

RESEARCH ARTICLE

A Glance to the Past: Subfossils, Stable Isotopes, Seed Dispersal, and Lemur Species Loss in Southern Madagascar

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The Spiny Thicket Ecoregion (STE) of Southern and southwestern Madagascar was recently home to numerous giant lemurs and other “megafauna,” including pygmy hippopotamuses, giant tortoises, elephant birds, and large euplerid carnivores. Following the arrival of humans more than 2,000 years ago, dramatic extinctions occurred. Only one-third of the lemur species which earlier occupied the STE survive today; other taxa suffered even greater losses. We use stable isotope biogeochemistry to reconstruct past diets and habitat preferences of the recently extinct lemurs of the STE. We show that the extinct lemurs occupied a wide range of niches, often distinct from those filled by coeval non-primates. Many of the now-extinct lemurs regularly exploited habitats that were drier than the gallery forests in which the remaining lemurs of this ecoregion are most often protected and studied. Most fed predominantly on C3 plants and some were likely the main dispersers of the large seeds of native C3 trees; others included CAM and/or C4 plants in their diets. These new data suggest that the recent extinctions have likely had significant ecological ramifications for the communities and ecosystems of Southern and southwestern Madagascar. *Am. J. Primatol.* 73:25–37, 2011. © 2010 Wiley-Liss, Inc.

Key words: seed dispersal; stable isotope; subfossil lemur; Spiny Thicket Ecoregion

INTRODUCTION

If there is any tropical location on earth where one might be surprised to see nonhuman primates that place would be the “Spiny Thicket Ecoregion” (STE) of Southern and southwestern Madagascar [Burgess et al., 2004]. This “spiny desert” covers 44,300 km², extending from the Mangoky River on the west coast to the Anosyennes Mountain chain in the southeast. Owing to an extreme rain shadow effect, the STE dry season lasts up to 11 months of the year, and in the driest areas, annual rainfall may not exceed 350 mm. Its vegetation is diverse, and varies depending on water sources and the porosity and chemistry of the soils [DuPuy & Moat, 1996]. It includes grasslands, spiny bush, bushy scrub, and denser (but usually narrow) gallery or riparian forests bordering streams or rivers, few of which are perennial. The more open “spiny” forests are usually only 3–6 m in height, with rare emergent trees exceeding 10 m, creating a discontinuous canopy.

This ecoregion is renowned for its unusual flora with special adaptations to store water or minimize water loss. Plant endemism is higher here than anywhere else in Madagascar [Burgess et al., 2004]. The most dominant plants are succulents (Didieraceae, Crassulaceae, and Euphorbiaceae) that use the Crassulacean Acid Metabolism (CAM) photosynthetic pathway [Kluge et al., 1991, 1995, 2001;

Winter, 1979]. Additionally, there are patches of endemic or native C4 grasses [Bond et al., 2008; Bosser, 1969]. C3 trees also thrive in the STE; these too may have extreme adaptations to aridity, such as swollen trunks or roots for water storage, or reduced leaves. Whereas many of the CAM plants have tiny seeds that are adapted for wind dispersal, some C3 trees have large seeds that are adapted for dispersal via endozoochory.

Today, half a dozen lemur species belonging to four families inhabit the STE. Four of these, ranging in size from 60 g to slightly more than 3 kg, are common. In the recent past, there were eight additional lemur species, ranging in size from just over 10 kg (*Mesopropithecus globiceps* and *Pachylemur insignis*) to around 85 kg (*Megaladapis edwardsi*).

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These species belonged to Madagascar's famed "mega-fauna," known through their bony remains at "sub-fossil" (late Pleistocene and Holocene) sites throughout the island. The megafauna, which also included giant ratites, giant tortoises, and pygmy hippopotamuses, disappeared after humans arrived roughly around 2,000 years ago [Burney et al., 2004]. All Madagascar's extant lemur families (Cheirogaleidae, Lepilemuridae, Indriidae, Lemuridae, and Daubentoniidae) and extinct families (Megaladapidae, Palaeopropithecidae, and Archaeolemuridae) are represented in subfossil deposits throughout the STE.

Burney et al. [2003] have shown that pre-settlement megafaunal biomass was high in what were then open areas, likely reflecting an abundance of giant tortoises, pygmy hippopotamuses, and elephant birds. However, the remains of giant lemurs have been found alongside those of non-primates, and the extent to which primates were key elements of the various ecosystems of the extreme South and Southwest is unknown. Although extant species have mainly been studied in gallery forests (e.g. Beza Mahafaly Special Reserve Parcel I; Berenty), lemurs survive today in the STE outside the gallery forests, and giant lemurs may have done the same. Furthermore, whereas there is clear anatomical evidence that even the largest-bodied extinct lemurs were adept tree-climbers [Godfrey et al., 2006], none could have been strictly arboreal, even in gallery forests. At Beza Mahafaly, which has both gallery and spiny forest habitats, three of the four locally surviving lemur species (*Lemur catta*, ring-tailed lemur; *Propithecus verreauxi*, Verreaux's sifaka; and *Microcebus griseorufus*, grey-brown mouse lemur) regularly descend to the ground [Brockman et al., 2008; Youssouf Jacky & Rasoazanabary, 2008]. Only the sportive lemur, *Lepilemur leucopus*, is strictly arboreal.

Here, we use stable isotope biogeochemistry to explore lemur paleoecology in the STE. Specifically, we investigate whether the extinct lemurs of this region occupied niches likely not filled by sympatric species and how their loss may have affected plant communities. Stable isotope ratios can be expected to differ among animals belonging to different trophic groups, consuming plants using different photosynthetic pathways or living in different habitats. We suggest that, by carefully combining what have hitherto been rather separate fields—subfossil lemur paleobiology, isotope geochemistry, lemur ecology, and floristic surveys of Madagascar's habitats—we might be able to unravel traces of the still-reverberating effects of the lemur extinctions.

BACKGROUND: INTERPRETING ISOTOPIC SIGNALS

When an element has variable numbers of neutrons, the different phases are called "isotopes."

Many lighter elements, such as carbon and nitrogen, have isotopes that are stable and do not radioactively decay. A standardized "δ" notation is used to refer to the relative proportion of heavy-to-light isotopes, where $\delta = ((R_{\text{sample}}/R_{\text{standard}})-1)*1,000$, and $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. Values are measured in parts per thousand (‰). The standards for carbon and nitrogen, respectively, are Pee Dee Belemnite (a marine carbonate fossil) and air. The different number of neutrons in isotopes of the same element results in different masses, causing each isotope to behave slightly differently during chemical and physical reactions; these differences lead to relatively more or less heavy isotope in different materials. When relatively more of the heavy isotope is present, the "δ" value is higher; when relatively less is present, it is lower.

Carbon

Carbon isotope ratios have been used to differentiate C3 and C4 plants, or the relative contribution of these plant groups to animal diets. Carbon isotope ratios for C3 and C4 plants range from -21 to -35 ‰ (average ~ -28 ‰), and from -10 to -20 ‰ (average -14 ‰), respectively [O'Leary, 1988]. Plants utilizing the CAM photosynthetic pathway have intermediate $\delta^{13}\text{C}$ values, depending on the degree of succulence and water stress [Ehleringer & Monson, 1993]. Nonphotosynthetic plant tissues, such as fruits and flowers, tend to have $\delta^{13}\text{C}$ values 1–1.5‰ higher than leaves [Codron et al., 2005; Cernusak et al., 2009], but this pattern has not been observed in Madagascar's dry forests [Crowley et al., submitted; Dammhahn & Kappeler, 2009]. Collagen $\delta^{13}\text{C}$ values in herbivores are roughly 5‰ higher than those in consumed plants, and consumers that eat animal matter should have slightly higher $\delta^{13}\text{C}$ values than sympatric herbivores [Koch, 1998].

Average foliar $\delta^{13}\text{C}$ values for C3 and CAM plants from the gallery forest at Beza Mahafaly are -29.6 ± 2.6 ‰ and -15.3 ± 0.9 ‰, respectively, and their ranges do not overlap (from -37.3 to -23.5 ‰ for C3 plants, and from -17.0 to -13.2 ‰ for CAM plants) [Crowley et al., submitted]. Carbon isotope ratios have not been measured for any C4 plants from the Beza region, but their $\delta^{13}\text{C}$ values should be higher than those in CAM plants. Assuming a fractionation of ca. 5‰ between lemurs and plants [Crowley et al., submitted; Koch, 1998; Krueger & Sullivan, 1984], lemurs eating pure C3 plant diets in gallery forest habitat should on average have $\delta^{13}\text{C}$ values ca. -24.6 ‰, whereas lemurs incorporating some CAM or C4 foods into their diets should have more positive $\delta^{13}\text{C}$ values.

However, animals living in water-stressed environments would be expected to have higher $\delta^{13}\text{C}$ values than animals living in more mesic environments [Ehleringer, 1989; Farquhar et al., 1989]. Although

mean $\delta^{13}\text{C}$ values for CAM plants ($-14.7 \pm 1.4\text{‰}$ with a range of -17.4 to -11.4‰) from the spiny scrub west of the gallery forest at Beza Mahafaly are similar to those from the gallery forest [Crowley et al., submitted], C3 plants from the spiny scrub do, indeed, have average $\delta^{13}\text{C}$ values that are higher than those in the gallery forest ($-26.4 \pm 2.8\text{‰}$, with a range of -30.9 to -22.8‰). Thus, the difference in the mean carbon isotope ratios between C3 and CAM plants is reduced in the spiny scrub but still substantial. Even in the arid South of Madagascar, C3 plants have $\delta^{13}\text{C}$ values lower than ca. -23‰ . This is ecologically important because, in Madagascar's arid South (and likely elsewhere), it seems that all plant species that produce nutritious fruits and, therefore, likely depend on endozoochory for seed dispersal also use the C3 photosynthetic pathway [Crowley et al., submitted; Crowley, unpublished data]. Thus, carbon isotope ratios may provide a means to distinguish consumers of plants that depend on endozoochory from consumers of plants that use other dispersal methods. Modern lemurs with $\delta^{13}\text{C}$ values higher than -21.4‰ (i.e. -26.4‰ plus 5‰) likely incorporate some CAM or C4 foods into their diets.

Because of ^{13}C changes in atmospheric carbon dioxide owing to the burning of fossil fuels over the past 150 years, subfossil $\delta^{13}\text{C}$ values are approximately 1.2‰ higher than modern values [Chamberlain et al., 2005]. We, therefore, use a conservative cut-off of -20.2‰ ($\sim 1.2\text{‰}$ higher than the -21.4‰ used for modern animals in xeric environments) to differentiate subfossil species that must have consumed at least some CAM or C4 matter from those that need not have included such plants in their diets.

Nitrogen

In most terrestrial systems, plants obtain their nitrogen from soil nitrate and ammonium, and their $\delta^{15}\text{N}$ values are higher than air (which is $\sim 0\text{‰}$) [Muzuka, 1999; Schmidt & Stewart, 2003]. C3 plants with symbiotic nitrogen-fixing bacteria have $\delta^{15}\text{N}$ values close to 0‰ because the bacteria fix nitrogen directly from the atmosphere [e.g. Schmidt & Stewart, 2003]. Nitrogen isotope ratios are useful for inferring trophic relationships within a community, as herbivore collagen $\delta^{15}\text{N}$ values are $3\text{--}5\text{‰}$ higher than the plants they consume, and secondary consumers have collagen nitrogen isotope ratios that are $3\text{--}5\text{‰}$ higher than herbivores [DeNiro & Epstein, 1981; Fox-Dobbs et al., 2007]. It follows that $\delta^{15}\text{N}$ values in a consumer's tissues should increase with increased faunivory, and decrease with the consumption of plants that have symbiotic nitrogen-fixing bacteria [Ambrose, 1991; Schoeninger et al., 1997]. However, these general principles tend to break down in arid and coastal systems. Decreasing rainfall and increased salinity often correlate with increasing plant $\delta^{15}\text{N}$ values in non-N fixing plants [Ambrose,

1991; Heaton, 1987; Sealy et al., 1987; Swap et al., 2004; Vitousek et al., 1989]. Finally, nitrate-bearing sea spray can increase foliar $\delta^{15}\text{N}$ values [Heaton, 1987].

The results of moisture stress in animals are similar to those in plants. Mammals can be classified as "obligate drinkers" or "drought-tolerant" [Ambrose & DeNiro, 1986]. Obligate drinkers take in adequate amounts of water and do not recycle their urea to the same extent as drought-tolerant animals. Consequently, they excrete more ^{15}N and their tissues tend to be ^{15}N -depleted relative to drought-tolerant animals [Ambrose, 1991]. Severe nutritional stress can lead to animals catabolizing their own tissues, which also increases tissue $\delta^{15}\text{N}$ values [Hobson & Clark, 1992; Hobson et al., 1993; Voigt & Matt, 2004]. Thus, although elevated $\delta^{15}\text{N}$ values could be the result of consumption of animal matter, they also could reflect differences in nutritional or moisture stress experienced by animals, or isotopic differences in consumed plants.

There are other possible explanations for variation in $\delta^{15}\text{N}$ values. For example, fire frequency is inversely correlated with foliar $\delta^{15}\text{N}$ values in semi-arid and mesic Australian savannas [Cook, 2001]. Additionally, increased animal biomass could result in elevated $\delta^{15}\text{N}$ values. Trampling and ammonia volatilization associated with urine input (which results in preferential loss of ^{14}N from the soil) can affect soil, and consequently plant, $\delta^{15}\text{N}$ values [e.g. Frank & Evans, 1997]. However, there is some debate about the degree to which herbivores affect plant $\delta^{15}\text{N}$ values and even the directionality of the presumed effect [e.g. Frank & Evans, 1997; Schulze et al., 1999]. Finally, consumed plant part could also affect $\delta^{15}\text{N}$ values; mean values in fruit can be higher than those in leaves [Codron et al., 2005; Crowley et al., submitted; Dammhahn & Kappeler, 2009].

In summary, subfossil lemurs that lived in drier localities and consumed more fruit or animal matter should have higher $\delta^{15}\text{N}$ values than lemurs that lived in moist localities and ate less meat or fruit.

METHODS

We sampled $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for 141 individuals (104 primates and 37 non-primates) from subfossil sites in the STE. To our own data, we added earlier reported $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for 2 individuals and $\delta^{13}\text{C}$ values for an additional 11 individuals from the STE [Burney et al., 2004; Burney, personal communication]. Four extant and all eight extinct lemur species were sampled from sites spanning the STE from its easternmost (Andrahomana) to its southern (e.g. Anavoaha) and western (e.g. Andolononby) borders. One subfossil site, Taolambiby, is located well inland (in a region today characterized by both spiny thicket and gallery forests); all other

TABLE I. Species Sampled at Each Subfossil Site Included in This Study

Site	Specimens sampled (with sample size)
Anavoha	1 <i>Daubentonia robusta</i> , 2 <i>Hadropithecus stenognathus</i> , 3 <i>Megaladapis edwardsi</i> , 1 <i>M. madagascariensis</i>
Andolonomby	1 <i>Lepilemur leucopus</i> , 7 <i>Archaeolemur majori</i> , 3 <i>Lemur catta</i> , 2 <i>M. edwardsi</i> , 5 <i>M. madagascariensis</i> , 2 <i>Pachylemur insignis</i> , 14 <i>Palaeopropithecus ingens</i>
Andrahomana	2 <i>H. stenognathus</i> , 10 <i>L. catta</i> , 6 <i>Microcebus</i> spp., 3 <i>M. edwardsi</i> , 2 <i>P. insignis</i> , 10 <i>Geochelone</i> spp., 3 <i>Hypogeomys australis</i>
Ankazoabo Grotte	6 <i>L. leucopus</i> , 2 <i>Mesopropithecus globiceps</i> , 2 <i>P. ingens</i> , 1 <i>Cryptoprocta spelea</i>
Beavoaha	1 <i>M. edwardsi</i>
SW near Nosy Ve	1 <i>Geochelone</i> spp.
Taolambiby	2 <i>A. majori</i> , 3 <i>L. catta</i> , 4 <i>L. leucopus</i> , 2 <i>M. edwardsi</i> , 7 <i>M. madagascariensis</i> , 4 <i>P. insignis</i> , 8 <i>P. ingens</i> , 9 <i>Propithecus verreauxi</i> , 3 <i>Tenrec ecaudatus</i> , 7 <i>Geochelone</i> sp., 8 <i>Hippopotamus lemerlei</i> , 2 <i>C. spelea</i>
Tsiandroina	1 <i>M. globiceps</i>

sites are on or near the coast. Among extinct STE primates, neither *Hadropithecus stenognathus* nor *Daubentonia robusta* has been found at Taolambiby, although both have been found at inland sites in other ecoregions. Only one specimen of *Mesopropithecus* has been found at Taolambiby, and it was not sampled. Thus, these three species were sampled only at coastal sites. Table I and Figure 1 summarize our samples by taxon and location.

Collagen from subfossil bone was isolated and analyzed following standardized procedures [Crowley & Godfrey, 2009; Fox-Dobbs et al., 2006]. Approximately 200 mg of subfossil bone were cleaned, fragmented, decalcified in 0.5M EDTA, rinsed 10 times in ultrapure water, and gelatinized in 0.01N HCl at 57°C. The gelatin solution was filtered using a 1.5 µm glass-fiber filter and dried under vacuum. After preparation, 0.7 mg of collagenous extracts were weighed into tin boats, combusted, and analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values on a ThermoElectron (Finnigan) Delta+XP continuous flow system connected to an Elemental Analyzer at the University of California, Santa Cruz Stable Isotope Lab. Analytical precision ($\pm 1\text{SD}$) based on 21 IAEA Acetanilide replicates was $-29.1 \pm 0.08\text{‰}$ for carbon and $1.1 \pm 0.1\text{‰}$ for nitrogen. Collagen integrity was determined using a combination of collagen yield, C:N ratios, and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values [Ambrose, 1991; Fox-Dobbs et al., 2006].

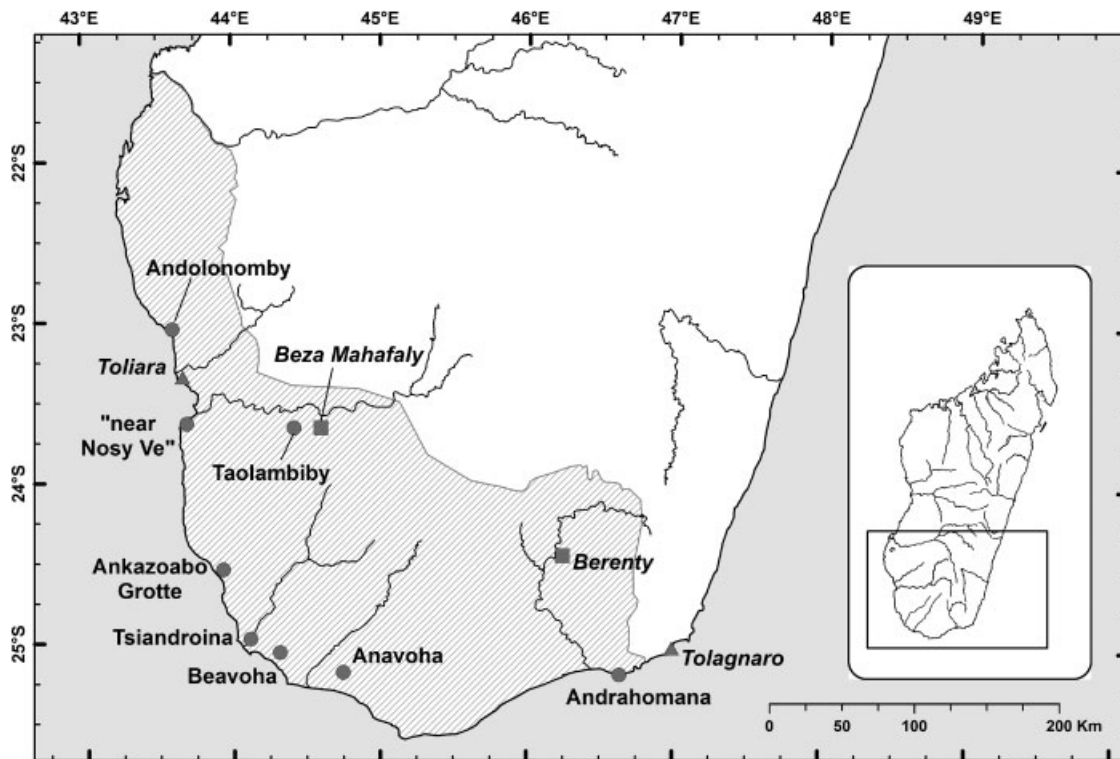


Fig. 1. Map of the Spiny Thicket Ecoregion of Madagascar, showing the subfossil sites sampled for this study (circles), protected forests (squares), and main cities (triangles).

Statistical analyses were conducted using SPSS 14. For multiple group comparisons, we used ANOVA with Tukey's tests of Honestly Significant Differences (HSD), to test the significance of differences between particular pairs. Independent sample *t*-tests were also used for comparisons of two samples. Significance was set at $\alpha = 0.05$.

RESULTS

Table II presents descriptive statistics for each species sampled. Looking first at differences among orders (primates, tortoises, hippos, carnivorans, tenrecs, and rodents) across the entire STE, we found no significant differences in $\delta^{13}\text{C}$ values. Carbon isotope ratios in primates overlap those of non-primate groups because three extinct species, *D. robusta*, *M. globiceps*, and *H. stenognathus* have very high $\delta^{13}\text{C}$ values, equaling or (for *H. stenognathus*) well surpassing those of non-primates (Table II, Fig. 2B). All other extinct primates in the STE (*Archaeolemur majori*, *M. edwardsi*, *M. madagascariensis*, *P. insignis*, and *Palaeopropithecus ingens*) have $\delta^{13}\text{C}$ values at or below our -20.2‰ cut-off for distinguishing diets, including CAM or C4 foods. Additionally, variability in $\delta^{13}\text{C}$ values is greater in coastal sites than inland (cf. Fig. 2A,B) because *D. robusta*, *M. globiceps*, and *H. stenognathus* were sampled only at coastal sites. At the inland site (Fig. 2A), primates have significantly lower $\delta^{13}\text{C}$ values than hippos, tenrecs, and carnivorans (ANOVA: $F = 5.1$, $df = 4$, 53 , $P < 0.01$). (The same applies to primates vs. non-primates at coastal sites

if *D. robusta*, *M. globiceps*, and *H. stenognathus* are removed.)

Ordinal differences in $\delta^{15}\text{N}$ values are highly significant, whether considered across the entire STE, inland only (at Taolambiby) or along the coast (ANOVA for all sites: $F = 7.1$, $df = 5$, 128 , $P < 0.001$; for inland only: $F = 2.8$, $df = 4$, 51 , $P < 0.05$; for coastal sites only: $F = 8.9$, $df = 3$, 74 , $P < 0.001$). In general, $\delta^{15}\text{N}$ values are highest at coastal sites (Fig. 2C and D). *H. stenognathus* has the highest $\delta^{15}\text{N}$ values, but high values are also found in many other extinct lemurs as well as carnivorans and tenrecs (Table II). At the inland site (Taolambiby), tenrecs and carnivorans have the highest $\delta^{15}\text{N}$ values and hippos and tortoises have the lowest; primates are intermediate. Post hoc tests across orders reveal that carnivorans have significantly higher $\delta^{15}\text{N}$ values than primates, tortoises, hippos, and rodents but not tenrecs, and primates have significantly higher $\delta^{15}\text{N}$ values than hippos. At coastal sites, carnivorans have the highest $\delta^{15}\text{N}$ values and rodents and tortoises the lowest, with primates intermediate (post hoc tests confirm significant differences between carnivorans and both rodents and tortoises), and primates have significantly higher $\delta^{15}\text{N}$ values than rodents (no hippos were sampled at coastal sites).

To better understand trophic variation, we grouped tortoises, hippos, and rodents as herbivores, and tenrecs and carnivorans as faunivores. A consistent pattern emerges, whether at coastal or inland sites (Table III; Fig. 3): $\delta^{13}\text{C}$ values are lower in primates than in non-primate herbivores and faunivores, although differences among groups approach significance only inland, at Taolambiby (ANOVA: $F = 2.9$, $df = 2$, 55 , $P = 0.06$). Variation in

TABLE II. Isotope Data for Subfossil Lemurs (Extinct and Extant) and Associated Fauna

Species	Family	Order	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
			<i>N</i>	$X \pm \text{SD}$	<i>N</i>	$X \pm \text{SD}$
Extant						
<i>Lepilemur leucopus</i>	Lepilemuridae	Primates	7	-20.0 ± 2.0	7	10.3 ± 2.2
<i>Lemur catta</i>	Lemuridae	Primates	14	-18.6 ± 1.2	14	10.9 ± 2.4
<i>Propithecus verreauxi</i>	Indriidae	Primates	9	-19.4 ± 1.4	9	8.6 ± 2.0
<i>Microcebus griseorufus</i>	Cheirogaleidae	Primates	6	-17.8 ± 1.0	6	11.0 ± 1.3
<i>Tenrec ecaudatus</i>	Tenrecidae	Afrosoricida	2	-17.7 ± 0.9	2	12.1 ± 1.9
Extinct						
<i>Archaeolemur majori</i>	Archaeolemuridae	Primates	9	-20.3 ± 1.2	9	12.5 ± 0.9
<i>Daubentonia robusta</i>	Daubentoniidae	Primates	1	-16.3	1	12.5
<i>Hadropithecus stenognathus</i>	Archaeolemuridae	Primates	4	-9.2 ± 0.6	3	15.6 ± 1.4
<i>Megaladapis edwardsi</i>	Megaladapidae	Primates	6	-20.3 ± 2.0	6	11.7 ± 2.3
<i>M. madagascariensis</i>	Megaladapidae	Primates	13	-20.2 ± 1.1	12	11.4 ± 1.5
<i>Mesopropithecus globiceps</i>	Palaeopropithecidae	Primates	2	-16.7 ± 3.7	2	13.2 ± 2.6
<i>Pachylemur insignis</i>	Lemuridae	Primates	8	-20.8 ± 1.2	8	13.0 ± 1.2
<i>Palaeopropithecus ingens</i>	Palaeopropithecidae	Primates	26	-20.4 ± 0.7	24	13.3 ± 2.0
<i>Cryptoprocta spelea</i>	Eupleridae	Carnivora	6	-18.7 ± 1.4	6	14.7 ± 1.1
<i>Geochelone</i> spp.	Testudinidae	Testudines	18	-18.9 ± 2.8	16	10.4 ± 1.4
<i>Hippopotamus lemerlei</i>	Hippopotamidae	Artiodactyla	8	-18.5 ± 1.2	8	8.9 ± 2.1
<i>Hypogeomys australis</i>	Nesomyidae	Rodentia	3	-18.9 ± 2.0	2	7.9 ± 0.1

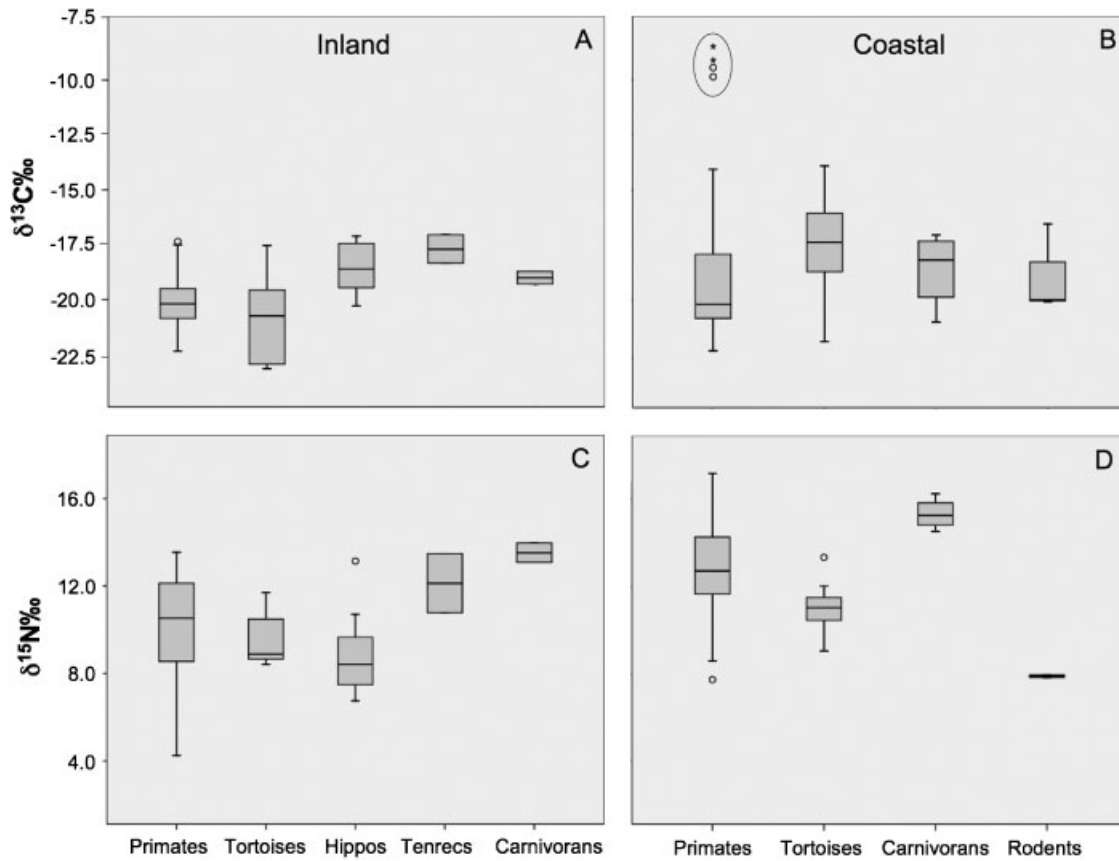


Fig. 2. Box and whiskers plots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for subfossil primates and associated fauna at inland (=Taolambiby) and coastal sites in the STE. Note the extremely high $\delta^{13}\text{C}$ values of *Hadropithecus stenognathus* (circled, panel B).

TABLE III. Trophic Group Comparisons, with ANOVAs

Comparison	Isotope	Primates		Herbivores		Faunivores		ANOVA <i>F</i> (df), <i>P</i>
		<i>N</i>	$X \pm \text{SD}$	<i>N</i>	$X \pm \text{SD}$	<i>N</i>	$X \pm \text{SD}$	
Entire STE	Carbon	104	-19.4 ± 2.5	29	-18.8 ± 2.3	8	-18.5 ± 1.3	1.0, (2,138), NS
	Nitrogen	100	11.9 ± 2.4	26	9.7 ± 1.8	8	14.1 ± 1.7	14.4, (2,131), <0.001
Coastal only	Carbon	65	-19.0 ± 3.0	14	-17.9 ± 2.3	4	-18.6 ± 1.8	0.8, (2,80), NS
	Nitrogen	63	12.8 ± 2.0	11	10.4 ± 1.7	4	15.3 ± 0.7	10.8, (2,75), <0.001
Inland only	Carbon	39	-20.1 ± 1.2	15	-19.7 ± 2.1	4	-18.3 ± 0.9	2.9, (2,55), 0.06
	Nitrogen	37	10.3 ± 2.2	15	9.2 ± 1.7	4	12.8 ± 1.4	5.2, (2,53), <0.01

$\delta^{15}\text{N}$ values is highly significant, both inland and on the coast, with primates consistently falling between herbivores (which have lower values on average) and faunivores (which have higher values). Pairwise comparisons of these groups are all significant according to Tukey's HSD ($P = 0.02$ for primates vs. faunivores; $P < 0.001$ for primates vs. non-primate herbivores and for faunivores vs. herbivores). Mean $\delta^{15}\text{N}$ values are higher in all three groups at coastal than inland sites, although significantly so only for primates ($P < 0.001$) and faunivores ($P = 0.02$).

DISCUSSION

Did the Extinct Lemurs of the STE Occupy Niches Likely not Filled by Other Taxa?

It is evident, on the basis of $\delta^{13}\text{C}$ values that most of the now-extinct lemurs of the STE exploited C3 foods (despite the abundance of CAM plants in the STE), and that the greater exploitation by lemurs of these resources distinguished these species from most sympatric species (herbivores and omnivores alike). Among the extinct lemurs, committed

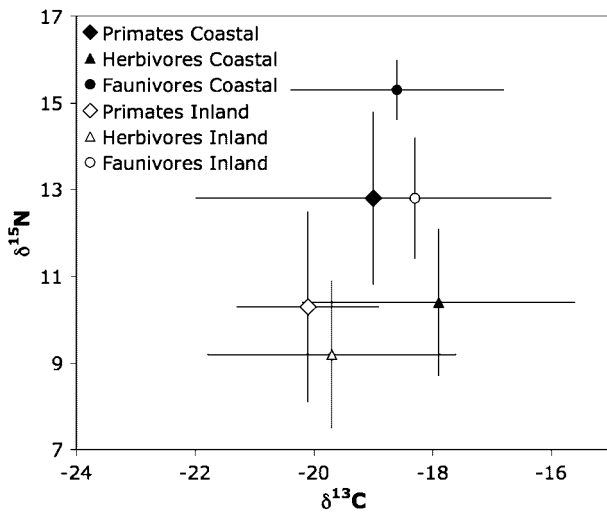


Fig. 3. Isotopic separation of trophic groups at coastal and inland sites, showing means and standard deviations for individuals.

C3 consumers apparently included *P. insignis*, *A. majori*, *P. ingens*, *M. edwardsi*, and *M. madagascariensis* (these taxa all had $\delta^{13}\text{C}$ values $< -20.2\text{‰}$). Both *A. majori* and *P. insignis* have been earlier reconstructed as likely large-seed dispersers [Godfrey et al., 2008a]. However, any of these large-bodied species could have played an important role in large seed dispersal.

Across the STE, all non-primate taxa have mean $\delta^{13}\text{C}$ values that are higher than -20.2‰ . This pattern holds for *Cryptoprocta spelea* and *Tenrec caudatus*, whose $\delta^{13}\text{C}$ values likely reflect some animal consumption, and *Hippopotamus lemerlei*, *Hypogeomys australis*, and *Geochelone* spp., whose $\delta^{13}\text{C}$ values likely reflect some CAM or C4 plant consumption. However, some subfossil lemurs also have mean $\delta^{13}\text{C}$ values above -20.2‰ . This is true for the extinct *D. robusta*, *M. globiceps*, and *H. stenognathus* as well as the still-extant *L. catta*, *M. griseorufus*, and *P. verreauxi*. Higher $\delta^{13}\text{C}$ values are unsurprising for subfossil *M. griseorufus* and *D. robusta*, which were both undoubtedly omnivorous, as *Microcebus* spp. and *D. madagascariensis* are today [Martin, 1972; Harding, 1981; Sauther et al., 1999; Sussman, 1999]. Modern *L. catta* are also somewhat omnivorous [Sauther et al., 1999], and in certain localities (e.g. Tsimanampesotse and Cap St. Marie), regularly consume CAM plants [Loudon et al., 2008; Kelley, personal communication]. *M. globiceps*' dental anatomy suggests it was highly folivorous and it is, therefore, likely that this species' elevated $\delta^{13}\text{C}$ values reflect utilization of C4 or CAM plants rather than ingestion of animal matter. The extremely high $\delta^{13}\text{C}$ values of *H. stenognathus* (whose teeth also suggest dominant herbivory) indicate it was likely an obligate C4 or CAM plant consumer [Godfrey et al., 2005, 2008b; Ryan et al., 2008].

TABLE IV. Possible Explanations of Elevated $\delta^{15}\text{N}$ Values in Subfossil Lemurs

Explanation	Notes and caveats
1. Lemurs were extremely protein stressed and were catabolizing their own tissues	Unlikely. There is little reason to believe that all lemurs sampled from subfossil communities would have been protein deprived
2. Lemurs occupied habitats with high animal biomass	Debatable. Although animal biomass was likely much higher in the past than it is today, how this would have affected nitrogen isotope ratios is uncertain; furthermore, this cannot account for differences between lemurs and other species
3. Fire frequency in the STE was low in the past	Cannot account for differences between lemurs and other species
4. Lemur diets were rich in animal matter	Unlikely, with some possible exceptions (<i>Daubentonia</i> , <i>Archaeolemur</i>)
5. Lemur diets were rich in fruit	Plausible. Anatomical evidence suggests that leaves were the primary staple for many of the extinct lemurs of the STE. However, at least three extinct species, <i>P. insignis</i> , <i>A. majori</i> , and <i>D. robusta</i> , likely consumed large quantities of fruit
6. Lemurs were able to thrive in habitats where access to water was limited	Plausible. The degree to which members of different orders may have been drought tolerant needs further investigation
7. Lemur diets were rich in plants that grew in dry and/or saline soils; they consumed relatively few plants with symbiotic nitrogen-fixing bacteria	Plausible. At inland sites, water-stress related to dry soils is more likely than water-stress related to salinity. However, soil salinity may help to explain very elevated nitrogen isotope values at coastal sites

What remains to be explained is why extinct primates of the STE tend to have elevated $\delta^{15}\text{N}$ values relative to co-occurring herbivorous non-primates, such as tortoises, hippos, and rodents. Table IV offers possible explanations for this phenomenon, some of which we regard as more likely than others. Increased soil salinity resulting in water-stressed plants may explain high $\delta^{15}\text{N}$ values in animals at coastal sites. But elevated $\delta^{15}\text{N}$ is not limited to coastal sites; lemurs living far from the ocean (e.g. Taolambiby) also have elevated $\delta^{15}\text{N}$ values, and the isotopic differences between taxa are

consistent across sites. It is also unlikely that severe nutritional stress elevated $\delta^{15}\text{N}$ values in all subfossil lemurs sampled.

Charcoal records attest to large fires across Madagascar for at least the past 40,000 years, but human arrival was accompanied by increased fire [Burney, 1987; Burney et al., 2004]. Assuming that the subfossil communities sampled here largely predate human arrival, an increase in fire frequency following human arrival could help to explain the elevated $\delta^{15}\text{N}$ values in subfossil animals. It is also possible that a greater megafaunal biomass on the landscape in the past is partly responsible for the elevated $\delta^{15}\text{N}$ values in subfossils (problems with this argument are discussed above). However, neither of these explanations can account for the observed differences between primates and hippos at the same subfossil sites.

The $\delta^{15}\text{N}$ difference between subfossil lemurs and non-primate herbivores might be explained if lemurs routinely consumed more fruit and animal matter; certainly, this may account for some of the observed differences. However, elevated $\delta^{15}\text{N}$ values characterize lemurs with very different reconstructed diets, including those that likely depended on leaves from C3 plants (*Palaeopropithecus*, *Megaladapis*), C4 or CAM plants (*Hadropithecus*, *Mesopropithecus*), and fruit and animal matter (*Pachylemur*, *Archaeolemur*, *Daubentonia*). Alternatively or additionally, the observed isotopic differences could reflect physiological differences across orders, i.e. that extinct lemurs were as capable as, or more capable than, non-primate herbivores with regard to surviving in dry, non-riparian habitats, without frequent drinking. For example, African hippos are obligate drinkers [Sealy et al., 1987], and one can surmise that Madagascan hippos would have been the same. In general (though not universally), obligate drinkers have lower $\delta^{15}\text{N}$ values than sympatric drought-tolerant species [Ambrose & DeNiro, 1986; Sealy et al., 1987]. Higher $\delta^{15}\text{N}$ values in extinct lemurs could, therefore, reflect drought tolerance—or an ability to survive in very dry habitats. If Madagascan hippos were more dependent on water and vegetation near water, one could expect that their $\delta^{15}\text{N}$ values would be lower than those in lemurs that survive on food resources located further from water.

The notion that extinct lemurs may have thrived in very dry (non-riparian) habitats gains further support from data collected on the fur of modern mouse lemurs across Madagascar [Crowley et al., submitted]. As a rule, variation in mouse lemur $\delta^{15}\text{N}$ values is pronounced, with individuals living in drier habitats having more positive $\delta^{15}\text{N}$ values than individuals living in moist environments. Differences across habitats in mouse lemur fur $\delta^{13}\text{C}$ values are much smaller. Moreover, $\delta^{13}\text{C}$ values for giant lemurs suggest that at least some were feeding on CAM or C4 foods, which are dominant elements in

open habitats but less so in riparian forests [Sussman & Rakotozafy, 1994]. The C3 plants of the spiny thickets themselves, through their anachronisms, reveal the habitat preferences of certain giant lemurs; many have special adaptations to counter overexploitation by *climbing* animals. Furthermore, it is likely that large-seeded C3 trees located far from water must have depended on climbing animals for seed dispersal. We discuss several examples below.

Effects of Lemur Species Loss

Primates likely have a positive overall effect on plant community diversity [Cowlshaw & Dunbar, 2000]. However, their effects on individual plants and plant species can be destructive (predatory) or beneficial (assisting reproduction). Folivory, or the consumption of other structural parts or underground storage organs, can reduce a plant's ability to reproduce by forcing a diversion of resources to the replacement of lost structures and/or depleting banked resources. Consumption of flowers can also be detrimental, as it reduces the plant's seed set, and granivory directly destroys seeds. Beneficial effects include pollination (which increases seed set) and seed dispersal (transport and deposition of viable seeds, sometimes with increased germination rates). Ecosystem-level benefits also exist. For example, consumption of terrestrial herbs and grasses can decrease fire frequency or intensity by reducing uncropped vegetation [Flannery, 1990; Johnson, 2009], particularly in dry, fire-prone habitats. The effects of primate extinction on individual plant species will, therefore, include both the removal of destructive and beneficial activities. Because the net effect will certainly vary among plant species, extinctions will alter plant community balance. Demonstrating effects of primate extinctions on STE plant communities is difficult, fundamentally because we are left with no "control plots" in which giant lemurs were spared from extinction. In addition, plant species' extinctions which followed lemur extinctions may be so far undetected. One strategy is to look for ecological "mismatches" (or "missing matches") in extant ecosystems.

Seed Dispersal: Ecological Mismatch in an Ecosystem Service?

Seed dispersal is often cited as one of the most important ecosystem services provided by primates [Chapman & Russo, 2006]. Unfortunately, interpretations of temporal changes in seed dispersal in the STE are hampered by the fact that there have been very few systematic studies of seed dispersal by modern lemurs in this ecoregion. In general, however, compared with primates in other tropical forests, Madagascar's lemurs play a disproportionately large role in seed dispersal owing to the relative lack of frugivorous birds [Voigt et al., 2004].

Whereas this dynamic may have been different in the past when the elephant birds were still alive, lemurs (including the giant species) likely played a major role in dispersing seeds of plants that did not attract elephant birds. We know that, in addition to the “wire-plants” that are likely adapted to defend their leaves against consumption by elephant birds, there are many succulent and broad-leaved trees in the STE, some of which are structurally adapted to defend their leaves against consumption by climbing animals [Bond & Silander, 2007]. Because plants build defenses against actual consumers, one can infer that many plants with defenses (such as spines) against climbing animals are today (or were in the past) exploited by lemurs, and that some of them, despite defending their leaves against predation, may actually depend (or have depended) on lemurs to disperse their seeds.

A list of STE tree genera that could have depended on giant lemurs for seed dispersal might include *Adansonia*, *Cedrelopsis*, *Commiphora*, *Delonix*, *Diospyros*, *Grewia*, *Pachypodium*, *Salvadora*, *Strychnos*, *Tamarindus*, and *Uncarina*. C3 photosynthesis has been confirmed for most of these genera [Crowley, unpublished data; Kluge, personal communication], but the seed dispersal biology for only a few is known. Some (such as *Cedrelopsis*) are known to be primate-dispersed outside the STE [Voigt et al., 2004]. Others (such as *Delonix*) have protein-rich edible pods [Grant et al., 1995]. The seeds of the spiny-trunked *Pachypodium* are today dispersed by wind, but may have had other means of dispersal in the past [Rapanarivo et al., 1999]. *Adansonia*, which has fruit with large seeds and nutritious pulp, may have been dispersed by *A. majori* or *P. insignis* [Baum, 1995; Godfrey et al., 2008a].

Regarding extant lemurs of the STE, Simmen et al. [2006] showed that *L. catta* consume and pass whole seeds of ripe *Tamarindus indica* fruit and that seeds in feces germinate. This is unsurprising, as comparative data from neighboring ecoregions confirm that other lemurid species disperse seeds, and that larger lemurid species tend to disperse larger seeds [Ganzhorn et al., 1999; Ralisoamalala, 1996]. Among the other lemurs, *Microcebus* spp. and *Cheirogaleus* spp. disperse small-seeded species. *P. verreauxi*, a folivore with long gut passage time, likely destroys most seeds but may occasionally disperse seeds.

Understanding the seed dispersal services of lemurs in the STE is complicated by two factors that differ from most primate communities elsewhere. First, the discontinuous canopy necessitates a great deal of terrestrial travel. Some extant species are quite devoted to terrestrial locomotion [*L. catta* spend 30–35% of their time on the ground; Sussman, 1974], whereas most other lemur species regularly come to the ground to cross open areas [*P. verreauxi*, Brockman et al., 2008; *M. griseorufus*, Youssouf

Jacky & Rasoazanabary, 2008]. Given the large body masses of all the extinct lemurs of the Spiny Thicket (ca. 10–85 kg), there can be no question that all of them, including the most arboreal, would have done the same. Indeed, both *A. majori* and *H. stenognathus* have been reconstructed as largely terrestrial. This means that small bushes as well as tall trees are candidates for lemur dispersal.

Second, most reviews of primate seed dispersal consider mainly endozoochory [e.g. Chapman & Russo, 2006], which seems to be a major mode of dispersal in most primate communities. However, in Madagascar’s STE, epizoochory must be considered as well. The generally water-stressed environment likely translates into an increased relative cost of producing fleshy fruits adapted for endozoochory; perhaps as a result, some small trees and shrubs in this ecoregion [e.g. *Uncarina* spp., all endemic to Madagascar; Humbert, 1971] have developed fruits adapted for epizoochory, with large spines and hooks for attachment to hair or feathers. Whereas strictly arboreal locomotion often involves contact with bare trunks and branches, semi-terrestrial locomotion likely involves more incidental contact with leaves, flowers, and fruit in the forest understory and in open areas with abundant shrubs. This would facilitate the attachment of fruits to primates’ hair. Indeed, it is common to see *Uncarina* fruits attached to the hair of extant lemurs [*L. catta* at Beza Mahafaly; Sauther, personal communication].

Although extant lemurs still certainly disperse seeds (both via endo- and epizoochory), there are indications that the quality of seed dispersal and the plant species affected have changed. First, home ranges of extant lemurs are small in the STE compared with other ecoregions. The home ranges of even the two largest STE species are small by primate standards [*L. catta*, less than 10 ha, Sussman, 1974; *P. verreauxi*, 1–7 ha, Richard, 1978; Jolly, 1966] and that of the smallest species (*M. griseorufus*) can be as small as 1 ha [Génin, 2008]. The larger extinct lemurs must have had larger home ranges. However, the contrast might not be that great—the largest STE lemurs (*M. edwardsi* at ~85 kg and *P. ingens* at ~40 kg) were likely dedicated folivores and not important seed dispersers, and the subfossil lemurs that were likely the most important seed dispersers (*P. insignis* and *A. majori*) weighed only 11.5 kg and ~18 kg, respectively [Jungers et al., 2008]. Godfrey and Irwin [2007] reconstructed home ranges of between 20 and 40 ha for both *M. edwardsi* and *A. majori* (the largest folivore and frugivore), using Milton and May’s [1976] regressions based on body size and diet. These may be overestimates, as giant lemurs were less agile than the anthropoids for which Milton and May [1976] generated their equations [Godfrey et al., 2006; Walker et al., 2008], but *Archaeolemur* was the most monkey-like and likely the most agile of all extinct lemurs [Walker et al., 2008].

Second, and probably more importantly, the small size of extant lemurs relative to extinct lemurs means that some fruits adapted for primate endozoochory may currently be too big for dispersal by any extant species. We know from studies in rain-forest sites that *Varecia variegata* can swallow seeds with diameters of more than 30 mm [Dew & Wright, 1998] and *Eulemur fulvus* can swallow seeds more than 20 mm diameter [Birkinshaw, 2001]. As an animal of more than twice the mass of *V. variegata*, it is likely that *Pachylemur* would have easily swallowed seeds in this range—perhaps, considerably larger [Godfrey et al., 2008a]. Thus, those plant species adapted for endozoochory and bearing large-seeded fruits are likely candidates for disruption of dispersal services following lemur extinctions. Species with smaller fruits but adapted for endozoochory, or with fruits adapted for epizoochory, may be experiencing reduced effectiveness or distance of dispersal as well [Johnson, 2009].

There is some evidence that trees in western Madagascar currently experience limited dispersal services compared with other ecoregions. Voigt et al. [2009] used methods based on population genetics to determine that the endozoochorous Madagascan dry forest tree *Commiphora guillaminii* exhibits high genetic differentiation among subpopulations at the local scale relative to a congener in South Africa, but both species have similar genetic differentiation among populations at the regional scale. The high local differentiation suggests a much shorter dispersal distance in the recent past for the Madagascan species, but the similar differentiation at the larger scale suggests similar levels of historic gene flow. This observed pattern might at least partially reflect the fact that the South African species is still dispersed by the large animals with which it is coadapted (frugivorous birds, including hornbills), but the Madagascan species may have lost its main dispersers (giant lemurs). Similar studies for other species will be crucial for identifying plant species whose dispersal relied mainly on extinct lemurs and is now disrupted.

Introduced animal species might act as potential dispersers and offset the losses of primate extinctions. Cattle, sheep, and goats regularly graze natural habitats. The wild pig *Potamochoerus larvatus*, thought to be introduced or recently arrived from Africa, is also a likely disperser of some seeds. It will thus be important to consider the attributes of native fruits that might predispose them to dispersal by introduced and domesticated animals. Unfortunately, it is also important to consider mounting evidence that introduced animals, especially ungulates, tend to facilitate the dispersal of invasive plants to the detriment of native species [Vavra et al., 2007].

In summary, our research on the stable isotope biogeochemistry of primates in the STE in the recent

past suggests that: (1) the extinct lemurs occupied a wide range of niches, often distinct from those filled by non-primate taxa; (2) some extinct lemur species included CAM and/or C4 foods in their diets, but most fed predominantly on C3 foods, and may have been the main dispersers of seeds of endemic and native C3 trees; and (3) many extinct lemurs regularly exploited arid habitats and were likely not restricted to gallery forests in which the surviving extant lemur species are most often protected and studied today. The combination of elevated $\delta^{15}\text{N}$ values coupled with low $\delta^{13}\text{C}$ values can best be explained as resulting from exploitation of C3 plants (rather than CAM plants) in dry habitats.

Deciphering the specific effects of recent lemur extinctions will require further research. The following hypotheses, when tested, will refine our understanding of the ecological roles of extinct lemurs: (1) Plants whose seeds were mainly dispersed by extinct lemurs, and whose dispersal has not been co-opted by introduced animals, should be rare and/or declining relative to species that use modes of dispersal other than endo- or epizoochory, or whose seeds are dispersed either by still-extant lemurs or by introduced animal species; and (2) such plants should also have signatures of reduced dispersal distances in their population genetic structure. Specifically, there should be a discordance between scales, with genetic similarity of populations on a regional scale (representing past dispersals) showing relatively low interpopulation differences, but with genetic differentiation on the local level (e.g. short parent-offspring distances within a single site) reflecting relatively short dispersal distances in recent generations.

Finally, it is important to point out that much more research attention has been paid to the effects of habitat changes on lemurs than the opposite—lemurs' effects on habitats [see Dausmann et al., 2008]. Undoubtedly, the uncertainty facing efforts to reconstruct the effects of past lemur extinctions derive largely from the lack of comparative studies today. Because of the unique nature of Madagascar's STE flora, we urgently need more quantitative studies of seed dispersal (gut passage time, dispersal distance, and seed sizes) in this region before we are to make more specific inferences about the seed dispersal services of extinct lemurs. One potentially productive area of research is in using local extirpation of extant species as an analogy for extinction. It will be imperative, not just for academic interest but for conservation of imperiled floras, to compare similar habitats with and without extant lemur species. It will also be important to increase our knowledge of ecological differences between lemurs surviving in gallery forests and drier spiny thicket. With an improved understanding of how primate extirpations change ecosystem processes, such as herbivory, pollination, and seed dispersal, we can develop better models for community change which

we can then apply to past lemur extinctions. This would be especially informative if coupled with studies of the effects of grazing by domestic animals, such as cattle, sheep, and goats.

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