, sometimes quite spectacular and up to 10 m between trees.

The members of **genus *Avahi*** are also known as Woolly lemurs (Picture 11). This common name refers to the curly or woolly appearance of the dense fur, while the

generic name is a transcription of the animal´s high-pitched vocalization (Thalmann, 2003). Frequently they rest closely huddled together in pairs or small family units of three or four. Current number of described *Avahi* species is nine but there are strong indications that still more species remain to be discovered.

Picture 11: Western woolly lemur (*Avahi occidentalis*), photo by E.E. Louis Jr.

The sifakas or simponas, **genus *Propithecus***, have long tails and legs relative to their trunk and arms (Picture 12). Movement is mainly by leaping which begins with a rapid extension of the legs that propel the animal upward and outward to the next vertically-oriented support. Some species also regularly descend to the ground, where they hop and bound bipedally, often with their arms held above the head. The name "sifaka" is derived from the characteristic threat behavior in which the animal issues an explosive, hiss-like "shee-faak" and jerks its head rapidly backwards, often several times in succession. The diet is seasonally variable but consists principally of leaves (most important food on dry season) fruit (during wet season) and flowers.

Picture 12: Coquerel´s Sifaka (*Propithecus coquereli*) photographed at the Duke Lemur Center by Gloria Fernández Lázaro.

They live in moderate-size groups of three to nine individuals, often with more than one breeding female. Females generally lead the group progressions and are dominant over males in access to food. They normally give birth to one infant. Until further researches on their taxonomy are posted we believe that the most appropriate approach is to recognize all nine *Propithecus* taxa as full species.

The **genus *Indri*** only has one species the *Indri indri* which can be either black-and-white or almost entirely black (Picture 13). It is different from almost all the lemurs by its size and especially by its unique vestigial tail. Many organisms possess [vestigial structures are those that have no apparent function, but that resemble structures their presumed ancestors had], the only living lemur with such a short tail. The name "indri" actually comes from the 18th century French naturalist Sonnerat, who recorded this term when his Malagasy guide pointed out an animal in the field. "Indri" is nothing more than a corruption of the malagasy word "iry" or "ery", which simply means "there", meaning in this case "there it is". A common Malagasy name is "babakoto", which translates as "father of Koto" or "old man".

They are folivores that subsists on a diet mostly of immature leaves, but also includes seeds, fruit and flowers. Its signature vocalization is a loud, drawn out, wailing territorial call. The adult pair of a family social group typically leads the chorus, and neighboring groups often call sequentially in response to one another.

Reproduction is highly seasonal, with the birth of a single offspring in May or June.

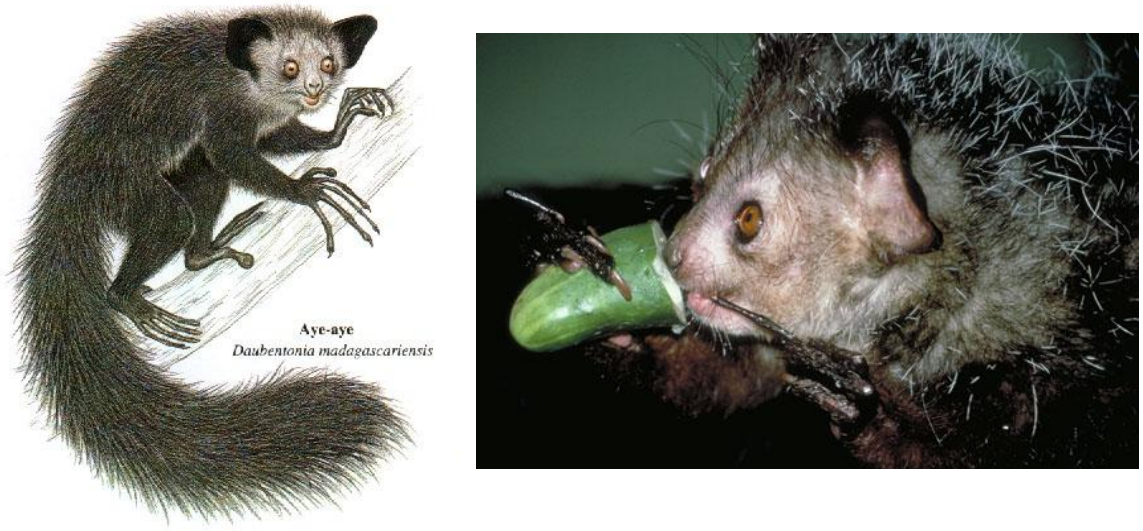
Picture 13: *Indri indri* (photo: Wildlife Madagascar).

Concerning their conservation status, in the 2011 IUCN Red List assessment, two species of sifakas are classified as Critically Endangered (*Propithecus candidus* and *Propithecus perrieri*), two species of *Avahi*, five species of *Propithecus* and the *Indri* as Endangered, two species of *Propithecus* as Vulnerable, none as Near Threathened and

one species of *Avahi* as Least Concern. The rest of species of *Avahi* are considered Data Deficient.

5.- AYE-AYES (Family Daubentoniidae)

This family has just one living species, the aye-aye (*Daubentonia madagascariensis*) and one extinct species (*Daubentonia robusta*). The living species is a very unusual nocturnal animal (the largest nocturnal primate in the world), that differs from other lemurs in many anatomical and behavioral specializations, among them its distinctive dental formula, the continually growing incisor teeth (which led to it being considered a rodent during part of the 19th century), the large ears that are almost certainly used to locate insect larvae in decayed wood, and the thin skeletal middle finger that is used to extract such prey. The animal´s total length is almost doubled by the big bushy tail, which is similar to a squirrel´s (Picture 14).



Picture 14: At the right one aye-aye eating using the middle finger (photo courtesy of David Haring) and at the left an illustration by Stephen D. Nash.

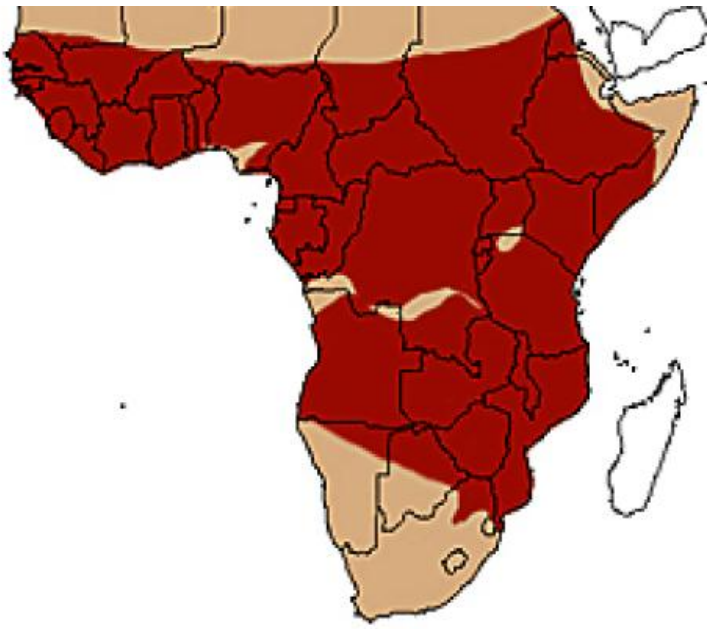
The aye-aye spends up to 80% of the night travelling and feeding. During the day it sleeps in nests, tree forks or vine tangles (Petter and Petter, 1967, Petter, 1977). It may not be as solitary as originally believed, with some studies suggesting foraging behavior in tandem and differing relationships between animals of the same sex (Sterling and Richard, 1995). There does not appear to be a restricted mating season, and a single infant is produced once every two to three years (Petter and Peyriéras, 1970). Its reputation as omen or portent of death is not helped by its taste for coconuts and sugar cane, which brings it into conflict with farmers. In 1935, the aye-aye was declared extinct, only to be rediscovered in 1957. The species is now known to have a wide but sparse distribution in eastern Madagascar. In the 2011 IUCN Red List assessment it is classified as Near Threatened but some authors consider this status as incorrect, believing that it should be placed in the Endangered category (Mittermeier *et al.*, 2010).

PROSIMIANS OF AFRICA AND ASIA

In addition to the diverse radiation of strepsirhines on Madagascar, there is a smaller mainland radiation represented by the galagos in Africa and the lorises in Africa and Asia. Galagos and lorises are nocturnal and arboreal but the two families are extremely different in their postcranial morphology and locomotor behavior.

6.- GALAGOS -OR BUSHBABIES (Family Galagidae)

Galagos or bushbabies received this common name because their calls resemble to the sound of a human infant. They are found in forests and woodlands across most of sub-Saharan Africa. Traditionally this family consisted of three genera: the Greater Galago (*Otolemur*), the Lesser Galago (*Galago*) and the Needle-clawed Galago (*Eutocius*). Most recent data recognize five genera and 24 species some of them not well defined yet although they are expected to be described in the next years. All of them live throughout sub Saharan Africa.



Range of the galagos

The genus *Sciurocheirus* has three species (*Sciurocheirus alleni* with two subspecies, *Sciurocheirus gabonensis* and another new species not well defined yet). The genus *Galagoides* (dwarf bushbabies) has eight species well defined and four new. The genus *Galago* has four species, genus *Euoticus* (Needle-clawed bushbabies) has two species and the genus *Otolemur* (grater bushbabies) has three species.

The confusion regarding the taxonomy of these primates is largely a consequence of their secretive, nocturnal habit and because separate species may look superficially similar, but their behavior is usually distinctly different; so only recently such differences are being used to help refine the taxonomy (Picture 15).

Picture 15: Similarities even within genera: at the left genus *Euoticu* and at the right genus *Sciurocheirus*

Bushbabies are amazing agile, they can easily cling onto vertical as well as horizontal supports and can use their powerful back legs to leap across gaps in the canopy up to 6 meters wide, using their long bushy tail as an aerodynamic stabilizer. This form of locomotion is more commonly used by the smaller ones. Larger species like the genus *Otolemur* (the biggest in the family) can also jump but seem to prefer running along branches (Picture 16). Also they used to take more fruit in the diet than the smaller species which catch more insects. They detect prey with their large, mobile ears and good nocturnal vision, catching it with their hands. Every species, however, eat some tree gum scraped from bark using the tooth-comb and in the genus *Euoticus* also by the clawlike nails. This ability enables bushbabies to survive the long winters in South Africa when fruits, flowers and insects are scarce. One new species, recently discovered in south-eastern Tanzania, drinks nectar from flowers, and may act as pollinator in its woodland habitat.

Picture 16: *Galago moholi* at the right and *Otolemur garnetti* at the left (courtesy of David Haring).

Many bushbaby species, especially males, urinate on their hands and rub the urine on their feet so that they leave a scent trail. Also they appear to be able to recognize individuals by their call. Up to 25 separate calls have been recorded for them (Petter and Charles-Dominique, 1979). Vocalization helps to maintain the social matrix and this is true over long distances as well as in close ranges interactions. They spend the night foraging, feeding and interacting with other animals and they meet to go to sleep in a nest, tree holes or in a branch. Males have large home ranges overlapping those of several related females, which occupy smaller home ranges. Females can act aggressively toward females from neighboring or unrelated sleeping clusters. Males are also aggressive towards rivals, but may tolerate smaller, low-ranking males. They can have twins or even triplets offspring and the mother carry the infants in her mouth if she must move them.

The 2011 IUCN Red List assessment classifies one species *Galagoides rondoensis* as Critically Endangered, none as Endangered or Vulnerable, one (*Galagoides orinus*) as Near Threatened. Two *Euoticus*, four *Galagos*, five *Galagoides*, two *Otolemur*, and two *Sciurocheirus* are listed as Least Concern. *Galagoides nyasae* appear as Data Deficient. Some recently discovered species (one *Sciurocheirus* and four *Galagoides*) and one species of *Otolemur* (*Otolemur monteiri*) don´t appear yet in the IUCN Red List.

7.- LORISES, POTTOS AND ALLIES (Family Lorisidae).

In contrast with galagos that are leapers, members of this family clamber along and between branches with slow, deliberate movements. They have long been characterized as "nocturnal and creepers" without much of a social life, but in recent years, new studies have started to throw more light on these secretive animals.

Many of the species appear very similar to human eye and it´s only with detailed DNA studies that the complexity of their taxonomy is being revealed, so it seems likely that more new species will be described. Nowadays available information recognize four genera: genus *Loris* (Slender Loris) with two species and maybe five subspecies, genus *Nycticebus* (Slow Loris) with five species, genus *Arctocebus* (Angwantibo) with two species (Figure below) and genus *Periodictius* (Potto) with one species.

Genus *Arctocebus*, figures redrawn from photos by S. Bearder (www.loris-conservation.org).

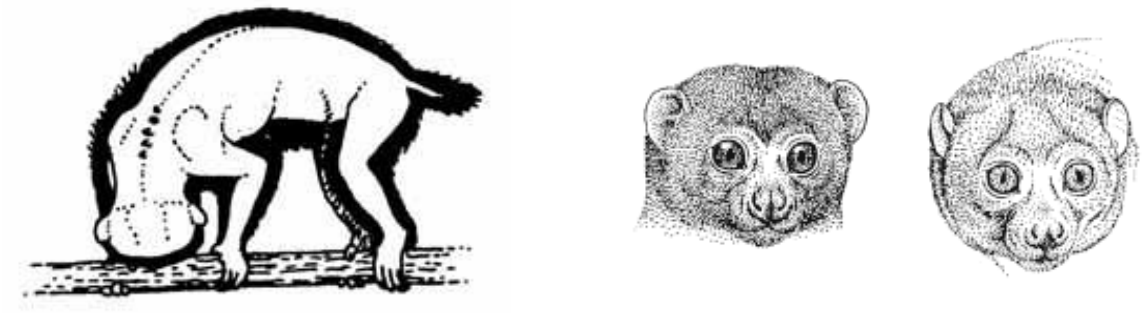
Loris, Pottos and Angwantibos have a powerful grip and can cling to branches for hours without tiring. They have very short tails and their arms and legs are of similar length.

Being slow-moving does limit the kind of prey these primates can catch. Pottos and Angwantibos eat ants, millipedes and caterpillars, apparently immunes to their chemicals defenses. Pottos and slow lorises also feed on fruit and gum. In contrast, Slender lorises, are among the most predatory primates, with up to100 per cent of their food being animal prey (Picture 17).

Picture 17: Slender loris or *Loris tardigradus* (photo courtesy of David Haring).

Members of this family have really unusual systems of defense. Slow lorises lick the inside of their arms where glands secrete a sweat-like substance that contains toxins to paralyze their prey. The saliva activates these toxins, so a bite from a loris can be doubly dangerous. Not only do lorises have long razor-sharp canine teeth that inflict deep lacerations, but some people overreact to loris saliva by going into an anaphylactic shock.

Pottos have apophyseal spines [a natural swelling, projection, or outgrowth of some vertebrae]. Several of their neck vertebrae (numbers 3 to 9) have long, bony parts that actually protrude from skin as spines. When threatened, a Potto tucks in its head to present the spines, and above them its shoulder blades form a bony shield (see figure below).



Redrawn of photos of genus *Periodictius* (Potto). At the left the defensive posture showing the nuchal spines (www.loris-conservation.org).

They are usually alone when encountered, but behavioral studies are beginning to reveal a more complex social life than previously suspected. Adults have been seen spending varying amounts of time with other adults and male slender lorises often play with infants from neighboring sleeping groups.

Male territories are larger than those of females and include more than one female territory. Females announce their receptivity by urine-marking and, in some species by giving calls. Pottos and angwantibos give birth to a single infant, lorises to one or two (Picture 18). There are sometimes two births in a year. After foraging all night they sleep in tangled thickets or forked branches, sometimes in male-female pairs with offspring.


Picture 18: A pair of Pygmy slow lorises (*Nycticebus pygmaeus*), photos courtesy of David Haring, DLC photographer.

None of the species are classified by the 2011 IUCN Red List as Critically Endangered. One species of *Loris* and one species of *Nycticebus* are listed as Endangered, four species of *Nycticebus* as Vulnerable, and the rest of the family (one species of *Loris*, the two *Arctocebus* and the *Periodictius*) as Least Concern.

II.- PROSIMIANS´ WELFARE IN CAPTIVITY

One of the most widely accepted definition of animal welfare is that it comprises the state of animal´s body and mind, and the extent to which its nature (genetic traits manifest in breed and temperament) is satisfied (Duncan and Fraser, 1997). However, the three aspects of welfare sometimes conflict, and this presents practical and ethical challenges. Disagreement occurs because the form of compromise that is acceptable to different animal-oriented social groups or collectivities depends on their values (Fraser *et al.*, 1997; Fraser, 1995) and it becomes farther complicated because of the additional need to consider eventual legitimate human interests.

This chapter II will describe the best practice for the maintenance of prosimians in captivity according to the experience of the Duke Lemur Center where no damage to the animals is caused. The Center, as stated in the introduction, was established in 1966 and today is the world´s largest sanctuary for rare and endangered prosimian primates. Nestled on 85 acres in Duke Forest (Durham, North Carolina), the Lemur Center houses 240 animals, including 225 Lemuriforms (family Lemuridae, Cheirogaleidae, Indriidae and Daubentonidae) and 15 Lorisiforms (family Galagidae and Lorisidae).

Although there are many ways to analyze how welfare conditions, which always overlap with health-veterinary conditions, are to be established and checked, we will analyze first the housing requirements and afterwards those related to nutrition, practice on groups composition and breeding, enrichment program and veterinary care.

1.- HOUSING CONDITIONS

General housing requirements need to:

- Allow for normal physiological and behavioral needs of the animals.
- Make it possible for animals to remain clean and dry.
- Allow adequate ventilation.
- Allow access to food and water and permit easy filling, changing and cleaning.
- Provide a secure environment that does not allow escape or accidental entrapment of animals or their appendages between opposing surfaces or structural openings.
- Be free of sharp edges or projections that could cause injury.

Normal maintenance of enclosures must pay attention in providing the correct cleaning to prevent the accumulation of pathogenic organisms, attach firmly heat lamp to prevent burns of shelves and animals, replace rusting or oxidized materials, remove sharp objects, be careful with items that are small enough for animals to ingest, etc.

But, in particular, the captive maintenance of strepsirhines also must take into account some peculiarities of this suborder.

The first and most important of them all is the need to adjust to the cycles of light.

In contrast with other primates, most of them are primarily nocturnal, some are diurnal, crepuscular (active only at dawn and dusk), others are cathemeral (being sporadically active throughout the 24 hour light cycle), and a few display some flexibility in circadian rhythms [the physical, mental and behavioral changes that follow a roughly 24-hour cycle, responding primarily to light and darkness.] The activity of some species appears to be seasonally influenced by photoperiods and directly modulated by nocturnal ambient luminosity (Donati and Borgognini-Tarli, 2006). Some institutions maintain reverse light cycles for nocturnal species by using regular lighting at night and red illumination during the day so that caregivers can observe them during their active period. In the Duke Lemur Center the night enclosures have the Northern Madagascar photoperiod, the hours of light and dark change over the seasons and they adjust the lights on time every two weeks. Unless absolutely necessary, white lights are never turned on during the animals' dark cycle and when observing them in dark, flashlights or white penlights are used, instead of bright white flashlights.

The well-developed rhinarium of strepsirhines makes the olfactory communication a very important part of their behavior. Most of the species scent-mark and urine-mark their surroundings, so the ability to maintain these marks in their environment is important for the general well-being of the animals. Behavioral observations and several experimental studies have shown that secretions yield detailed information about scent

donors species, subspecies, gender, individuality, and hormonal status as well as the age of the scent mark (Colquhoun, 2011; Lewis, 2006,2005; Hayes *et al.*, 2004; Fisher *et al.*, 2003; Buesching *et al.*, 1998). This information is important in many contexts, such as territoriality, reproduction, and social hierarchies. Frequent and too-thorough cage cleaning might be highly disruptive to their wellbeing. In the Duke lemur Center sanitization (process of 99.9% elimination of all microorganisms) takes place every 6 weeks the nocturnal enclosures (mouse and dwarf lemurs, aye-ayes, lorises and galagos), every 4 weeks the indoor enclosures for the rest of the species and every 8 weeks the outdoors enclosures. They have found this periodically cleaning to be effective in maintaining sanitation and providing essential species odors.

To minimize cross contamination, cleaning equipment such as dustpans, brooms, scrub brushes, rubber gloves, and nets, are section-specific and should not be moved between sections. For the nocturnal enclosures daily cleaning include manually cleaning cage bottoms to remove feces, urine soaked shavings and left over food. All water bowls, bottles and cloth items are sanitized weekly. Every two weeks caretakers sweep the floor, hose with dilute bleach, foam and water and replace pine shavings. Every six weeks, except during breeding season, animals are removed from the rooms and complete sanitation of the cages is performed.

Heat treated pine shavings are used as floor covering for nocturnal indoor animal areas instead of hardwood mulch because it has been proved to be superior for animal health avoiding fungal spores and with significantly less dust and fine particulate.

Other animal facilities at the center provide heated indoor areas associated with larger outdoor runs connected with parts of the wood delimitated with electrical fences where the lemurs are free ranging (See picture 19).

Picture 19: Outdoor run connected with the woods

These areas are called Natural Habitat Enclosures (NHE) and their daily care and maintenance loads are likely to vary seasonally. Only animals that are 100% reliable at

lock up from August 1st through September 30th may be allowed into the NHE to free range in winter season (which ends April 15) on days when the night-time temperature is 40º F (4,4º C) or above for three consecutive nights, unless otherwise directed for medical reasons or for groups with young infants. To be eligible, individual animals must have resided in the same NHE during the summer months as the one they are released into during the winter months. 100% reliable is defined as being locked into a secure area within one hour after receiving an auditory cue, or other conditioning stimulus, 100% of the time. If an animal does not maintain 100% reliability (from August 1 through the winter season ending April 15), that individual animal loses the option for the NHE winter free ranging privileges for that winter season. During periods when the animals are free ranging for more than 3 consecutive nights, conditioning for lock up continues twice weekly on the same schedule as during the summer for each NHE. On days when the nighttime temperature is predicted to be below 40ºF, free ranging animals must be confined/locked-up by noon that day and locked into heated space for that night.

Daily cleaning of indoor enclosures include washing of shelves, walls and floors and swept of food items. Disinfection occurs monthly and bedding must not be used on the floor, exceptions may be made when young infants are present.

Outdoor compartments have daily raking and spot cleaning, and disinfection is every two months. Cage branching of nocturnal enclosures as well as the indoor and outdoor enclosures has to ensure that branches are changed with sufficient frequency to be in good repair and has to be sanitized adequately.

Variances among families do exist. True lemurs (*Lemur, Eulemur* and *Varecia*) are well in large outdoor enclosures. Indoor rooms may also be used, although lights must kept on a timer or on a manual light cycle designed to stimulate seasonal photoperiod changes to ensure breeding (Bernstein *et al.*, 1998). Heated shelters should be provided with resting and feeding shelves. Under severe weather conditions, lemurs might need to be secured in sheltered housing. It should be furnished with vertical and horizontal natural substrates, such as vines, bamboo and branches. Large forested enclosures can hold species of all lemurids genera, inasmuch as these species are not hostile or competitive toward each other.

Family Cheirogaleidae (mouse and dwarf lemurs) specimens are conveniently housed in cages containing family groups of pairs or trios of animals plus the season´s juveniles. For breeding, cages partitioned in several chambers that can be connected are recommended (see Picture 20, next page) because for example, females of *Microcebus* can reply aggressively to male solicitations before the female becomes receptive (Andres *et al.*, 2001). "Nest boxes" composed of plastic tubing of various diameters to accommodate larger and smaller cheirogaleids are recommended.

Sifakas have highly developed leaping abilities and they need more space and more vertical supports than do other strepsirhines. Small families have been successfully maintained in indoor rooms. In warmer climates, these animals can be released into large outdoor enclosures, but they need access to heated shelter boxes during colder weather. Heat lamps should be in more than one location because males are sometimes excluded from choice sleeping sites.

Picture 20: Mouse lemur cages for breeding purpose at Duke Lemur Center, photo by Gloria Fernández Lázaro.

Aye-ayes gnaw cage structures with their large front incisors and require sturdier housing than other strepsirhines. They have bred successfully in indoor rooms with vertical and horizontal branches as well as ropes for climbing. They require a nest box for rest periods and daytime sleeping (Picture 21). Aye-ayes stuff their nest boxes with branches, leaves, or straw as available. They normally live independently in the wild, but when provided with two or more next boxes, mixed-sex pairs can live together after a period of adjustment.

Picture 21: Aye-aye in a nest box at the Duke Lemur Center, photo by Gloria Fernández Lázaro.

Lorises and galagos require nest boxes for daytime sleeping.

The slow-moving lorises require multiple horizontal branches and platforms for resting and marking. Maximizing surface area by providing many pathways of varying size and making visual barriers will make the most of any space. They like to vary sleep sites, so leafy cover in branches and boxes on the floor can also provide other options. Because lorises have a "dropping response" when startled, floors should be well cushioned with shavings or other suitable material. Temperature range can be 65.5 F (18.5 ºC) min – 85.5 F (30.5 ºC). The lighting requirement is approximately 12hours/day. Unless they are outdoors in natural light, full spectrum illumination is suggested. Relative humidity should be maintained between 40% and 60% (Fitch-Snyder and Schulze, 2001).

Bushbabies are active jumpers and need enclosures to allow them for leaping furnished with tree branches. Large cages and cages partitioned into several chambers connected, that can be variously be closed off, can be used for breeding. Males should be separated from newborn infants because they sometimes attack. They can be kept in groups of females and young together with a single male. Mature males will fight and such battles may even end in the death of the loser if they are not separated. In the wild, confrontations are usually avoided but in captivity aggression is most common between members of the same sex while affinitive behaviors are mostly seen between the sexes (Nash and Flinn, 1978). Bushbabies sleep in nests so they need tree hollows or another structure to use during the inactive period.

3.- NUTRITION

Prosimians are a highly diversified group of animals with extraordinary variation in dietary requirements, no general diet can be recommended. Many of their dietary needs are still not well understood; therefore, maximizing dietary variety might prevent nutritional imbalance. One thing that they have in common is that, unlike anthropoids primates, for which the fruit is a primary source of vitamin C, strepsirhines can synthesize their own vitamin C (Nakajima *et al.*, 1969). They should not be fed with a lot of citrus fruits. Diets high in vitamin C have been thought to cause hemosiderosis, a type of iron overload disorder, since vitamin C increases iron absorption (Spelman *et al.*, 1989). However, as of today, there is not sufficient information to determine whether dietary levels of vitamin C contribute to the development of hemosiderosis in lemurs. Current findings suggest that iron overload in lemurs may be more complex than was previously believed (Glenn *et al.*, 2006). Some authors have found significant differences in various iron metabolites in lemurs, suggesting that normal reference values may need to be developed on a species by species basis (Williams *et al.*, 2006).

Vitamin D deficiency is a common problem in primates housed exclusively indoors. It causes weak or deformed bones and fractures. It is most common in young growing animals that do not get enough of the nutrient from their diet and have no exposure to natural sight. At the Duke Lemur Center, Vitamin D supplementation is provided for young aye-ayes (begin at 3 month of age) and after a year, they have consumed enough vitamin in the gruel so supplements are no longer necessary.

Food handling procedures are designed to ensure food safety and prevent the spread of food borne illnesses to the animals. Surfaces of fruit and vegetables may contain bacteria, fungal spores, parasite ova, and viral contaminant that can make animals ill if they are ingested. So at the DLC all fruits and vegetables are soaked in a dilute chlorine bleach solution prior to feeding.

True lemurs can be maintained on a diet of monkey chow (Old World Monkey chow) mixed with alternating selections of chopped fruits (banana, apple, melon, grapes…) and vegetables (corn, carrot, cucumbers, green beans, broccoli, sweet potato…). Some species, such as *Eulemur mongoz*, should use folivore chow.

Ruffed lemurs need more fruit in the diet because they are frugivorous and have a simple stomach (they are not rumiants). They can eat a lot of food very quickly and have a very short small intestine. Gut transit is approximately two hours, which means that they do not have a lot of processing time. They put a lot of food in because they do not process it so much. Foods that require bacterial fermentation do not work well with ruffed lemur digestive tracts. They assimilate nutrients that are easy to assimilate (Less *et al.*, 2009).

Ring-tailed lemurs are omnivorous and have a longer digestive tract, therefore gut transit time (7 to 8 hours) is slower. Caecum, where fiber is broken down by bacteria, is similar in size to that of the ruffed lemur. Volatile fatty acids are produced by breakdown of fiber in caecum and are absorbed by the mucosal wall. Because of their longer gut, they can be fed high-fiber diets (Less *et al.*, 2009).

Bamboo lemurs, as their name suggests, need big amounts of several bamboo species in their diet with folivore chow, fruits, and vegetables.

Mouse and dwarf lemurs can be fed with a combination of cracked monkey chow mixed with fruit and vegetables. Insects must be provided during the summer diet. In winter no insects must be provided for dwarf lemurs and only one insect for mouse lemurs. Obesity will result if winter diets are not reduced. Giant mouse lemurs (genus *Mirza*), in contrast, do not enter in torpor and no dietary change is required.

Sifakas are folivores specialized in detoxifying various classes of leaf compounds, such as tannins. As a consequence their captive diets require much more attention than that of other lemurs. Leaf fiber (mango, sumac, mimosa, sweet gum and tulip poplar) appears to be critically needed for their health. The seed pods and flowers of such plants as mimosa, redbud and maple are also important food items, but they might cause diarrhea if introduced abruptly in large amounts. Peanut and oak nuts should be limited because of their high fat content, but they are preferred foods, so they might help a sick animal to recover from illness. Sifakas seem reluctant to drink water, and few will use a water bottle. Open bowls are more likely to be used and browse can be sprayed with water.

Daily standard aye-aye diet should include 250 grams of gruel, 1 fruit, 1 veggie and other less critical items. The gruel recipe, ideally fed 1-2 hours before other diet items, should have 700 grams of folivore chow, plus 700 grams of cracked old world chow with 16-17 cups of warm to hot water (this should make enough to feed 19-20 animals): the other items provide the flavoring. The daily aye-aye diet rotation can be the following

DAY	GRUEL FLAVOR	FRUIT	VEGGIES	ITEMS
1	Honey	Optional	Corn	Nuts
2	Peanut Butter	Banana	Carrot	Meal Worms
3	Syrup (maple or fruit) or jam	Orange	Turnip	Coconut
4	Almond Butter	Melon	Cucumber	Egg *
5	Apple Sauce	Pear	Corn	Meal Worms
6	Cashew Butter	Apple	Sweet potato	Nuts
7	Vanilla Extract	Grape	Optional	Egg *

*(Eggs should be soft-boiled)

Aye-ayes are not seen to drink often either, but they will lick water from a bowl by using their specialized third digit (Napier and Napier, 1985).

Lorises are adequately maintained with cracked monkey chow combined with chopped fruit and vegetables, crickets and occasional mealworms. Yogurt and additional food should be provided to pregnant and lactating females. Lactation lasts for 6 months, but juveniles begin to eat solid food at 2-3 months.

Galagos diet is similar to that of the lorises but the two need hard elements in their diet to remove tartar or plaque from their tooth combs and canines. If they are not provided or if the teeth are not cleaned, these accumulations can cause severe gingivitis, tooth loss, and eventual death. The presence of too much protein in the diet can promote kidney disease. Also galagos are gum feeders; they consume gums exuded from trees. Gums are a highly-energy food source composed mainly of water, complex polysaccharides, calcium, and trace minerals (iron, aluminum, silicon, potassium, magnesium, sodium –and specially calcium) (Nash, 1986). The calcium-to-phosphorous ratio is high in gums which offsets its ratio in insects, which is low. Because in the wild gummivores also include insects in their diet, combining both in captivity, may approach a desired nutritional balance (Nash, 1986). Gum Arabic, the hardened exudate of *Acacia senegal* and *Acacia seyal*, is widely available through primate food suppliers and other vendors (Huber and Lewis, 2011).

2.- GROUP COMPOSITION AND BREEDING

Nocturnal prosimians (Families Cheirogaleidae, Daubentonidae, Galagidae and Lorisidae) have been considered generally solitary foragers that sleep together in small groups. Recent data shows that their social organization is more complex than originally thought, so it is very important to have these patterns into account in order to make the proper group composition.

Careful monitoring of reproductive status, pregnancies, and infant births are essential to the success of the breeding program. Animals may be handled for visual inspection for signs of estrous in females and testicle size in males.

Mouse lemurs live within a dispersed yet complex social structure and are not solitary although they forage alone (Radespiel, 2000; Kappeler and Rasoloarison, 2003;

Dammhahn and Kappeler, 2005). Within this dispersed multi-male/multi-female system exist social relationships in which individual mouse lemurs are able to personally recognize other mouse lemur individuals and establish relationships with them (Radespiel, 2000). They have communal sleeping sites but there is variation between species as well as seasons (Radespiel, 2006; Atsalis, 2008). During breeding season there are morphological changes in the genitals of both sexes (Atsalis, 2008). The female vagina is closed except during estrus and birth, and at the beginning of an estrus cycle will exhibit changes in color and morphology (Perret, 1990). Mouse lemurs females can have more than one estrus cycle per breeding season (Blanco, 2008). Copulation can be detected because males deposit sperm plugs. In captivity *Microcebus murinus* males form a dominance hierarchy, with higher ranking males showing more sexual behaviors than lower ranking males and mating more often (Andrès *et al.*, 2001). As dwarf lemurs they usually have litters of two or more offspring.

At the Duke Lemur Center, the parturition and birth protocols for mouse lemurs take into account the following patterns which have proven to be really effective for their breeding:

- No new nestboxes, nesting material or enrichment should be introduced into the birth cages.
- First thing in the morning, when the lights are on, a thorough visual check of the cage, the cage below and any loose nesting material on the cage floor should be done, looking for a stranded or injured infant.
- The female should not be tempted out of the nestbox with food, neither during the light nor dark phase. She should be left undisturbed and with the most quiet environment as possible.
- Nestboxes should not be touched unless something is obviously wrong.
- The female´s diet should be increased by 50% on the day infants are discovered (or suspected).
- Infants should not be removed from the nestbox for at least the first 48 hours.
- Gloves should be used hen the animals are handed or taken out of the nestbox.

The strong seasonality of breeding found in mouse and dwarf lemurs depends on the variable "photoperiod", or day length. In the DLC they breed from mid April to July and gestation period is 58-62 days. Mothers of dwarf lemurs give birth in the nest-boxes and generally will keep their infants hidden inside these shelters. If they need to move their offspring, they do so by carrying them in their mouth but infants are capable of independent locomotion when they are two months old. On average mouse and dwarf lemurs reach sexual maturity at one year of age, although females generally are not capable of giving birth until they are 18 months of age. In the wild, juvenile dwarf lemurs tend to enter their first period of dormancy later than adults, perhaps providing the youngest with a period of reduced feeding competition in which to put on additional pre-torpor weight.

Aye-aye male/female pairs and their single infant might coexist peacefully for years in captivity. Breeding can occur at any time of the year. Males might join the female for a few days at the time of ovulation and even sleep on or near her nest. Gestation period is

around 170 days and in the wild infants are weaned as early as 7 months. In captivity, they will continue nursing as long as they remain housed with their mothers (infants might still be nursing even at 1.5 years of age). In captivity, females give birth every 2-3 years. At the DLC captive born female bred at age 3.5 years, indicating that this is the age of sexual maturity in this species.

In the wild Loris male home range overlap with that of several females. Optimally, separated cages that individuals can rotate into when they cannot be housed together, is preferable. Lorises are usually best housed as a breeding pair or mother with immature offspring. If an individual has to be separated for any length of time, visual and olfactory access should be allowed. Lorises have been shown to do well in same sex and extended family groups, but individual situations may not allow this. They are solitary by nature but not asocial. Maximizing space by housing multiple individuals together allows individuals more stimulation (Fitch-Snyder and Schulze, 2001). Horizontal branches are especially important for breeding purpose, because copulation usually takes place in a suspended position from a horizontal branch. Slender loris females typically exhibit signs of estrus at approximately one year of age, and slow and pygmy lorises can produce their first offspring at two years of age. The gestation period for both slow and pygmy lorises range between 176 and 198 days; this translates approximately in six months. The slender loris gestation period is shorter, between 166 and 169 days. Signs of estrus in females include frequent vocalizations and the enlargement and reddening of the vaginal area (Fitch-Snyder and Schulze, 2001). Timing of estrus and birth may be influenced by environmental factors such us lighting.

The social fabric of the lives of bushbabies is as varied as their habitats and for many species, incompletely known. Because some of the activity period is spent solitary, social groupings are perhaps best quantified through sleeping group size, which can vary between solitary sleeping up to ten individuals per sleeping site (Bearder, 1987; Bearder *et al.,* 2003). Males will sleep with each individual whose range overlaps his, mostly one or several adult females and immature per night (Harcourt and Nash, 1986) but this can change between species. Female bushbabies exhibit estrus swelling of the sex skin and the vagina is closed at all other times other than estrus (Lipschitz *et al.*, 2001). Estrus and the mating period lasts 1-3 days and females may mate with more than one male (Pullen *et al.,* 2000). Presence of branches is really important because some species practice suspensory copulation. Gestation lengths range from around 111 to 142 days and sexual maturity is reached between 8 and 18 months of age (Charles-Dominique, 1977; Nekaris and Bearder, 2007). Usually one infant is born per pregnancy, with the possibility of twins and, extremely rarely, triplets (Bearder *et al.*, 2003; Nash, 1983). Females lactate for an average of around 100 days after the birth of their infant with weaning at 10-14 weeks of age (Zimmermann, 1989).

True lemurs live in multi-male/multi-female groups but some species such as *Eulemur mongoz* or *Eulemur rubriventer*, also breed in single pairs. They exhibit female philopatry (females remain in the group, and maintain its cohesion, and males leave at sexual maturity) and the social group is matrilinear (Jolly, 2003). There is a dominance hierarchy among females according to matrilineality and adult females are always dominant over adult males. Some species can exhibit ovarian synchronicity so that all of the adult females are in estrus at roughly the same time. Females are sexually receptive for one to two days each year. Reproduction is highly seasonal and varies between genera.

Ruffed lemurs (genus *Varecia)* breed in Madagascar between May and July and most infants are born after a 102 days gestation period. At the DLC breeding usually occurs in December or January with births in April or May. Females can give birth to litters of up to six in well constructed nests, but infant mortality appears to be very high (65% of infants failing to make three months of age).

Ringtailed lemurs (genus *Lemur*) give birth first at three years of age and produce offspring annually thereafter. In the wild, mating begins in mid-April with infants born in August and September. They usually give birth to singletons, rates of twins and triplets being higher in captivity (Sussman, 1991). Weaning happens at 5-6 months of age.

Breeding season in Madagascar for genus *Eulemur* ranges from April to June (in the northern hemisphere locations most breeding occurs in November and December with births of one or two infants in March, April or June). Males and females are likely to mate with more than one other group member, making paternity determination difficult. Gestation period lasts between 120 to 135 days and sexual maturity is reached between 18-20 months.

The Parturition protocol of the DLC for *Lemur, Eulemur* and *Varecia* determines that the mother and the new infant be separated from the rest of the group members one or two days, being always better if they maintain visual contact with the family group.

Lemur catta is the most social species and separation of mother and infant for 24 hours is likely sufficient if all goes well. It should be recorded if the infant is nursing. The position and the response of the mother is also important during the first day as well as to obtain the birth weight in order to compare it to the weights in subsequent days.

Bamboo lemur females come into estrus once a year and single infants, very rarely twins, are born after a gestation period of some 140 days. Unfortunately, lesser bamboo lemur breeding pairs have been unsuccessful at producing infants at the DLC for years. The last successful birth of a bamboo lemur occurred in October 1998. Current DLC population is down to 7 individuals (2 males, 5 females) with little hope for future increase. Worldwide, population in captivity of *Hapalemur griseus* is only 16 individuals, none of which are currently breeding.

Sifakas live in social groups of between 3 and 10 individuals and age and sex composition of the groups vary widely. Females are dominant to males, which gives them the preferential access to food and the choice of with whom to mate. At the DLC Coquerel´s sifakas are maintained in family groups of up to six members. Breeding occur in late summer to early fall and single infants are born in late winter to early spring. Gestation period is about 162 days and at 5-6 months of age infants are weaned. Other adult females and juveniles might interfere with newborns, so it is advisable to separate periparturient females for a week. It is however desirable to maintain visual and olfactory contact with other members of the group. Young become sexually mature at around the age of 3.5 years. At this point at the DLC, they might be removed from their family groups for formation of new breeding pairs.

Attention must be paid when there are mixed species groups because although they can provide companionship for social outcast, make exhibits more interesting, maximize use of space and provide better educational opportunities, they have also the disadvantage of the complexity of managing groups´ diets and diseases. For example, lorises housed with provost´s squirrels (*Callosciurus prevostii*) have died from infectious diseases contracted from the squirrels; and lorises carry a parasite that is fatal to callitrichids [the smallest of the anthropoid primates, they are one of five families of New World monkeys, including genera such as the marmosets and tamarins] (Less *et al.*, 2009).

4.- ENRICHMENT PROGRAM

The goal of the enrichment program is to maximize the physical and psychological wellbeing of prosimians housed at the DLC given the limitations that all captive environments imply. The plan strives to encourage the expression of a wide range of species-typical behavior, to provide cognitive stimulation, and to minimize self-injurious and stereotypic behaviors.

Enrichment must be documented daily on the "enrichment sheet" and for every animal. All animals should be enriched a minimum of three times per week. Single animals should be enriched a minimum of four times per week to decrease boredom frequently associated with living alone. Animals that have free ranging privileges need to be enriched if locked in for three or more days.

The veterinary department is responsible for providing enrichment to hospitalized and quarantined animals. The veterinarian will determine enrichment frequency based on need and health status.

The DLC has seven categories of enrichment and the approved items used for them are different for small nocturnal, aye-aye and diurnal prosimians.

Categories:

1. Environmental:
   Different structures and substrates for the cage should be used such as sand, pine, natural branches, plastic chairs or pvc pipes for diurnals and small nocturnal and rotten logs, bamboo or pine boughs and needles for making nests for aye-ayes.

2. Foraging and manipulation:
   Diurnals and small nocturnals use rubber tubs, pvc tubes, paper bags, phone books, kongs [the firm rubber dog toys shaped like the top portion of a soft-serve ice cream cone, with a hole through its core which can be stuffed with dog biscuits, peanut butter, and other food items] laundry baskets, cardboard boxes, pinecones, etc. Aye-ayes use metal box feeders, bamboo tubes, cardboard boxes or paper items (bags, envelopes, phone books).

3. Sensory:

   Prosimians need olfactory or auditory stimulation. For diurnals and small nocturnal spice, food flavor extracts, perfume or cage washing are used for olfactory stimulation and radio, playbacks and soothing CD´s for auditory stimulation. For aye-ayes the same items are used for olfactory stimulation and calming music, playbacks and soothing CD´s for auditory stimulation.

4. Social:

   This kind of enrichment can be done with a new cage mate, new neighbor, keeper interaction or with a stuffed animal.

5. Training:

   The animals should get used to any operant conditioning involving an animal. For example training of female sifakas to let them touch the belly so technician can take the baby when they have them to control the weight or to recognize a shape assigned to each individual (Picture 22) [Operant conditioning (or instrumental conditioning) is a form of learning in which an individual's behavior is modified by its consequences; the behavior may change in form, frequency, or strength. Operant conditioning is distinguished from *classical conditioning* (or *respondent conditioning*) in that operant conditioning deals with the modification of "voluntary behavior" or operant behavior. Operant behavior operates on the environment and is maintained by its consequences, while classical conditioning deals with the conditioning of reflexive (reflex) behaviors which are elicited by antecedent conditions. Behaviors conditioned via a classical conditioning procedure are not maintained by consequences.]

6. Research:

   Any research project involving an animal.

7. Novel food items:

   Diurnals can use browse but not sifakas because is part of their diet. Other approved items are for example honey, jelly, fruit syrup, peanut butter, dried fruit, coconut milk, cereal as corn flakes or air popped popcorn. For small nocturnal: crickets, browse, hard boiled eggs and ice cub treats, and for aye-ayes a mix of them: crickets, syrup, sunflower seeds, peanut butter, sugar cane, dried fruit, Gatorade, fresh garlic, jelly, etc.

Picture 22: Training at DLC with sifakas in which they have different shapes and they have to touch the one assigned to the animal. If they do it correctly the technician plays the whistle and they are rewarded with a peanut (photo by Gloria Fernández Lázaro).

5.- VETERINARY CARE

The DLC has some animal first aid procedures that should be administrated if immediate action is needed to save the animal. When an animal has been wounded or is in distress, it is important to evaluate the seriousness of the situation, call for help to the veterinarian and always wear latex or nitrile gloves before administering first aid.

Lemurs can suffer heat stress. In their natural environments are adapted to temperatures ranging from approximately 40 to 100 ºF (5 - 38 ºC) depending on their specific range in Madagascar. The ability to tolerate temperatures at the high or low end of the extreme depends on the species, age, health and the period of time they have been allowed to physically acclimatize to the new temperature range. Lemurs have evolved a variety of physiological mechanisms to deal with high ambient temperatures including decreasing activity, increasing evaporative cooling by licking their hands, and seeking cool locations on the ground, next to a cement wall, or beside a fan.

Injury illnesses, or in extreme cases, death, result if rectal temperature exceeds 104º F (40º C).

Hypothermia is present if the body temperature is lower than 96ºF (35, 5 ºC) and the animal is lethargic or impaired. In the case of small nocturnals, particularly those in

torpor (Cheirogaleus), may have rectal temperatures several degrees below 96ºF, so this is not an indication of hypothermia in these animals.

Every two years all the animals are subject of a complete exam at the DLC, taking fecal examinations (flotation and smear [in a smear test, a sample is smeared over a microscope slide to be studied for any pathology]), weights, blood samples, test of the liver function, etc. Nevertheless consistent weight data provides critical information regarding the health status of the animal, so weight measures are done frequently, the time span depending on the animals. At the DLC caged and free ranging diurnal lemurs are weighted at least every two months or any time they are caught. Sifakas and small nocturnal are weighted monthly and infants of all species are weighted more frequently if they are not gaining weight well or as directed by the veterinarian.

Contraception of selected animals is a routine tool used to manage total numbers in captive animal colonies. Several species or individual animals currently housed at the DLC are not of interest to researchers and are not of genetic importance to endangered species protection programs. Separation of the sexes during the breeding season is not feasible as many prosimians are social in nature and isolation is stressful. It may also result in permanent fragmentation of social groups. The methods described below may be applied to any individual animal of any species as determined by management needs. Different types of contraception can be distinguished depending on whether they are reversible or not:

- Reversible methods for females: Depo-Provera injections and Megestrol Acetate implants (MGA).
- Reversible methods for males: Gonadotropin releasing hormone analogs (GNR analogs) – Deslorein or leuprolide.
- Non-reversible methods for females: Tubal ligation and Ovariohysterectomy.
- Non-reversible methods for males: Vasectomy and Castration.

Depo-Provera: (the method consists in injectiing Progesterone in the muscle). In the DLC it is done every 40 days for ring-tailed lemurs and bamboo lemurs and every 60 days for *Eulemur*, *Varecia* and Sifakas. Side effects may include increased aggression in females, change in coat coloration, gain of weight and abnormal lactation.

MGA implants: In the DLC they are obtained from the Wildlife Contraception Center in St. Louis (Missouri) of the Association of Zoos and Aquariums (AZA -previously American Zoo and Aquarium Association, and originally American Association of Zoological Parks and Aquariums) at a determined dose based on the weight of the animal. Implants are placed subcutaneously between the shoulder blades in June, July or August depending on the species. Implants must be gas sterilized prior to placement. Animals are anesthetized and the skin at the implant site is clipped and prepped for sterile surgery. A 0.5 cm incision is made in the skin, the subcutaneous tissue is loosened with blunt dissection [surgical separation of tissue layers by means of an instrument without a cutting edge or by the fingers during surgical procedures]. and the implant is placed. Skin sutures close the wound unless surgical skin glue is used to close the incision. Finally, the animal is recovered. Sutures are removed at 10-14 days if still present. Implants last usually 2 years following placement. They should be removed in the spring of the second year following the breeding season. Side effects may include:

weight gain or coat color change. MGA implants are not reliable or recommended in ring-tailed lemurs as they tend to spontaneously back out given the very tight skin over the inter-scapular region in this species.

GNRH analogs- deslorelin or leuprolide: Deslorelin is delivered in an implant form that lasts for 8-9 months. Leuprolide is a repository injection lasting approximately 3-4 months. Both are administrated once to males at the beginning of the breeding season in August. Deslorelin implants are placed in males in a similar manner and location to the MGA implants in females. They do not need to be removed as they are completely absorbed in 8 months.

Tubal ligation: Animals are anesthetized according to standard techniques and a ventral midline incision is made. The abdominal cavity is entered and the uterus and ovaries identified. Double ligatures of 4-0 non-absorbable sutures or hemoclips are placed around the fallopian tubes bilaterally taking care to preserve the arteria blood supply to the uterus and ovaries. The uterus is replaced in normal position and the abdomen closed. Post-operative analgesia is given for 48 hours following surgery. The incision site is checked the day following surgery. Behavior and appetite are monitored daily thereafter.

Ovariohysterectomy: The animal is anesthetized and prepared for surgery. Once the uterus and ovaries are identified they are exteriorized. The ovarian and uterine arteries are double ligated and cut. The ovarian ligaments are broken to free up the ovaries. The uterine body is double ligated as caudally as possible prior to being separated. Care must be taken to preserve the ureters and the blood supply to the bladder. The abdomen is evaluated to ensure adequate hemostasis [the process which causes bleeding to stop, meaning to keep blood within a damaged blood vessel (the opposite of hemostasis is hemorrhage)] and then closed. Post-operative analgesia and monitoring is as described above for tubal ligations.

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Vasectomy: The animal is anesthetized and prepared to surgery. The spermatic cord and associated structures are palpated in the inguinal region. A 1-2 cm incision is made in the skin immediately over the spermatic cord. The vas deferens is doubled ligated and transected distal to the sutures. One centimeter of the vas is removed and the distal segment of the vas is left open. The skin incision is closed and, when possible, subcuticular sutures are done instead of skin sutures to minimize the potential for animals chewing at skin sutures. The procedure is repeated on the opposite side. Post-operative analgesia and monitoring is conducted as described above.

Castration: Preparation of the animal as above. A pre-scrotal incision is made in the skin. The right testicle is exteriorized through the incision and the spermatic cord isolated. The spermatic cord and associated structure are doubled ligated and a clamp placed distal to the ligatures. The spermatic cord is transected distal to the ligations. Hemostasis of the stump is ensured prior to its replacement into the inguinal region. The procedure is repeated on the opposite side. The skin is closed and post-operative analgesia and monitoring are conducted as described above.

Old lemurs can suffer liver cancer, kidney failures or ocular problems such as glaucoma or cataracts. The better the diets and the better the husbandry practices, the more cases of cancer will there be since cancer is often an old-age disease. The digestive system is

the one usually most affected, with the liver being the most affected organ. Its most common tumor is a hepatocellular carcinoma (Less *et al.*, 2009). They can also suffer Diabetes. The most likely seen in lemurs is Type 2 and it can be controlled with a proper diet: removal of simple sugars and starches and increase of the dietary fiber. It is helpful to split the diet into three feeding periods. There is a strong correlation between weight and insulin resistance so it is important to keep the animals within their weight range. Attention must be paid because lemurs are very prone to developing stress hyperglucemia which does not mean they are diabetic, but only that they are stressed or scared (Less *et al.*, 2009).

Dental diseases in general can be prevented by feeding prosimians with biscuits with Dentaguard. Tooth root abscesses are the most common. If the pulp cavity gets exposed, bacteria can form abscesses. Antibiotics will relieve swelling temporarily in order to remove the tooth or get a root canal (Less *et al.*, 2009).

Ocular problems occur most commonly in the nocturnal prosimians. Cataracts are a clouding of the lens and can get opaque. It can be partial or complete. If the cataract starts leaking protein into the eyeball, this causes inflammation. This can lead to glaucoma which is an increase in pressure in the fluid of the eye (if production of fluid is greater than drainage, it leads to glaucoma). Retinal degeneration is age related change of the retina. Retinal detachment and blindness may be associated with hypertension and renal disease. It is good to cultivate a relationship with a veterinary ophthalmologist (Less *et al.*, 2009).

Lemurids are also sensitive to Toxoplasma [*Toxoplasma gondii* is a species of parasitic protozoa which can be carried by many warm-blooded animals (birds or mammals, including humans; toxoplasmosis is the disease of which *T. gondii* is the causative agent, usually minor and self-limiting but that can have serious or even fatal effects on an immuno-compromised mammal; its effect on endangered species non-exposed to the protozoan during the evolutionary development of the species can be lethal, see e.g. for the Southern California Sea Otter, an analysis in a similar Franklin Institute "friends of Thoreau program" Case Study: A. Recarte Vicente-Arche, 2004] and Yersinia [a genus bacteria, family Enterobacteriaceae), which is ubiquitous and usually causes acute gastroenteritis and mesenteric lymphadenitis in children and arthritis, septicemia, and erythema nodosum in adults; the variety *Y. pestis* causes plague in humans and rodents, is transmitted from rats to humans by the rat flea, and from person to person by the human body louse; *Y. pseudotuberculosis* causes disease in rodents and mesenteric lymphadenitis in humans; yersiniosis is referred to as an infectious disease marked by diarrhea, enteritis, ileitis, pseudoappendicitis, erythema nodosum, and sometimes septicemia or acute arthritis]. Efforts should be made to keep these organisms out of the enclosures of prosimians. Fecal material from cats and poultry are likely vectors, and Yersinia thrives in standing pools of water. Yersinia can be controlled by preventing the formation of standing water pools in runs. All lemurs are not equally affected, some species have more problems than others. Ring-tailed lemurs can experience respiratory problems and ruffed lemurs can develop titers but not get sick. Treating it is hard; there are a variety of antibiotics that may or may not work, so prevention is the best thing to try (Less *et al.*, 2009).

Depending on the species special consideration should be taken to different issues:

a.- Dwarf and mouse lemurs (family Cheirogaleid) usually have very powerful odors. Coquerel´s mouse lemur has a particularly pungent and penetrating odor which implies that the odor *per se* does not indicate unsanitary conditions.

b.- Kidney disease affects bamboo and mouse lemurs more. You need 25% of your kidney capacity to survive, it is hard to know and cause injury to kidneys. At DLC 58% of animals has kidney disease (Less *et al.*, 2009). They have polycystic kidney disease where kidneys develop clear fluid filled cysts. This can be due to genetic predisposition but it is not yet well known.

c.-Aye-ayes usually have teeth problems, with the age the lower teeth stop growing and infections can develop in the jaw.

d.- Sifakas are very susceptible to changes in the diet, which can lead to diarrhea. They usually have digestive problems. Much of their body fluid is extracted from ingested plants and anorexia quickly leads to dehydration, electrolyte imbalance and death. Sifakas are also extremely sensitive to acepromazine [the psychotropic drugs, used in animals as a means of chemical restraint for the quietning and calming of frightened and aggressive animals] which causes almost immediate apnea and should never be used for sedation.

e.- Lorises and galagos need fairly high relative humidity (50-60%) to avoid the peeling and cracking of the skin. If their cages have insufficient or inappropriate surfaces for marking, lorisids suffer urine burns. Ventilation and drainage holes should be drilled into the bottom of PVC nest tubes. Lorises and galagos might urinate in these tubes while sleeping and can develop urine scald if the urine is not allowed to drain. For that reason, some caregivers prefer sleeping boxes made of wood, which is more absorbent. Also as mentioned above, if they are not provided with hard elements in the diet or the teeth are not cleaned, they can develop tartar or plaque that can cause severe gingivitis, tooth loss and eventual death (Picture 23).

Picture 23: Veterinary at Faunia (Madrid) trying to remove tartar from the teeth of a Loris, photo by Gloria Fernández Lázaro.

III.- REGULATIONS AND PROTOCOLS IN THE U.S.

The Animal Welfare Act (AWA) was signed into law in 1996 (it was built upon the previous Laboratory Animal Welfare Act of 1966, P.L. 89-544; since then it has been modified several times: in 1970, 1976, 1985, 1990, 2002, 2007 and 2008). It is the only Federal statute in the U.S. that regulates the treatment of animals in research, exhibition, transport and trade by dealers. Other statutes, regulations, policies and guidelines may include additional species-oriented coverage or specifications for animal care and use, but all refer to the Animal Welfare Act as the minimum acceptable standard. The Act is enforced by the United State Department of Agriculture (USDA), through its Plant Health Inspection Service (APHIS) and the Animal Care agency.

The AWA requires that all facilities conducting research with animals are registered and that they submit for review all proposals in which animals are used in research so that they are approved by a committee whose membership includes, at a minimum, an experienced scientist, a veterinarian and a individual whose not affiliated with the institution (such as a local veterinarian, minister, or employee of the local Society for the Prevention of Cruelty for Animals). Membership on this committee must also include a veterinarian with specific experience in laboratory animal care. These committees, called Institutional Care and Use Committees (IACUCs), must review, approve or disapprove protocols, and monitor and inspect every research study to help ensure that the animals are not subject to unnecessary pain and distress. Review committees are charged with keeping abreast with, and requiring research scientist to use, state of the art methodology to prevent pain in laboratory animals.

The AWA also requires institutions to report the number of animals used in research and the number of animals that experience not only pain, but also distress, along with an explanation of why the research had to be performed in this manner.
APHIS officials inspect research facilities annually to ensure compliance with the AWA. On these unannounced inspections, APHIS officials review the facility´s animal enclosures and husbandry practices, programs of veterinary care, animal acquisition records, research protocols, and IACUC records. If the officials find any items that are not in compliance with the law, they document them and give the facility a timeframe for correction, or, in cases of severe neglect or repeated violations, they immediately initiate enforcement actions. Such actions can include monetary penalties, corrective cease and desist orders, or confiscation of the animals. APHIS may consider innovative settlements for facilities that show an interest in improving their animal´s conditions. These settlements allow facilities to invest part or all of their monetary sanctions in facility improvements, independent reviews of their animal care programs, or employee training.

In addition to the AWA, those who receive funding from the Public Health Service or are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) must also comply with the Guide for the Care and Use of Laboratory Animals, which is based on a performance standards approach. The Guide is intended to assist institutions in caring for and using animals in ways judged to be scientifically, technically, and humanely appropriate. AAALAC is a private nonprofit organization that promotes the humane treatment of animals in science through a voluntary accreditation program administrated by veterinarians specialized in laboratory animal medicine, among other experts. Accreditation is a complex process requiring months or years but its benefits are significant because accreditation is indirectly mandatory for all practical purposes in many situations and some federal agencies require it to apply for grants.

For non-human primates, in the 1985 amendments to the AWA (Improved Standards for Laboratory Animals Act), Congress included the requirement of their “psychological well-being”. In 1989, the APHIS responded to the new AWA amendments by drafting regulations based on the advice received from a group of 10 primate experts. These proposed regulations contained requirements for social housing, inanimate enrichment

items, and exercise for nonhuman primates. APHIS eventually amended the regulations with more general language after receiving public comments on the proposed standards and they became a final rule in 1991 and still exist today (9 CFR Sec. 3.81, see Box below). See Kulpa-Eddy et al., 2005.

Title 9, Code of Federal Regulations, Subchapter A – Animal Welfare
Part 3 Standards, Subpart D Specifications for the Humane Handling, Care, Treatment, and Transportation of Nonhuman Primates, Section 3.81

Sec. 3.81 Environment enhancement to promote psychological well-being.

Dealers, exhibitors, and research facilities must develop, document, and follow an appropriate plan for environment enhancement adequate to promote the psychological well-being of nonhuman primates. The plan must be in accordance with the currently accepted professional standards as cited in appropriate professional journals or reference guides, and as directed by the attending veterinarian. This plan must be made available to APHIS upon request, and, in the case of research facilities, to officials of any pertinent funding agency. The plan, at a minimum, must address each of the following:

(a) Social grouping. The environment enhancement plan must include specific provisions to address the social needs of nonhuman primates of species known to exist in social groups in nature. Such specific provisions must be in accordance with currently accepted professional standards, as cited in appropriate professional journals or reference guides, and as directed by the attending veterinarian. The plan may provide for the following exceptions:

(1) If a nonhuman primate exhibits vicious or overly aggressive behavior, or is debilitated as a result of age or other conditions (e.g., arthritis), it should be housed separately;
(2) Nonhuman primates that have or are suspected of having a contagious disease must be isolated from healthy animals in the colony as directed by the attending veterinarian. When an entire group or room of nonhuman primates is known to have or believed to be exposed to an infectious agent, the group may be kept intact during the process of diagnosis, treatment, and control.
(3) Nonhuman primates may not be housed with other species of primates or animals unless they are compatible, do not prevent access to food, water, or shelter by individual animals. and are not known to be hazardous to the health and well-being of each other. Compatibility of nonhuman primates must be determined in accordance with generally accepted professional practices and actual observations, as directed by the attending veterinarian, to ensure that the nonhuman primates are in fact compatible. Individually housed nonhuman primates must be able to see and hear nonhuman primates of their own or compatible species unless the attending veterinarian determines that it would endanger their health, safety, or well-being.

(b) Environmental enrichment. The physical environment in the primary enclosures must be enriched by providing means of expressing noninjurious species-typical activities. Species differences should be considered when determining the type or methods of enrichment. Examples of environmental enrichments include providing perches, swings, mirrors, and other increased cage complexities; providing objects to manipulate; varied food items; using foraging or task-oriented feeding methods; and providing interaction with the care giver or other familiar and knowledgeable person consistent with personnel safety precautions.

(c) Special considerations. Certain nonhuman primates must be provided special attention regarding enhancement of their environment, based on the needs of the individual species and in accordance with the instructions of the attending veterinarian. Nonhuman primates requiring special attention are the following:

(1) Infants and young juveniles;
(2) Those that show signs of being in psychological distress through behavior or appearance;
(3) Those used in research for which the Committee-approved protocol requires restricted activity;
(4) Individually housed nonhuman primates that are unable to see and hear nonhuman primates of their own or compatible species; and
(5) Great apes weighing over 110 lbs. (50 kg). Dealers, exhibitors, and research facilities must include in the environment enhancement plan special provisions for great apes weighing over 110 lbs. (50 kg), including additional opportunities to express species-typical behavior.

(d) Restraint devices. Nonhuman primates must not be maintained in restraint devices unless required for health reasons as determined by the attending veterinarian or by a research proposal approved by the Committee at research facilities. Maintenance under such restraint must be for the shortest period possible. In instances where long-term (more than 12 hours) restraint is required, the nonhuman primate must be provided the opportunity daily for unrestrained activity for at least one continuous hour during the period of restraint, unless continuous restraint is required by the research proposal approved by the Committee at research facilities.

(e) Exemptions.

(1) The attending veterinarian may exempt an individual nonhuman primate from participation in the environment enhancement plan because of its health or condition, or in consideration of its well-being. The basis of the exemption must be recorded by the attending veterinarian for each exempted nonhuman primate. Unless the basis for the exemption is a permanent condition, the exemption must be reviewed at least every 30 days by the attending veterinarian.

(2) For a research facility, the Committee may exempt an individual nonhuman primate from participation in some or all of the otherwise required environment enhancement plans for scientific reasons set forth in the research proposal. The basis of the exemption shall be documented in the approved proposal and must be reviewed at appropriate intervals as determined by the Committee, but not less than annually.

(3) Records of any exemptions must be maintained by the dealer, exhibitor, or research facility and must be made available to USDA officials or officials of any pertinent funding Federal agency upon request.

(Approved by the Office of Management and Budget under control number 0579-0093)

After five years of enforcing the regulations on environmental enrichment for nonhuman primates, APHIS surveyed their Animal Care (AC) inspectors about the implementation of enrichment plans at research facilities, exhibitors, and dealers. The consensus among AC inspectors was that most facilities did not understand how to develop an adequate environmental enrichment plan that would promote the well-being of nonhuman primates. So in 1999 the Final Report on Environment Enhancement to Promote the Psychological Well-being of Non-human Primates was published. It was drafted by a committee of experts from the areas of research, teaching, regulation and exhibition. which was included in a draft policy and issued for public comment on July 15, 1999 (Federal Register /Vol. 64, No. 135 /Thursday, July 15, 1999 / Proposed. See the section Links to Online Resources of this Case Study). Although the report included a draft policy, which was submitted to public comment, ultimately, the policy was not implemented. However, the draft policy and Final Report did provide a great deal of science-based information for facilities housing nonhuman primates and many began implementing aspects identified under five general elements (social grouping, social needs of infants, structure and substrate, foraging opportunities, and manipulation).

Recently the AWIC (Animal Welfare Information Center) has published a new document that covers literature published from 1999 to March 2011 updating the information refers to enrichment of non-human primates overall.

But not only these regulations affect prosimians, also more general ones that deal with animals in zoos, also included in the AWA (see K. S. Grech, 2004), and in particular the self-regulating guidelines and protocols of the zoological parks themselves.

Founded in 1942, the Association of Zoos and Aquariums (AZA) (previously American Zoo and Aquarium Association, and originally American Association of Zoological Parks and Aquariums) is a nonprofit organization dedicated to the advancement of zoos and public aquariums in the areas of conservation, education, science, and recreation.

It was established among other reasons, to foster continued improvement in the zoological park and aquarium profession.

One of its most important roles is to provide a forum for debate and consensus building among its members, the intent of which is to attain high ethical standards, especially those related to animal care and professional conduct. The stringent requirements for AZA accreditation and high standards of professional conduct supposedly surpass the USDA-APHIS requirements for licensed animal exhibitors. AZA member facilities must abide by a Code of professional Ethics: a set of standards that guide all aspects of animal management and welfare.

As a matter of priority, AZA institutions should acquire animals from other AZA institutions and dispose of animals to other AZA institutions.

Its Wildlife Conservation and Management Committee (WCMC) works collaboratively with other Committees and is responsible for facilitating the professional and scientific management of the animals cared for in AZA-accredited institutions. The WCMC develops, oversees, promotes, evaluates, and supports the cooperative animal management, conservation, and scientific initiatives of the AZA. It is responsible for the formulation and communication of the various guidelines and protocols essential to Species Survival Plans (SSPs), Taxon Advisory Groups (TAGs), Regional Collection Plans (RCPs), Studbooks and Scientific Advisory Groups (SAGs).

a.- The mission of each **Taxon Advisory Group (TAG)** is to examine the conservation needs of an entire taxa, and to develop recommendations for population management and conservation based upon the needs of the species and AZA-accredited Zoos and Aquariums, Certified Related Facilities and Approved Non-Member Participants. Prosimian TAG promotes and participates in efforts to conserve prosimians and to advance towards the highest levels of animal welfare.

b.- The main responsibility of the TAG is the **Regional Collection Plan (RCP)** which describes a list of species recommended for management in AZA zoos and aquariums, the level at which those species should be managed, detailed explanations on how those recommendations should be developed, and an evaluation of how much space should be dedicated to each species. The TAG is in charge to develop taxon-specific Animal Care Manuals and to manage the population with the Species Survival Plan (SSP) Programs and the Studbooks.

c.- The mission of an AZA **Species Survival Plan Program** is to cooperatively manage specific, and typically threatened or endangered, species populations within AZA-accredited Zoos and Aquariums, Certified Related Facilities, and Approved Non-Member Participants. The goal is to provide a genetically viable situation. In January 2011 AZA Policy on sustainable Populations and Species Survival plans has changed and population sustainability of each species is classified so that available populations of each species is paced in one of the following three levels: Green, Yellow, or Red, following genetic and demographic assessment of current populations:

1.- Green SSP: The species population size must be equal to, or greater than, 50 individuals and have a sustainability retaining 90% gene diversity at 100 years or at 10 generations. Of all of the prosimian species held in North American institutions, none have been classified as Green SSps.

2.- Yellow SSP: The species population size must be equal to, or greater than, 50 individuals but does not have a sustainability retaining 90% gene diversity at 100 years or at 10 generations. Only 6 species of prosimians in North American institutions are at the Yellow program level (*Pygmy loris, Mongoose lemur, Ring-tailed lemur, Red ruffed lemur, Black and White ruffed lemur and Coquerel´s sifaka*).

3.- Red SSP: Populations with fewer than 50 individuals. These populations are considered unsustainable. The rest of prosimians species held in North American institutions (20 species) have been assessed as Red and will no longer be considered SSP programs. The program is managed as an official AZA Studbook if the Taxon Advisory Group recommends the species in the Regional Collection Plan.

d.- An AZA **Studbook** dynamically documents the pedigree and entire demographic history of each individual in a population of species. These collective histories are known as the population´s genetic and demographic identity and are invaluable tools that track and manage each individual as part of a single ex-situ population. Most of the studbooks are North American Regional Studbooks, but some can be international in scope. Non-AZA institutions are also included in the studbook. Each studbook is maintained by a Studbook Keeper whose primary functions are:

- The creation/maintenance of a current studbook developed in coordination with the Population Management Center (PMC). The AZA Population Management Center, located and hosted by the Lincoln Park Zoo in Chicago, is responsible for conducting the genetic and demographic analyses needed to develop and distribute population management recommendations.
- The presentation of general biology and species ecology data.
- The presentation of status and distribution information about in-situ populations.
- The development of a bibliography of relevant publications.
- The monitoring and documenting ex-situ all death, births and transfer information.
- The maintenance of an accurate data base that allows detailed genetic and demographic analyses.
- The recommendations on breeding decisions to enhance genetic diversity.
- The assessment of the population status (stable, increasing or decreasing).

The DLC is member of the AZA community and is also accredited by the AAALAC. All the animal procedures performed at the center, require the approval of the DLC Research Committee and Duke´s IACUC.

The Mission of the DLC is to promote research and understanding of prosimians and their natural habitat as a means of advancing the frontiers of knowledge, to contribute to the educational development of future leaders in international scholarship and conservation and to enhance human condition by stimulating intellectual growth and sustaining global biodiversity.

The DLC commits to achieving these goals through:

1.- Conducting and facilitating research on prosimian behavior and physiology.

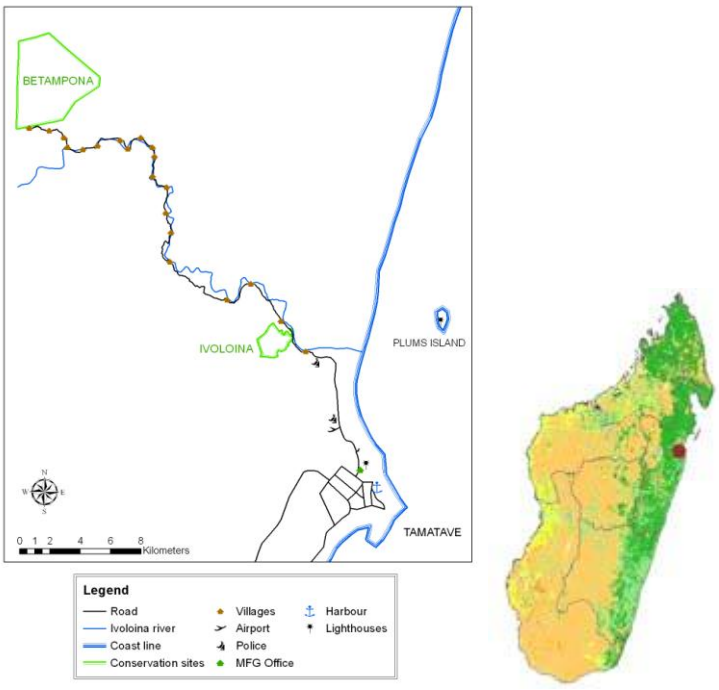
2.- Furthering undergraduate, graduate and professional education in multiple disciplines.

3.- Encouraging efforts to preserve prosimians and tropical biodiversity through international collaboration.

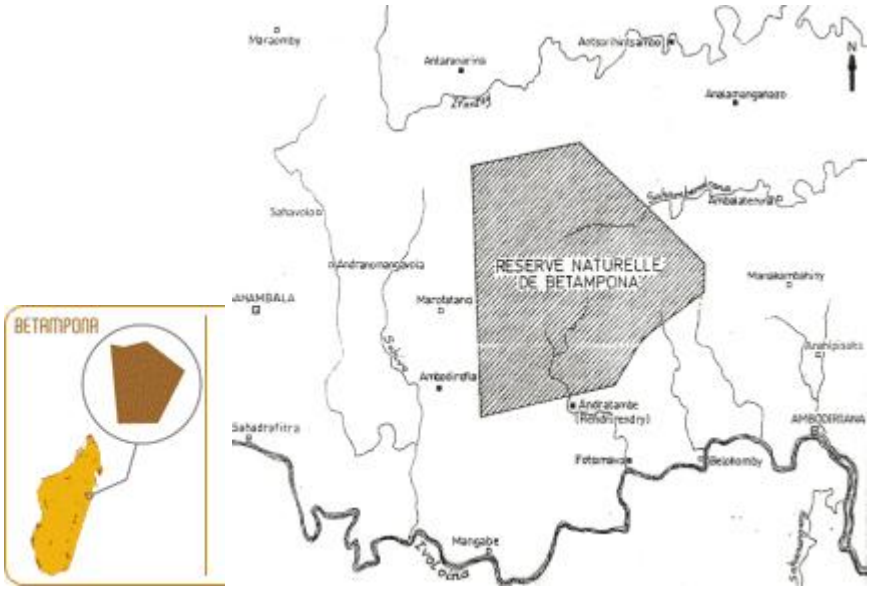
4.- Serving as a national and international center for the dissemination of information on prosimians and their natural habitat.

Concerning its contribution to in situ (on site) conservation, the DLC is a founding and managing member of the Madagascar Fauna Group (MFG) which is a consortium of zoos and other institutions interested in supporting conservation in Madagascar.

Through the MFG, the DLC conducts projects in Madagascar which include Betampona Natural Reserve and Parc Ivoloina.



The 5,500-acre **Betampona Natural Reserve** is one of the last patches of lowland rainforest in eastern Madagascar, with remarkably rich plant and animal diversity including many threatened species (among which are 11 lemur species), where the DLC conducts its **lemur re-stocking program (**the MFG/Duke Lemur Center program to return captive-bred lemurs to the wild.)



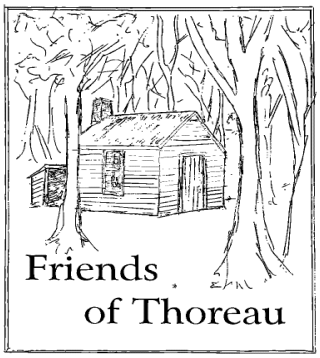
Since 1997, 13 black and white ruffed lemurs (*Varecia variegata variegata*) have been released into the reserve. Ten survived for longer than one year, and two (DLC-born Tany and Masoandro) are known to be still living in the reserve and have integrated with wild groups. Four offspring have been born to or sired by the released lemurs, and all were parent-raised and continue to thrive. All together, four of the captive-born ruffed lemurs have contributed to improving the genetic diversity of Betampona's ruffed lemur population. The project's conservation agents have monitored the released lemurs, their offspring and the wild groups continuously since 1997, providing a wealth of data about their adaptation and life in the wild.

The lemur re-stocking program has provided the foundation to transform Betampona into an important and valued site for conservation research on many different plant and animal species. In addition to their observations on the released ruffed lemurs, the conservation agents also conduct regular inventories on the reserve's birds, reptiles and amphibians, other lemur species and other mammals. This program has also provided training opportunities for university students, field research assistants and local agents in methods to inventory and monitor biological diversity.

Parc Ivoloina is situated about 7 miles north of the city of Tamatave, on the east coast of Madagascar. The Park has many attractions with a small zoo featuring Malagasy species, several free-ranging lemur groups, many wild birds and a beautiful Environmental Education Center. The land is protected as a forestry station and is also the site of agroforestry, tree nursery and reforestation activities to help teach local

villagers about alternatives to destructive slash-and-burn rice cultivation. The station also includes a recently completed Training Center consisting of a large meeting room, a laboratory, a dormitory, and a dining hall.

It is now recognized as the region's most important environmental education resource and spark-plug for biodiversity conservation. Multi-faceted education programs include special environmental classes for primary schoolchildren, teacher training workshops, adult outreach activities and training of Malagasy graduate students.



Prosimians in U.S. Ex-Situ Institutions: the Duke Lemur Center as an Example of Animal Welfare Science and its Contribution to Biodiversity Conservation.

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SCHOLARS' DEBATE

As we have seen prosimians, scientifically referred to as strepsirhines, are not only much different from what we imagine as a primate. However, the information available about them is quite poor, in particular in the case of the nocturnal species. Also, they are really endangered and most species are protected, which is "good news".

1.- Prosimians research and research on prosimians in the U.S.

The "bad news" are that being within the order of primates their similarity to humans is relatively close, and their small size, less weight, earlier reach of sexual maturity, production of more offspring in a short period of time, relative short-lived of some species… theoretically enables more rapid research colony growth and development, which encourages their use in biomedical research (Fischer and Austad, 2011).

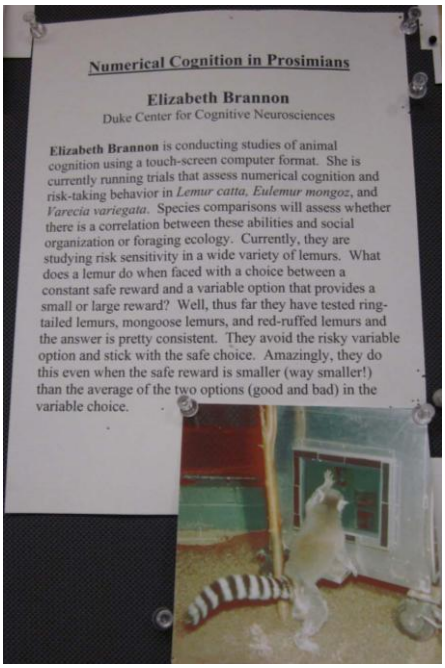
It true, though, that there are some drawbacks in their use, as their small body size results in reduced samples of blood and tissue, and there is also evidence that important metabolic and biochemical traits may differ from those of humans more than it is the case with larger simians Old World species.

For example, growth hormone in the *Galago senegalensis* is more similar to nonhuman primate growth hormone than to human growth hormone (Adkins, *et al.*, 2001).

In addition, several species, as the grey mouse lemur, appear to be particularly susceptible to stresses associated with captivity, and this stress can affect their physiological systems such as the immune system (Rogers et al., 1998) and their reproductive function (Bethea et al., 2008). Could it be that these effects are due to the less well developed husbandry techniques for prosimians?

The Annual Report of Animals used in research in all the states, done by the United States Department of Agriculture (USDA) in 2010, counts a total of 71.317 non human primates. This number includes all families of primates and all types of research (invasive and non invasive). Can we can suppose that strepsirhines are also used?

It is clear that they are used in non-invasive research because many there are open publications about it, including those of Duke University (Duke Lemur Center-DLC) (Picture 24), but not so much information is provided concerning invasive research.



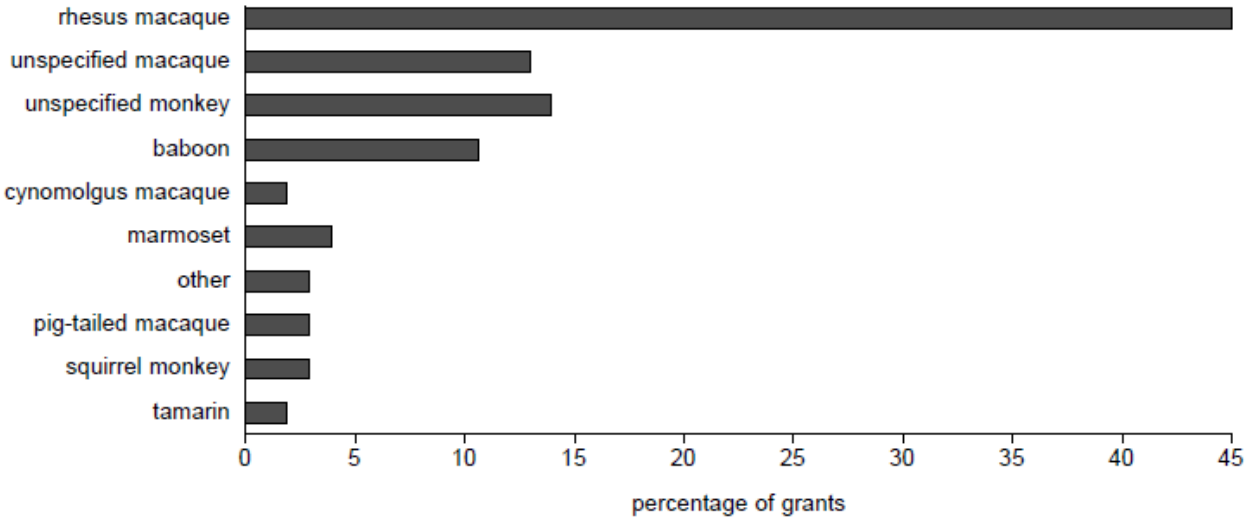
Picture 24: Research about numerical cognition in prosimians at the Duke Lemur Center (photo by Gloria Fernández Lázaro).

Nowadays the primate rights and the medical dilemma are having much impact in the U.S. because is one of the only two countries in the world (the other is the central African nation of Gabon) that conducts invasive research on chimpanzees. Chimps´ similarity to humans makes them valuable for research, but at the same time inspires intense sympathy. To research scientist they may look like the best chance to cure terrible diseases, but to many people, they look like relatives behind bars (Gorman, 2011).

Hepatitis is the field of biomedical research for which chimpanzees are most commonly used (it helped produce a vaccine for hepatitis B, and research is aiming at another one for hepatitis C, which infects 170 million people worldwide). Other common research areas (biomedical and otherwise) include HIV, behavior, reproduction (as a model for

human reproduction), genetics, malaria, respiratory viruses, infectious disease and drug testing (Conlee, Hoffeld and Stephens, 2004).

Defining invasive research as inoculation with an infectious agent, surgery or biopsy conducted for the sake of research and not for the sake of the non-human primate itself, and/or drug testing, 59% of the research conducted in the U. S with chimpanzees and 77 % with monkeys, is invasive according to the data obtained by Conlee, Hoffeld and Stephens in 2004. The species analysis of this 2004 work indicates that 45% of the total number of grants dedicated to monkey research by the U.S involved rhesus macaque. As in the case of the USDA report, Conlee, Hoffeld and Stephens don´t address the use of prosimians either (see figure below).



Species of monkeys used in federally funded biomedical research and testing by percentage of grants, based on 20% of monkey research grants (Conlee, Hoffeld and Stephens, 2004).

Does this silence mean that there is not invasive research in U.S with prosimians? If there is, for what purpose are they used? And also, are strepsirhines the best –o even a good- model to use?

Conlee, Hoffeld and Stephens, conclude in their work that non-human primates are used extensively in research in the U.S., the majority of which is invasive and that this scale of use clearly prompts the need for a cost-benefit analysis of non-human primate research in order to determine whether non-human primates are the only, or most effective strategy for biomedical progress.

Two of the U.S government principles for the utilization and care of vertebrate animals used in testing, research and training assert that:

> "*Procedures involving animals should be designed and performed with due consideration of their relevance to human or animals health, the advancement of knowledge or the good of society"*

"The animals selected for a procedure should be of an appropriate species and quality and the minimum number requires to obtained valid results. Methods such as mathematical models, computer simulation, and in vitro biological systems should be considered"

What do you think about that? Why if invasive research on apes has been forbidden in other countries it is not forbidden in the U.S.? Are there alternative methods for research on hepatitis?

In the U.S., the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) tries to advance in their acceptance and normal use but, despite the great effort made in recent years to seek alternative methods that could substitute animal testing, there are not many of them fully accepted by regulatory authorities. Ii is difficult to introduce them because there is some reticence and it takes time to develop the method and to get if validated and afterwards accepted by the authorities (Vinardell Martínez-Hidalgo, 2007).

So, what should be the policy during this period of acceptance, or if no alternative method is found? Can –or should- endangered species be used? Or, to the contrary, should research be put on hold when there is a clear case of threat to human lives for which the continuation of invasive research could provide a short o medium term remedy?

Whatever final decision is taken by the U.S. on the use of chimps, this example is only a tiny piece of a bigger debate. This is, as Kathleen Conlee (senior director for animal research issues at the human society) says, it needs "*the kind of rigorous analysis we should be applying to all animal research"*.

And what is the situation in Europe? Are strepsirhines used for invasive research? And for other scientific purposes? The recent new Directive 2010/63/UE of the European Parliament and of the Council of 22 September 2010, "On the protection of animals used for scientific purpose", mandates the following:

"Specimens of non-human primates shall not be used in procedures, with the exception of those procedures meeting the following conditions:

1.- The procedure has one of these purposes:

Translational or applied research with the aim of avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality or their effects in human beings, animals or plants, and for the development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feed-stuffs and other substances or products. Also for basic research and research aimed at preservation of the species.

2.- There is a scientific justification to the effect that the purpose of the procedure cannot be achieved by the use of species other than non-human primates.

The use of great apes, as the closest species to human beings with the most advanced social and behavioral skills, should be permitted only for the purposes of research

aimed at the preservation of those species and where action in relation to a life-threatening, debilitating condition endangering human beings is warranted, and no other species or alternative method would suffice in order to achieve the aims of the procedure"

Taking these mandates into account, do you think that strepsirhines can be often used in research? And paying attention at the last paragraph, it is true the usual belief, social understanding, and common assertion that invasive research with apes is forbidden in Europe?

2.- The impact of the Great Ape Project in the U.S. and elsewhere.

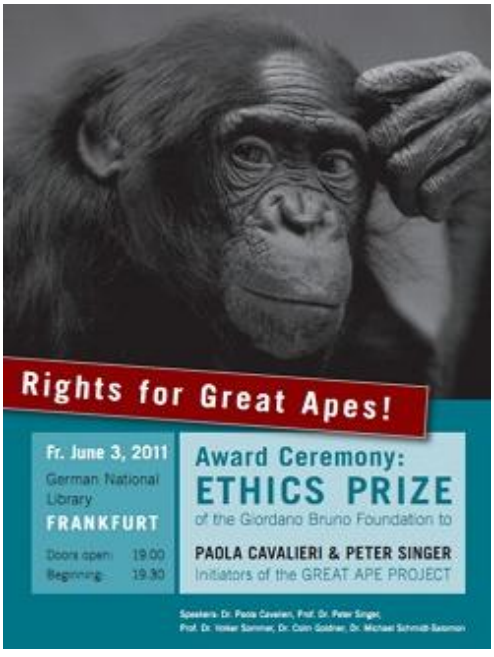
Great apes, as the closest species to human beings with the most advanced social and behavioral skills have provided the rationale for developed a big "revolution" concerning their ethical use by humans through the "Great Ape Project" (see A. Recarte Vicente-Arche, 2001).

This international organization of primatologist, anthropologist, ethicists, and other experts founded in 1994, advocates for a United Nation Declaration of the Rights of Great Apes that would confer basic legal rights on chimpanzees, bonobos, gorillas and orangutans (see figure below). The basic rights suggested are: the right to life, the protection of individual liberty, and the prohibition of torture.

It is argued that from the biological point of view, between two human beings there can be a difference of 0.5% in the DNA. Between a man and a chimpanzee this difference is only 1.23%.

This difference is an important argument? What do you think? Considering that mice for example, share as much as 94% of their DNA with humans, what do you consider are the main reasons to not include other primates in the Nation Declaration of Rights? Can be the physical similarity a reason because of what humans feel more empathy to anthropoids than with other primates?

Campaign poster of the Great Ape Project.



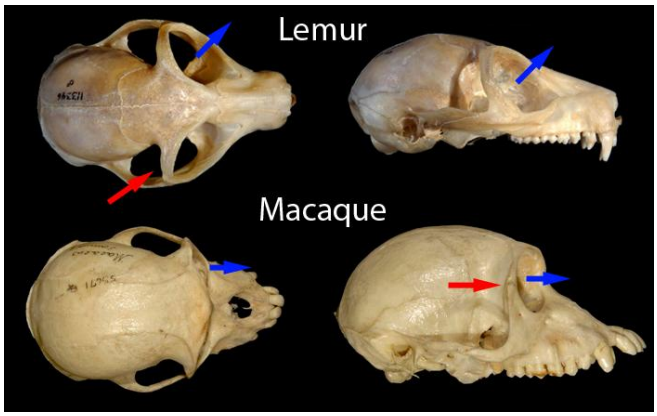
3.- The intricacies of science: the case of a singular species within a group.

Primates are an extraordinarily diverse and successful order of mammals that include species as different as gorillas, macaque, mouse lemurs and humans. So what are the key characteristics that all of them have in common?

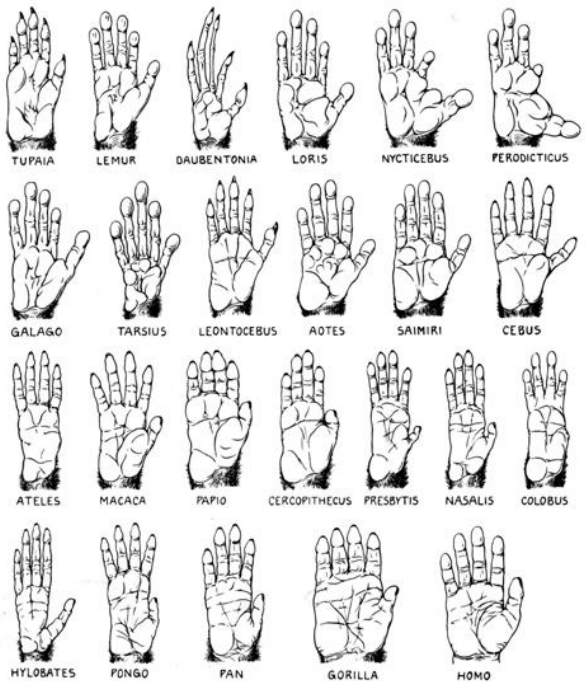
Most of them have:

- Forward-facing eyes: both eyes point in the same direction, allowing primates to perceive a three dimensional view of whatever they look at, although they are unable to look at what is behind them without turning their head.
- Eye sockets: The eyeball sits inside a solid, protective ring of bone called the orbit (see figure below).
- Grasping hands: One of the digits grips against the other four, enabling a firm grasp (see figure below).
- Nails: Fingers and toes end in a flat nail to protect the sensitive tip.
- Fingerprints: The skin under the fingertips and underside of the hand is bare and covered in a pattern of tiny ridges, which are unique to each individual.
- Large brains: Large cerebral hemispheres (compared with other mammals) give primates higher intelligence, the ability to learn and complex repertoire of behaviors.

There is a species that does not have them all? Which one? What does it have as an alternative?



Primates hands at the right and skulls of a Lemur and a Macaque at the left.



4.- Interspecies diversity within families of prosimians.

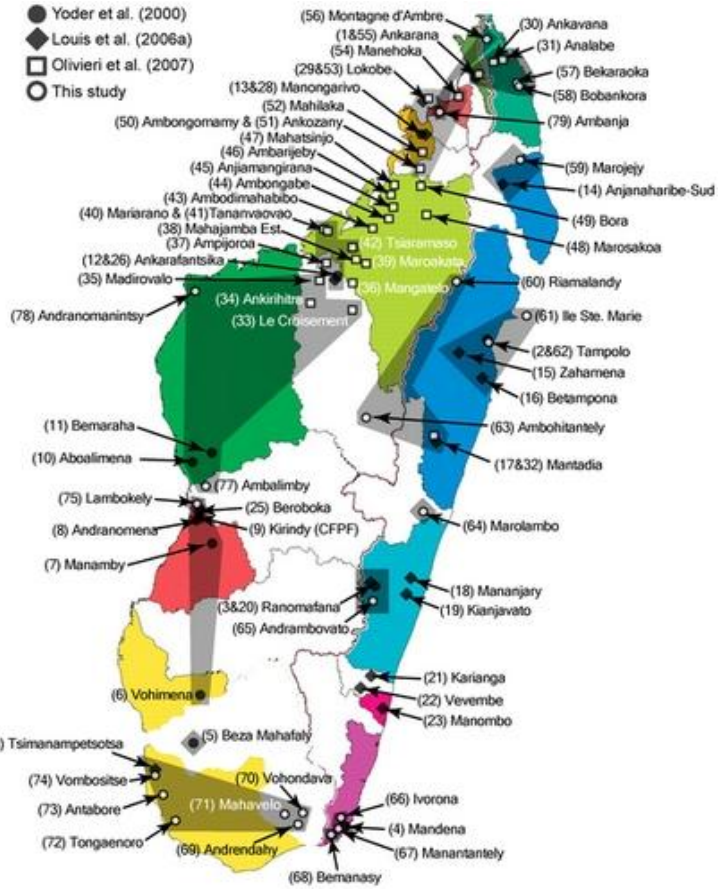
Stunning examples of that diversity within the order of primates are prosimians.

Malagasy strepsirhines evolved and diversified into a variety of forms including the 97 or so species endemic to the island today. What are the main reasons to such diversity? And why they are endemic?

New studies suggest that there are even more species yet to be found. Species are commonly distinguished from their relatives on the basis of physical characteristics, but genetic studies suggest that there are a number of cryptic lemur species which are only identifiable by unique genetic signatures in particular populations.

A new lemur species will get its start as a population which is physically identical to, but genetically isolated from, a population of a parent species. This means that the genetic makeup of a diverging lineage will begin to change before physical appearances do.

A team of researchers led by David Weisrock looked at genetic samples from 286 individual mouse lemurs, most of them coming from wild populations and found that there was more genetic disparity between certain mouse lemur populations than might be apparent just by looking at them using classic taxonomic techniques. While the authors note that their hypothesis is provisional, the end result of the analysis recovered as many as 16 genetically distinct mouse lemurs populations (see figure below), some of which fit in with known species while others may represent diverging lineages. The question is whether each of the newly discovered cryptic lineages represents a new species.



Geographic positions for sampled *Microcebus* localities presented in the study (Weisrock, *et al.*, 2010).

What do you think? What is the definition of species? Do you agree with the genetic criteria? Or as other authors do you prefer criteria based upon reproductive isolation, physical characteristic, or other indicators that show up later after divergence?

The International Code of Zoological Nomenclature (ICZN or ICZN Code) is the most widely accepted convention in zoology that rules the formal scientific naming of organisms treated as animals. Its rules mainly regulate: 1) how names are correctly established in the frame of binominal nomenclature; 2) which name has to be used in case of conflicts among various names; and 3) how names are to be cited in the scientific literature. But the listing of species as such is not a clear question. The measures used are morphology, ecological niche or similarity of DNA. Some biologists may view species as statistical phenomena, as opposed to the traditional idea, with a species seen as a class of organisms. In that case, a species is defined as a separately evolving lineage that forms a single gene pool. Although properties such as DNA-sequences and morphology are used to help separate closely related lineages, this definition has fuzzy boundaries. However, the exact definition of the term "species" is still controversial (De Queiroz K, 2007; H. Koch, 2010 for Malagasy invertebrates).

How does this debate affect prosimians nomenclature?

5.- The mystery of the arrival of prosimians to Madagascar.

How did strepsirhines arrive to Madagascar? Documenting the paths of animals during geological history is not an easy task.

Madagascar began to split from eastern Gondwana and the present-day African coast 180-160 million years ago (see figure A next page).

During the late Cretaceous Madagascar was home to dinosaurs, but 65 million years ago they died out, along with the other dinosaurs in the rest of the world. At this time Madagascar was already separated from the rest of Africa, but this did not stop it from being colonized by mammals. Studies of the genetics of Madagascar´s living inhabitants have indicated that the ancestors of its modern-day fauna, such as the primates that gave rise to lemurs, started to arrive soon after the extinction of the dinosaurs. The ancestors of lemurs were among the first to arrive, between 60-50 million years ago, followed by tenrecs between 42-25 million years ago, carnivorans between 26-19 million years ago, and rodents between 24- 20 million years ago (see figure B next page). [Tenrecidae (common name tenrecs) is a family of mammals found in Madagascar and other parts of Africa that resemble hedgehogs, shrews, opossums, mice and even otters, as a result of convergent evolution, occupying aquatic, arboreal and terrestrial environments, and some of which can also be found in the Madagascar dry deciduous forests, including the greater hedgehog tenrec. See picture 25 two pages below].

One hypothesis suggests the presence of a **land bridge** through which they could have arrived to the island.

Other authors argue the **sweepstakes hypothesis**. As articulated by paleontologist G.G. Simpson, this was a kind of "sweepstakes" in which creatures would be cast out to sea on floating mats of plants matter and of those wayward animals a few might be washed up in a new habitat able to support them. From these few survivors of tropical storms entirely new ecologies could become established.

Fig. A

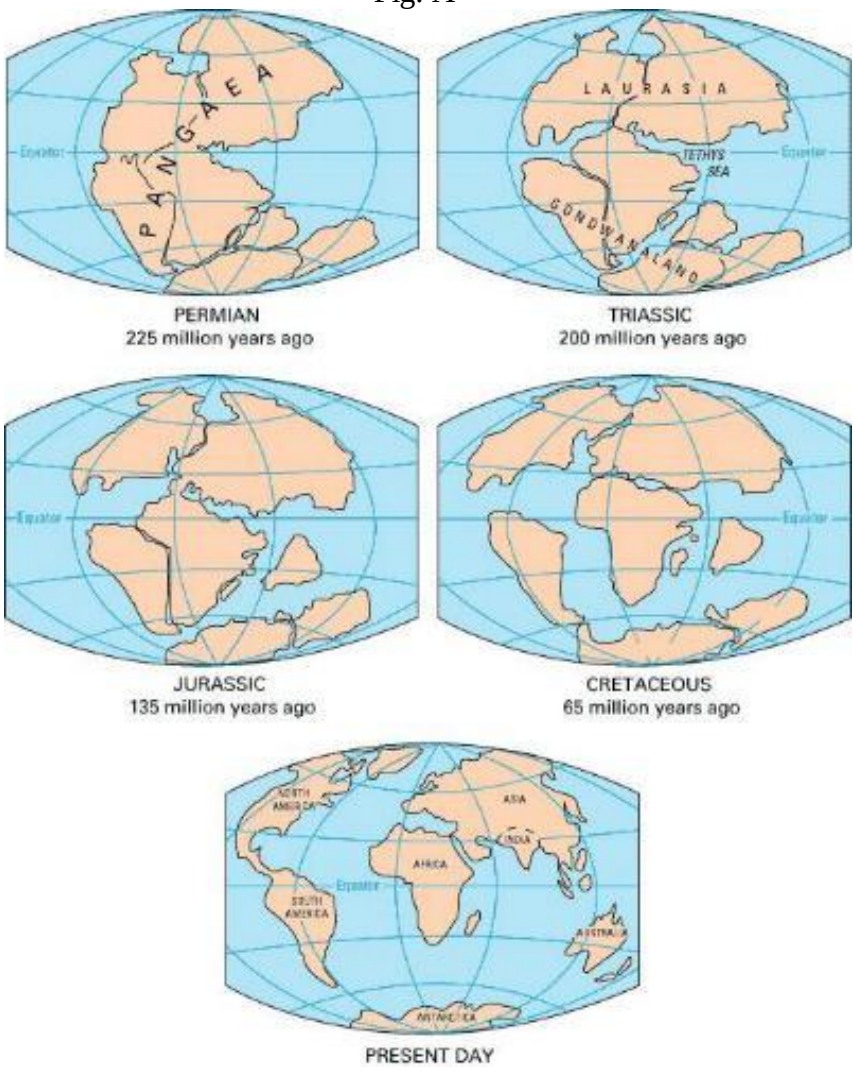
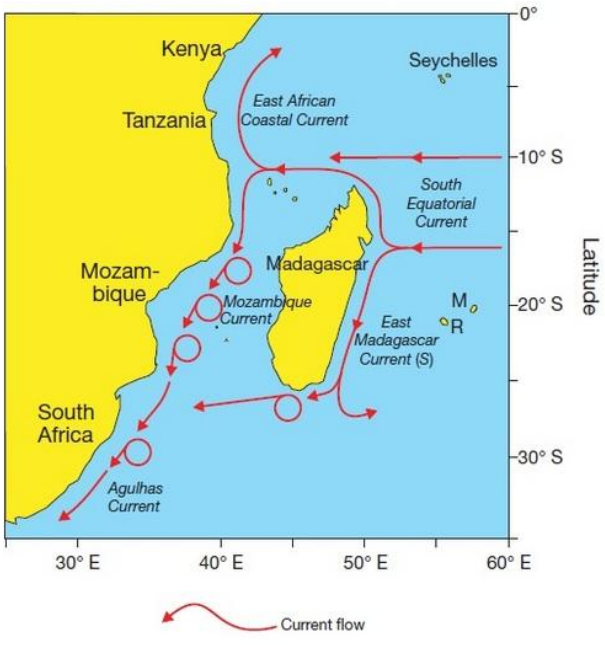


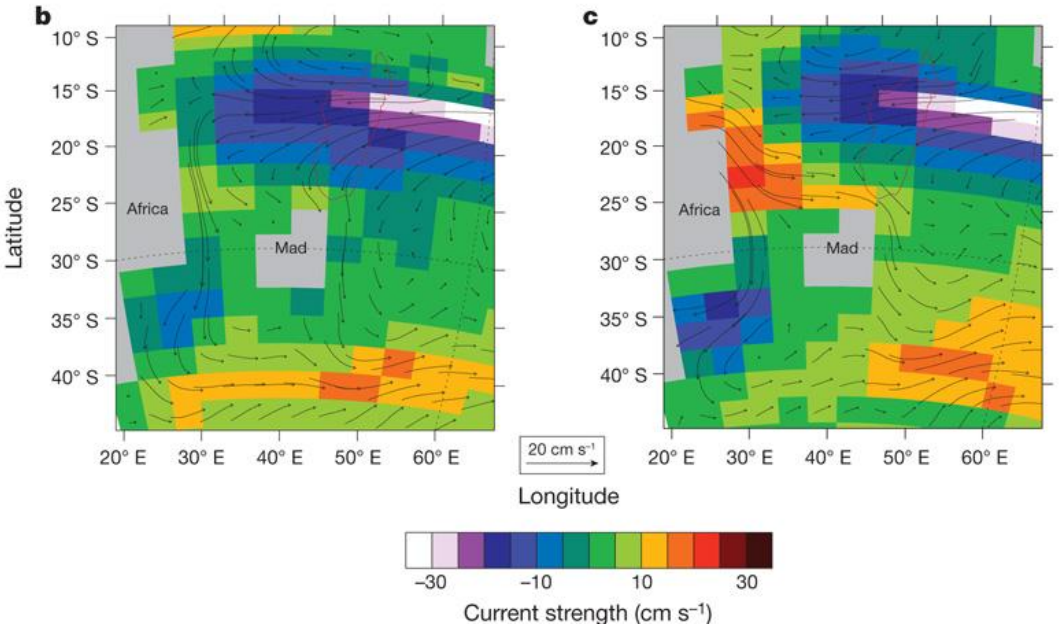
Fig. B: Arrival of mammals at Madagascar

What of the two hypothesis do you think best explains it? Why?

Picture 25: Greater Hedgehog Tenrec

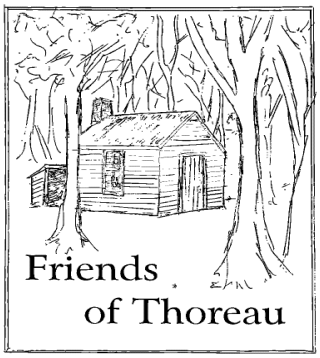
Critics of the rafting or sweepstakes hypothesis have cited the present currents and winds. They move south-southwest and thus would prevent rafts from making it to the eastward island (see figure below). If the same situation was true in the past, then it could be safely assumed that whatever early primates found themselves adrift would be deposited back along the African coast, if they returned to shore at all. But, there is some reason to believe that the winds and currents around Madagascar have remained constant during the 120 million years that the island has been separated from the African continent? Unfortunately the intricacies of prehistoric ocean currents cannot be observed directly. However, a new study published in Nature in 2010 simulated the behavior of past currents using computer modeling (see figure next page).





In the previous page, the figure shows the currents (red lines) currently surrounding Madagascar and at the top of this page (figures b and c) show the Eocene ocean currents (Ali and Huber, 2010).

Looking at the figures, do you think the rafting hypothesis is a good theory to explain the arrival of mammals at the island?



Prosimians in U.S. Ex-Situ Institutions: the Duke Lemur Center as an Example of Animal Welfare Science and its Contribution to Biodiversity Conservation.

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GUIDING STUDENTS' DISCUSSION

Prosimians, scientifically referred to as strepsirhines, are really unique animals not only among primates but throughout our planet. They are in serious danger of extinction, but why? What are their threats?

The threats can be divided into three main categories: habitat destruction, hunting for food or other purposes, and live capture.

1.- Habitat loss.

The impacts of the three abovementioned factors varies considerably from species to species and from region to region, but habitat destruction is usually the major threat.

More than 90% of the world´s primates reside in tropical forest habitats, and strepsirhines are no exception. It is estimated that more than 90% of Madagascar´s original forest cover has already been converted for agriculture and pasture, for mining, for the extraction of precious hardwoods, for firewood and other products, and for a variety of other uses (Mittermeier *et al.*,2010).

The human population in the island as of 2009 is about 20 million people in an area of 587,041 km2, not very high compared to many other developing countries. Can you compare this ratio with the number of people in your country?

Nonetheless, it is growing 3% annually, with a doubling time of 25 years; definitely a cause for concern. Perhaps more telling is the fact that there are only 30,000 km2 of arable land in a country that is 80% dependent on small-scale agriculture (rice, coffee, vanilla, spices), meaning that there is already great land-use pressure. Another variable that needs serious consideration is that the Malagasy people are relative newcomers to their island, having arrived there form southeast Asia, Africa, and intermediate points, as recently as 1.500-2.000 years ago and bringing with them a mix of land-use practices not well adapted to Madagascar´s delicate ecosystems, including rice-growing from southeast Asia and cattle-raising from East Africa (Mittermeier *et al.*,2010).

Rice is the mainstay of life in Madagascar, and its cultivation is the primary livelihood of 70% of the country´s population. About 70% of the rice is grown in paddies (see picture 26 below) which can be maintained year after year and have relatively high productivity. Rice paddies are located in the valleys and low plains but the remainder rice is grown as rain-fed rice through slash-and-burn cultivation or "tavy" (the traditional name given to Madagascar´s slash-and-burn technique used to clear brush and forest for crop production). Where this involves primary forest, it is not only disastrous for lemurs and other forest wildlife. It also exacerbates erosion and destroys the watershed for the cultivation of paddy rice in the valleys below. The widespread use of inappropriate land practices has led to some of the most dramatic examples of erosion in other parts of our planet. Can you think in some of them? Sadly, restoring such land to forest is difficult and costly, and would likely take more than a generation to achieve even if resources were available. So, what solutions do you think can be done to solve this problem?

Picture 26: Rice paddies in Madagascar´s Central Plateau (photo by Rhett A. Butler).

Another land use issue in Madagascar is fuelwood.

Every year, large areas of natural forest are cut down to provide firewood and charcoal for cooking. The problem is especially severe in the southern spiny desert region, where very poor tribesmen convert large areas of slow-growing spiny forest into bags of charcoal and stacks of firewood for sale to townspeople. Could other alternative sources of economic activity for them be found?

Picture 27: Madagascar "Spiny Desert" (Courtesy of Wayne Ranney).

2.- Hunting and bushmeat.

Hunting or also known as bushmeat trade. It is the second most important threat to primate populations.

Primates are hunted around the world as food items, as bait, for medical purposes, as crop pests, for their skins and other body parts as ornamentation, as remedies against evil omens or for other quasi-religious reasons, and often simply for sport (Mittermeier, 1987).

Hunting pressure generally increases with the size of the species; larger animals simply provide more meat, skin, bait or other products, while small ones barely recompense the hunter for the effort involved. This is probably why all the lemurs that have already gone extinct were larger than any of the extant taxa (Godfrey and Irwin, 2007). ["Extant" is a term commonly used in biology to refer to taxa, such as species, genera and families, that are still in existence, meaning still alive].

Fortunately, in some parts of Madagascar there are strong taboos, known in Malagasy language as "fady", against hunting certain species of lemur. However, despite such local traditions, the subsistence hunting of lemurs for food is widespread, and recent studies suggest that may be much more significant than previously recognized (Golden, 2009).

In China, slow lorises are eaten and the bones are used for medical uses, and fur for local hunting bags (Lan, 1999). The illegal trade is common for this group of prosimians being found for sale in Indonesian and Cambodian markets (See figure below). In Vietnam, collection for medical purposes results in captured animals being dried or placed in rice wine (Streicher *et al.*, 2002) and some people believe that the collection of a loris eyeball may help the person´s eyesight (Medhi *et al*., 2004).

In Tanzania, bushbabies are not actively hunted and rarely are they found in bushmeat markets. However, even one of the smallest bushbabies (*G. demidoff*) is reported to be eaten in Bioko Island (in Europe traditionally called Fernando Pó, Equatorial Guinea) in the western coast of Africa (Albrechtsen *et al.*, 2006). This means that even the smallest species are not immune from the threats posed by hunting and bushmeat trade.

Transport and treatment of lorises in illegal trade.
(www.loris-conservation.org).

Increased monitoring and better enforcement practices are necessary, but also what do you think is more effective? Better livestock raising practice? Introduction of alternative protein sources? … Why?

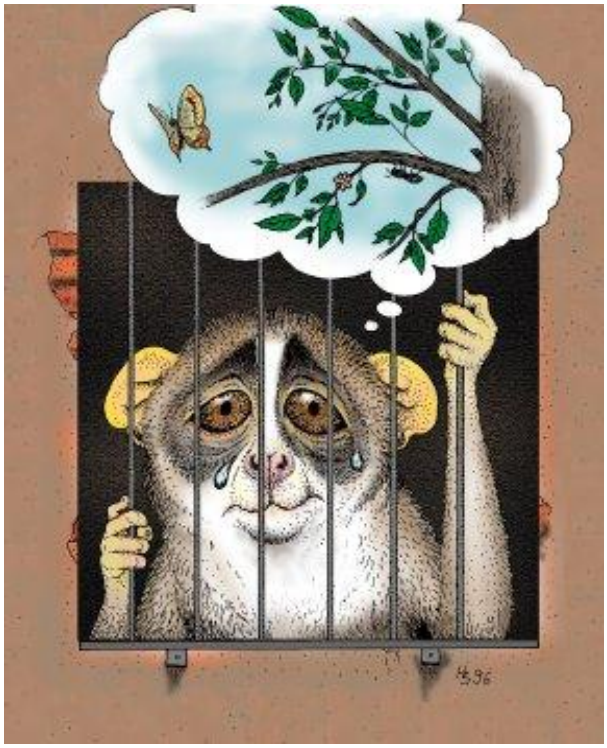
3.- Prosimians as pets: local and foreign markets.

Live capture of strepsirhines as pets is another serious threat.

Sometimes they are kept as pets by local people, and in Madagascar usually this is not a major problem. One exception is the frequent capture of live *Hapalemur alaotrensis* by villagers in the Lac Alaotra area (this lake is the largest in Madagascar; it is located in Toamasina Province, in the northern central plateau). This practice is apparently quite common there, and is probably a major factor in the decline of this now Critically Endangered species (Mittermeier *et al.*, 2010).

Lemur exports also do not appear to be a serious issue. All lemurs are protected by law in Madagascar, and all species are listed in the appendices of CITES, the Convention on International Trade in Endangered Species. Indeed, the only documented lemur exports over the past two decades have been for scientific purposes, particularly for conservation-oriented captive breeding programs such as those at the DLC and at Durrell, on the island of Jersey in the UK (formerly, the Durrell Wildlife Conservation Trust). These programs have taken only a very small number of individuals from the wild and do not constitute a threat in any way. Nonetheless, there are rumors that several lemur species are being kept in private collections in parts of Asia, where their importation was illegal.

For lorises, this is a bigger problem; they are one of the most commonly traded protected primates in southeast Asia (see drawing below). In some areas, the trade is so intense that devoted animal rescue centers are overwhelmed (Nekaris and Jaffe, 2007). Although buying and keeping them as pets is illegal in almost all counties, many people want them as pets. Do they know that they are increasingly danger in the wild and buying them contribute to that? What do you think? Can you offer reasons to not have them as pets?



Drawing of a loris in captivity
(www.loris-conservation.com)

Students should be asked if they know that having lorises as pets in their country is or not illegal. And, if it is illegal, there is some punishment?

The U.S. is a party to the Convention on International Trade in Endangered Species of Wild Fauna and Flora, commonly known as CITES, since its very beginning (the Senate ratified the treaty in July 1975). The USDA is responsible for enforcing regulations specific to the import and export of plants regulated by CITES under the Endangered Species Act (ESA). Under such statute, what would be the consequences of importing a prosimian into the US as a pet?

4.- Zoos and captive species of prosimians.

Under article 2 of the Convention on Biological Diversity (CBD), "Ex-situ conservation" means the conservation of components of biological diversity outside their natural habitats.

The international rules concerning ex situ conservation are quite precise under the CBD (article 9. See box below).

Article 9. Ex-situ Conservation

Each Contracting Party shall, as far as possible and as appropriate, and predominantly for the purpose of complementing in-situ measures:

(a) Adopt measures for the ex-situ conservation of components of biological diversity, preferably in the country of origin of such components;

(b) Establish and maintain facilities for ex-situ conservation of and research on plants, animals and micro- organisms, preferably in the country of origin of genetic resources;

(c) Adopt measures for the recovery and rehabilitation of threatened species and for their reintroduction into their natural habitats under appropriate conditions;

(d) Regulate and manage collection of biological resources from natural habitats for ex-situ conservation purposes so as not to threaten ecosystems and in-situ populations of species, except where special temporary ex-situ measures are required under subparagraph (c) above; and

(e) Cooperate in providing financial and other support for ex-situ conservation outlined in subparagraphs (a) to (d) above and in the establishment and maintenance of ex- situ conservation facilities in developing countries.

The DLC certainly is an ex-situ institution. But also are such institutions other zoological parks in the U.S. and abroad.

Students should be guided to consider the role of zoos in general. What should the role of zoological parks be under the CBD? They act as centers of education and

conservation with the species they host? Or are they still almost exclusively entertainment centers? Why?

What is the big difference between what you know about the DLC and other zoos in the U.S. concerning lemurs and other prosimians?

Reading carefully article 9, the role of such institutions is only "predominantly for the purpose of complementing in-situ measures", which implies measures to conserve in situ ("In situ conservation" being defined under article 2 of the CBD as "*the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties*").

They are differences between countries? Does the DLC achieve this standard?

Do other U.S. zoos achieve it?

Have in mind, though, that the U.S. is one of the very few countries of the world which is not a Party to the CBD (the only other countries of the world being Andorra, the Holy See, and Southern Sudan). Does this mean that the U.S. is totally opposed to the ex-situ institution rules of article 9 of the CBD?

Captive colonies provide a safety net against possible extinctions in the wild, they are a source of animals for possible future reintroduction programs, they serve a very important public awareness and conservation education function, and they should be a focal point to research into diet, reproductive behavior, handling, transport, and genetic management that complement and augment field-based research activities.

Do you know if the zoological parks of your city have a research program? And if they have, do you think is a good thing to promote better than other activities? Does the public consider these activities at the time of visit a zoo?

What is the role of zoos in educating the public? Do they really educate about the natural ecosystems of the place of origin of the species?

For example, do children know perfectly that clown fish need an anemone to live by because they have learned it in an aquarium or because they have seen the film "Finding Nemo"? or that lemurs live in an island called Madagascar because they have seen prosimians in the zoo or because they watched the movie "Madagascar" at it was described in the introduction in page 3 -Main Page- of this Case Study?

Do you think that these films promote the education about animals and respect for nature better than seeing live specimens in zoos?

Perceptions and attitudes of many people about and toward zoos are often negative because animals are enclosed in facilities. Do you think that attitudes to conservation and awareness of the lives of animals can be improved when people have a positive zoo experience?

A positive zoo experience implies provide naturalistic, well-designed cages and suitable environmental enrichment making more likely that zoo visitors will see active animals doing natural behaviors in an appropriate habitat (Hosey, 2005): *"Zoo visitor attitudes can become more positive and people´s interest in the animals increased when they encounter free-ranging primates, and in this respect the animals act also as ambassadors furthering the conservation cause"*.

Do you agree with the definition of zoo animals as "ambassadors" for the conservation cause? And what is a free-ranging facility? Have you ever been in some one? Do they have any special consideration (both for animals and for the public) compared to classic facilities?

Touring the Duke Lemur Center

With strepsirhines the positive zoo experience is quite hard to achieve. In particular for nocturnal species because they have to be habituated to reverse night cycle if the zoo wants that the active period of the public to be the same as that of the animals. But this implies additional conditioning. For example, flash photos should be totally forbidden in these enclosures, and visitors must keep silent. Can it be achieved by the zoos? Or this kind of animals, if in a zoo, unavoidably suffer more stress? If a nocturnal strepsirhine is in a breeding program, can it be visited by the public?

Nevertheless, nocturnal species are not so common in zoos, but other prosimians such as ring tailed lemurs (*Lemur catta*) are really common animals. Why? The activity period of this species is mostly diurnal; they are the most terrestrial of all lemurs; and they are opportunistic omnivores, which means that their diet include a wide range of things from fruits, vegetables to insects and small mammals. But may be the most important reason to their success in captivity is that they can reproduce easily usually having twins and triplets. They live in multi-male/multi-female groups and the social system is a matrilinear, which means that there is a dominance hierarchy among females and a single adult female is in the top-ranking of the group. This position usually changes and can be not easy to identify the new top-ranking female.

But even in this cohesive social structure rejection of individuals, especially males which are low ranking, can happen quite quickly. What solution do you propose to solve this problem if you have to take a male to the veterinarian clinic? To detect these

problems and use the better solution a carefully monitoring of the group has to be done by a specialist which could recognize all the individuals, do you think these tasks are performed in all zoos and by real experts in all of them?

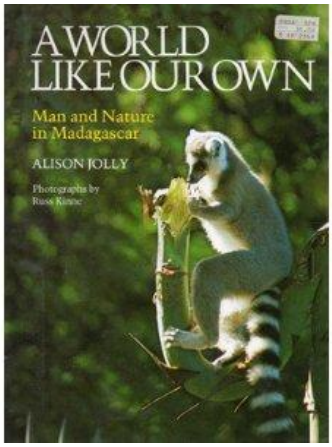
Each strepsirhine species pose particular problems to its maintenance in captivity but it is clear that better information is needed to provide the best welfare to these animals from the common species to the rarest ones.

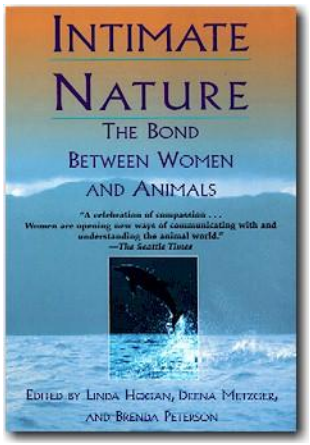
5.- Women primatologists: what postmodern analysis has shown to the new generation of American women scientists.

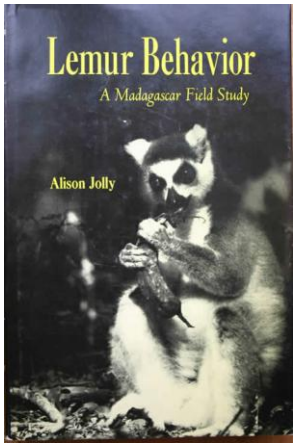
As Linda Hogan, Deena Metzger and Brenda Peterson have asserted not too long ago, "*it has been women in science, largely, who have revised ideas about is considered knowledge and fashioned a new approach to this knowledge*", referring to the previous assertion that "*as forbidden concepts it has seemed in scientific scrutiny, love for other species must always be part of the equation. (..) In their work these women [scientists] integrate receptivity as well as objectivity. When these women watch animals, they also engage their hearts in what Jane Goodall calls `compassion… a heightened moral responsibility for beings who are so like ourselves´ (…) This strong sense of compassion that many women bring to the study, celebration, and love of animals has been world changing and visionary. We can say now that the old guard of detached science is being replaced with the new guardians , many of them …women".* (Hogan, L., Metzger, D., and Peterson B, 1998).

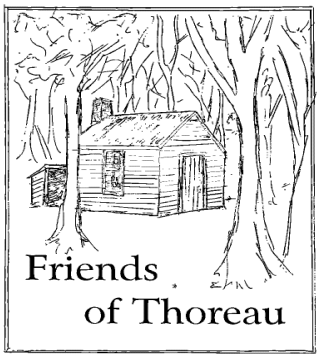
These words could be applied without any limitations to the "discoverers" of prosimians for science. The story of the Research conducted by Alison Jolly, has even been used to analyze postmodern gender, race, decolonization, and class relations both in the field and in the internal "power" structures of research institutions by Women´s Studies specialists such as Donna Haraway (see the Works Cited Section of this Case Study).

Is it true that the personal lives of these "women silent revolutionaries" of the late 21st Century did produce such a revolution? Compare Haraway´s analysis with Alison Jolly´s own account on the history of ecological studies of Malagasy lemurs (A. Jolly, & Sussman, R. W. , 2007).









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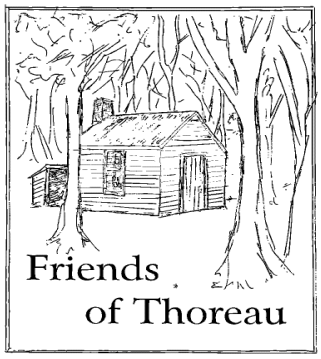

Prosimians in U.S. Ex-Situ Institutions: the Duke Lemur Center as an Example of Animal Welfare Science and its Contribution to Biodiversity Conservation.

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- IUCN Red List of Threatened Species: http://www.iucnredlist.org/
- Nocturnal Primate Research Group: http://www.nprg.org/home.shtml
- Oxford Brookes University, Department of Social Science, Nocturnal Primate Research Group: http://www.social-sciences.brookes.ac.uk/research/centres/accend/nprg/
- Duke Lemur Center: http://lemur.duke.edu/

- On the DLC Parc Ivoloina program: http://lemur.duke.edu/parc-ivoloina/
- On the DLC New Project: "The Ivoloina Training Center": http://lemur.duke.edu/new-project-the-ivoloina-training-center/
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- USDA webpage on the animal welfare laws and guidelines concerning laboratory animals: http://awic.nal.usda.gov/research-animals
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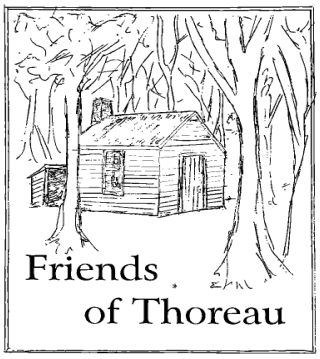
- On the Betampona Reserve and the Ruffed Lemur Re-stocking Program of the DLC: http://lemur.duke.edu/betampona-reserve-and-the-ruffed-lemur-re-stocking-program/



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ACKNOWLEDGEMENTS & ILLUSTRATION CREDITS

I would like to thank all the team at Duke Lemur Center for their incredible help when hosting me during and for providing most of the information needed to conduct the Case Study. I am very grateful for allowing me not only to visit the Center but also to provide me with a really good experience when meeting the animals, and for their enthusiasm in conveying to me their long term experience in the fields of animal welfare, conservation, education and research with strepsirhines. Especial thanks to GREG DYE for all his assistance and support during my visit, ANDREA S. KATZ and CHARLES R. WELCH for sharing their outstanding professional experience with the conservation of lemurs in Madagascar and for providing me with recent information about the status of these animals and the intricacies of colony management in captivity, CATHY V. WILLIAMS for the information about veterinary care, DAVID HARING for his incredible photos of the animals, SARAH ZEHR for her information about the research program, BRITT KEITH for her information about the enrichment program and animal housing, and JULIE MCKINNEY for her information about nocturnal buildings.

Also special thanks to RON E. BANKS, Director of the Office of Animal Welfare Assurance, Duke University, in particular for his information about animal welfare in U.S. and the exceptional reception on my visit.

Illustration Credits

Pictures of the species, courtesy of:
Wildlife Madagascar: http://www.wildmadagascar.org/

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Some of them are courtesy of David Haring, Duke Lemur Center photographer.

Some have been taken by R.A. Mittermeier, E.E. Louis Jr., Tony King and Stephen D. Nash.

Pictures of philippino tarsiers, courtesy of Roberto Verzo, Chocolate Hills, Bohol, Philippines: http://www.flickr.com/photos/verzo/1009489040/.

The rest of the pictures were taken by Gloria Fernández Lázaro (US-EU Project on Animal Welfare Science, Research Institute of North American Studies, University of Alcalá de Henares, Madrid) in the Duke Lemur Center or in Faunia.

Other pictures and figures, courtesy of the Duke Lemur Center, the Madagascar Fauna Group (MFG) and:

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Photo of Madagascar "Spiny Desert" by Wayne Ranney.

Geographic distribution of mouse lemur species:
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0009883

Figures of currents surrounding Madagascar:
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