A comparative evolutionary approach to gum-feeding in *Galago moholi* and *Microcebus griseorufus*

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General Abstract

Gums are soluble plant exudates rich in complex carbohydrates. In primates, the consumption of gum (gummivory) has been described as a primitive, fall-back diet exhibited when other food sources become scarce, particularly during dry periods. In apparent support for this interpretation, gummivory is often observed in nocturnal strepsirhines (tooth-combed primates) believed to have retained many primitive features. The complex carbohydrates in gums, however, are also known to be difficult to digest, and require particular alimentary adaptations. The hypothesis of a primitive diet predicts that gummivorous strepsirhines should use homologous digestive strategies, while the presence of different digestive adaptations in different lineages would suggest convergent evolution. I compared the digestive adaptations to gummivory in two small strepsirhine taxa, African lesser bushbabies (*Galago moholi*) and Malagasy reddish-grey mouse lemurs (*Microcebus griseorufus*). Both taxa digest gum primarily by fermentation, and have enlarged caeca for this process, but only *G. moholi* has an ansa coli in which digestion can be continued. In captive feeding experiments, the faeces of wild-caught *G. moholi* and *M. griseorufus* showed no significant difference in their digestive efficiency of gum compared with a control food (banana), and the banana and gum samples showed no significant difference in nutrient concentration and overall composition. To gain a broader understanding of the origins of gummivory in strepsirhines, I used a phylogenetic method to reconstruct their dietary evolution. My results indicate that gummivory evolved convergently in several primate lineages, apparently in response to environmental hypervariability. I conducted biochemical analyses of the secondary compounds found in gums that are regularly consumed, and preliminary results show that *Commiphora* spp. have a number of compounds, while *Acacia* spp. show no such traces. The absence of secondary compounds from *M. griseorufus* faeces suggests that the animals have physiological means for either converting them into digestible products or detoxifying and excreting them in their urine. Finally, I compared the distribution patterns of *G. moholi* and *M. griseorufus* with climatic parameters; both study taxa inhabit regions in which the dry season is characterised by little to no rainfall, a drought that may persist for months. Similar climatic regions are occupied by other gum-feeders, including the marsupial gliders (Petauridae) of Australia.

Kewords: digestion, gummivory, hypervariability, phylogeny, secondary compounds

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DECLARATION

The work described in this dissertation was carried out by me in the School of Biological and Environmental Sciences, University of Fort Hare, Alice campus, and Ithumela Primate Sanctuary, Pretoria, South Africa and Berenty Private Reserve, Madagascar between January 2012 and December 2013 under the supervision of Dr. Fabien G.S. Génin and Prof. Judith C. Masters. I declare that this is my own work, and all the results included were collected by me unless explicitly stated, in which case the source is identified. I have never submitted this thesis before for any degree or diploma at any tertiary institution.

I, Curswan Allan Andrews, declare that the research reported in this dissertation, except where otherwise stated, and is my original work. This dissertation does not contain another person's data, pictures, graphs or other information, unless otherwise stated and referenced. In cases where the exact words of others have been reproduced, such writing appears in italics inside quotation marks and the reference has been supplied.

Signed…….

at…… on……

DECLARATION OF RESEARCH ETHICS CLEARANCE

I, Curswan Allan Andrews, student number, 200804393, hereby declare that I am fully aware of the University of Fort Hare's policy on research ethics and I have taken every precaution to comply with the regulations. I have obtained an ethical clearance certificate from the University of Fort Hare's Research Ethics Committee and my reference number is the following: SMAS011SAND01.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Feeding adaptations of primates

Mammals exhibit a wide variety of dietary patterns and more often than not, their classification is based on a particular dietary feature. Mammals can be carnivorous, like members of the order Carnivora, or herbivorous like members of the Perrisodactyla and Ruminantia. Mammals also include insectivorous taxa, like the Tubulidentata and Microchiroptera, frugivorous taxa like Megachiroptera, and nectarivorous taxa like the marsupial honey possums of the order Diprotodontia (Feldhamer *et al.* 2007; Raven *et al.* 2008).

Associated with each diet is a suite of behavioural and morphological adaptations that permit the harvesting and processing of the dietary components. Carnivore dentition includes canines capable of piercing and tearing meat and carnassial cheek teeth with sharp shearing cusps. Most herbivores, on the other hand, (with the exception of hippos) lack canines, and have cheek teeth that are modified for shearing and grinding large quantities of abrasive plant material, both through hypsodonty (i.e. constantly erupting teeth with sharp enamel shearing crests) and through the extension of the occlusal area by molarised premolars (Feldhamer *et al.* 2007). The insectivorous mammals, with the exception of anteaters that have no teeth, have pointed cusps that allow them to pierce through the exoskeletons of insects (Springer and Holley 2013). Teeth are particularly important for the study of evolution, because many fossils are known largely or only from teeth which allow palaeontologists to make inferences about ancient diets.

When considering the gastrointestinal tracts of mammalian orders, variation in size is quite apparent, and with this variation in size comes specialization. Ruminantia have multi-chambered stomachs and are foregut-fermenters, while most non-ruminant herbivorous mammals have simple stomachs and are hindgut-fermenters. Carnivorous mammals share the feature of a simple stomach with most other mammals, and have caeca that are considerably smaller than those of other mammals (Feldhamer *et al.* 2007). Unfortunately, intestinal tracts do not fossilize often, with the exception of the fossil site Grube Messel, where the digestive tracts of birds (*Strigogyps sapea*; Mayr and Richter 2011), bats and the most complete primate fossil to date, *Darwinius masillae* (better known as Ida; Franzen *et al.* 2009), have been discovered. To study the evolution of digestive adaptations, researchers cannot rely on fossil evidence alone, and must use indirect methods, such as comparing fossils with living primate analogues. Another method used for herbivores consists of comparing the co-evolution of animals and the plants they consume, because this process often involves the development of defence systems in the plants (antagonistic inter-specific relationships) or the evolution of rewards from the plants in exchange for services like pollination and seed dispersal.

Mammalian orders are generally restricted to one or two dietary categories (insectivory, carnivory or herbivory) and it is rare that an order includes representatives of several dietary modes. Therefore, dietary adaptations are often diagnostic of an evolutionary lineage, a fact that suggests that feeding adaptations are particularly important in evolutionary divergence. Members of the order Primates, however, have more diverse feeding ecologies, perhaps as a result of their mostly arboreal lifestyles. Most primates consume a variety of plant parts (herbivory), which may include leaves (folivory), fruits (frugivory), seeds (granivory), and nectar and exudates (exudativory, including gummivory) (Richard 1985); but some species feed on animal prey, ranging from insects (mouse lemurs) to small

vertebrates (tarsiers) to other primates (chimpanzees). Therefore, reconstructing "the" ancestral primate diet is particularly difficult, and may indicate a degree of generality in the diets of ancient primates.

1.2. Primate origins and the evolution of primate dietary specializations

Diet co-evolves with body size and locomotion, and these additional characteristics can inform our interpretations of fossils. For instance, fossil primates found in the Fayum Depression of Egypt have features which suggest that Eocene prosimians were insectivorous, frugivorous, both insectivorous and frugivorous, and folivorous, all of which required specialist adaptations (Kirk and Simons 2001). Unfortunately, the fossil record for primates is largely incomplete, and the gaps include some key periods of the evolution of primates. For instance, we know very little of the early evolution of primates (prior to the Eocene; Silcox *et al.* 2007), and we know very little of the transition between the first real primates (Euprimates), represented by the extinct Adapiforms of the Eocene, to modern primates that seem to appear suddenly in the Neogene. Soligo and Martin (2007) have suggested that there are too many gaps in the primate fossil record (about 25 million years missing) to reconstruct the origins of primates adequately.

As a consequence, the origins of primates and their subsequent dispersal is one of the most contested subjects in primate evolution. Several authors have contributed to the development of theories relating to the adaptive origins of primates (Smith 1912; Jones 1916; Szalay 1968, 1972; Cartmill 1974, 1992; Szalay and Dagosto 1988; Sussman 1991), proposing different models to explain the evolution of the unique combination of primate characteristics and how they influenced the extant primate radiation. In almost all recent models, diet plays the central role – not so surprisingly, as dietary evolution is one of the corner-stones for explaining the emergence of mammalian lineages. The only recent model

that does not refer to dietary adaption is that of Szalay and Dagosto (1988), who proposed that grasping extremities and nails on the digits evolved together with leaping adaptations to facilitate grasp-leaping locomotion. In all other models, the defining primate characteristics are viewed as feeding adaptations, usually for a single "ancestral diet", despite the diversity and versatility of modern primate dietary adaptations. Below I discuss three scenarios of primate dietary evolution that enjoy support today.

1.2.1. Cartmill's model of a small insectivorous primate ancestor

Cartmill (1974, 1992) proposed the visual predation hypothesis, inspired by Smith (1912), Jones (1916) and Charles-Dominique and Martin (1970). In his model, primate characteristics like grasping hands and feet, the transition from claws to nails, forward-pointing orbits and stereoscopic vision, and the enlargement of the brain were viewed as adaptations not only to an arboreal lifestyle, but to a primarily insectivorous diet that involved a peculiar form of prey capture characteristic of extant small-bodied strepsirhines. Cartmill's **visual predation hypothesis** posits that ancestral primates hunted prey by gripping tree branches with their feet, and launching their bodies forward to capture flying insects with their hands, in much the same way that living cheirogaleids and bushbabies do. This model of a small, nocturnal, insectivorous ancestor for all primates was thus directly inspired by the two groups of primates that form the subject of my study: the Malagasy mouse lemurs (*Microcebus* spp.) and the African Galagidae.

Criticisms of Cartmill's scenario (Soligo 2006; Masters *et al.* 2007; Soligo and Martin 2007) are based on phylogenetic reconstructions of ancestral body size which contradicted the hypothesis of a small-bodied ancestor. In particular, Masters *et al.* (2013, in press) have published evidence that Malagasy cheirogaleids, often used as living analogues for ancestral

primates, evolved relatively recently from a larger ancestor, and reduced their body sizes over time (dwarfing).

1.2.2. Sussman's model of diffuse co-evolution and frugivory

Cartmill's model was advanced in opposition to Szalay's (1969, 1972) earlier proposal that primates diverged from their insectivorous ancestors by developing molars capable of crushing plant material, particularly fruit. This idea was developed further by Sussman (1991), who hypothesized that the ancestral primates had co-evolved diffusely with the early lineages of flowering plants or angiosperms (the **angiosperm co-evolution hypothesis**); their grasping extremities and the presence of nails on their digits, therefore, evolved for the gathering and consumption of fruit and plant parts. Sussman supported his interpretation with the observation that modern primates evolved contemporaneously with frugivorous bats, plant-feeding birds and herbivorous mammals.

The concept of co-evolution is based on the fact that food is not only a resource, but at least part of another living organism which is also a product of evolution. Interactions between animals and their food may be strictly exploitative and antagonistic with no reciprocal benefit for the food species, in which case plants and insects will evolve to defend themselves against predators by developing chemical (toxicity and deterrent taste) or mechanical (spines and other armaments) defences. Over time, exploitative relationships can evolve towards more reciprocal (co-evolved) relationships. Co-evolution was first defined as strict one-to-one associations between species, but "diffuse co-evolution" involves multiple partners and can affect entire lineages (Janzen 1980). Sussman (1991) proposed that many primate characteristics, including their social systems, evolved as products of such co-evolution, based on the temporal coincidence between the evolution of euprimate characteristics and the first modern angiosperms bearing large, fleshy fruits. Fruit pulp is

likely to have evolved as a reward for potential seed dispersers, while the low energy yield (Hladik 1978) and sometimes toxicity of leaves and flowers may have evolved as defences against folivores. The latter proposal is difficult to test, as the putative defences have variable effects on different herbivorous "predator" species (Cornell and Hawkins 2003).

Sussman's angiosperm co-evolution hypothesis was based on a literal reading of the fossil record, which yielded a relatively recent date for primate origins (Palaeocene/Eocene boundary), compared with estimations derived from molecular dating. Consequently, early primate evolution is likely to have ante-dated the co-evolution of angiosperms and frugivores.

Rasmussen (1990) synthesized the theories of Cartmill and Sussman, theorizing that grasping evolved primarily for the location and harvest of fruits, while orbital convergence evolved secondarily to aid in the visual predation of insects (Sargis 2002).

1.2.3. Nash's hypothesis of ancestral exudativory and its inverse

In addition to the hypotheses described above, I discuss two other insights into the dietary evolution of primates, as they have particular relevance for my study. The first is the hypothesis of Nash (1986), who based her scenario on Cartmill's hypothesis that the ancestral primate was a small-bodied gummivore-insectivore like a mouse lemur, and that gummivory served as the possible precursor to folivory. The alternative hypothesis was recently proposed by Masters *et al.* (2013, in press): that gummivorous strepsirhines – and mouse lemurs in particular – are likely to be descended from folivorous ancestors, so that adaptations for folivory may have served as an essential precursor to a gummivorous diet.

Primates consume only one of the three major categories of exudates, i.e. soluble gum, usually transparent or amber in colour. The other two categories include resins (hydrocarbon compounds secreted mostly by coniferous trees) and latexes (milky fluids

exuded when a plant is cut, that coagulate when exposed to the air). Most gums consist mainly of complex β-linked carbohydrates (polysaccharides), and are scentless and tasteless, to humans to and *Otolemur crassicaudatus* (a major gummivore) (J. Ward, pers. comm.). They are exuded by trees and lianas of certain families, in particular Fabaceae and Combretaceae, as a result of internal infestations by wood-boring larvae. By contrast, resins are not soluble in water and contain toxic alkaloids; they are used by the trees to protect wounds from external attack. The distinctive smell of most resins comes from terpenes, which are highly toxic in concentrated doses. Primates may consume the resinous gums of trees belonging to families like the Burseraceae, but these compounds differ from most resins in that they have both a soluble and an insoluble fraction. Latexes, used by certain plants such as *Euphorbiaceae* to protect photosynthetic parts from herbivores, are never consumed directly by primates. Latexes are white or yellow, viscous and caustic (Table 1.1).

Very little is known about the interactions between gummivores and their plant hosts. Gouging bark and making a tree vulnerable to invasion by disease vectors is unlikely to be beneficial to the tree, but gum-scraping could carry associated benefits: gum-scrapers could assist by feeding on xylophagous larvae and actively hunting the adult insects while they are laying their eggs through the bark. Gums are usually poor in protein, and most gummivores also consume insects and small prey (Hladik *et al.* 1980).

Nash (1986) proposed an evolutionary transition to folivory from gummivory, because these diets require similar digestive adaptations (e.g. fermentation chambers), and because gummivorous primates are generally viewed as more representative of the ancestral primates than folivores are. Gummivory is restricted to two vertebrate groups: the gliding and striped possums of the Petauridae family of Australia, which are primarily insectivorous but occasionally consume the gum of *Acacia* and *Eucalyptus* spp. (Ashwell 2010), and primates.

Among the latter, gum-feeding is practised by members of six strepsirhine genera (*Microcebus*, *Allocebus*, *Phaner*, *Galago*, *Nycticebus* and *Otolemur*; Nash 1986), and of three New World monkey genera (*Cebuella*, the pygmy marmosets and *Callithrix* spp., both of which are more specialized gummivores than *Saguinus* spp., which consume gum facultatively). In addition, a few Old World monkeys (species of *Papio*, *Erythrocebus* and *Cercopithecus*) have been observed consuming gum on occasion (Nash 1986). In this study, I focussed on two gum-scraping strepsirhine species: the southern lesser bushbaby (*Galago moholi*) and the reddish-grey mouse lemur (*Microcebus griseorufus*).

Table 1.1. Examples of plant families and genera in which at least some species produce exudates of three types

Latex	Resinous gums	Gums
Apocynaceae	Anacardiaceae	Combretaceae
Rauvolfia	*Operculicarya*[e], *Poupartia*	*Terminalia*, *Combretum*
Asclepiadiaceae	Burseraceae	Fabaceae
Cynanchum	*Commiphora*, *Canarium**	*Acacia*, *Alantsilodendron*[e],
Euphorbiaceae	Didiereaceae	*Albizia*, *Delonix*[e]
Euphorbia	*Didierea*	Malvaceae
Moraceae	Hypericaceae	*Adansonia*
Ficus	*Callophyllum**	Meliaceae
Sapotaceae		*Quivisianthe*[e]
Sideroxylon		

*Not present in Berenty; [e]Madagascar endemic

Génin *et al.* (2010) and Masters *et al.* (2013, in press) have questioned the ancestral nature of gummivory from two angles. Firstly, specialized gummivory seems to have evolved convergently in at least two groups of mammals under climatic conditions that developed

relatively recently, in the Neogene; and secondly, two of these cases of convergent gummivory in primates are associated with a reduction in body size (cheirogaleids, callitrichines), probably as a response to environmental unpredictability. In Cheirogaleidae (mouse and dwarf lemurs, *Phaner*), gummivory appears to have evolved from folivory, whereas in Callitrichinae (marmosets and tamarins), the ancestral diet is difficult to assess, but probably involved fruit and/or hard seed consumption. These two groups exhibit very different foraging strategies: marmosets and tamarins gouge bark to induce gum flow, while Cheirogaleidae, like their African relatives the Galagidae, scrape at already exuding gum with their tooth-combs (Richard 1985; Génin *et al.* 2010).

The first obvious correlate of gummivory is relatively small size, allowing the animals to cling to trunks and branches while foraging. All gummivorous strepsirhine primates are relatively small in body size, ranging from 60 g (*Microcebus*) to 800 g (*Otolemur*) (Nekaris and Bearder 2007). The strepsirhine tooth-comb, made up of narrow, horizontally-orientated and closely approximated lower incisors and canines, is used in grooming but makes an excellent gum-scraper (Nekaris and Bearder 2007). Specialist gummivores have particularly long tooth-combs (see *Euoticus* and *Phaner* in Figure 1.1). Gum-flow may be induced by active gouging of the bark (Power 2010), but in strepsirhines this requires robust upper canines and anterior premolars, rather than the scissor-like incisors of callitrichines.

Once gum has been acquired, the challenge is to derive adequate nutrition from it, as it is viewed as nutritionally deficient due to the presence of β-linkages (also in cellulose) which require bacterial enzymes for digestion (Nash 1986). To facilitate this, the gastro-intestinal tracts of gummivores have been extensively modified to allow caeco-fermentation. The caecum is enlarged and this, along with its commensal microbial flora, ensures adequate digestion of gum (Power 2010). In this feature, the gastro-intestinal tracts of primate

gummivores resemble those of leaf-eating primates, in which the caecum is also extensively modified and enlarged to accommodate the fermentation process (Nash 1986). Research into gum digestion has shown that gum has a longer retention time than other food items (Nash 1986), and the proximal hindgut may comprise up to 34% of the total gut (Caton *et al.* 2000). In *Galago senegalensis* and *G. moholi* the selective retention of the fluid phase and particulate digesta in the proximal hindgut before they are moved into the caecum and ansa coli, ensures adequate fermentation and subsequent absorption of the gum (Caton *et al.* 2000).

In addition to the adaptations described above, pointed nails or claws often play an important role in gummivory, allowing the animals to cling safely to vertical surfaces while feeding. In strepsirhine primates, sharply pointed nails are found in obligate gummivores like *Euoticus* and *Phaner*, as well as in the folivorous Lepilemuridae (Masters *et al.* 2013, in press) and in the insectivorous *Daubentonia*. Among Haplorhini, such claw-like nails are found only in the gummivorous Callitrichinae. No galagid gummivore, with the exception of *Euoticus*, has keeled nails, although the nails of *Galago matschiei* are sharply pointed. Other galagos employ behavioural strategies, like shifting body posture to facilitate the acquisition of gums (Nash 1986). Like most small animals, gummivorous primates could fall prey to larger animals while feeding, and to reduce this risk, the colouration of the pelage aids in the animals' concealment. Nocturnal gummivores like *Galago* are grey to grey-brown, and blend with the tree bark while feeding. Similarly, *Cebuella* and *Saguinus* from the Neotropics show patterns of cryptic colouration, reducing predation and allowing for increased foraging time (Nash 1986). *Phaner*, *Nycticebus* and the Australian marsupial sugar gliders have a dorsal stripe that aids in this pattern of cryptic colouration (Nash 1986).

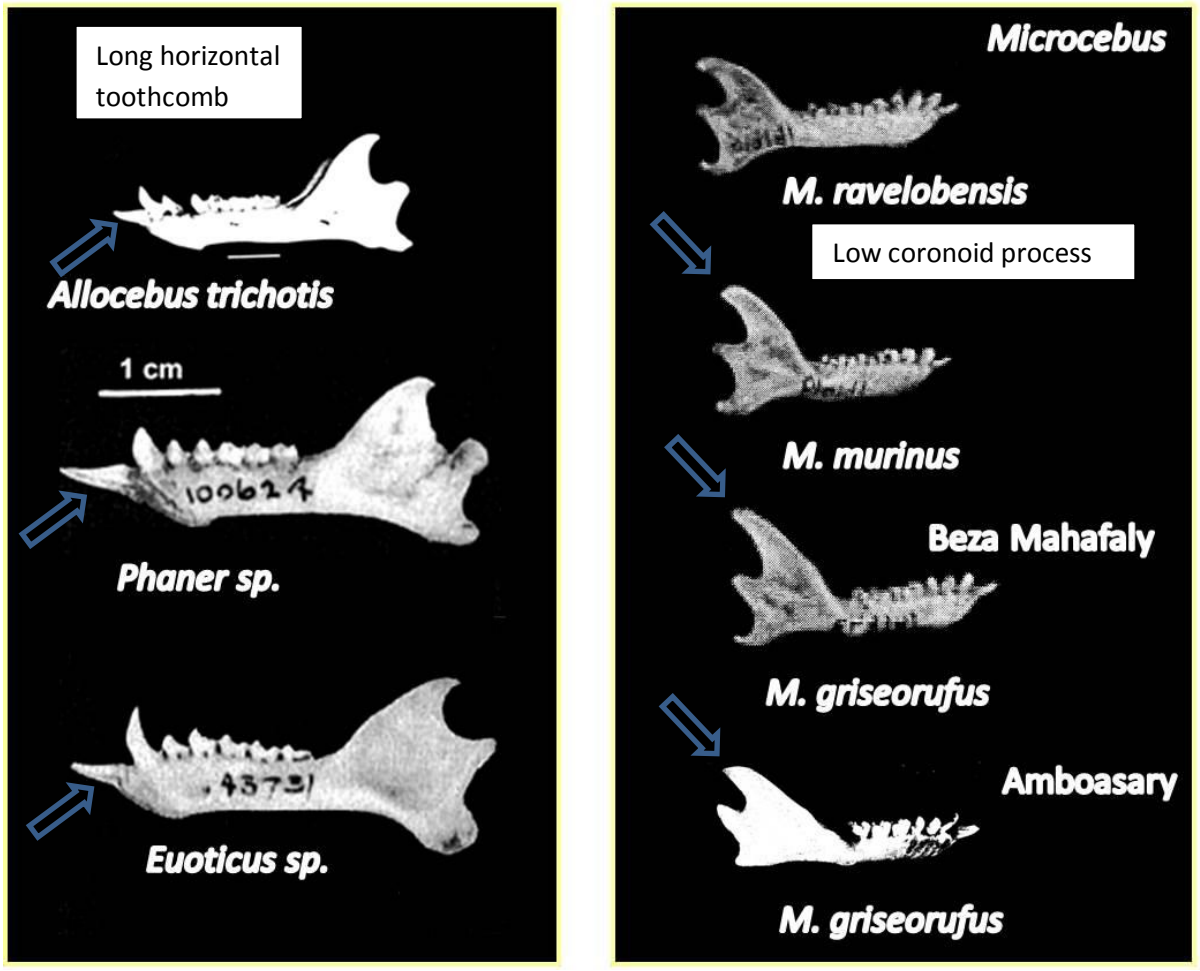

Figure 1.1. Mandibular form in gummivorous primates (from Schwartz and Tattersall 1985)

1.3. Research rationale and motivation

Nash (1986) reviewed the adaptations for gummivory found in primates (updated in Nash & Burrows, 2010), including dietary, morphological and behavioural characters, and listed the primate taxa categorized as gummivores. Her paper summarized research into gummivory to that date, and since then, gummivory has received more focussed attention. Topics of research have ranged from skull form (Vinyard *et al.* 2003; Ravosa *et al.* 2010), through dental ecology (Burrows and Nash 2010; Mork *et al.* 2010), to the digestive strategies of gummivores (Isbell 1998; Heymann and Smith 1999; Caton *et al.* 2000; Swapna *et al.* 2010).

Bearder and Martin (1980) described and defined the different types of exudates to eliminate the use of terms like gum and sap interchangeably, as they differ in composition and mode of extrusion. Even though both are soluble in water, sap is composed of minerals and photosynthate (Power 2010), while the composition of gum depends on a number of factors like soil type and vegetation (Bearder and Martin 1980). It is known that mammals do not consume sap but may feed on sap-based insect exudates (e.g. Flatidae secretions). Previous studies of the use of gum by different primate species have involved direct observations and focal sampling of Bengalese slow lorises (*Nycticebus bengalensis*; Swapna *et al.* 2010), tamarins (*Saguinus mystax* and *S. fuscicollis*; Heymann and Smith 1999) and Goeldi's marmoset (*Callimico goeldii*; Porter *et al.* 2009).

Two hypotheses regarding the evolution of gummivory have been proposed: Nash (1986) interpreted it as a primitive diet, possibly the precursor to folivory, while Génin *et al.* (2010) viewed it as a recent phenomenon that evolved in response to the environmental unpredictability that accompanied the relatively recent establishment of the El Niño/La Niña climatic cycles. Taking this idea to its logical conclusion, folivory could have been the precursor to gummivory, i.e. the reverse of Nash's (1986) hypothesis. These two explanations have different predictions.

1. If gummivory is an ancestral fall-back diet, adaptations for gummivory must have evolved early in the history of primates. Gummivores should therefore share suites of plesiomorphic features for exploiting this food source. With regard to biogeography, this hypothesis gives no clear indication as to where gummivores are most likely to be found – other than where gum-producing trees occur. More specifically, if adaptations to gummivory are homologous at least across strepsirhines, then mouse lemurs and

galagos should use the same digestive strategies and feed on gums of similar composition.

2. If gummivory has evolved only recently in evolutionary time (i.e. in the Neogene), it must have been acquired convergently in lineages that were already phylogenetically separated from one another. The adaptations that facilitate gummivory in diverse taxa should be shared only eclectically, or clearly derived from different ancestral states. In addition, the distribution of gummivores and gum trees should occur mainly in geographic areas that are strongly affected by environmental hypervariability. These areas are likely to be the ones most affected by global climatic changes, since relatively small changes in global climate have marked effects in hypersensitive regions.

1.4. Aims and objectives

The objectives of this study were to investigate the evolutionary history of gummivory in strepsirhine primates and to provide a clearer understanding of the evolutionary forces influencing its emergence. To this end I focussed on two gummivorous strepsirhine taxa that have been separated for at least 60 million years (Masters *et al.* 2013): *Galago moholi* in Africa and *Microcebus griseorufus* in Madagascar. The aims of my study were:

1. To reconstruct the evolutionary history of gum-feeding in Strepsirhini and in the primate clade using a phylogenetic approach;

2. To compare the efficiency of digestion of gums consumed in nature by *G. moholi* and *M. griseorufus*;

3. To plot the geographic distributions of my two study species in relation to environmental parameters (i.e. rainfall predictability and vegetation).

CHAPTER 2: RECONSTRUCTING THE EVOLUTIONARY HISTORY OF GUM-FEEDING IN *GALAGO MOHOLI* AND *MICROCEBUS GRISEORUFUS*

2.1. Introduction: phylogenetic reconstruction of primate ancestral states

Ancestral state reconstruction has long been a goal of evolutionary biologists, but the great advance in phylogenetic philosophy and methodology in recent decades – particularly since the development of molecular systematics – has revolutionized this practice. Phylogenetic reconstructions vary widely in their applications, from trying to identify the primate clade's closest living relatives (Janečka *et al.* 2007) to estimating the trends in the evolution of brain size (Montgomery *et al.* 2010). The comparative method, with the assistance of molecular phylogenetics, has provided an exceptional tool for deciphering the past. In this section I trace the evolution of gummivory in the primate order, using a well-supported phylogeny and phylogenetically independent contrasts to reconstruct the diets of various primate ancestors: i.e. the ancestor to the primate clade, the strepsirhine ancestor, and the ancestors to the most gummivorous extant species. I also use molecular phylogenetic data to estimate the timing of the evolution of gummivory in my two subject species.

2.2. Methods

2.2.1. Dietary categorization

I used anatomical adaptations, specifically locomotor, craniodental and alimentary adaptations, to delineate primate dietary categories. I followed the spirit rather than the letter of Sussman's (1991) angiosperm co-evolution hypothesis to describe four fundamental dietary syndromes (rather than strict diets because of multiple overlaps, see section 2.2.2.): faunivory, folivory, frugivory and exudativory. Despite this precaution, several species could

not be characterized by a single dietary syndrome and were considered to have mixed diets (see Appendix I).

2.2.2. Definitions of dietary syndromes

Faunivory including insectivory. Living faunivorous primates exhibit a variety of adaptations that are unlikely to be symplesiomorphic because they are associated with at least three kinds of specialized faunivory. The first is associated with very small size and sharp teeth adapted to the killing and consumption of live insects and small vertebrates, and is observed in *Tarsius*, *Galagoides* and *Microcebus*. All three taxa have been observed catching insects in flight with their hands, although *Tarsius* is also known as a formidable hunter of vertebrates, including toxic reptiles (Niemitz 1984). Charles-Dominique and Martin (1970) proposed that mouse lemurs (*Microcebus* spp.) and dwarf galagos (*Galagoides demidoff*) share a suite of characteristics related to their small body size, involving anatomy, diet, locomotion and social behaviour, which are likely to be primitive retentions from the common primate ancestor, making this kind of faunivory potentially plesiomorphic. The two other kinds of primate faunivory involve specializations for the ambush capture and consumption of slow, potentially toxic insects (*Perodicticus*, *Arctocebus* and *Loris*), or cryptic wood-borers (*Daubentonia*).

Folivory. Etymologically, folivory refers to the consumption of leaves; however, leaves and flowers have the same embryological origin, and folivores often consume flowers as well. This may be an ancient dietary pattern. The first angiosperms had either very small flowers or large edible flowers and small fruits (Friis *et al.* 2010, 2011). Ancient *Magnolia* trees, for instance, may use their fleshy petals to attract pollinators in the same way that more recently evolved angiosperms use edible pulp to attract seed dispersers. Most living folivorous primates also consume unripe (often toxic) fruits [e.g. proboscis monkeys (*Nasalis*

larvatus) and golden langurs (*Trachypithecus geei*); Das *et al.* 2008; Meijaard *et al.* 2008]. My working definition of folivory, therefore, was based on a suite of adaptations to a diet rich in leaves, including large teeth cheek with sharp shearing crests, and long guts.

Frugivory including granivory. Fruits and seeds are often consumed simultaneously and many frugivorous primates also consume the seeds of non-fleshy fruits (e.g. *Trachypithecus vestulus;* Hladik 1977). Because they are full of energy reserves, seeds are likely to have been attractive dietary options for mammals prior to the evolution of fleshy fruits in the Eocene. Extant angiosperms defend their seeds against predators, using either chemical (toxicity) and/or mechanical (thick coat) strategies. The diversity of these defences indicates that they evolved convergently a number of times (i.e. thick seed coats may involve very different structures). Most living frugivorous primates also consume buds and young leaves. Therefore, my working definition of frugivory was based on a suite of adaptations to a diet rich in fruits, including relatively small cheek teeth with bunodont cusps, and relatively short guts.

Exudativory including gummivory. Exudates include gum, but also a variety of other liquids, such as nectar, honey and the sap-based secretions of moth-bugs (Flatidae, Homoptera). Exudativory, therefore, is also more a dietary syndrome than a restrictive diet. Gum exudation is limited to a few angiosperm families such as Anacardiaceae, Burseraceae, Combretaceae, and Fabaceae, all of which belong to the subclass Rosidae (Génin *et al.* 2010). Resinous gums like those produced by Burseraceae seem to function as barriers to protect wounds against external attacks, while true gums serve to expel xylophagous larvae that infest the trees.

Gummivorous primates either gouge bark with robust anterior tooth batteries to induce gum flow (callitrichines and slow lorises) or scrape gum already exuded after prior damage (bushbabies and cheirogaleids) (Richard 1985; Génin *et al.* 2010). Lesser bushbabies (*Galago moholi*) and mouse lemurs (*Microcebus griseorufus*) consume insects and extensive amounts of gum, which is available year-round. Access to the latter, however, may be dependent on factors like recent damage to trees by other organisms, and precipitation (since gum is a soluble polysaccharide, and may be dissolved by rain). Gum may be produced as a response to wood-borer infestation (Fabaceae, like *Acacia* and *Alantsilodendron*) or as the result of external wounding (Bursearceae, like *Commiphora*). My working definition of gummivory, based on the ecological convergences observed between scrapers (bushbabies and cheirogaleids) and gougers (marmosets and slow lorises), involved dental specializations for gum-gathering and digestive adaptations for gum digestion (e.g. capacious caeca).

2.2.3. Scenarios of dietary evolution in primates

From a co-evolutionary viewpoint, the evolution of plants and plant eaters is likely to have involved three steps: (1) the antagonistic evolution of leaves and leaf-eaters; (2) co-evolution between flowers and pollinators; (3) co-evolution of fleshy fruits and seed dispersers. I devised four potential evolutionary scenarios of primate dietary evolution from the models of Cartmill (1974, 1992), Sussman (1991), Nash (1986), and Masters *et al.* (in press) (Figure 2.1.). Most evolutionary transitions were considered reversible because of the numerous species with mixed or intermediate diets. Irreversible transitions were only considered in the reconstruction based on Masters *et al.* (in press) with respect to the faunivory-folivory and the folivory-gummivory transitions, because their reversals would involve considerable changes in body size, as well as digestive and dental innovations. The

result of this reconstruction was compared with a null hypothesis of equiprobable transtions (i.e. the control).

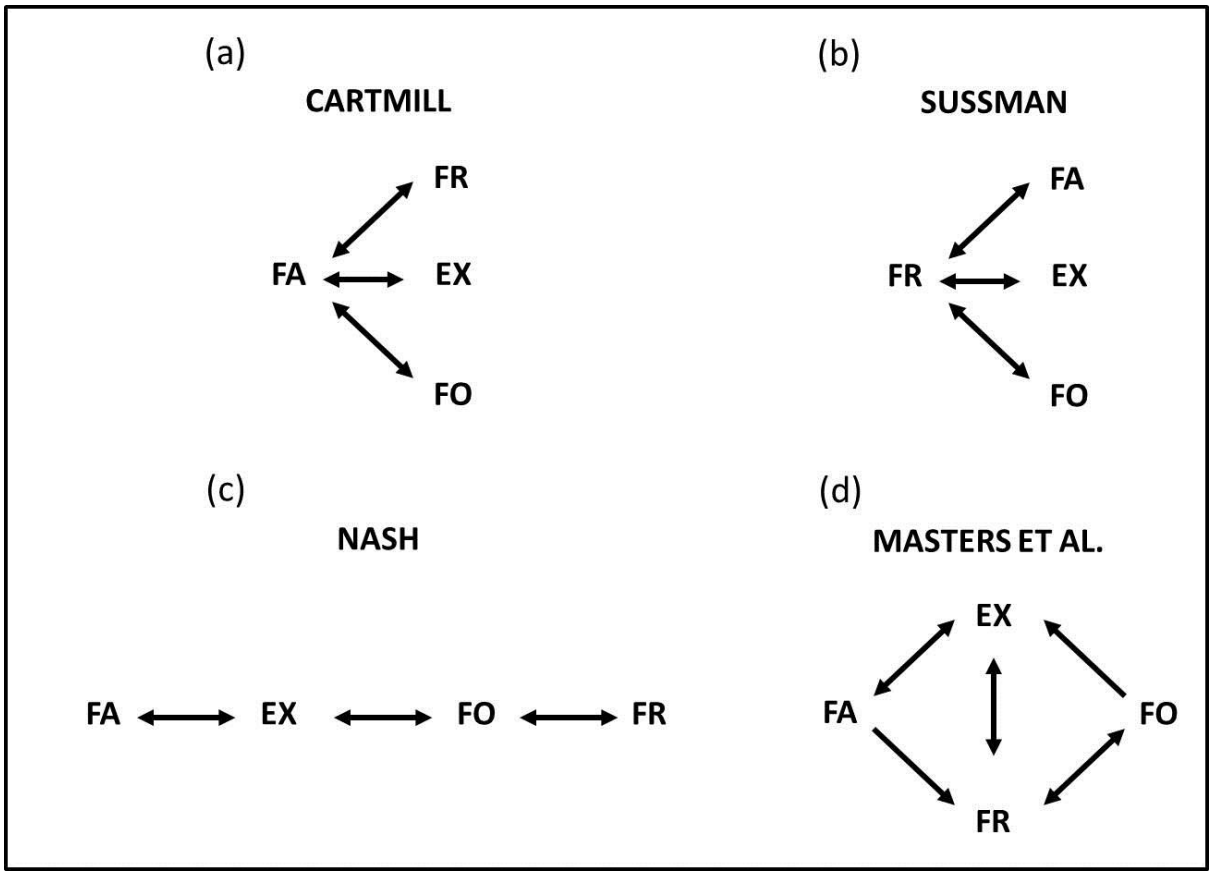

Figure 2.1. Illustration of possible dietary evolution scenarios: (a) Cartmill's small-bodied insectivorous ancestor; (b) Sussman's angiosperm co-evolution theory (frugivorous ancestor); (c) Nash's exudativorous ancestor; and (d) model reconstructed for this study based on the hypothesis of Masters *et al.* (in press). [FA: faunivory; EX: exudativory; FO: folivory; FR: frugivory].

2.2.4 Ancestral reconstruction

I used four primate phylogenetic trees taken from Springer *et al.* (2012) to accommodate for uncertainties associated with divergence times. I estimated Pagel's lambda using the Phytools package (Revell 2012) in R (R Core Team 2013) in order to assess the phylogenetic signal inherent in the data. A lambda value of 0 indicates that the data contain no phylogenetic signal, while a lambda close to 1 indicates a strong signal. I performed an ancestral reconstruction using the Multistate-Markov chain Monte Carlo (MCMC) method

implemented in the BayesTraits (software available from www.evolution.rdg.ac.uk) (Pagel *et al.* 2004).

Like any other Bayesian analysis, the estimation of priors is crucial for the reconstruction. First, I gave a subjective and wide pre-prior with a uniform distribution varying between 0 and 100. That pre-prior was used to estimate the prior for the reconstruction. In order to estimate the prior, the chain was run 1,010,000 times with the first 10,000 iterations discarded as burn-in. The chain was sampled every 1000^{th} iteration. The transition rates between dietary syndromes were fixed for one run but allowed to change from one iteration to the next. The 10,000 trees sampled from the 1,000,000 iterations were used to evaluate the distribution of the priors for use in the reconstruction. Priors were estimated separately for each model of evolution. The priors used for the ancestral reconstruction have a uniform distribution. The boundaries of the distribution were defined by the whole number immediately below the minimum estimated prior, and the whole number immediately above the maximum estimated prior.

I re-ran the MCMC as before, but this time using the estimated prior. I used the Most-Recent-Common-Ancestor approach (MRCA) to reconstruct the diet at internal nodes and to track dietary evolution within the primate clade (Pagel *et al.* 2004). Bayes factors (BF) are calculated as the harmonic means of the all the likelihood values generated during the 1,000,000 iterations of the model minus the likelihood values generated during the 1,000,000 iterations of the control (null) model. The significance and meaning of the BFs are reported in Table 2.1. The reconstruction model that had the best fit (i.e. had the highest likelihood value), was analysed further, and the reconstruction resulting from this model was represented graphically using the Ape package (Paradis *et al.* 2004) in R (R Core Team, 2013). Time of divergence was estimated from a consensus tree built by BayesTrees (Meade and Pagel 2011) from the 4 trees analysed.

Table 2.1. Log Bayes factors and the significance they confer on the models compared

Log Bayes factor	**Significance**
<2	Weak difference
>2	Positive difference
5-10	Strong difference
>10	Very strong difference

Log BF = 2(log [harmonic mean (complex model)] – log [harmonic mean (simple model)]

2.3. Results

My dietary data contained a strong phylogenetic signal (lambda = 0.987), giving validity to the reconstructions. Tables 2.2 and 2.3 and Figure 2.3 summarize the results of my reconstructions and show that the control (null) model, allowing unrestricted transitions between dietary syndromes, fitted the phylogeny better than the two classical models (visual predation and angiosperm co-evolution; Table 2.2, no restriction: Lh = -68.5). While my results confirm Cartmill's hypothesis of a faunivorous ancestor, they do not indicate the small body size required to hunt insects in the "fine branch niche" at the tips of tree branches. Further, my reconstruction suggests that all four dietary syndromes constrain future evolutionary trajectories, i.e. only certain transitions from one syndrome to another are allowed, while others are prohibited, introducing irreversible transitions (Lh = -63.4; Table 2.2). Because exudativory is associated with relatively small size and dental adaptations rather different from those required for eating leaves, it is unlikely to have acted as a precursor to folivory, contradicting Nash's hypothesized gummivory-folivory transition. Likewise, folivory and frugivory are often associated with relatively large size, long guts and specialized dentition that are unlikely precursors for faunivory. Finally, faunivory did not evolve directly into folivory, for similar reasons.

The proposition that the primate ancestor was very small in size is contradicted by the reconstructed ancestral body weight between 1-2 kg (Table 2.3) (see also Soligo and Martin 2006; Masters *et al.* 2007). This argues against the scenario of an agile hunter moving among fine branches, favouring and instead the scenario of a medium-sized ancestor with a slow metabolism and the ability to digest food items that require considerable processing (i.e. the exoskeletons of insects that consist of complex polysaccharides).

My reconstructions also suggest that the lemuriform ancestor, like the primate ancestor, had a mixed frugivorous/folivorous diet, with an estimated body mass close to 1 kg, while the ancestors of the *Lepilemur*-Cheirogaleidae clade (with *Lepilemur* and *Phaner* as sister taxa) and the Indriidae were folivorous. If this is accurate, then exudativory must have evolved from folivory at least in the case of *Phaner*, but may have evolved from at least partial frugivory in other Cheirogaleids. The Lorisidae-Galagidae ancestor was reconstructed as a faunivore weighing approximately 500 g, suggesting that exudativory in this group evolved from faunivory and frugivory, as is observed in living *Otolemur* (Table 2.3).

Table 2.2. Results of the phylogenetic reconstruction of primate diets indicating the rates of transition simulated for the three tested models, using 5 different models, and indicating the statistical support of the model (Lh).

Model	Lh	Lh (Harmonic Mean)	log BF	Prior transition rate	Posterior transition rate*											
					FR-EX	FR-FA	FR-FO	EX-FR	EX-FA	EX-FO	FA-FR	FA-EX	FA-FO	FO-FR	FO-EX	FO-FA
No restriction	-68.5	-69.2	control	uniform 0 - 1	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Masters *et al.*	-63.4	-64.6	9.1	uniform 0 - 2	0.69	0	0.69	0.69	0.69	0	0.69	0.69	0	0.69	0.69	0
Sussman	-70.6	-71.4	4.4	uniform 0 - 2	0.63	0.63	0.63	0.63	0	0	0.63	0	0	0.63	0	0
Nash	-74.8	-75.8	13.2	uniform 0 - 3	0	0	1.55	0	1.55	1.55	0	1.55	0	1.55	1.55	0
Cartmill	-82.6	-85.4	32.4	uniform 0 - 4	0	1.52	0	0	1.52	0	1.52	1.52	1.52	0	0	1.52

*FR: frugivory; EX: exudativory; FA: faunivory; FO: folivory; Lh : average of log likelihood of the 1000 sampled trees; Lh harmonic mean: mean of the log likelihood for all 1,000,000 iterations; Log BF : log Bayes factor calculated from the formula Log BF = 2(log[harmonic mean(complex model)] – log[harmonic mean(simple model)].

Table 2.3. Phylogenetic reconstruction of ancestral diets showing the probabilities for four types of diets ($P < 0.05 \approx 0$) and body sizes (values) of Cheirogaleidae and Galagidae indicating the dates of divergence estimated from Chaterjee *et al.* (2009) and Springer *et al.* (2010)

Node ancestral state	Frugivory	Exudativory	Faunivory	Folivory	Resolution[1]	Body mass (g)[2]	Dates (My)
Primates	0.07	0.11	0.80	0	X	1974	>67.8
Strepsirhini	0.15	0.15	0.64	0.06		894	53.1-54.2
Lemuriformes	0.54	0	0	0.46		967	49.4-50.0
Cheirogaleidae-Lepilemuridae-Indridae	0.13	0	0	0.87	X	[3]	30.6
Cheirogaleidae-Lepilemuridae	0.56	0.07	0	0.36		953	30.6-38.9
Cheirogaleidae without *Phaner*	0.63	0.27	0.09	0		398	27.6-28.7
Mirza-Microcebus	0.31	0.18	0.50	0		203	13.2-19.0
***Microcebus* first divergence**	0.34	0.15	0.50	0		92	6.9-9.8
Lepilemur-Phaner	0.08	0	0	0.88	X	779	22.3-32.1
Lorisidae-Galagidae	0	0.20	0.80	0	X	493	34.7-38.5
***Euoticus* divergence**	0	0.46	0.53	0		338	22.7-26.1
***Otolemur* divergence**	0.96	0	0	0	X	866	6.9-8.8

[1]$P > 0.75$ (see Fig. 2.3 for colour codes indicated when $P > 0.25$); [2]from Masters *et al.* 2007, Masters *et al.*, in review; [3]not a clade in Chatterjee *et al.* 2009

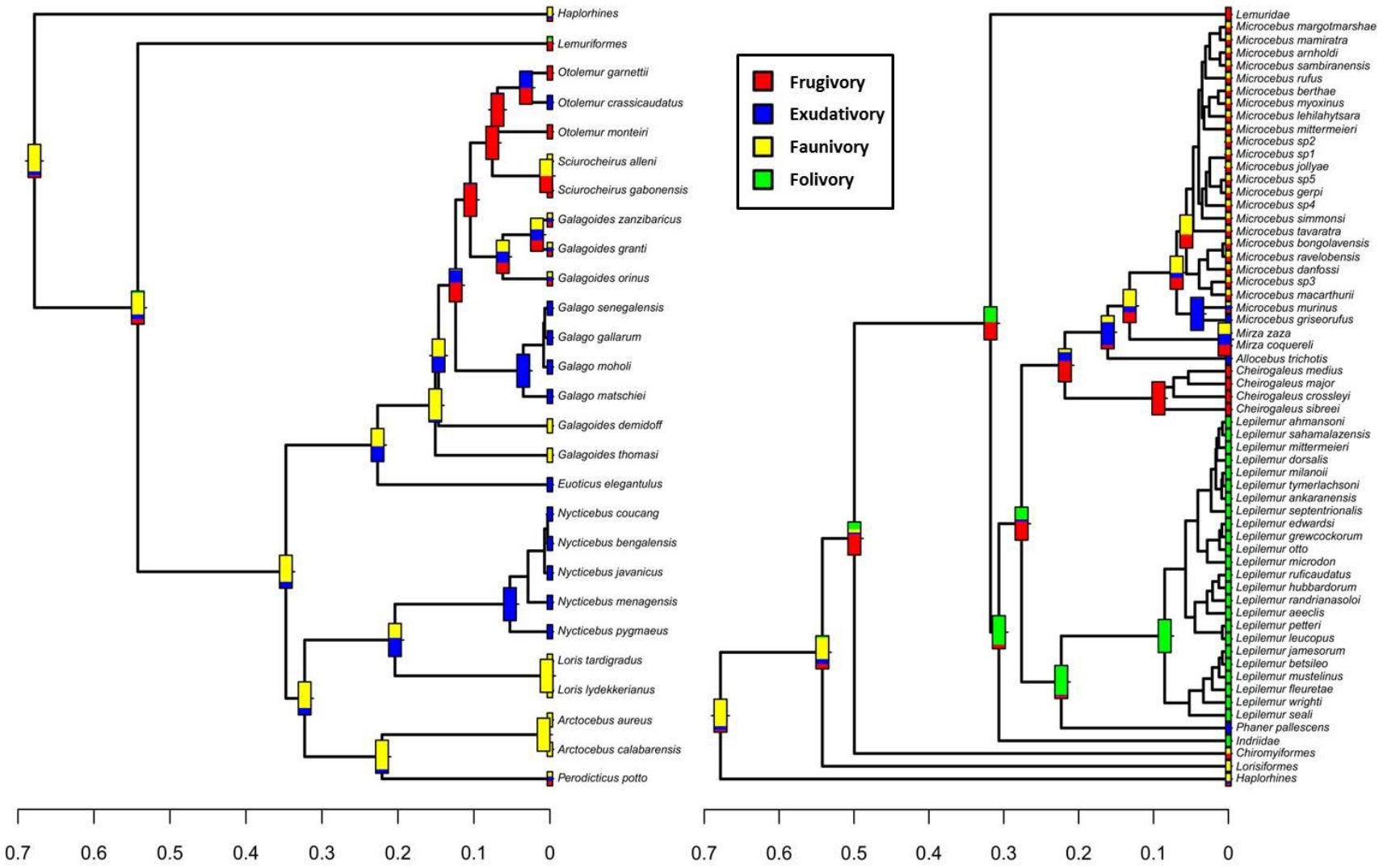

Figure 2.2. Phylogenetic reconstruction of ancestral dietary patterns of (left) Afro-Asian Lorisoidea and (right) Malagasy Lepilemuridae-Cheirogaleidae

2.4. Discussion

The unresolved nodes (see groups not marked with 'x' in Table 2.3, i.e. those for which no dietary syndrome makes up $\geq$ 75%) suggest that a mixed dietary preference is something that has been evident throughout the phylogenetic history of the primates, and still characterizes many extant primate genera and families. My reconstruction suggests a faunivorous ancestor for all Lorisiformes and indicates that exudativory in the Galagidae probably evolved from frugivory in the case of *Otolemur*, and faunivory in the remaining galagid genera. The gouging gummivory observed in slow lorises is also likely to have evolved from faunivory or faunivory-gummivory; while scraping gummivory in cheirogaleids and galagids evolved convergently from folivory (cheirogaleids) or faunivory. Phylogenetically it is likely that this peculiar diet evolved recently, in dwarfed lineages, during the dry periods of the Neogene, in association with the emergence of gum trees like Mimosoidae (Oligocene) or the diversification of others like Combretaceae (Miocene-Pliocene) (Maurin 2009; Bouchenak-Khelladi *et al.* 2010).

The diet reconstructed for the Lemuriformes ancestor may reflect a mixed frugivorous/folivorous diet. Similarly, the ancestral cheirogaleids may have had a mixed frugivorous/exudativorous diet; and the ancestral galagids a mixed faunivorous/exudativorous diet. Mixed diets may also reveal evolutionary transitions/divergences. For instance, a possible ancestral diet for Cretaceous primates may include large insects and seeds, as is observed in living aye-ayes (*Daubentonia*). The high level of specialization of *Daubentonia*'s adaptations to foraging on well-protected foods is likely to be extremely derived, but the same types of food may have been much easier to obtain at the time of the *Daubentonia*-lemur divergence.

Dietary evolution is linked to the evolution of body size, and various rules have been proposed to describe this relationship. Kay's rule states that a body weight of approximately 500 g is the upper limit for insectivores and the lower limit for folivores because of energetic constraints (Ankel-Simons 2007), and Cope's rule holds that body weights within animal clades tend to get larger over evolutionary time (Alroy 1998). With regard to body size, *Daubentonia* does not hold to Kay's rule and is the largest insectivore, weighing approximately 2 kg. The evolution of body size is unlikely to have been unidirectional in primates, and evidence for repeated dwarfing exists, with the Cheirogaleidae as a pertinent example. These dwarfing events appear mainly to have been associated with forest fragmentation and possible drought (Masters *et al.* in press). Changes in dietary preferences are a necessary concomitant of dwarfing episodes because of the relationship between body size and metabolism; hence, as the mouse lemurs grew smaller, their diet probably changed from folivory to a mixture of faunivory and exudativory. This shift in diet was physiologically possible as the gastro-intestinal specializations required for digesting insects and gums would have been derived from those adapted for the difficult digestion of leaves and other plant material, which are also known to require fermentation.

It is important to mention that ancestral reconstruction should be interpreted with caution, as the characters and dietary syndromes used in the models are based on extant families and could over- or underestimate the dietary preferences of extinct taxa. This is particularly relevant in the case of primates that consume plant material, as these associations are likely to have evolved as a result of long-term animal-plant interactions and co-evolution.

As the evolution of dietary syndromes must always have had a complex relationship to body size, another important factor to consider is the source of these syndromes, in particular, the consumption of plant exudates. Is exudativory a fall-back diet, as it is often viewed, or is it the result of phytochemical co-evolution involving an active role for the

consumers – e.g. in plant defence against insect invasion – in much the same way as fruit flesh provides a reward to the consumer in return for seed dispersal? The concept of a reward for services rendered is supported by the fact that all gummivores consume non-toxic gum, even though alkaloids are present in some exudates, and in some cases where gum is exuded, insects are present. Hence, gum could be a reward for the removal of insects that infest a particular tree. In a similar vein, the early stages of the primate-angiosperm interaction may have involved an association between trees infested with insects and the ancestral primates that consumed them, as well as various plant parts (i.e. exudates, fruits).

CHAPTER 3: COMPARING THE DIGESTIVE EFFICIENCY OF *GALAGO MOHOLI* AND *MICROCEBUS GRISEORUFUS*

3.1. Introduction

Gummivory has been considered a fall-back feeding strategy employed in the face of persistent adverse environmental conditions and dietary scarcity (Lambert 2007; Marshall and Wrangham 2007; Marshall *et al.* 2009; Rosenberger 2013). If gummivory was indeed a fall-back diet that contributed to the survival of certain lineages, then it must have led to the evolution of the necessary adaptations seen in modern primate gum-feeders, and over time, have become a staple instead of a fall-back food to some taxa. It is necessary, therefore, to distinguish specialist gummivores, which possess these adaptations, from occasional gummivores, which do not. My two focal species, the southern lesser galago (*Galago moholi*) and the reddish-grey mouse lemur (*Microcebus griseorufus*), are among the most frequent consumers of gum in their respective families, but both have highly specialized gum-feeding relatives [needle-clawed galagos (*Euoticus* spp.) and fork-marked lemurs (*Phaner* spp.)], the skulls and dentitions of which have become extensively and convergently modified to allow gum scraping. Thus, lesser galagos and mouse lemurs are essentially semi-specialists, and excellent models on which to test the fall-back diet hypothesis.

The digestive tracts of all specialized gummivorous primates have enlarged, capacious caeca to allow the fermentation of gum (Nash 1986; Power 2010, Smith 2010). Gum contains β-linked polysaccharides (Bearder and Martin 1980; Heymann and Smith 1999), which are particularly difficult to break down during digestion (Porter *et al.* 2009; Swapna *et al.* 2010). Studies have been conducted of the gastro-intestinal tracts of primates, both to compare the differences between primates and other mammals (Chivers and Hladik 1980), and to explore the adaptations of specific gut morphologies (Caton *et al.* 2000).

Fall-back foods have been described as either low in quality, but available when more desirable food is not (Bearder and Martin 1980; Lambert 2007; Marshall and Wrangham 2007; Porter *et al.* 2009; Rosenberger 2013), or high in quality, but rare (Lambert 2007). More recent research, however, indicates that gums are not necessarily lower in energy content than fruit, although the gums of different tree species may vary widely in mineral composition (Génin *et al.* 2010), and may confer health benefits; e.g. pygmy slow lorises in captivity show ill health when their diets lack exudates (Starr and Nekaris 2013). As mentioned in Chapter 1, the gastro-intestinal tracts of *G. moholi* and *M. griseorufus* differ by the presence of an ansa coli in *G. moholi* only, that acts as an additional fermentation chamber (Caton *et al.* 2000). This led me to question whether or not this structure influences digestive efficiency in the two study species, and whether *G. moholi* extracts more nutritional value from gum than *M. griseorufus*. I tested this hypothesis using captive feeding experiments.

3.2. Description of the study species

I focussed on two taxa that are believed to be only distantly related, but both practise frequent gum-scraping, and both occupy habitats that are subjected to hypervariable climatic regimes. The *Galago* and *Microcebus* lineages shared an ancestor that was at least Early Eocene or even Palaeocene in age, approximately 60 Mya (Chatterjee *et al.* 2009). The suborder Strepsirhini contains most of the primate taxa with specializations for gum-feeding. As their colloquial name implies, the tooth-combed primates share a modification of the anterior dentition which includes a procumbent "tooth-comb" in the lower jaw consisting of incisors and incisor-like canines (Merrit 2010), plus a cartilaginous sublingua under the tongue which serves to keep the tooth-comb free of detritus. The tooth-comb is used both in grooming and feeding, when it may be referred to as a "tooth-scraper" (Vaughn 1986). The upper incisors

are often reduced markedly in size and the medial teeth are separated by a relatively wide gap which contains the vomeronasal organ (Martin 1990).

Within the Lorisoidea (the African bushbabies and Afro-Asian lorises), most species consume at least some gum. All lorisoids are nocturnal and arboreal, and southern African bushbabies take refuge during the day in holes in *Acacia* and mopane trees (Skinner and Chimimba 2005). While they may sleep in family groups, they usually forage alone (Vaughn 1986; Skinner and Chimimba 2005). *Galago moholi* (A Smith 1836) (Figure 3.1) inhabits savanna woodland in Africa (Figure 3.2) and follows a diet that includes insects and other invertebrates, fruits and gums. Insects and fruits are mostly available in the warm, wet summer months (October – March), while gum is consumed throughout the year but makes up a much greater part of the diet during the cold, dry season (April – September; Vaughn 1986; Skinner and Chimimba 2005).

Figure 3.1. *Galago moholi*

Figure 3.2. Distribution of *Galago moholi*

The Malagasy Cheirogaleidae (mouse and dwarf lemurs) comprise five genera (*Allocebus*, *Cheirogaleus*, *Microcebus*, *Mirza*, and *Phaner*), and > 30 species have been described to date for the family (Mittermeier *et al.* 2010). Cheirogaleids are all nocturnal, use

quadrupedal locomotion coupled with varying degrees of vertical clinging and leaping, and sleep either in tree holes or in leaf-nests on branches (Mittermeier *et al.* 2010). The reddish-grey mouse lemur *Microcebus griseorufus* (Kollman 1910) (Figure 3.3.) inhabits the spiny thicket and forest of southern Madagascar, from the Mikea forest south to the Toliara region (Mittermeier *et al.* 2010) and in the extreme south to Tsimanampetsotsa, Berenty and Petriky (Andrainarivo *et al.* 2008; Figure 3.4).

The diet of *M. griseorufus* consists primarily of fruits, gums and arthropods, with the occasional consumption of nectar and moth bug secretions (Garbutt 2007; Génin 2008; Bohr *et al.* 2011), and varies seasonally; Bohr *et al.* (2011) found that more gum is consumed at the beginning of the dry season, when fruit consumption is correspondingly reduced. Of the gummivorous Cheirogaleidae, *M. griseorufus* appears to be the most specialized (Génin *et al.* 2010).

Figure 3.3 *Microcebus griseorufus*

Figure 3.4 Distribution of *Microcebus grisorufus*

3.3. Gum composition

Gum is a dietary fiber with complex β-linkages between constituent sugars (Nash 1989), making it, for the most part, indigestible by mammalian enzymes. In order to overcome this, gummivorous animals require intestinal flora and microbial digestive enzymes to ferment and extract the nutrients (Power 2010). Major nutrients found in gum include protein, calcium, carbohydrates and magnesium (Bearder and Martin 1980). Gum composition varies not only in accordance with tree species, but also with season, type of injury, soil type and the age of the tree (Bearder and Martin 1980).

3.4. Gut morphology of *M. griseorufus* and *G. moholi*

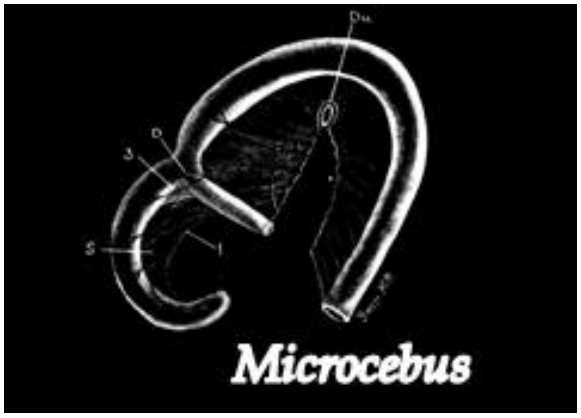

Figure 3.5 Caecum of genus *Microcebus* (Hill and Rewell 1948)

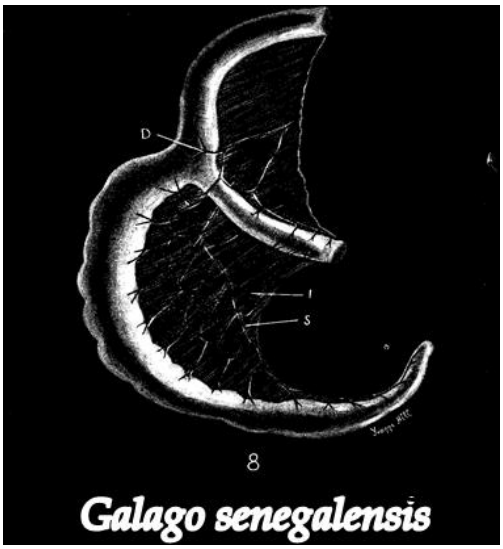

Figure 3.6 Caecum of *Galago senegalensis* (Hill and Rewell 1948)

Gummivory is a specialized feeding strategy that requires various adaptations, particularly to the gut. Generally an enlarged or expanded caecum is evident in gummivorous primates (Nash and Burrows, 2010.). *Microcebus* and other members of the Cheirogaleidae have relatively simple guts, and fermentation occurs in the caecum (Hill and Rewell 1948, see Figure 3.5). Lesser galagos, in contrast, use caeco-ansal fermentation for digesting the

complex β-linked polysaccharides found in gum and the exoskeletons of insects. While they have elongate caecum (Hill and Rewell 1948, see Figure 3.6) Caton *et al.* (2000) also found that fermentation took place in the proximal colon and ansa coli (see Figure 3.7).

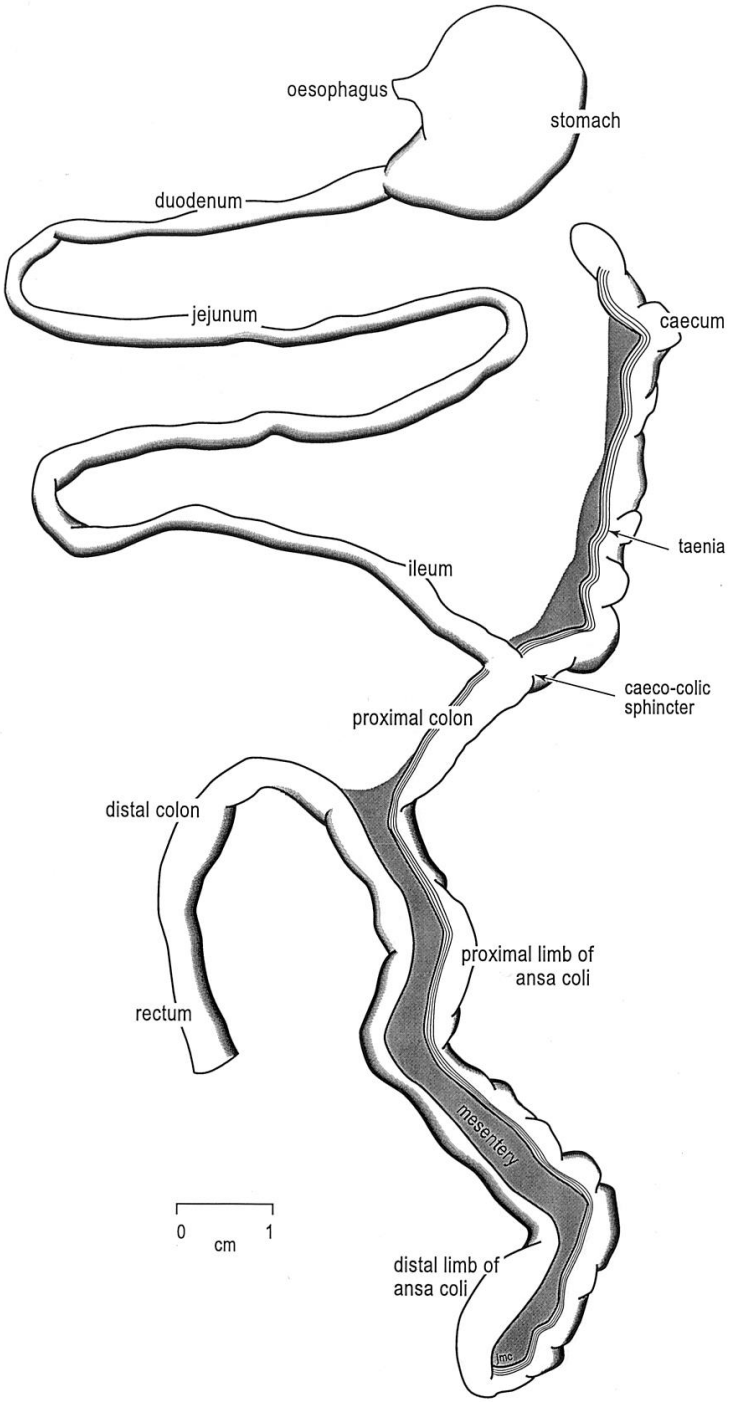

Figure 3.7. Entire gastro-intestinal tract of a lesser galago (probably *Galago moholi*; from Caton *et al.* 2000).

3.5. Materials and Methods

3.5.1. Description of study sites

The Ithumela Primate Sanctuary (Figure 3.8) is situated in Buffelsdrift in the north of Pretoria, South Africa. A private conservancy owned by Tom van Niekerk and Marti Koen, Ithumela contains both an academy for training courses and a sanctuary, which houses rescued and often injured indigenous primates, including vervet monkeys and bushbabies. Rehabilitated animals are often released back into the wild (see http://www.ithumela.co.za/page4.html).

Figure 3.8. Map illustrating Ithumela Primate Sanctuary in Buffelsdrift, South Africa.

Berenty Private Reserve (BPR) (Figure 3.9) in south-eastern Madagascar has been a nature reserve protected from hunting and burning, as well as extensive grazing by livestock and clearing of forests for over 75 years (Jolly *et al.* 2002; Génin 2008; Rambeloarivony and Jolly 2012).

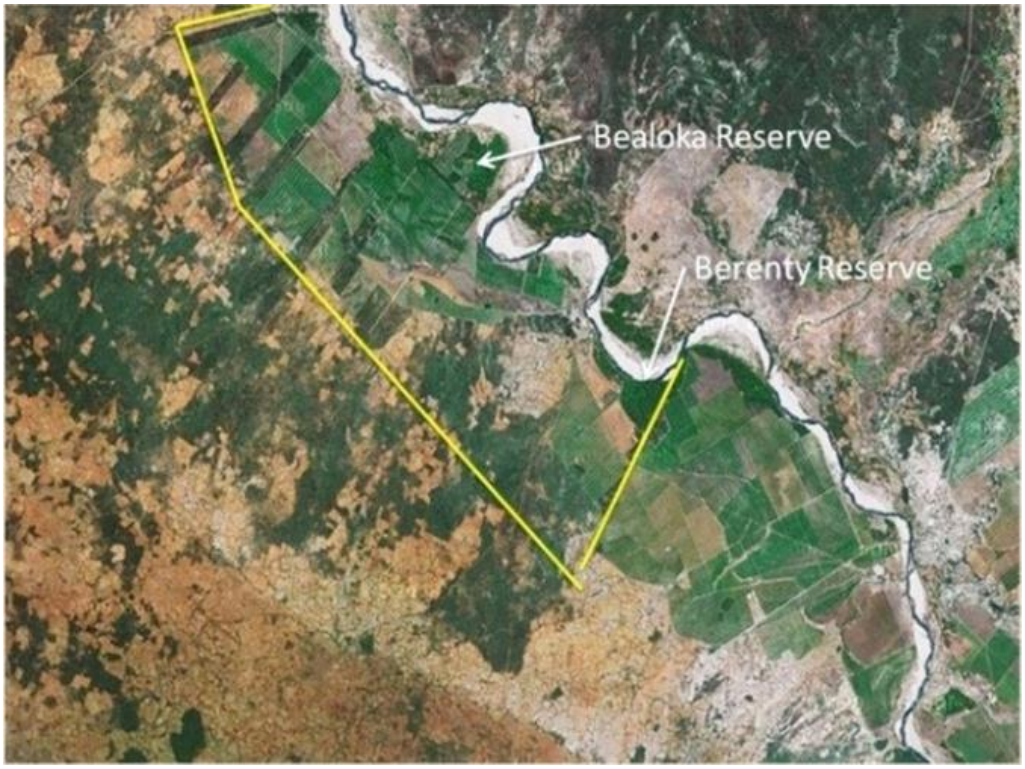

Figure 3.9. Map illustrating Berenty Private Reserve and neighbouring Bealoka Reserve in south-eastern Madagascar.

The reserve is owned by the de Heaulme family and contains ca. 1000 ha of protected forest that includes two patches of gallery forest at Berenty and Bealoka, and three patches of spiny forest at Berenty (where the study took place), Rapily and Anjampolo. The climate of BPR is characterized by long dry seasons (March – October) and short rainy seasons (November – February), with a mean annual rainfall of 513±13 mm (Génin 2008).

3.5.2. Gum collection

In South Africa, gum for feeding to *G. moholi* was collected in the Roodeplaat Nature Reserve (RNR), 22 km north-east of Pretoria. The gum samples were all taken from *Acacia karroo*. For Malagasy *M. griseorufus*, gum was collected in the spiny forest where the mouse lemurs are found. In addition to the patch of forest where the animals were trapped, a second patch just across the road also yielded some gum samples. Patches of spiny forest at the edges of the reserve were also sampled. All gum samples were taken from *Commiphora* spp.

3.5.3. Field observations and trapping

To collect *M. griseorufus* individuals for the feeding experiments, Sherman traps baited with banana were set along a trail in the spiny forest. On the first night, 24 traps were set and 12 animals were captured (see circles in Figure 3.10 A); 7 individuals were retained for the experiments. These individuals were weighed and sexed, and their tails were marked by trimming some hair to avoid their repeated inclusion in the experiments. The animals were also checked for markings on the ears made by previous researchers, as identification tools to track the dynamics of the population. Animals that were not part of the experiment were released at dusk the following evening at the site where they were trapped, to facilitate their re-adjustment.

A second night's trapping was conducted approximately a week after the first, and the sample size was reduced from 7 animals to 4 because of the scarcity of gum after the rain. The traps were set according to the plan in Figure 3.10 B. To avoid re-trapping the 7 individuals from the first captures, the animals were kept at the camp until the following evening, and then released. A total of 19 Sherman traps were set, and baited with banana as before. Ten animals were captured (see circles in Figure 3.10 B), weighed and sexed, and 4

were retained and marked for inclusion in the experiments. The remaining animals were released.

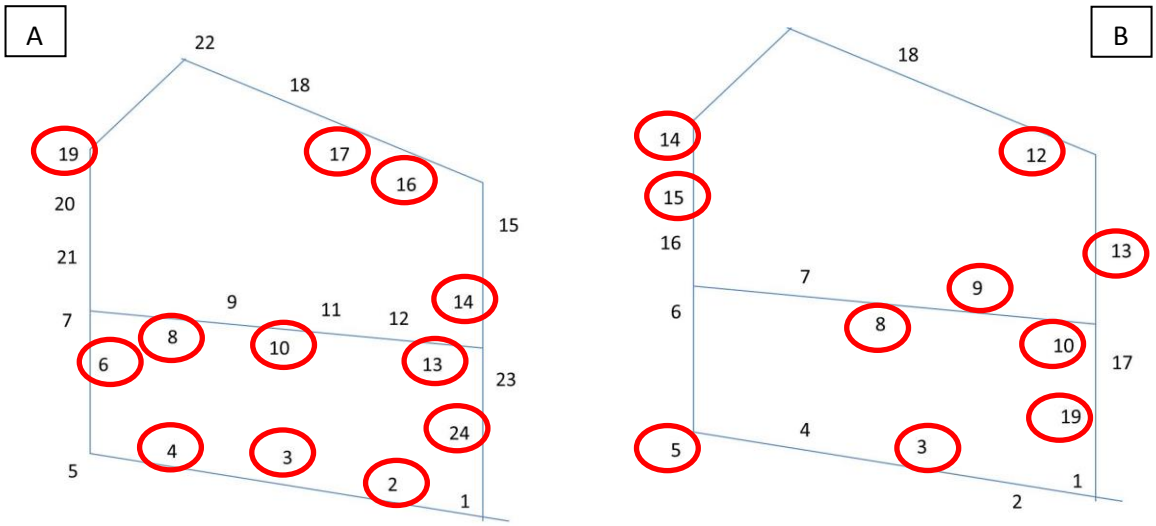

Figure 3.10. Sherman traps baited and placed for trap nights 1 (A) and 2 (B)

3.5.4. Digestive efficiency experiments

At Ithumela Primate Sanctuary, 6 captive lesser bushbabies were transferred to cages (1 animal/cage), each fitted with a nest box and a plastic tray to facilitate the collection of faecal matter without disturbing the animals (Figure 3.11). The 6 individuals were fed over 5 nights (06/09/12- 10/09/12) with gum, using an environmental enrichment technique following Huber and Lewis (2011), and one night as a control (11/09/12), with banana. The food items along with the subject animals were weighed before the feeding trials, and the subsequent faeces were collected, weighed, and put in a drying oven at 43°C for 30 hours to ensure complete desiccation of the samples. After desiccation, the faeces were weighed again and stored in airtight bags in the refrigerator for further analyses.

Figure 3.11(a) The cage with the fitted nest box and plastic tray, **(b)** water and feeding bowl, **(c)** log feeding device.

In Berenty Private Reserve, The first feeding experiment trial with *M. griseorufus* was performed over a period of 5 days. The subjects were fed with banana for 2 nights (09/05/13-10/05/13), which served as the control as well as to allow the animals to adjust to their captivity. On the third night the animals were fed *Acacia* spp. gum, using the same environmental enrichment technique as used for the galagos, for 3 nights (11/05/13-13/05/13). Before each subsequent feeding trial after the first night, any faeces that had been produced were collected, as well as any food items remaining in the cage. These were weighed and recorded, and the faeces were preserved as for *G. moholi*.

The same procedure was followed for the second feeding experiment trial, 15/05/13-20/05/13. The animals were fed a diet of banana for the first 3 nights, after which they were

fed *Commiphora* spp. gum for 3 nights. Faecal samples were collected as before, as well as any food items remaining in the cage. These were weighed and recorded.

3.5.5. Calorimetric analyses of samples

Gum and banana samples were prepared for the micro-calorimeter by compression into pellets. Samples were pre-weighed, inserted into the machine and compressed. Every pellet produced was weighed again and subjected to calorimetric combustion to determine its energy content. In addition to the food items, faecal samples were analysed for energy (MJ/kg). Digestive efficiency was calculated as follows:

Digestive Efficiency [%] = Gross Energy Feed – Gross Energy Faeces/ (Gross Energy Feed-Gross Energy Faeces)/Gross Energy Faeces *100

3.5.6. Nutrient composition analyses

The food items and faeces were subjected to 4 biochemical assays to assess the nutrient composition. These tests determined the total nitrogen and sugar content of the items as well as the condensed tannin and phenolic concentrations.

3.5.6.1. Analysis of total nitrogen. For nitrogen extraction, 1 g of each gum and banana sample was weighed and placed in a reaction vial, which was marked appropriately. A replicate was prepared for each sample, and the vials were labelled A and B. To the sample in the vial, a Kjedahltab was added, a capsule comprising the catalyst. A 20 ml aliquot of 98% sulphuric acid was added to each vial, which was placed in the extraction reservoir connected to the Turbosog and water cooling system, and switched on. The temperature of the reservoir was set to 200°C, and was raised in 25°C steps from 0°C to 200°C, then increased to 300°C, and finally to 430°C. At the final temperature, the sample was heated for an hour and left to cool overnight.

This step was followed by distillation, for which a titration vessel was prepared. The cooled Kjedahl-vial was placed into the Vadopest machine alongside an Erlenmeyer flask containing 40 ml 0.1 N sulphuric acid. NaOH was added to the reaction vial, resulting in the production of NH_3, which was transferred to the Erlenmeyer flask. Once the Vadopest machine had run for 3 min, the contents of the Kjedahl-vial were discarded. A few drops of indicator were added to the Erlenmeyer flask, and the mixture was titrated. After titration, the total nitrogen concentration was calculated as follows:

Nitrogen concentration [%] = $(V_0 - V_1) * c * 0.014 * 100/M$ where:

V_0= Volume (ml) of NaOH used for the "Blind"

V_1= Volume (ml) of NaOH used for the sample

c= Concentration (mol/l) of the NaOH

M= Sample weight (g)

The product was converted to % crude protein as follows:

Crude Protein [%] = Nitrogen Concentration [%] * 6.25

3.5.6.2. Analysis of total sugar. A 50 mg sample of banana or gum was weighed and placed in a test tube. This was extracted using 50% methanol in a two-step process: 2.5 ml methanol was added and the samples were well-mixed using a vortex. A further 2.5 ml was added and vortexed, and the test tubes were covered with aluminium foil for 24 h. After the extraction, 0.5 ml of the extracted samples was pipetted into a reaction vial, into which 0.5 ml Phenol-reagent was pipetted, followed by 2.5 ml of concentrated sulphuric acid. The reaction vial was incubated in darkness for 1 h. After the incubation period, the extinction of the sample was measured at 490 nm using a photometer, and the percentage of sugar was calculated as follows:

Sugar [%] = $\frac{Extinction * 860}{Sample\ Weight\ (mg)}$

3.5.6.3. Condensed tannins. Extraction of samples to assess condensed tannins followed the same protocol as for the analysis of total sugar. To each extract, 2.5 ml tannin-reagent (i.e. 5 ml 37% hydrochloric acid in 95 ml n-butanol) was added and mixed using a vortex. The solution was incubated for 160 min in an 80°C warm water bath, and cooled for a few minutes. Once the sample had cooled adequately (about 5 min), the extinction of the sample was measured at 540 nm using a photometer, and the percentage of condensed tannins was calculated as follows:

Condensed Tannins [%] = $\frac{Extinction * 540}{Sample\ Weight\ (mg)}$

3.5.6.4. Phenolics. A 100 mg subsample of each banana and gum sample was weighed and placed in a reaction vial, to which 10 ml of distilled water was added. The reaction vials were covered with aluminium foil and placed in a warm water bath at boiling temperature for 40 min. Thereafter, the vials were cooled and the extract filtered.

An aliquot of 500 µl of each extract was pipetted into a vial to which 500 µl of Folin-Ciocalteus prepared solution was added, followed by the addition of 9 ml 20% $NaCO_3$, and set aside for 6 min. After this period, the extinction was measured at 750 nm using a photometer. Using the extinction and a calibration curve, the percentage of condensed tannins was calculated as follows:

Total Phenolics [%]: $\frac{Extinction + c}{Sample\ Weight\ (mg) * b}$ where:

c= intercept (0.0078)

b= slope (0.0173)

3.6 Statistical analyses

Statistical tests were performed using the SYSTAT package. The measures of the replicates used in the chemical analysis were centred (value – average) to allow a single statistical test (Repeated Analysis of Variance). I used the same test of Repeated Analysis of Variance to compare the digestive efficiency of banana (trial 1) and gum (trial 2) in the two species. $P < 0.05$ was considered the level of statistical significance.

3.7. Results

Table 3.1 presents the results of the different biochemical tests performed on the food items, gum and banana. Each sample has two replicates (A and B).

Table 3.1. Measures of replicates used in chemical analyses

Method of Chemical Analysis	**Food Items**	**Nutrient Constituent**	**Measured replicates for each biochemical test (g)**	
			A	B
Kjedahl	*Commiphora*	Protein	1.0123	1.0219
	Acacia	Protein	1.0194	1.0138
	Malagasy banana	Protein	1.0103	1.0857
	SA banana	Protein	1.0809	1.0463
Phenol-Sulphuric acid	*Commiphora*	Carbohydrates	0.0555	0.0547
	Acacia	Carbohydrates	0.0561	0.055
	Malagasy banana	Carbohydrates	0.0512	0.0545
	SA banana	Carbohydrates	0.0555	0.0541
Phenol-Sulphuric acid + Tannin reagent	*Commiphora*	Condensed Tannins	0.0555	0.0547
	Acacia	Condensed Tannins	0.0561	0.055
	Malagasy banana	Condensed Tannins	0.0512	0.0545
	SA banana	Condensed Tannins	0.0555	0.0541
Folin-Ciocalteau-Reagent	*Commiphora*	Phenolics	0.1002	0.1001
	Acacia	Phenolics	0.1001	0.1006
	Malagasy banana	Phenolics	0.102	0.1029
	SA banana	Phenolics	0.1002	0.1008

The results of the analyses of the nutritional content of gum and banana are presented in Table 3.2, and compared with the nutritional values of other food items either consumed by prosimians or not consumed at all.

Table 3.2. General chemical characteristics of gum and fruit including those consumed by *G. moholi* and *M. griseorufus.*

Gum	*Crude Protein (%)*	Carbohydrates (%)	Tannins (%)	Phenolics (%)	Energy Content (MJ/Kg)	Reference
Acacia karroo	1.08	59.63	0.24	0.09	14.0	This study
Acacia Senegal	2.13					Ali *et al*. 2012
Acacia senegal var. *Senegal*	1.94		0.40			Mhinzi 2004
Acacia senegal var. *leiorhachis*	3.00		0.57			Mhinzi 2004
Acacia sieberana var. *woodii*	2.44		0.24			Mhinzi 2004
Acacia seyal	1.31					Ali *et al*. 2012
Acacia polyacantha	2.06					Ali *et al*. 2012
Acacia laeta	2.00					Ali *et al*. 2012
Commiphora sp.	2.92	52.45	0.05	0.18	16.5	This study
Commiphora orbicularis	11.30	55.90			64.3	Génin *et al*. 2010
Commiphora aprevalii	6.60	43.50			48.0	Génin *et al*. 2010
Commiphora lamii	5.00	31.40			34.9	Génin *et al*. 2010
Commiphora humbertii	2.30	39.60			40.0	Génin *et al*. 2010
Commiphora sp.	15.30	30.80			44.0	Génin *et al*. 2010
Commiphora sp.	4.40	74.50			75.4	Génin *et al*. 2010
Terminalia mantaliopsis	1.40	25.20			25.4	Génin *et al*. 2010
Terminalia mantaliopsis	5.20					Hladik *et al*. 1980
Terminalia mantalis	2.40	26.10			27.2	Génin *et al*. 2010
Terminalia tricristata	3.30					Hladik *et al*. 1980
Delonix decary	3.90	47.60			49.2	Génin *et al*. 2010
Albizia mainaea	11.30	28.40			37.9	Génin *et al*. 2010
Allantsilodendron alluaudianum	21.00	29.50			48.3	Génin *et al*. 2010
Rhopalocarpus sp.	3.90	38.20			40.2	Génin *et al*. 2010

Fruit						
Banana (SA)	3.9	60.04	0.25	0.18	14.5	This study
Banana (MD)	5.1	73.02	0.00	0.26	14.6	This study
Phylloctrenium decaryi	7.3	55.5			60.9	Génin *et al*. 2010
Maerua ruda	17.5	4			20.5	Génin *et al*. 2010
Operculicarya gummifera	9.5					Hladik *et al*. 1980
Physena sessiliflora	8.3					Hladik *et al*. 1980
Strychnos clecussata	7.1					Hladik *et al*. 1980
Grewia glandulosa	6.0					Hladik *et al*. 1980
Euonymus plurostyloides	5.8					Hladik *et al*. 1980
Insect Secretions						
Dried secretion from *Flatida coccinea*	1.4					Hladik *et al*. 1980

The differences between carbohydrate and protein content, as well as the concentration of secondary compounds, are extremely small between gum and banana. Sample replicates (A and B) showed no significant difference ($P > 0.05$) for each sample across all tests performed, validating the repeatability of the methods (Table 3.3).

Table 3.3. Results of Repeated Analysis of Variance on the replicates used for each chemical analysis (protein, carbohydrates, tannins and phenolics). Replication indicates the overall statistical comparison between the two replicates, while analysis indicates the possible interaction between replicates and the four analyses.

Source of Variance	Test of Hypothesis	Df	F	P	Significance
Replication	Hypothesis	1	0.250	0.626	**NS**
	Error	12			
Analysis	Hypothesis	3	0.224	0.878	**NS**
	Error	12			

Table 3.4. Results of Repeated Analysis of Variance on digestibility of food items by the two study species

Source of Variance	Test of Hypothesis	Df	F	P	Significance
Species difference in digestibility (gum and banana)	Hypothesis	1	0.022	0.885	**NS**
	Error	8			
Banana versus gum	Hypothesis	1	0.330	0.581	**NS**
	Error	8			
Banana versus gum: species comparison	Hypothesis	1	2.251	0.172	**NS**
	Error	8			

Neither *G. moholi* nor M. *griseorufus* showed a significant difference in their ability to digest food ($P > 0.05$) (Table 3.4). Similarly, there was no significant difference in the digestive efficiencies scored for banana or gum ($P > 0.05$) or in the interaction between the two factors ($P > 0.05$) (Table 3.4).

The ability to digest gum and banana in *G. moholi* (93.5 %) and *M. griseorufus* (89.1%) illustrates a slight variation (Figure 3.12), but because of the small sample size, this is not statistically significant (see Table 3.4).

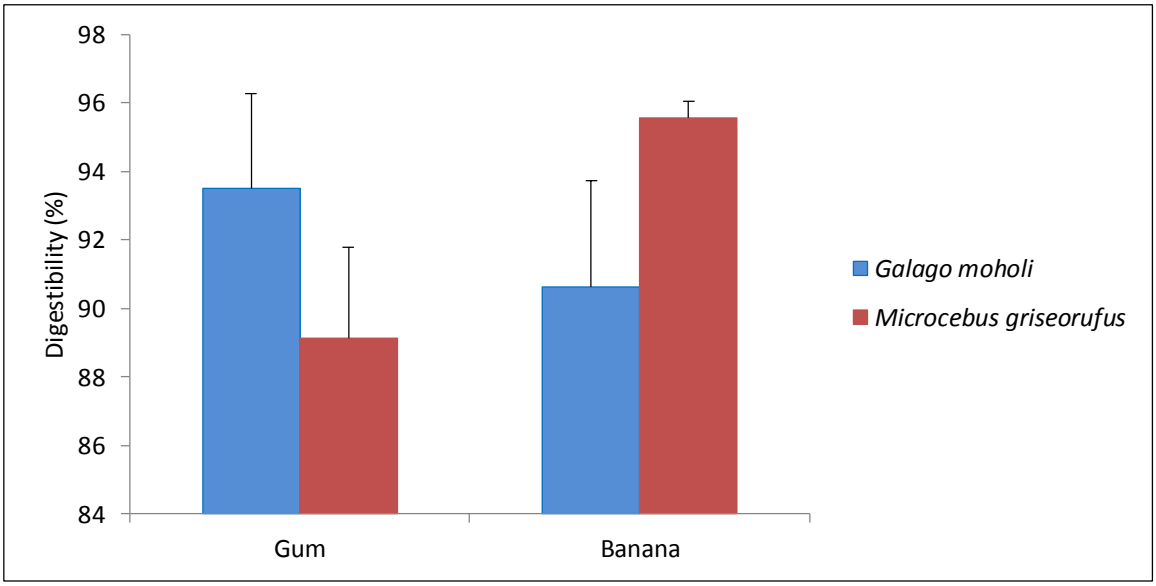

Figure 3.12. Digestibility of gum and banana in *Galago moholi* and *Microcebus griseorufus*.

3.8. Discussion

My research question was whether *G. moholi* and *M. griseorufus* showed different efficiencies in their ability to digest gum, with fruit (banana) serving as a control food item. Since *G. moholi* has an ansa coli (Caton *et al.* 2000) in addition to an enlarged caecum, providing an additional fermentation chamber to prolong the retention and digestion of complex carbohydrates, it could be presumed that lesser galagos have a higher digestive efficiency than mouse lemurs. In their study of *G. moholi* digestion, Caton *et al.* (2000)

found that the mean retention times (MRTs) of digestive markers exceeded 24 hours before appearing in the faecal material, and that the selective retention of fluid digesta is a common trait in small mammals that exhibit caecum fermentation. My experiments on digestibility of gum and banana in galagos and mouse lemurs, however, indicated no significant difference in the digestibility of either food item in the two taxa.

Even though I did not use digestive markers in my study, direct observation led me to conclude that, in both species, retention times were longer when the animals were fed gum than when they were fed fruit. For instance, when the animals were fed the control food item (banana), the animals would already have faecal material in their cages before the next feeding time, 24 h later, whereas when the animals were fed gum, the time between feeding and the first appearance of faecal material was much longer. In the case of *G. moholi*, gum was fed over a period of 3 nights, after which no faecal material appeared. The animals were left with the gum fed on the third night, and after a period exceeding 36 h (including a 24 h period of starvation), faecal material was collected and processed for analysis. Similarly, when *M. griseorufus* was fed gum over a 2 night period, the first appearance of faecal material was after a period exceeding 36 h, indicating prolonged retention of digesta in the gastro-intestinal tract for adequate fermentation. This suggests that the retention time and not the number of fermentation chambers is important for the adequate digestion of gum.

Determining the possible separation of fluid and particulate phases would, however, have to be tested using digestive markers. This is an obvious next step in the case of *M. griseorufus*, as Caton *et al.* (2000) have already investigated this for *G. moholi* and for the common marmoset (*Callithrix jacchus*; Caton *et al.* 1996). My observations that retention times are prolonged in animals that practice caecal fermentation are consistent with the findings of Power and Oftedal (1996), who compared the digestive efficiencies of captive

callitrichines. They found that the transit time of digesta was longer in marmosets (*Cebuella pygmaea* and *Callithrix jacchus*) – which have capacious caeca – than in tamarins (*Saguinus fuscicollis*, *S. oedipus* and *Leontopithecus rosalia*), which are occasional gummivores, and do not have such extensive gut areas for gum fermentation (see illustrations in Hill and Rewell 1948).

All gums require long gut retention times for adequate digestion, but some appear to present more of a challenge than others. Porter *et al.* (2009) investigated the selection of exudates by *Callimico goeldii*, and proposed that exudates that were more difficult to digest were eaten later in the day and digested overnight. Heymann and Smith (1999) drew similar conclusions regarding gum-feeding in two *Saguinus* species (*S. mystax* and *S. fuscicollis*), in that gum-feeding generally occurred later in the day.

All of my feeding experiments were conducted in the late afternoon/early evening, since this suited the natural feeding pattern of my nocturnal species. Although the gum was presented at the beginning of the animals' activity period, it was left in the cages for 24 h, and the exact timing of their major consumption was not recorded. The experiments conducted on *M. griseorufus* in Berenty, Madagascar, were run in two phases. In the first trial, involving 7 animals, subjects were offered *Acacia* gum from South Africa, as it was proving difficult after the rains to find exuding *Commiphora* gum. Although the mouse lemurs sniffed and licked briefly at the gum, it was essentially left uneaten. In the second trial, involving 4 animals, subjects were offered *Commiphora* gum, which they consumed readily.

Acacia gum is a foreign substance that the animals were not accustomed to encountering in their natural environment, and their avoidance of this food could have been influenced by neophobia. Mouse lemurs generally, however, showed little neophobia: when offered unknown fruits (banana), they consumed them readily. Hence, the gum's

physicochemical nature could have contributed to the animals' lack of interest in it. In Chapter 4, I present the results of my analyses of *Acacia* and *Commiphora* gum for secondary compounds. No traces of secondary compounds were found in *Acacia* gum, whereas *Commiphora* spp. contained several secondary compounds, including one that identifies *Commiphora* as a resinous gum. Hence, from my feeding experiments I conclude that the presence of secondary compounds does not render *Commiphora* gums undesirable food sources to reddish-grey mouse lemurs.

CHAPTER 4: TOXICITY OF GUMS AND PLANT-ANIMAL INTERACTIONS

4.1. Introduction

4.1.1. Theories of co-evolutionary biology

Thompson (2005) defined co-evolution as "the process of reciprocal evolutionary change between interacting species driven by natural selection". In Thomspon's view, species evolve largely by manipulating other species, and these relationships are generally mutualistic to some degree. Most co-evolutionary relationships are hypothesized to involve one-on-one species interactions, but Janzen (1980) proposed the concept of "diffuse co-evolution", in which either or both of the reciprocating parties are represented by, not just one species interacting with another, but by groups of populations/taxa/clades that influence the evolution of one another. Diffuse co-evolution formed the basis of Sussman's (1991) hypothesis of the co-evolution of euprimates and angiosperms.

Co-evolutionary relationships range from exploitative to mutualistic (see 1.2.2). While interactions between frugivores and fruiting trees are likely to involve a degree of mutualism, nothing is known of possible interactions between gum-producers and gum-feeders. According gum the status of a fall-back food implies that these interactions are exploitative to neutral, and superficial in terms of shared evolutionary histories. The function of gum exudation is obscure, but it appears to be comparable to bleeding and cicatrisation/ scar formation, because gums are transparent and fluid when first exuded, hardening over time. The process of hardening of gum is often associated with oxidation, as the gum turns amber in colour. Exudates may have evolved to expel xylophagous larvae infesting the trees (F. Génin, pers. comm.). During this phase, gummivores may be of service to the trees, both by maintaining the flow of gum and by consuming both the insect larvae and the adults responsible for the infestations. Gummivores and gum trees would form associations

comparable to those observed between cleaner birds and fish and their "clients" (Poulin and Grutter 1996).

Resinous gums have a soluble gum fraction and a non-soluble resinous fraction, and are often used in traditional medicine to activate cicatrization in Asia, Africa and Madagascar, as well as in religious rituals in the form of incense. Famous examples are the Biblical myrrh (*Commiphora myrrha*) and balm (*C. gileadensis*), medicinal gums used in Middle Asia. *Commiphora* has more than 200 species distributed mainly in sub-arid regions of Middle Asia, Africa and in Madagascar (Steyn 2003), *Commiphora* gum is an important resource for cheirogaleids, particularly mouse lemurs. By contrast, Fabaceae, such as *Acacia*, *Albizia*, or the Malagasy endemics *Delonix* and *Alantsilodendron*, produce soluble gums, also frequently consumed by mouse lemurs.

4.1.2. Secondary compounds of plants

Plant secondary compounds are more than simple end-products of metabolism; they may also play a role in the primary metabolism of the plant. For example, Seigler (unpublished data, cited in Seigler and Price 1976) found that the seeds of the Mexican buckeye (*Ugnadia speciosa*) contained cyanolipids which disappear during germination, suggesting that the secondary compound influences seed viability. Additionally, they may serve as stores of nitrogen and carbon, as these elements are prominent in chemical compounds that are considered toxic. Their most commonly cited role, however, is their function as insect repellents (Seigler and Price 1976; Glander 1982). Because seeds have evolved to nurture and protect plant embryos, they are highly nutritive for seed predators, and hence vulnerable to predation. Toxic secondary compounds along with thick seed coats (e.g. *Ugnadia*) can help to deter plant predators.

Secondary compounds are ubiquitous in plants, and this makes it highly likely that they influence food choices in primates (Glander 1982). The exact number of known secondary compounds has not been determined, but it is probably $\geq$ to the number of living plant species, i.e. in excess of 10 000. Examples of secondary compounds that may deter feeding activity include condensed tannins, phenolic resin, hypericin, saponins, cardenolides and alkaloids (Rhoades and Cates 1976, cited by Glander 1982).

4.1.3. Primate foraging selectivity

Primate species consume a wide variety of plant foods, including some that are considered high in nutritional quality (i.e. fruits) and those that are considered nutrient deficient (i.e. leaves and gum) (Nash 1986; Krishnamani and Mahaney 2000; Lambert 2007). The types of foods that primates consume depend on a variety of factors, such as the preferred habitat (including the home range area), energy needs, specific nutrients, body size and anatomical and behavioural adaptations (Krishnamani and Mahaney 2000).

Glander (1982) provided a comprehensive overview of the influence secondary compounds are likely to have on the selectivity of primate foraging, and of the possible co-evolution of these compounds, first as defences against insects, and secondarily as defences against primates. Herbivorous primates may actively avoid certain plant parts that contain high levels of secondary compounds, especially alkaloids and phenolics. Glander (1981, cited in Glander 1982) examined the feeding behaviour of mantled howler monkeys (*Alouatta palliata*) to determine whether they practised such avoidance behaviour. The results of chemical analyses showed that the animals indeed avoided plant material that was rich in secondary compounds, and at the same selected foods that yielded high nutritional returns. Similarly, a study of black colobus monkeys (*Colobus satanus*) (McKey *et al.* 1978, cited in Glander 1982) revealed that the animals only ate new leaves of certain species, while mature

leaves in general, and the new leaves other plant species, were avoided. The monkeys' leaf preferences coincided with lower levels of phenolics. Chimpanzees appear to prefer fig species with lower tannin and higher sugar content (Reynolds *et al.* 1998), as well as younger leaves with lower tannin levels over mature leaves with higher tannin levels. Food preferences are not always consistent across a species, however. Study groups of wild mountain gorillas were characterized in terms of their food preferences (Ganas *et al.* 2008): one group consumed fruits with high condensed tannins and sugar, while the other consumed fruits with low condensed tannin and high sugar content. *Lemur catta* has been shown to tolerate quinine, possibly as an adaptation to ingest foods containing alkaloids and other bitter-tasting toxins that are present in the leaves it consumes, although the animals are less tolerant of tannic acid (Simmen *et al.* 2006).

In addition to studies of foraging selectivity by primates, some studies have been undertaken to document the influence that secondary compounds have, when consumed, on the digestion of particular plant material; e.g. several studies have investigated the effect that condensed tannins have on digestion (Reynolds *et al.* 1998; Carrai *et al.* 2003; Rothman *et al.* 2009). Some secondary compounds decrease the nutritional profile of certain foods while, in turn, some nutrients decrease the effects of certain plant secondary compounds.

Even though many primate species that consume plant material avoid secondary compounds, others seem to make food choices that take no account of secondary compound concentrations, and a few seem to select plant foods specifically for their high levels of secondary compounds. Many primates, therefore, can not only tolerate these compounds, they are quite capable of digesting them. Huffman (1997) has suggested that such plant compounds may be used in self-medication. Carrai *et al.* (2003) observed such active choice in female Verreaux's sifakas (*Propithecus verreauxi verreauxi*), which increased their tannin

consumption significantly between pregnancy and birth. The authors proposed that increased tannin consumption provides prophylactic benefits in female sifaka reproduction (Carrai *et al.* 2003). It is further possible that some primates (the slow loris *Nycticebus* in particular) are capable of either sequestering toxic compounds from their foods, or synthesizing them *de novo*, for use in chemical defence against predators or ectoparasites (Altermann 1995; Nekaris *et al.* 2013).

Primate insectivores may imbibe their secondary compounds by way of their insect prey. Insects that feed on plant material are likely to ingest secondary compounds (Glander 1982), and, since most primate insectivores consume their prey items entirely, it is a reasonable assumption that they have evolved the necessary physiological abilities to tolerate or detoxify the toxins ingested by insects.

Little is known of the role of secondary compounds in the selectivity of gums by gummivores, and in this chapter I investigate the presence of such compounds in samples of gum regularly consumed by strepsirhine primates.

4.2. Materials and Method

Samples were prepared for gas chromatography-mass spectrophotometry (GC-MS) by weighing 100 mg of each sample (gum samples and gum faecal samples) and placing it into a 25 ml vial. To each sample, 5 ml hexane was added and the vials closed with a lid. The entire vial was wrapped in aluminium foil and placed in a shaker for 2 h, after which the samples were refrigerated for 24 h. After refrigeration, the samples were placed in the GC-MS. Each sample had a running time of 50 min. The mass spectra of all major compounds were compared with spectra in the MS library, based on the peak detection output.

4.3. Results

GC-MS outputs are illustrated and tabulated below. No major peaks were detected in the outputs from either *Acacia* spp. gum or from analyses of faeces produced after the animals had been fed on *Acacia* gum, indicating an absence of secondary compounds in any detectable quantities. Similarly, no major peaks were detected in the outputs from GC-MS analyses of faecal samples from mouse lemurs that had been fed on *Commiphora* gum. This was not the case, however, for the analyses of gum from *Commiphora* spp., in which several compounds were detected with retention times varying between 18.842 for α-copaene to 20.849 for Naphthalene (see Figure 4.1).

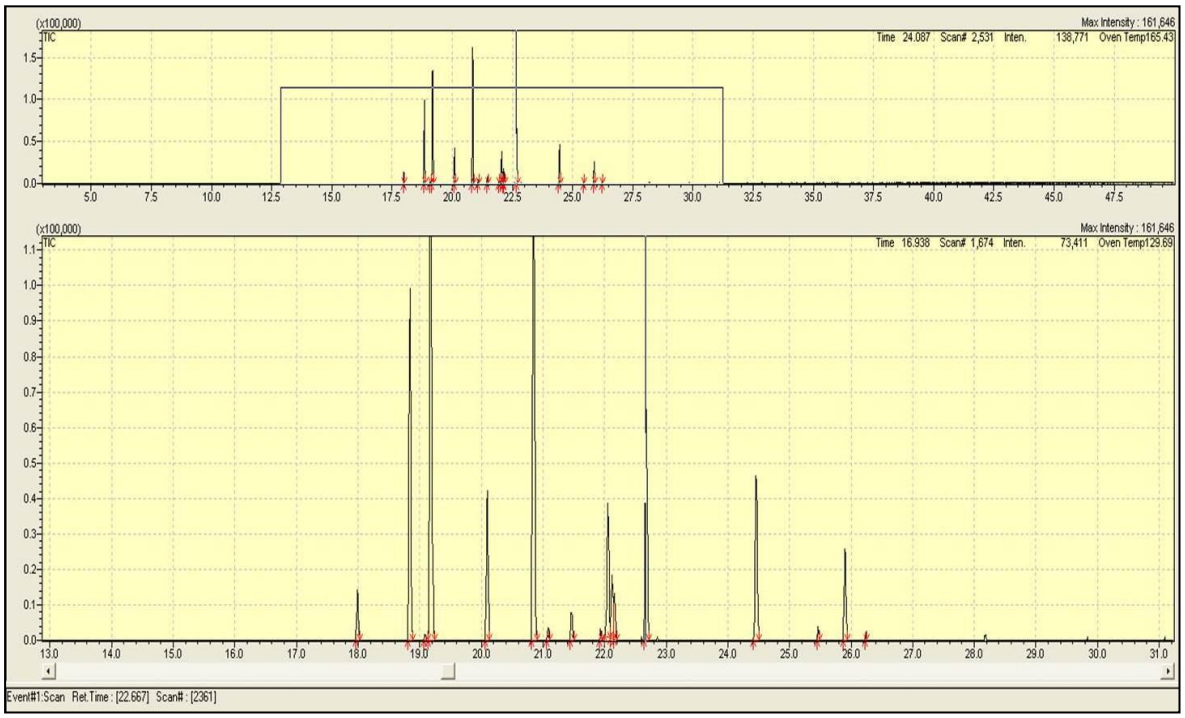

Figure 4.1. Mass spectra of compounds indicating peaks detected and retention time using GC-MS.

Table 4.1. Retention times, areas and heights of peaks detected using a GC-MS

Retention Time	Area	Area %	Height	Height %	Compound Name
18.842	208970	13.86	99041	14.58	Copaene <alpha>
19.180	333584	22.13	134093	19.74	1.6. Cyclodecadiene
20.849	363767	24.13	161646	23.81	Naphthalene
22.677	141958	9.42	65098	9.58	1.6. Cyclodecadiene

4.4. Discussion

Investigations into the influence of secondary compounds on primate dietary selectivity have focussed mostly on herbivorous and insectivorous species, with few previous studies (e.g. Wrangham and Waterman 1981) on gummivorous primates; there is some evidence that gum selection by monkeys was based primarily on the presence of tannins and phenolics; gums containing these compounds were avoided or consumed in small amounts.

The secondary compounds detected in the *Commiphora* sp. could play a role in the plant's natural defence mechanisms, or in its primary metabolism, or even both (Seigler and Price 1976). The compounds I detected (Table 4.1) all share the property of deterring insects. Naphthalene, in particular, is considered toxic, and one of its commercial uses is in the production of moth repellents. It occurs naturally in petroleum and coal, and is produced when these fossil fuels are burned. Exposure to naphthalene may have severe effects on humans, causing conditions ranging from haemolytic anaemia to diarrhoea and discolouration of the skin (the skin turns yellow). Laboratory tests on animals found that some subjects exposed to naphthalene vapour continuously over a period of 2 years developed nose tumours. When they were fed food mixed with naphthalene, however, the only effect

observed was a decrease in body weight. Based on these studies, naphthalene is considered a potential carcinogen for humans (U.S Department of Health and Human Services 2005).

The compound α-copaene, a tricyclic sesquiterpene found in small quantities in some plants, has been reported to be an effective sex attractant for the insect pest *Ceratitis capitata*, the Mediterranean fruit fly (http://en.wikipedia.org/wiki/Copaene), allowing for trapping and control of the insect. Germacrene occurs in a number of plant species, and is also known for its insecticidal and antimicrobial characteristics (http://en.wikipedia.org/wiki/Germacrene).

In ruminant mammals, moderate concentrations of condensed tannins aid in the increased absorption of amino acids in the lower gut through prolonging the microbial degradation of protein (Acamovic and Brooker 2005). This suggest that gummivores with simple stomachs, like *M. griseorufus*, may use secondary compounds to extend the retention times of the liquid and particulate phases of their digesta in the gastro-intestinal tract, to maximize the breakdown of proteins and the absorption of amino acids. Most secondary compounds are transformed in the liver into more digestible compounds, and if they are hydrophilic in nature, they are simply excreted in the urine (Acamovic and Brooker 2005). Hence, many animals do not actively avoid secondary compounds as long as they do not exceed certain levels. Iason and Villalba (2006) hypothesized that avoidance or reduction of consumption of secondary compounds must have co-evolved with an animal's ability to tolerate physiologically the ingestion and digestion of such compounds.

Despite the fact that *Galago moholi* has both a capacious caecum and an ansa coli within which to ferment gum, while *Microcebus griseorufus* has only a large caecum, the two species show similarly long retention times when they are fed gum. My study showed that the gums consumed by *G. moholi* (*Acacia karroo*) lacked appreciable quantities of secondary compounds, while the *Commiphora* spp. consumed by *M. griseorufus* contained several

compounds well known for their role in insect deterrence or attraction. In a similar manner to that in which secondary compounds serve to retard digestion and prolong gut retention of digesta in ruminant mammals, I propose that the secondary compounds found in *Commiphora* may serve to retard digestion and prolong gut retention of gum in *Microcebus*, offering a possible explanation for the similarity in digestive efficiency observed in *Galago* and *Microcebus*.

CHAPTER 5: HYPERVARIABILITY AND THE DISTRIBUTION PATTERNS OF GUMMIVOROUS PRIMATES

5.1. Introduction

Gummivory has been proposed as a food procurement strategy for animals living in hypervariable and unpredictable environments (Génin 2008), which are associated with unpredictable patterns of fruiting and flowering (Dewar and Richard 2007), and hence with strong fluctuations in food availability. In this chapter I investigate the distribution patterns of *Galago moholi* and *Microcebus griseorufus* in terms of environmental parameters.

5.1.1. Environmental hypervariability and El Niño/La Niña oscillations

Hypervariable environmental regions experience marked inter-annual and intra-annual variations in rainfall and temperature that may or may not be linked to El Niño oscillations (Kripalani and Kulkarni 1997). Although El Niño/La Niña events are not the sole source of environmental unpredictability, the regions influenced by these phenomena have often been used as model unpredictable regions. There are numerous definitions of the El Niño/La Niña phenonmenon (Trenberth 1997), but the most consistent is provided by Glantz (1996), and describes El Niño as a "name given to the occasional return of unusually warm water in the normally cold water [upwelling] region along the Peruvian coast, disrupting local fish and bird populations" and/or "used interchangeably with ENSO (El Niño–Southern Oscillation) which describes the basinwide changes in air–sea interaction in the equatorial Pacific region". For each El Niño event, certain anomalies can be observed and these vary according to the onset and the region affected (Figure 5.1). Southern Africa and regions of the western Pacific are especially prone to drought, which in some years can lead to increased precipitation and in others, marked rainfall decline (Kovats *et al.* 1999; Thompson *et al.* 2003). The events vary in strength (classified as weak, strong, or very strong; Glantz 1996) as

well as in frequency, duration, intensity and time of onset (Kovats *et al.* 1999). La Niña events occur after particularly strong El Niño events, generally reversing the climatic patterns (Figure 5.2).

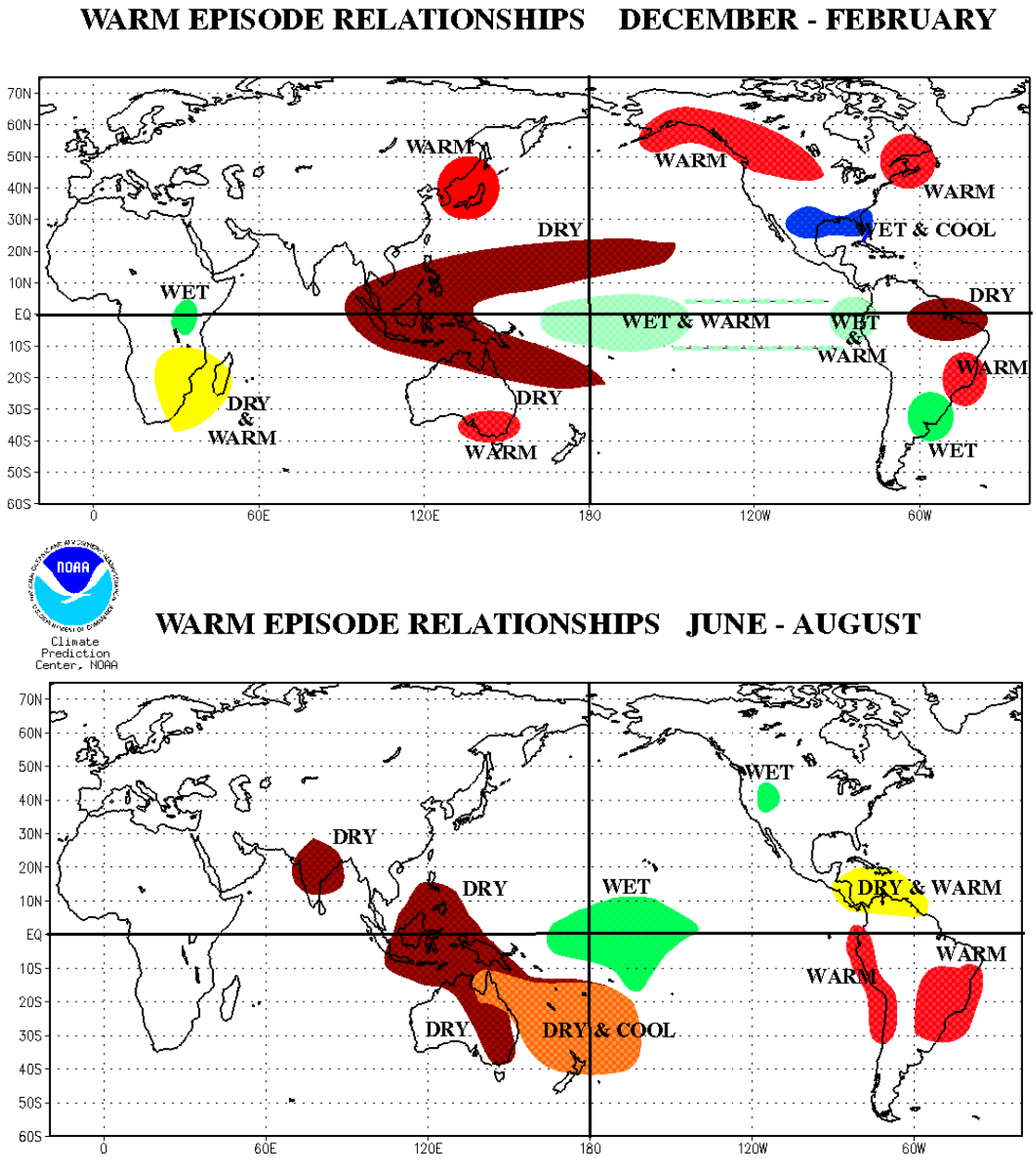

Figure 5.1. Anomalies associated with El Niño events (Kovats *et al.* 1999).

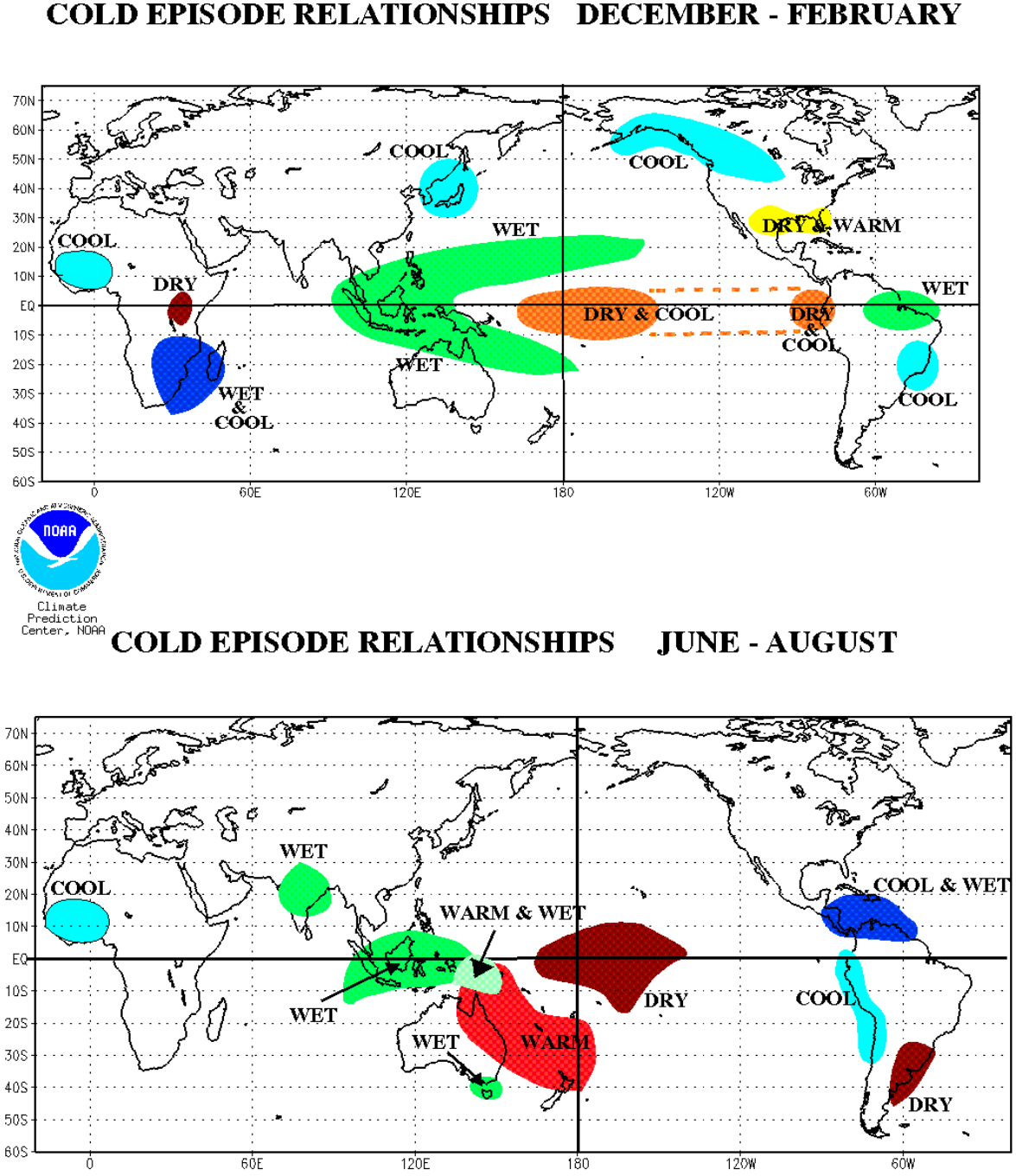

Figure 5.2. Anomalies associated with La Niña events globally (Kovats *et al*. 1999).

El Niño events occur every 2 to 7 years and can last from 7 to 22 months (Trenberth 1997; Kovats *et al.* 1999) (Table 5.1).

Table 5.1. Listings of El Niño and La Niña events after 1950 (data from Trenberth 1997; Kovats *et al.* 1999).

El Niño events		La Niña events	
Start	End	Start	End
Aug 1951	Feb 1952	Mar 1950	Feb 1951
Mar 1953	Nov 1953	Jun 1954	Mar 1956
Apr 1957	Jan 1958	May 1956	Nov 1956
Jun 1963	Feb 1964	May 1964	Jan 1965
May 1965	Jun 1966	Jul 1970	Jan 1972
Sep 1968	Mar 1970	Jun 1973	Jun 1974
Apr 1972	Mar 1973	Sep 1974	Apr 1976
Aug 1976	Mar 1977	Sep 1984	Jun 1985
Jul 1977	Jan 1978	May 1988	Jun 1989
Oct 1979	Apr 1980	Sep 1995	Mar 1996
Apr 1982	Jul 1983		
Aug 1986	Feb 1988		
Mar 1991	Jul 1992		
Feb 1993	Sep 1993		
Jun 1994	Mar 1995		

* Before 1950, El Niño events varying in intensity and duration were recorded for 1899-1900, 1902-1903, 1905-1906, 1913-1915, 1918-1920, 1923-1924, 1925-1926, 1930-1931, 1932-1933, 1939-1940, 1940-1941, 1941-1942 and 1946-1947.

5.1.2. Measuring environmental hypervariability

Various methods have been proposed to assess inter- and intra-annual variability in rainfall, such as the method used by Dewar and Wallis (1999) using cluster analyses (Figure 5.3.). In this study, I used the method of Colwell (1974) who designed three indexes to

measure intra-annual variability (constancy), inter-annual variability (contingency) and overall "predictability" (sum of constancy and contingency). Dewar and Richard (2007) based their study of the effect of rainfall hypervariability (low predictability index) on life history on Colwell's predictability index, using Madagascar as an example of a hypervariable region. Here I used the same predictability index to predict the occurrence of exudativory, as a test of the proposal of Génin *et al.* (2010) that exudate feeding is typical of hypervariable regions like Madagascar.

Hypervariability is not limited to unpredictability (high inter-annual variability) but also involves high levels of seasonality. Seasonal changes in resource abundance may be extreme but are predictable, and life histories can be adapted to accommodate recurrent environmental conditions. Unpredictability, on the other hand, has little effect on aseasonal animals, but has drastic effects on seasonal animals, as it disrupts predictable patterns of resource availability. For example, typical unpredictable regions like southern Madagascar are a challenge for small seasonal animals, because the onset and the offset of the rainy season are extremely variable.

Unpredictable regions and their associated irregular periods of drought are distributed throughout the world, including parts of Africa, Madagascar, Brazil, Australia and South-east Asia (red circles, Figure. 5.3) (Dewar and Wallis 1999). Génin *et al.* (2010) observed that the distribution of these regions matched the areas of occurrence of gummivorous mammals, including both primates and marsupials; in this chapter I test this suggestion by examining the habitat characteristics of my two study species.

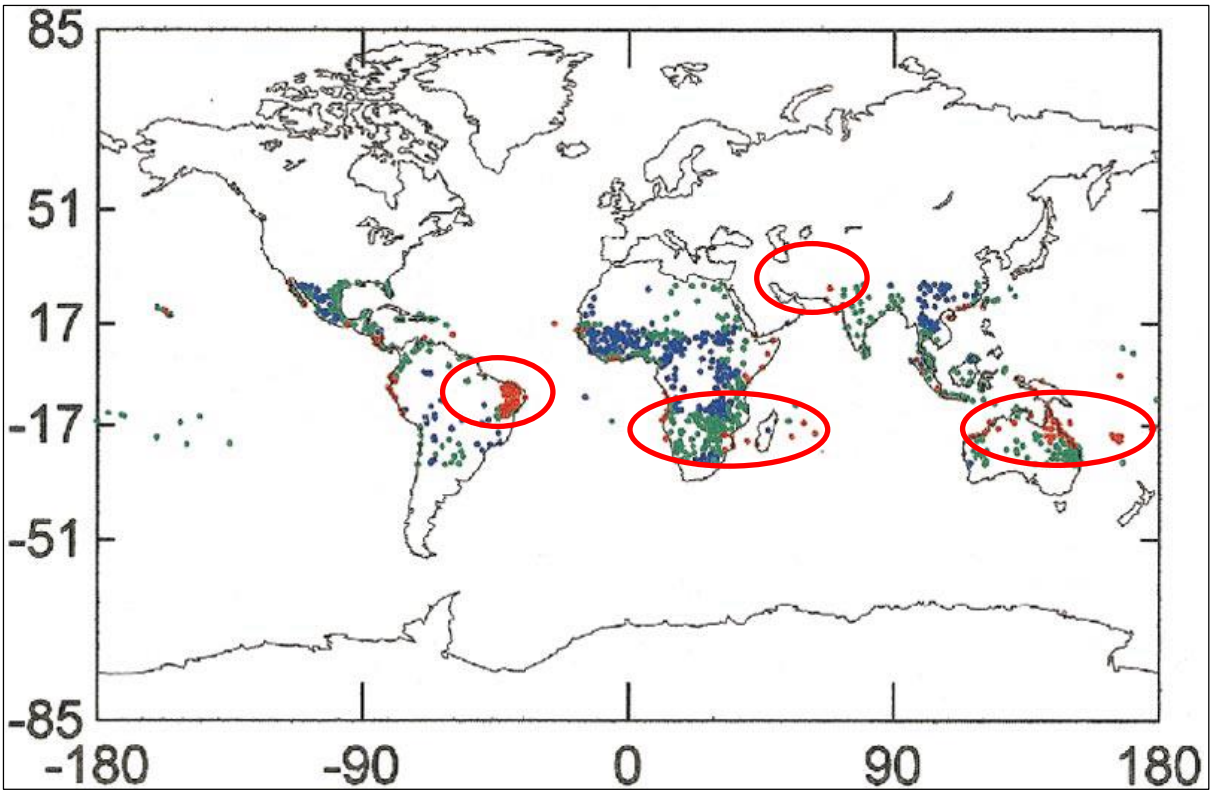

Figure 5.3. Global variation in the QU10 (cluster analysis using a 0.1 quantile) of Dewar and Wallis (1999). Red dots are the stations from the most variable regions, green from moderately variable regions, and blue from the least variable stations.

Africa has distinct zones of rainfall variability that range from low variability in the central and interior continental areas to high variability along the coasts (Dewar and Wallis 1999). South Africa has a small unpredictable region subject to El Niño oscillations, the extreme north-east of the country (Figure 5.3), and this is where the three bushbaby species native to South Africa occur. In particular, the lesser galago, *Galago moholi*, reaches the southernmost limit of its distribution in the drier part of this region of South Africa (Butynski *et al.* 2013).

Most of the island of Madagascar experiences hypervariable climatic conditions, particularly the south, the west and the north (Dewar and Richard 2007). The reddish-grey

mouse lemur (*Microcebus griseorufus*) is found exclusively in the driest and the most unpredictable part of the island, the southern xerophytic domain (Génin 2008).

5.1.3. Environmental variability and vegetation

Primates probably evolved in forests (continuous stands of trees at least 10 m tall with interlocking crowns, White 1983), but have also colonized more open habitats (e.g. papionids and *Erythrocebus patas*; Kingdon *et al.* 2008). This habitat shift is likely to have been an effect of forest regression in the Neogene, associated with the evolution of savannas (grasslands with dispersed trees, White 1983) and savanna woodlands (open canopies and developed grassy understories, White 1983), as a result of severe drying periods associated with global cooling. The specialized strepsirhine gummivores, *Phaner* and *Euoticus*, are found in forests (Andrainarivo *et al.* 2008; Bearder 2008), but the smaller, less specialized gummivorous species like *Galago* and *Microcebus* are found in drier, more open habitats including savannas and savanna woodlands, and xerophytic thicket.

The type of vegetation found in a given area is strongly influenced by climate and topography. Forests are expected to occur in regions of high rainfall (> 500 mm per year) under oceanic conditions or at mid-altitude. Grasslands (< 10% of woody vegetation in ground cover, White 1983) develop generally in seasonal regions where the rainfall is below 400 mm, often at high altitude (Osborne 2008). Dry, hypervariable regions are typically associated with xerophytic thicket and dry deciduous forest, and occur along the east coast of Africa, from Tanzania south to the Eastern Cape in South Africa, and in southern and south-western Madagascar. Floras with similar xerophytic adaptations, e.g. bottle-shaped trees, are found in Australia, north-eastern Brazil and on the island of Socotra. Africa and Madagascar also show some stark flora differences. In general, Madagascar is considerably woodier than continental Africa, and in particular, lacks natural savannas and grasslands (Carlquist 1974).

As a result, all lemurs are associated with forest or thicket, with exception of a single wetland specialist (*Hapalemur alaotrensis*) (Mittermeier *et al.* 2010). By contrast, Africa has several savanna-adapted primate species (Butynski *et al.* 2013). For instance, South Africa, largely dominated by open habitats, is home to four primate taxa adapted to savanna and thicket (*Papio ursinus*, *Chlorocebus aethiops*, *Galago moholi* and *Otolemur crassicaudatus*), and only two forest species (*Cercopithecus albogularis* and *Galagoides granti*) (Butynski *et al.* 2013; F. Génin pers. comm. for *Galagoides granti*).

The distribution of the southern lesser galago, *Galago moholi*, roughly matches the distribution of Miombo woodlands (at latitudes between 15º and 24º and altitudes > 600 m a.s.l.), characterized by species of *Brachystegia, Julbernadia, Isoberlinia* (all members of Fabaceae, and all potential gum trees) and *Uapaca* (Euphorbiaceae). The species reaches the southern limit of its distribution in South Africa and Botswana, where it is also found in dry savanna woodlands and riverine *Acacia* woodland in the driest areas (Skinner and Chimimba 2005). By contrast, the reddish-grey mouse lemur *Microcebus griseorufus* is limited to the southern xerophytic thicket and dry forest of Madagascar, dominated by *Alluaudia*, *Didierea* (Didiereaceae) and coraliform *Euphorbia* species (Euphorbiaceae) (Lowry *et al.* 1997).

5.2. Materials and methods

I began by plotting the distributions of my focal taxa in relation to vegetation cover. Once this had been accomplished, I assessed rainfall predictability within each of these species' ranges using the predictability indexes of Colwell (1974) and ArcGIS. I obtained climatic data from the South African Weather Service (SAWS) and WorldClim.org. The SAWS data included mean monthly maximum and minimum temperatures (°C) as well as monthly rainfall from 27 stations within the country, with 3 stations representing each of the 9 provinces. The data obtained from WorldClim.org included monthly precipitation (mm) for

the period 1950-2000. The data were global but for the purposes of this study I focussed on the Afrotropics, and mapping involved only continental Africa and the island of Madagascar. The data illustrating the distribution patterns of the subject species were obtained from the IUCN (pers. comm.).

Three predictability indexes were calculated for the South African stations according to the equation: $\boldsymbol{P = M + C}$, where $\boldsymbol{P}$ represents predictability, $\boldsymbol{M}$ is a measure of contingency (or inter-annual variation), and $\boldsymbol{C}$ is a measure of constancy (or as intra-annual variation). Additional index values for stations from mainland Africa and Madagascar were taken from Dewar and Richard (2007). All indexes were databased, categorised in terms of degree of predictability (low, moderate, high), and plotted along with the distributions of *G. moholi* and *M. griseorufus*.

5.3. Results

The distribution of *G. moholi* includes a mosaic of vegetation types that range from dry savannas to closed and open grassland areas and semi-deciduous forests, and extends from the east to the west of southern Africa (Figure 5.4). *M. griseorufus*, on the other hand, occupies a narrower range restricted to the south of Madagascar, with preferred regions characterized by xerophytic thicket and dry forest (forest-shrubland: Figure 5.5).

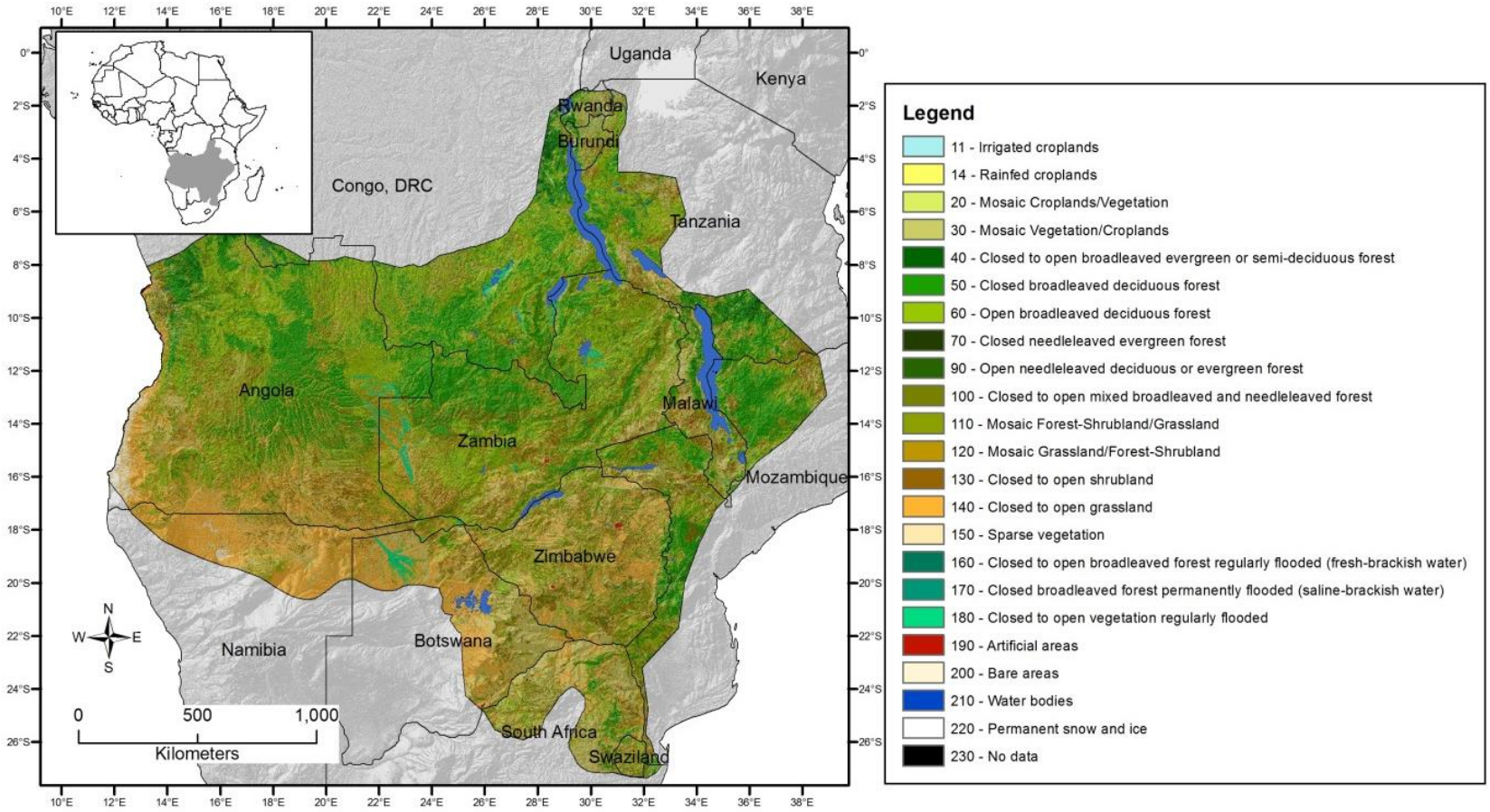

Figure 5.4. ArcGIS map of southern Africa illustrating the distribution of *Galago moholi* in relation to vegetation

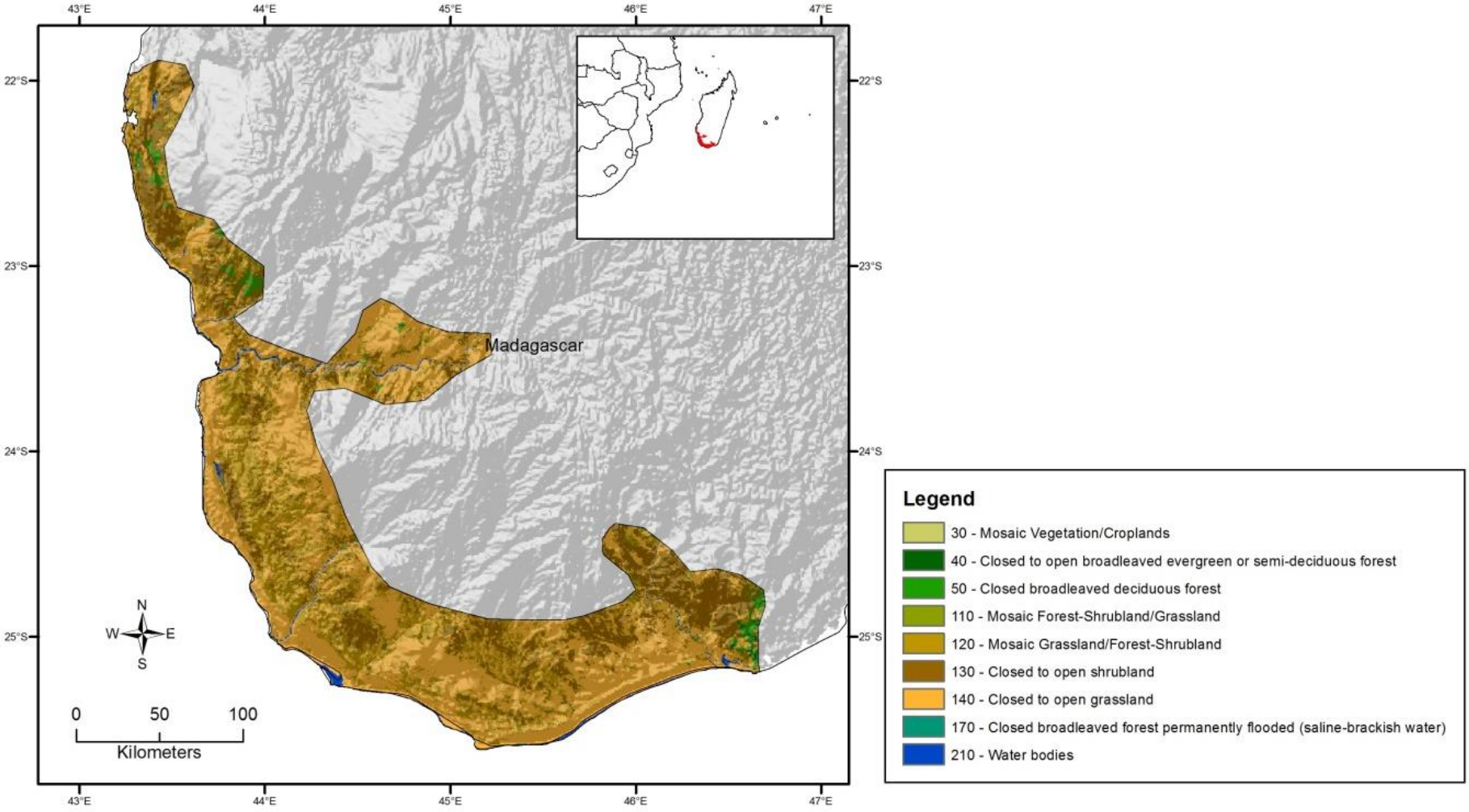

Figure 5.5. ArcGIS map of southern Madagascar illustrating the distribution of *Microcebus griseorufus* in relation to vegetation

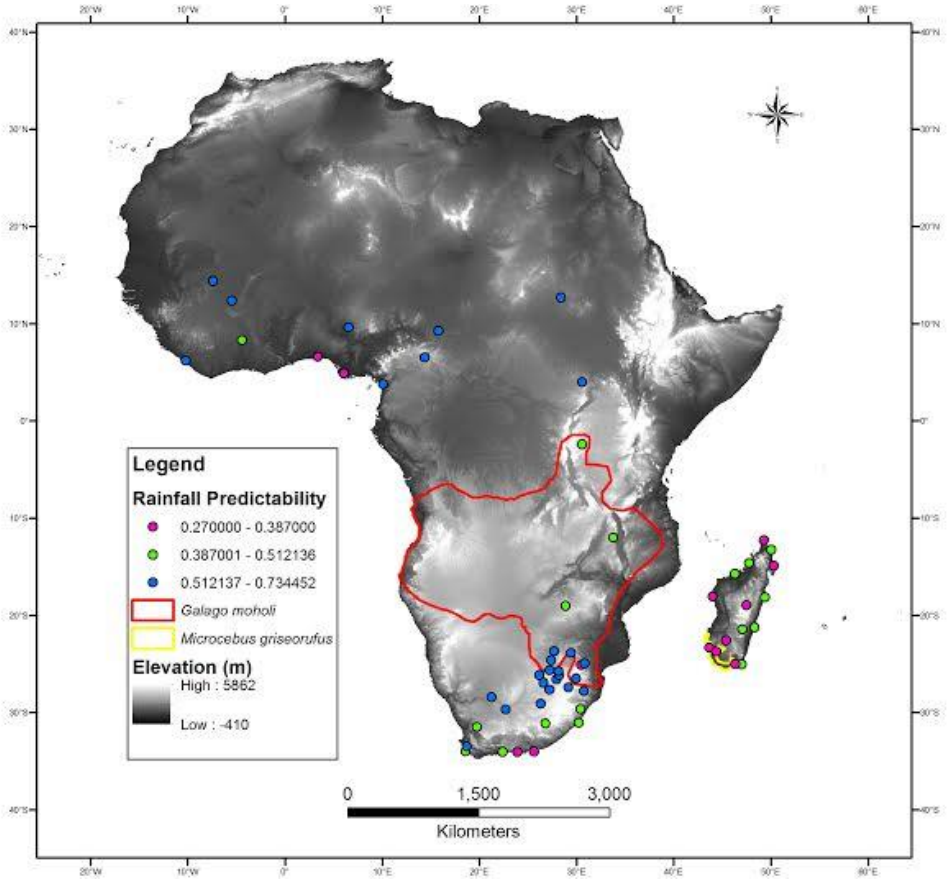

Figure 5.6. Map of Africa (including Madagascar) illustrating the distribution of *Galago moholi* and *Microcebus griseorufus* in relation to rainfall predictability indexes.

The predictability indexes were scattered across mainland Africa and Madagascar illustrating a variety of levels of predictability. The northern and central regions of South Africa are more predictable in terms of rainfall than regions closer to and along the south coast. Madagascar showed a blend of moderate to low variability across the country, with the stations distributed along the east coast (rain forest) and north of Mahajanga experiencing moderately predictable rainfall (Figure 5.6). The regions coinciding with the range of *M. griseorufus* are all areas of low predictability (pink dots).

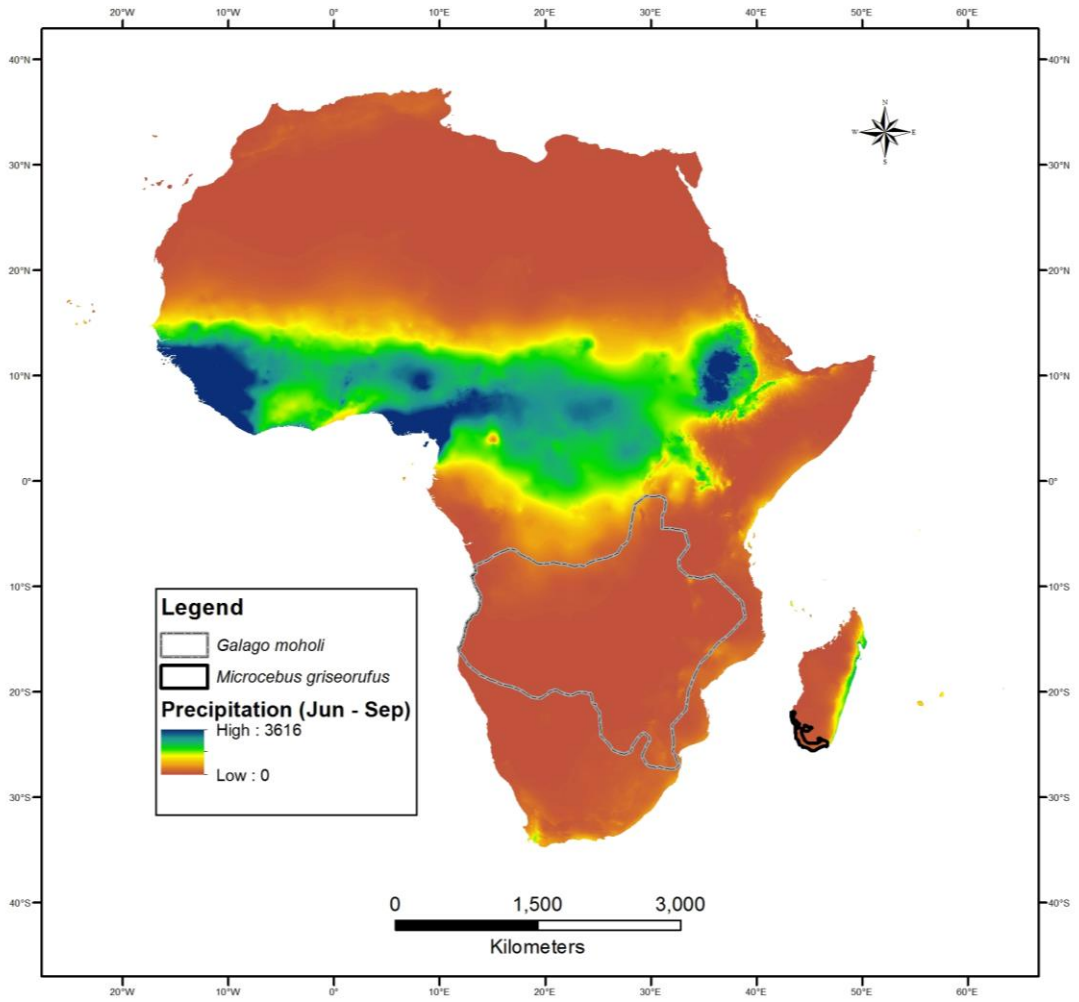

Figure 5.7. Map of Africa (including Madagascar) illustrating the distribution of *Galago moholi* and *Microcebus griseorufus* in relation to rainfall between June and September (data collected from 1950 to 2000).

The pattern of precipitation in Africa and Madagascar during the months June – September is illustrated in Figure 5.7. While the major portions of both landmasses receive little to no rainfall during this period, the rain forest of central to western sub-Saharan Africa receives high levels of rain. The central and western regions of Madagascar experience severe drought at this time, and moderate amounts of rain fall along the east coast (Figure 5.7). Hence, both study taxa are subjected to droughts at least from June to September, and these conditions may persist for longer periods in dry years.

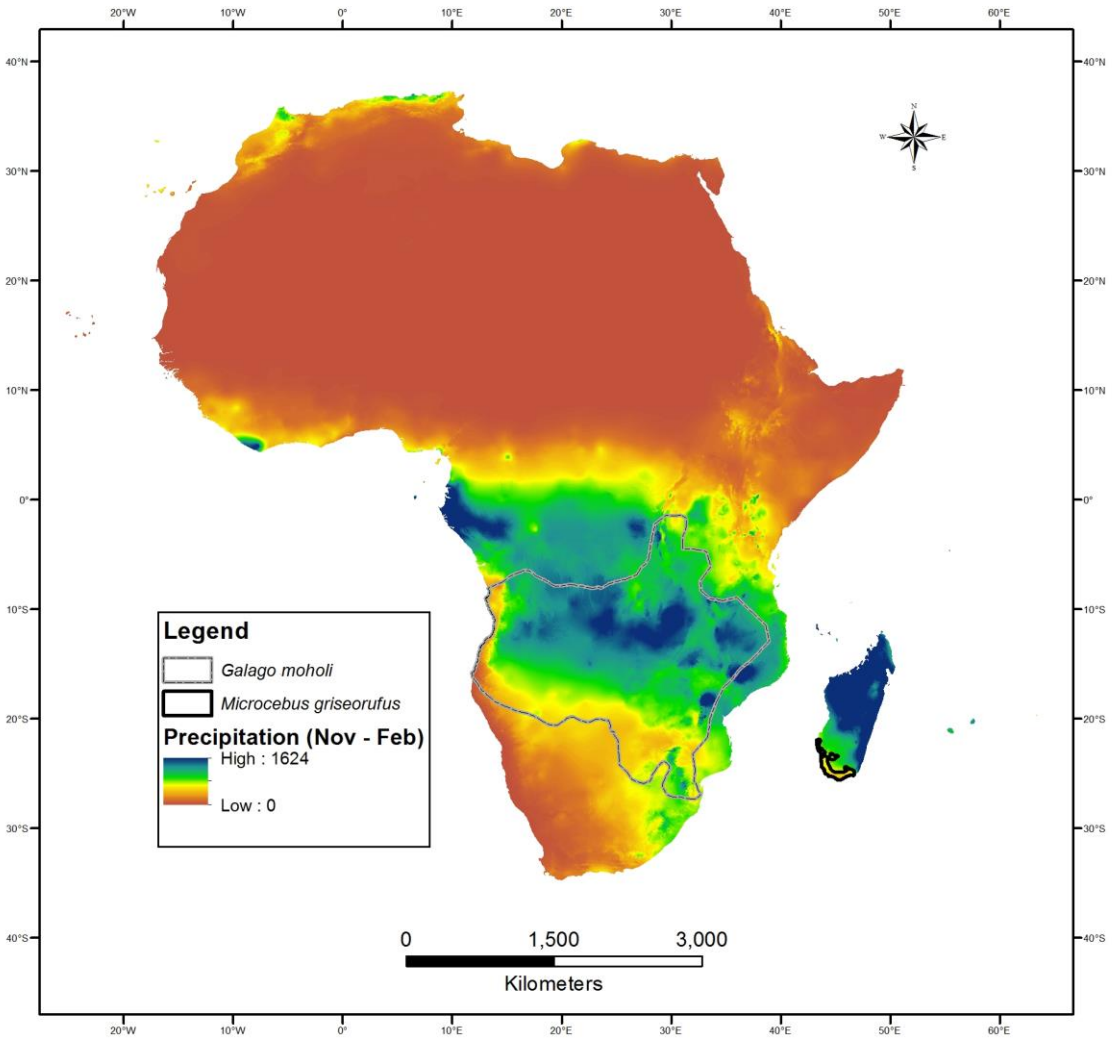

Figure 5.8. Map of Africa (including Madagascar) illustrating the distribution of *Galago moholi* and *Microcebus griseorufus* in relation to rainfall between November and February (data collected from 1950 to 2000).

During the months between November and February, the rainfall pattern changes dramatically (Figure 5.8). Most of central Africa – and most of the area occupied by *Galago moholi* – receives high to moderate levels of precipitation, while the east coast receives moderate amounts of rain. The south-western part of southern Africa (the Namib Desert, the Kalahari Desert, the Karoo and the Western Cape province of South Africa) experiences drought at this time. Southern Madagascar, and particularly the area occupied by *M. griseorufus*, continues to experience low levels of rainfall at this time, in comparison with regions to the north that receive considerable amounts of rain.

The influence of the ENSO is most prominent during the southern summer, December – February. El Niño events herald particularly dry periods with very low to no rainfall (Figure 5.1). Particularly harsh El Niño events may be followed by La Niña events that reverse the El Niño anomalies, bringing cooler, wetter conditions (Figure 5.2), although these may only occur 1 – 2 years after the El Niño event. The evolutionary significance of the ENSO is that it disrupts the predictable pattern of a cool, dry winter followed by a warm, wet summer. During an El Niño event, the expected summer rainfall is drastically reduced; instead of experiencing a humid summer with plenty of rain, the region is subjected to several more months of drought, followed by another dry winter. Resources become extremely scarce; fruiting and flowering of plants become unpredictable. Only animals that have access to resources that are continuously produced, like the gummivorous *G. moholi* and *M. griseorufus*, can survive such difficult periods. Hence, for both of my study taxa, gum becomes – not a fall-back food – but a staple during El Niño events; and gummivory is supported as a feeding strategy for animals that are periodically and unpredictably subjected to extreme climatic oscillations where food abundance and variety are suddenly reduced for extended periods.

5.4. Discussion

The southern lesser galago *Galago moholi* occurs predominantly in dry savannas, but extends its range into open to closed broadleaved evergreen or semi-deciduous forests. Its habitat preference can hence be characterized as a mosaic of dry forests and savanna woodland. The range of *G. moholi* extends from western Angola and northern Namibia all the way across the interior (Zambia, Botswana, Zimbabwe) to the eastern part of the continent, where the range peters out in western Mozambique, reaching its southern limit in northern South Africa and Swaziland (Figure. 5.4.). The range of *Microcebus griseorufus* is limited to the southern

Malagasy xerophytic thicket and dry forest (Figure. 5.5). The taxa thus share similar vegetative preferences, even though the plants that make up these continental and insular regions are taxonomically distinct.

With regard to patterns of precipitation in mainland Africa and Madagascar, the use of Colwell's predictability indexes confirmed that the reddish-grey mouse lemur (*Microcebus griseorufus*), the most gummivorous of the mouse lemurs (Génin *et al.* 2010), is indeed found in a hypervariable climatic region (Génin 2008). By contrast, the northern part of South Africa, where the lesser bushbaby (*G. moholi*) occurs, was found to be highly predictable, despite low annual rainfall. Most of the *G. moholi* range, however, extends into countries to the north of South Africa, for which little P index data were available; Dewar and Wallis (1999) described this region as moderately predictable (Figure 5.3), with the eastern part affected by El Niño oscillations. The western and southern parts of the range are dominated by sub-arid conditions, in which suitable habitats for *Galago moholi* may be restricted to riverine habitat. For instance, the Okavango delta receives limited, predictable rainfall, but seasonal flooding is caused by rivers that rise in more unpredictable eastern regions.

Under the influence of El Niño, if the phenomenon is strong enough, the areas occupied by both taxa may experience such drastically reduced rainfall during the anticipated rainy season from December to February as to incur substantial die-off among flowering and fruiting trees, and hence among the insects that visit them. It is therefore possible that ENSO events may have yielded the major selective pressures that influenced the evolution of gummivory in both taxa. On a global scale, the majority of gummivorous primates inhabit regions subject to ENSO events (Kovats *et al.* 1999; Génin *et al.* 2010). Gummivorous primates are distributed in Africa (*Galago* and *Euoticus*), South America (*Callithrix* and *Saguinus*), Asia (*Nyticebus*) and Madagascar (*Microcebus*, *Phaner* and *Allocebus*), and their

preferred habitats share several similarities: savanna grassland and woodland, dry tropical scrubland, and evergreen and semi-deciduous forests (Charles-Dominique and Petter 1980; Vaughn 1986; Skinner & Chimimba, 2005; Wiens *et al.* 2006; Raboy *et al.* 2008; Mittermeier *et al.* 2010). The exceptions in terms of habitat characteristics are the needle-clawed galagos, *Euoticus* spp. and *Galago matschiei*, both of which occur in rain forests. *Euoticus* has recently been reconstructed as a phylogenetically ancient lineage that dates back to the Eocene (Pozzi *et al.* in press), and clearly has a long, independent history.

Looking at gummivory from an evolutionary perspective, the distribution of the semi-specialist gummivore *Microcebus griseorufus* supports the hypothesis of Génin *et al.* (2010) that its diet evolved as an adaptive response to environmental hypervariability. The case of the lesser galagos is less clear, although several studies have suggested that the adaptive divergence and radiation of this group is relatively recent, probably Pleistocene in age (Masters 1998; Pozzi *et al.* in press). Federov *et al.* (2010) have traced El Niño-like climatic phenomena to the early Pliocene (5 – 3 Mya), indicating that these conditions would have been well established during the emergence of the *Galago* clade. Hence, both of my study species appear to have acquired their specific ecologies and diets under conditions of strong seasonality and high levels of environmental unpredictability, possibly linked to El Niño effects.

CHAPTER 6: SYNTHESIS AND CONCLUSIONS

The evolution of gummivory has received considerable attention in the last three decades, with attention focussed both on the physicochemical properties of exudates (Bearder and Martin 1980; Nash 1989; Génin *et al.* 2010) and on anatomical adaptations of gum-feeders (Caton *et al.* 1996; Caton *et al.* 2000; Vinyard *et al.* 2003; Burrows and Nash 2010). Gummivory appears to be phylogenetically restricted to primates and one family of marsupial mammals, the *Petaurus* spp. Few attempts have been made to reconstruct when this peculiar diet evolved, and it is unclear whether the anatomical traits associated with gummivory evolved in response to gum-feeding, or whether they were co-opted from adaptations that evolved to serve other functions, like grooming (Rosenberger 2010).

I reconstructed dietary evolution in strepsirhine primates using a phylogenetic approach based on four trees published by Springer *et al.* (2012). I categorized strepsirhines as specialized gummivores when they had dental and alimentary gum-feeding adaptations. The most likely ancestral diet for the lemuriforms included faunivory and granivory, as in modern *Daubentonia* (although the ancestor is unlikely to have shared the hyper-specialization of this living genus). The strepsirhine and lorisoid clades also had faunivorous ancestors. The ancestor of non-daubentoniid lemurs was reconstructed as frugivorous or folivorous, and the ancestor of the *Lepilemur*-Cheirogaleidae clade was reconstructed as frugivorous. The ancestor of the *Lepilemur-Phaner* sister-group was probably folivorous (Table 2.3), as predicted by Masters *et al.* (in press), suggesting that the cheirogaleids were pre-adapted to the digestion of gum by dint of their folivorous ancestor. Bushbabies may have been similarly pre-adapted to the digestion of gums by the specialist adaptations necessary to breakdown insect cuticles, which are rich in chemical complexes requiring digestion by commensal bacterial flora.

In my study I aimed to shed light on the evolution of gummivory in strepsirhines by comparing two distantly related taxa, *Galago moholi* and *Microcebus griseorufus*, by evaluating their digestive efficiencies in the light of their different alimentary anatomies. Gums are generally considered to be nutritionally deficient. Many strepsirrhines use caeco-ansal fermentation; i.e. in addition to a caecum, the animals have a U-shaped ansa coli in which the fermentation of foods that are difficult to digest [like gum that is composed of β-linked polysaccharides (Nash 1986)] takes place (Hill and Rewell 1948). An ansa coli does not appear to be present in dwarf or mouse lemurs (Caton *et al.* 2000). This suggests either that gum will not be digested efficiently by cheirogaleids, or that cheirogaleids have some additional means of enhancing digestive efficiency. My experiments suggest that gum is retained in the digestive tract of mouse lemurs for a similar period of time to that seen in lesser galagos, despite their lack of an ansa coli.

Gums consumed by both species were analyzed for the presence of secondary compounds, using a GC-MS and only the *Commiphora* spp. gum yielded results indicating the presence of secondary compounds. The faecal samples of *M. griseorufus* had no residues of such compounds, indicating that the animals have either a detoxifying mechanism by which the compounds are converted into digestible products, or that the compound residues are excreted in the urine, which was not measured in this study. Because secondary compounds can delay the passage of digesta through the alimentary tract, it is possible that the secondary compounds found in *Commiphora* spp. serve to retard digestion and prolong retention times of gum in *Microcebus*, explaining the similarity in digestive efficiency seen in *M. griseorufus* and *G. moholi*.

Finally, I compared the habitats of the two taxa on the basis of vegetation types and rainfall variability over a period of 50 years. I calculated predictability indexes and mapped

them geographically. The environmental parameters of the two species' ranges shared some similarities, but differed in the degree of rainfall unpredictability that they experienced. *G. moholi* occupied a wider range of vegetation types (including savanna and semi-deciduous forests), while *M. griseorufus* occurred only in regions dominated by xerophytic thicket. Both species' ranges are subjected to periodic droughts which are unpredictably intensified by ENSO events. The vegetation types occupied by *G. moholi* and *M. griseorufus* share similarities with those of other gummivorous primates globally, as well as those inhabited by most of the sugar-gliders of Australia; i.e. drier open forest and woodland. Few *Petaurus* spp. occupy rainforests and areas with very high rainfall (Menkhorst *et al.* 1988; Rowston *et al.* 2002; Rowston and Catterall 2004). Regions in central and north coastal New South Wales are characterized by unpredictable climates which influence food abundance (Quin 1995).

My results indicate that the evolution of gummivory in *Microcebus* and *Galago* in Madagascar and Southern Africa, respectively, occurred convergently during the Plio-Pleistocene, although gummivory may have evolved in lineages such as *Euoticus* and *Phaner* as far back as the Early Oligocene, in relatively larger animals (500 g). Both the Pliocene and Oligocene were dry and relatively cold periods, especially in Africa, where they were marked by more open, arid conditions (deMenocal 1995). They may also have witnessed the emergence of strongly unpredictable environmental conditions caused by the establishment of tropical cyclones (Federov *et al.* 2010). The convergent evolution of hyper-specializations associated obligate gummivory in *Phaner* and *Euoticus* is the subject of another study (D. Forbanka, pers. comm.).

I plan to extend my study to expand its scope to include both more study species and more sources of information (anatomical, physiological and behavioural), combining research on museum specimens with field studies of gum-feeding. This will be framed by a broader

collaborative study of the origin of primates, and strepsirhines in particular, evaluating existing and new hypotheses relating to dietary evolution and providing a more holistic approach to the study of the origins of gummivory. Another interesting research direction is the co-evolution of gum-producers and gum-feeders. Small gummivores are always partially insectivorous and consume the same insects that infest the trees, which results in gum exudation. This hypothesis would be an extension of Sussman's hypothesis of diffuse co-evolution, integrating the evolution of exudativory.

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Appendix I: DIETARY RECONSTRUCTION OF THE CHEIROGALEIDAE-LEPILEMURIDAE CLADE AND LORISIFORM CLADE

(*FR: frugivory; EX: exudativory; FA: faunivory; FO: folivory)

Node	FR	EX	FA	FO
Primate ancestor	0.070944	0.113726218	0.79967997	0.015650228
Haplorhines ancestor	0.180776	0.188400438	0.59220418	0.038619086
Strepsirhines ancestor	0.14655	0.149442333	0.64258424	0.061423041
Lemuriformes-Chyromiiformes ancestor	0.628618	0.051879622	0.09727658	0.222226188
Lemuriformes ancestor	0.536475	0.000765828	0.00051721	0.462242423
Cheirogaleidae-Lepilemuridae-Indridae ancestor	0.125248	0.001017956	0.00031455	0.873419532
Cheirogaleidae-Lepilemuridae ancestor	0.563815	0.069523875	0.00912652	0.357534742
Cheirogaleidae ancestor	0.626759	0.270306825	0.08755517	0.015378499
Allocebus-Mirza-Microcebus ancestor	0.114708	0.692367966	0.18856024	0.00436332
Mirza-Microcebus ancestor	0.313307	0.180316743	0.50323022	0.003145935
Microcebus ancestor	0.341612	0.154273551	0.50266266	0.001451285
Other *Microcebus* ancestor	0.411337	0.000172901	0.58845561	0.000034324
Microcebus griseorufus-murinus ancestor	0.023009	0.952208989	0.0235862	0.001195871
Mirza ancestor	0.332408	0.333792789	0.33379279	6.53E-06
Cheirogaleus ancestor	0.997406	0.000864511	0.00086451	0.000864511
Lepilemur-Phaner ancestor	0.084822	0.026669249	0.00454548	0.883963692
Lepilemur ancestor	0.000413	0.000000002	2E-09	0.999586829
Indridae ancestor	0.003115	0.000003433	3.433E-06	0.996878376
Lemuridae ancestor	0.944788	0.003261031	0.00326103	0.048690337
Lorisiformes ancestor	0.000601	0.195045456	0.80380471	0.000548504
Galagidae ancestor	0.004849	0.456866043	0.53358287	0.004702441
Node *G. thomasi* ancestor	0.0003	0.072888756	0.92678685	0.000024273
Node *G. demidof* ancestor	0.030994	0.443982543	0.52351946	0.001504048
Otolemur-Sciurocheirus-Galago-clade *G. granti* ancestor	0.581751	0.382130439	0.02584659	0.01027224
Otolemur-Sciurocheirus-clade *G. granti* ancestor	0.951152	0.021122195	0.02664022	0.001085698
Otolemur-Sciurocheirus ancestor	0.987132	0.003572925	0.00891559	0.000379578
Otolemur ancestor	0.961777	0.034468541	0.00187716	0.001877162
O. crassicaudatus-garneti ancestor	0.490172	0.49017227	0.00982773	0.00982773
Sciurocheirus ancestor	0.498794	0.000012531	0.50119058	3.141E-06
clade *G. granti* ancestor	0.319401	0.339907257	0.33990726	0.000784238
G.granti-zanzibaricus ancestor	0.329425	0.335231623	0.33523162	0.00011219
Galago ancestor	0.00023	0.999309965	0.00023	0.00023
Lorisidae Ancestor	0.002974	0.206799877	0.78782423	0.002401863
Asian loris ancestor	0.003425	0.562025059	0.43112465	0.003425137
Nycticebus ancestor	0.000714	0.997858003	0.00071401	0.000714012
Loris ancestor	0	5.228E-06	0.99999477	0
African loris ancestor	0.00702	0.110630942	0.88039404	0.001954612
Arctocebus ancestor	0	2.0218E-05	0.99997978	0

APPENDIX II: DATA FROM GUM AND BANANA FEEDING EXPERIMENTS ON *G. MOHOLI* AND *M. GRISEORUFUS*

				Gum Feeding (06-10/09/12)										
				Night 1			Night 2			Night 3				
Galago moholi	**Age (years)**	**Sex**	**Weight (g)**	**GBC (g)**	**GAC (g)**	**GE (g)**	**GBC (g)**	**GAC (g)**	**GE (g)**	**GBC (g)**	**GAC (g)**	**GE (g)**	**Gum Faeces collected after 48 hours (g)**	**Dried Gum Faeces (g)**
1	1	M	160	16.8	6.3	10.5	14.3	7.0	7.3	15.4	10.0	5.4	0.4	0.4
2	1	M	180	16.3	5.3	11.1	19.5	15.6	4.0	16.9	10.2	6.6	1.7	1.4
3	1	M	140	14.1	4.7	9.4	16.2	9.5	6.7	14.7	7.5	7.2	0.5	0.2
4	1	F	145	15.1	12.0	3.1	18.2	11.4	6.8	17.6	11.6	6.0	0.9	0.7
5	1	F	135	19.9	11.7	8.1	17.0	11.7	5.3	14.0	4.1	9.9	3.0	1.2
6	1	F	160	14.1	12.6	1.5	18.2	13.2	5.0	15.4	9.8	5.6	2.1	1.4

Note: Gum Before Consumption (GBC); Gum After Consumption (GAC); Gum Eaten (GE)

			Banana Feeding (11/09/12)					
Galago moholi	**Age (years)**	**Sex**	**Weight (g)**	**BBC (g)**	**BAC (g)**	**BE (g)**	**Banana Faeces (g)**	**Dried Banana Faeces (g)**
1	1	M	160	43.6	43.6	43.6	6.3	2.9
2	1	M	180	45.1	2.1	43.0	1.0	0.6
3	1	M	140	45.2	45.2	45.2	3.0	1.9
4	1	F	145	45.1	8.5	36.6	1.1	0.9
5	1	F	135	45.0	45.0	45.0	2.8	0.9
6	1	F	160	45.0	45.0	45.0	1.0	0.7

Note: Banana Before Consumption (BBC); Banana After Consumption (BAC); Banana Eaten (BE);

Banana Feeding																
Microcebus griseorufus	**Sex**	**Weight (g)**	**Night 1 (15/05/13)**					**Night 2 (16/05/13)**				**Night 3 (17/05/13)**				
			BBC (g)	**BAC (g)**	**BE (g)**	**FB (g)**	**FB (AD) (g)**	**BBC (g)**	**BAC (g)**	**FB (g)**	**FB (AD) (g)**	**BBC (g)**	**BAC (g)**	**BE (g)**	**FB (g)**	**FB (AD) (g)**
1	F	59	31.9	0.0	31.9	1.5	0.7	30.2	0.0	2.6	1.2	50.3	0.7	49.5	2.6	1.7
2	F	70	31.7	0.0	31.7	1.2	0.5	30.6	0.0	3.0	1.4	50.1	2.4	47.7	2.6	1.4
3	M	54	31.4	1.4	30.0	0.3	0.2	30.2	0.0	2.3	1.2	50.6	7.2	43.4	0.8	0.6
4	F	77	31.7	0.0	31.7	1.2	0.7	30.0	0.0	1.7	1.0	50.2	0.0	50.2	1.9	1.4

Note: Banana Before Consumption (BBC); Banana After Consumption (BAC); Banana Eaten (BE); Faeces Banana (FB); Faeces Banana After Drying (FB-AD)

Gum Feeding							
			Night 4 (18/05/13) - Night 7 (21/05/13)				
Microcebus griseorufus	**Sex**	**Weight (g)**	**GBC (g)**	**GAC (g)**	**GE (g)**	**FG (g)**	**FG (AD) (g)**
1	F	59	10.2	6.2	4.0	0.6	0.3
2	F	70	10.3	3.0	7.3	0.7	0.3
3	M	54	10.2	4.9	5.3	0.8	0.6
4	F	77	10.3	4.5	5.8	0.7	0.4

Note: Gum Before Consumption (GBC); Gum After Consumption (GAC); Gum Eaten (GE); Feaces Gum (FG); Feaces After Drying (FG-AD)