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Explaining geographical variation in the isotope composition of mouse lemurs (*Microcebus*)

Brooke E. Crowley^{1,2*}, Sandra Thorén³, Emilienne Rasoazanabary⁴, Erin R. Vogel^{5,6}, Meredith A. Barrett⁷, Sarah Zohdy⁸, Marina B. Blanco⁴, Keriann C. McGoogan², Summer J. Arrigo-Nelson⁹, Mitchell T. Irwin¹⁰, Patricia C. Wright¹¹, Ute Radespiel³, Laurie R. Godfrey⁴, Paul L. Koch¹² and Nathaniel J. Dominy^{1,13}

¹Department of Ecology and Evolutionary Biology, 1156 High Street, University of California, Santa Cruz, CA 95064, USA,

²Department of Anthropology, 19 Russell Street, University of Toronto, Toronto, ON M5S2S2, Canada, ³Institute of Zoology, University of Veterinary Medicine Hannover, Bünteweg 17, 30559 Hannover, Germany,

⁴Department of Anthropology, Machmer Hall, 240 Hicks Way, University of Massachusetts, Amherst, MA 01003, USA, ⁵Center for the Advanced Study of Hominid Paleobiology, Department of Anthropology, George Washington University, 2110 G Street Northwest, Washington, DC 20052, USA,

⁶Department of Anthropology, Rutgers University, New Brunswick, NJ 08901-1414, USA, ⁷Duke University Program in Ecology, Box 90338, Nicholas School of the Environment, Durham, NC 27708, USA,

⁸Institute of Biotechnology, Viikki Biocenter, PO Box 56, 00014 University of Helsinki, Helsinki, Finland, ⁹California University of Pennsylvania, Department of Biological and Environmental Sciences, 250 University Ave – Box 45, California, PA 15419, USA, ¹⁰School of Biological Sciences, University of Queensland, Brisbane, Qld 4072, Australia, ¹¹Department of Anthropology, Stony Brook University, Circle Road, Social and Behavioral Science Building, Stony Brook, NY 11794-4364, USA,

¹²Department of Earth and Planetary Sciences, 1156 High Street, University of California, Santa Cruz, CA 95064, USA, ¹³Department of Anthropology, 6047 Silsby Hall, Dartmouth College, Hanover, NH 03755, USA

*Correspondence: Brooke E. Crowley, Department of Anthropology, 19 Russell Street, University of Toronto, Toronto, ON M5S2S2, Canada.

E-mail: brooke.crowley@utoronto.ca

ABSTRACT

Aim We sought to quantify geographical variation in the stable isotope values of mouse lemurs (*Microcebus*) and to determine whether this variation reflects trophic differences among populations or baseline isotopic differences among habitats. If the latter pattern is demonstrated, then *Microcebus* can become a proxy for tracking baseline habitat isotopic variability. Establishing such a baseline is crucial for identifying niche partitioning in modern and ancient communities.

Location We studied five species of *Microcebus* from eight distinct habitats across Madagascar.

Methods We compared isotopic variation in C₃ plants and *Microcebus* fur within and among localities. We predicted that carbon and nitrogen isotope values of *Microcebus* should: (1) vary as a function of abiotic variables such as rainfall and temperature, and (2) covary with isotopic values in plants. We checked for trophic differences among *Microcebus* populations by comparing the average difference between mouse lemur and plant isotope values for each locality. We then used multiple regression models to explain spatial isotope variation in mouse lemurs, testing a suite of explanatory abiotic variables.

Results We found substantial isotopic variation geographically. Ranges for mean isotope values were similar for both *Microcebus* and plants across localities (carbon 3.5–4.0‰; nitrogen 10.5–11.0‰). Mean mouse lemur and plant isotope values were lowest in cool, moist localities and highest in hot, dry localities. Rainfall explained 58% of the variation in *Microcebus* carbon isotope values, and mean plant nitrogen isotope values explained 99.7% of the variation in *Microcebus* nitrogen isotope values. Average differences between mouse lemur and plant isotope values (carbon 5.0‰; nitrogen 5.9‰) were similar across localities.

Main conclusions Isotopic data suggest that trophic differences among *Microcebus* populations were small. Carbon isotope values in mouse lemurs were negatively correlated with rainfall. Nitrogen isotope values in *Microcebus* and plants covaried. Such findings suggest that nitrogen isotope values for *Microcebus* are a particularly good proxy for tracking baseline isotopic differences among habitats. Our results will facilitate future comparative research on modern mouse lemur communities, and ecological interpretations of extinct Holocene communities.

Keywords

Crassulacean acid metabolism, habitat variability, Madagascar, *Microcebus*, mouse lemur, rainfall, stable isotopes, trophic level, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$.

INTRODUCTION

Stable isotope ratios in consumers can serve as a useful tool for tracking the diet and habitat preferences of individuals and populations (Kelly, 2000). However, assessing the relative contribution of dietary and habitat signals can be difficult, particularly when dealing with palaeocommunities. Spatial differences in plant isotope values, which are reflected in the tissues of herbivores and higher-level consumers, are driven by both the abiotic (e.g. geology, elevation and climate) and biotic (e.g. canopy cover, plant species composition) aspects of a habitat (Heaton, 1987, 1999; Amundson *et al.*, 2003; Marshall *et al.*, 2007). When this variation is passed on to consumers, it could generate isotopic differences among animals that are erroneously attributed to dietary differences, leading to an overestimate of dietary breadth. This potential error can be mitigated by measuring the isotopic covariation between plants and an animal taxon spread across a wide variety of modern habitats. Such an approach has been applied to systems in Africa, Australia, Europe and North America (e.g. Sealy *et al.*, 1987; Cormie & Schwarcz, 1994; Miller *et al.*, 2005; Männel *et al.*, 2007).

Here we describe the isotopic covariation between mouse lemurs (Cheirogaleidae: *Microcebus*) and plants across diverse Madagascan habitats. *Microcebus* is a radiation of small-bodied, omnivorous primates that is well suited for studying habitat-driven variation in stable carbon and nitrogen isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively).¹ Species belonging to the genus have a fairly recent common ancestor [12 million years ago (Ma); Yoder & Yang, 2004] and are of comparable body size (Table 1), but they live in a wide range of habitats, ranging from rain forests to arid spiny thickets (Fig. 1). Additionally, individuals have small home ranges of 0.4–6.0 ha (Radespiel, 2006). Given small ranges and a shared diverse diet (in this case, small vertebrates, arthropods, homopteran secretions, plant exudates, fruits and flowers; see Martin, 1972; Radespiel *et al.*, 2006; Atsalis, 2008; Dammhahn & Kappeler, 2008; Génin, 2008; Thorén *et al.*, 2011), the isotope values of mouse lemur fur should track habitat vegetation and differences between lemur and vegetation isotopes should be roughly constant and predictable. Thus, the isotopic values of *Microcebus* should serve as a reliable proxy for habitat.

To test this hypothesis, we quantified the extent to which latitude, longitude, elevation, annual rainfall and ambient temperature (abiotic variables), and plant isotope values (which integrate abiotic and biotic factors) drive geographical isotopic variation among populations of mouse lemurs. We constructed two hypotheses. First, we predicted that mouse lemurs from drier habitats with lower rainfall and warmer temperatures would evince higher and more variable $\delta^{13}\text{C}$ and

$\delta^{15}\text{N}$ values than those living in wetter and cooler habitats. Second, we predicted that the isotopic values of *Microcebus* would covary consistently with those of plants. Quantifying this covariation will help tease apart the effects of habitat versus diet in studies of modern and extinct mammals.

Causes of isotopic variation

Factors that affect isotopic variation in plants

Plants fix atmospheric CO_2 , and their $\delta^{13}\text{C}$ values are influenced by their photosynthetic pathway. Globally, the average $\delta^{13}\text{C}$ values of C_3 and C_4 plants are *c.* -28‰ (range -21 to -35‰) and -14‰ (range -12 to -16‰), respectively (O'Leary, 1988). Depending on the degree of succulence, temperature and water stress, plants using the crassulacean acid metabolism (CAM) photosynthetic pathway can have $\delta^{13}\text{C}$ values similar to those in C_3 plants or C_4 plants, or more intermediate values (Ehleringer, 1989; Ehleringer & Monson, 1993). In most terrestrial ecosystems, plants obtain their nitrogen directly from soil nitrate and ammonium, resulting in $\delta^{15}\text{N}$ values ranging from slightly less than to much greater than 0‰ (Muzuka, 1999; Schmidt & Stewart, 2003). Some plants, such as numerous Fabales taxa, have symbiotic bacteria that fix nitrogen directly from the atmosphere, resulting in $\delta^{15}\text{N}$ values that resemble air (0‰) (Ambrose, 1991; Schmidt & Stewart, 2003).

On the basis of prior global studies, we expect that rainfall will be the dominant environmental factor affecting $\delta^{13}\text{C}$ values in C_3 plants (e.g. Diefendorf *et al.*, 2010; Kohn, 2010). We anticipate that plants from drier, warmer localities will have higher $\delta^{13}\text{C}$ values than those in moister, cooler localities. As water availability decreases, stomatal conductance tends to drop, decreasing water loss but also CO_2 uptake, which leads to ^{13}C -enrichment of the internal CO_2 pool and increased $\delta^{13}\text{C}$ values in leaf tissue (Ehleringer, 1989).

For nitrogen, prior global studies suggest that rainfall and, to a lesser extent, temperature will be the dominant environmental factors affecting the $\delta^{15}\text{N}$ values of soils and thereby of non- N_2 -fixing plants (e.g. Amundson *et al.*, 2003). In general, drier, hotter localities experience substantial loss of ^{15}N -depleted forms of mineral N via volatilization of ammonia and denitrification, resulting in soils and vegetation enriched in ^{15}N . Plants growing in drier, hotter soils may also preferentially take up ammonium, which is enriched in ^{15}N relative to nitrate (Amundson *et al.*, 2003).

Beyond the expected relationships between rainfall/temperature and plant isotope values, there are a host of other factors that might introduce variation. For carbon, any factor that influences water use efficiency, photosynthetic rate or the $\delta^{13}\text{C}$ value of local atmospheric CO_2 (e.g. light levels, soil salinity, elevation, canopy cover, etc.) may affect plant $\delta^{13}\text{C}$ values (Ehleringer, 1989; van der Merwe & Medina, 1989; Heaton, 1999). For nitrogen, other important factors include soil age and chemistry and mycorrhizal associations (Amundson *et al.*, 2003; Marshall *et al.*, 2007). With the exception of elevation,

¹We used a standardized ' δ ' notation to refer to the proportion of heavy to light isotope within a given substance relative to a standard, where $\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, and $R = ^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. Values are reported as parts per thousand (‰).

Table 1 Descriptive characteristics for sampling sites and localities for mouse lemurs (*Microcebus* spp.) in Madagascar, including geographical coordinates, elevation above sea level, and the mean annual rainfall, and temperature range. Climate variables are averaged over different years (see sources).

Site and location	Species and authority	Mean mass (range) (g)	<i>n</i>	Latitude (S)	Longitude (E)	Elevation (m)	Rainfall (mm)	Temperature* (°C)	Source†
AM-JBA	<i>M. murinus</i> (J.F. Miller, 1777)	M: 52.3 (36–67) F: 53.4 (43–69)	31	16.32	46.8	125–200	1503	20.7–32.6	1–4
	<i>M. ravelobensis</i> (Zimmermann <i>et al.</i> , 1997)	M: 58.2 (46–75) F: 60.1 (37–95)	28						
AM-JBB	<i>M. ravelobensis</i>	M: 53.8 (40–68) F: 60.9 (38–87)	30	16.30	46.81	60–95	1503	20.7–32.6	1–4
BRN	<i>M. simmonsi</i> (Louis <i>et al.</i> , 2006)	M: 47.6 (42–63) F: 46.1 (43–50)	32	17.59	49.23	275–650	3962	18.6–25.2	5–8
BM-P1	<i>M. griseorufus</i> (Kollman, 1910)	M: 44.6 (30–58) F: 51.3 (30–89)	40	23.65	44.62	154	584	17.5–34.8	9–12
BM-P2	<i>M. griseorufus</i>	M: 42.5 (34–63) F: 41.3 (30–60)	46	23.69	44.58	168	584	17.5–34.8	9–12
KM	<i>M. murinus</i>	M: 49.7 (42–59) F: 52.4 (44–60)	28	20.78	44.17	65	767‡	19.4–32.5	13–15
RNP	<i>M. rufus</i> (É. Geoffroy Saint-Hilaire, 1828 [1834])	M: 49.4 (35–73) F: 43.8 (34–63)	13	21.27	47.33	600–1513	3605	15.7–21.9	16–20
TSI	<i>Microcebus</i> sp. cf. <i>M. rufus</i>	M: 47.8 (38–74) F: 53.4 (39–91)	40	19.69	47.76	1300–1675	2008	6.6–27.3	21–23

AM-JBA, Ampijoroa Jardin Botanique A; AM-JBB, Ampijoroa Jardin Botanique B; BRN, Betampona Réserve Naturelle; BM-P1, Beza Mahafaly-P1; BM-P2, Beza Mahafaly-P2; KM, Kirindy Mitea; RNP, Ranomafana National Park; TSI, Tsingy; M, male; F, female.

*Temperature ranges were calculated using the mean daily maximum and minimum temperatures recorded across all complete annual periods for which data are available.

†Sources: (1) S.T. unpublished data (2007–08), (2) Rendigs *et al.* (2003); (3) K.C.M. & U.R., unpublished data; (4) Durrell Wildlife Trust, unpublished data (2007–08); (5) M.A.B., unpublished data (2008); (6) Glessner & Britt (2005); (7) Louis *et al.* (2006); (8) Madagascar Fauna Group, unpublished data (2004–06); (9) E.R., unpublished data (2004–06); (10) Ratsirason (2003); (11) B.E.C., unpublished data; (12) Beza Mahafaly Special Reserve, unpublished data (2004–08); (13) M.A.B., unpublished data (2008); (14) Sorg & Rohner (1996) (1979–87); (15) R.J. Lewis, University of Texas, unpublished data (2008–09); (16) M.B.B. and S.Z., unpublished data (2004–08); (17) Aisalis, 2008; (18) King *et al.*, 2005; (19) Yoder *et al.* (2000); (20) Centre Valbio, unpublished data (2004–09); (21) M.B.B., unpublished data (2006–09); (22) Irwin, 2008; (23) Irwin & Raharison, unpublished data (rainfall 2001–07, temperature 2002–05, 2007).

‡Annual rainfall data do not exist for Kirindy Mitea. We therefore used rainfall data from Kirindy Centre de Formation Professionnelle Forestière (CFPF) (Sorg & Rohner, 1996).

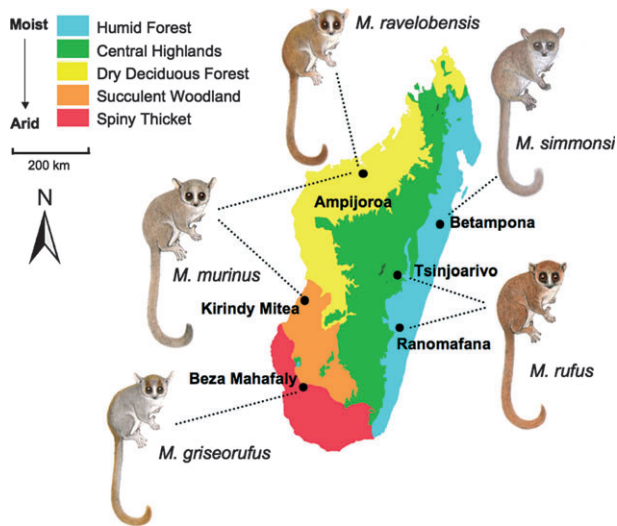


Figure 1 Geographical projection of the major Madagascan ecoregions, and mouse lemur (*Microcebus*) species and localities included in this study (adapted from Cornet, 1974 and Burgess *et al.*, 2004). Illustrations by Stephen D. Nash/Conservation International. Used with permission.

we are not able to control for these factors. Microhabitat variation and physiological differences in water use, photosynthetic efficiency and nutrient uptake among plant species may be particularly pronounced when plants are water- or temperature-stressed. As a result, isotopic variability can be greater in drier or warmer localities (Martinelli *et al.*, 1999; Schmidt & Stewart, 2003; Kohn, 2010).

Factors that affect isotopic variation in animals

Proteinaceous tissues in animals tend to be enriched in ^{13}C and ^{15}N relative to the tissues of consumed plants and prey. The increase in $\delta^{15}\text{N}$ value with each trophic step varies from 1.3 to 5.0‰ (DeNiro & Epstein, 1981; Schoeninger, 1985; Roth & Hobson, 2000; Fox-Dobbs *et al.*, 2007), but a 3‰ trophic step is often assumed. The $\delta^{13}\text{C}$ value of keratin from herbivores is 2–3‰ more positive than that of their diet, whereas herbivore-to-carnivore trophic ^{13}C enrichment ranges from 0.2 to 4‰ (DeNiro & Epstein, 1978; Roth & Hobson, 2000; Sponheimer *et al.*, 2003; Fox-Dobbs *et al.*, 2007; Hyodo *et al.*, 2010).

Isotope values in keratin may disproportionately reflect dietary protein rather than the whole diet (O'Connell & Hedges, 1999). With the exception of arthropods, the majority of *Microcebus* food items (fruits, exudates, homopteran secretions) contain only small amounts of protein (Hladik *et al.*, 1980; Dammhahn & Kappeler, 2010). By combining plant and mouse lemur isotope data, we can confirm the relative importance of animal and plant proteins in mouse lemur diets. Based on the results of recent research on Malaysian small mammals (Hyodo *et al.*, 2010), differences in $\delta^{13}\text{C}$ between consumers and plants should separate herbivores from consumers with some animal matter in their diets, and differences in $\delta^{15}\text{N}$ between consumers and plants

should clearly distinguish herbivores, trophic omnivores and carnivores. If arthropods provide the majority of dietary protein in mouse lemur diets, then we expect their keratin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values to be c. 5.4‰ and 6.4‰ higher than for plants, respectively, as they are for faunivorous bats and tree shrews. Conversely, if arthropods provide little to no protein, then we expect keratin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values to be c. 2.4‰ and 2.1‰ higher than for plants, respectively, as they are for herbivorous bats. If mouse lemurs obtain their protein from a mix of plant and animal resources, then we might expect their keratin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values to be c. 5.4‰ and 3.3‰ higher than for plants, respectively, like those of omnivorous Malaysian rodents.

In addition to being influenced by trophic enrichment, the $\delta^{15}\text{N}$ values of animals can increase with nutritional stress (Hobson *et al.*, 1993; Kelly, 2000). Accordingly, mouse lemurs that experience nutritional stress (perhaps at drier localities) might exhibit higher $\delta^{15}\text{N}$ values. Early studies suggesting ^{15}N -enrichment related to water stress (Ambrose & DeNiro, 1986; Heaton *et al.*, 1986; Sealy *et al.*, 1987; Ambrose, 1991) lacked a well-supported mechanism, and the basic pattern has not been supported by recent work (e.g. Murphy & Bowman, 2006; Hartman, 2011). As a consequence, we will not consider water stress on animals in our analysis or discussion.

MATERIALS AND METHODS

Study localities and species

Madagascar is divided into seven ecoregions based on climatic, floral and faunal compositions: Humid Forest, Succulent Woodland, Spiny Thicket, Subhumid Mid-altitude Forest (Central Highlands), Dry Deciduous Forest, Ericoid Thicket, and Mangrove (Burgess *et al.*, 2004). Each ecoregion is dominated by, but not limited to, its eponymous habitat. Two ecoregions, Ericoid Thicket and Mangrove, were excluded from this study. Although *Microcebus* has been sighted in mangrove forest, its occurrence there is minor, and its ecology in this habitat is unstudied (Hawkins *et al.*, 1998). The Ericoid Thicket lacks mouse lemurs. Within the remaining ecoregions, we sampled fur from five species of *Microcebus* occupying eight distinct habitats (Fig. 1; see descriptions below). The taxonomy of *Microcebus* is in flux; here we use the nomenclature of Mittermeier *et al.* (2010).

Humid Forest – Ranomafana National Park

We sampled fur from the brown mouse lemur, *Microcebus rufus*, in Ranomafana National Park (RNP). RNP is characterized by montane humid forest. The climate is cool and perennially moist, but there is marked variation in seasonal rainfall (Centre ValBio, unpublished data). The samples are from Talatakey, an area of RNP that was logged selectively until 1987 (Wright, 1995). Beetles and fruits, especially from the mistletoe *Bakerella*, are the primary dietary items of *M. rufus* (Atsalis, 2008).

Humid Forest – Betampona Strict Nature Reserve

We sampled fur from Simmons' mouse lemur, *Microcebus simmonsi*, in Betampona Strict Nature Reserve (Betampona Réserve Naturelle, BRN). Betampona protects a large tract of low-elevation rain forest. The park is extremely moist and lacks a clear dry season (Glessner & Britt, 2005). Little is known about *M. simmonsi* at Betampona. Until recently this population was classified as *M. rufus* (Louis *et al.*, 2006).

Central Highlands – Tsinjoarivo Forest

We sampled fur from *Microcebus* sp. cf. *M. rufus* in Tsinjoarivo (TSI), a mid-elevation rain forest. The climate at Tsinjoarivo is moist throughout the year with 65% of the annual rainfall occurring from December to March (Irwin, 2008). The samples originate from Andasivodihazo, a 228-ha forest fragment (Irwin, 2008). The foraging behaviour of this population is poorly studied. We assume it is similar to that of *M. rufus* at RNP.

Dry Deciduous Forest – Reserve Forestière d'Ampijoroa, Ankarafantsika National Park

We sampled fur from the grey mouse lemur, *Microcebus murinus*, and the golden-brown mouse lemur, *Microcebus ravelobensis*, in Ampijoroa (AM). The climate at Ampijoroa is strongly seasonal. There is a cool, dry season from May to October and a hot, wet season from November to April (Rendigs *et al.*, 2003). Most rainfall occurs in January and February.

Fur samples were collected from two habitats within Ampijoroa, Jardin Botanique A (JBA) and Jardin Botanique B (JBB). Despite similar temperatures and relative humidities (and a proximity of 1.5 km), JBA and JBB are distinctly different. JBA is a plateau featuring dry deciduous forest. JBB is a riparian forest bordering Lake Ravelobe, which partly floods during the wet season (Rendigs *et al.*, 2003). Both *M. murinus* and *M. ravelobensis* live at JBA, but they differ in preferred sleeping sites. *Microcebus murinus* mostly sleeps in tree holes, whereas *M. ravelobensis* routinely uses a variety of substrates for sleeping, including vine tangles and leaf nests (Radespiel *et al.*, 2003; Lutermann *et al.*, 2010). Females of both species eat similar quantities of arthropods, homopteran secretions, plant exudates and fruit during the dry season (Radespiel *et al.*, 2006). *Microcebus murinus* has not been observed at JBB, perhaps due to the lack of trees with suitable sleeping holes (Rendigs *et al.*, 2003).

Succulent Woodland – Kirindy Mitea National Park

We sampled fur from the grey mouse lemur, *M. murinus*, in Kirindy Mitea (KM), which is a new protected area and research site. Accordingly, few data exist on the vegetation composition, annual rainfall or temperature. Detailed annual and monthly measurements are unavailable, but KM is

reported to receive the majority of its rainfall in January and February (R.J. Lewis, University of Texas, pers. obs.), which is consistent with climatic data from the Kirindy Centre de Formation Professionnelle Forestière (CFPF), located c. 200 km to the north. Kirindy CFPF has a seasonal climate, with a pronounced rainy season from December to March and a prolonged dry season from April to November. Up to 37% of annual rainfall is in January and February (Sorg & Rohner, 1996). Our fur samples originate from a 1-km² block of dry deciduous forest at the centre of KM. The diet of *M. murinus* at KM is assumed to include arthropods, homopteran secretions, plant exudates and fruit, as in Kirindy CFPF (Dammhahn & Kappeler, 2008).

Spiny Thicket Ecoregion – Beza Mahafaly Special Reserve

We sampled fur from the grey-brown mouse lemur, *Microcebus griseorufus* (Heckman *et al.*, 2006) at Beza Mahafaly Special Reserve (BM). Beza Mahafaly is characterized by extreme seasonality, with 500–600 mm of annual rainfall occurring mainly in January and February (Table 1). The reserve is divided into two parcels: Parcel 1 (P1) is riparian forest along a seasonally dry river; Parcel 2 (P2) has xerophytic spiny thicket. The diet of *M. griseorufus* is dominated by plant exudates, arthropods and fruit, which is comparable to the diets of *Microcebus* at other dry forest localities. Mouse lemurs consume more plant exudates at P2 than at P1 during the wet season (Rasoazanabary, 2004, 2011).

Sample preparation and analysis

Fur samples were acquired in 2003–09 under protocols approved by the Institutional Animal Care and Use Committees of four universities (approval nos 0701.a3 to N.J.D. and B.E.C., 26-17-03 to M.B.B., 27-17-01 to E.R. and A282-07-10 to M.A.B.). The mouse lemurs were captured in Sherman live traps and their fur was cut and cleaned with methanol. To estimate the isotopic variability of plants in a habitat, we opportunistically collected, dried and homogenized tissue samples from common species at each locality (see Appendix S1 in Supporting Information).

Approximately 700 µg of fur and 5 mg of plant tissue were weighed into tin boats, combusted and analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values on a Finnigan ThermoElectron Delta+XP continuous flow system (Bremen, Germany) connected to a Carlo Erba elemental analyser (Milan, Italy) at the University of California, Santa Cruz Stable Isotope Laboratory. Raw isotope data for plants and mouse lemurs are presented in Appendices S1 & S2, respectively. For the plant samples, we also measured weight per cent C and N, and calculated atomic C:N ratios (Appendix S1). The carbon and nitrogen standards were Vienna Pee Dee Belemnite and Air, respectively. The analytical precision (± 1 SD) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was 0.2‰ and 0.08‰, respectively, based on 58 International Atomic Energy Agency (IAEA) acetanilide replicates. The average difference

between 12 fur samples run in duplicate was 0.2‰ for both carbon and nitrogen.

Data analyses

Locality comparisons

We compared mouse lemurs from all eight localities and plants from six: Ampijoroa-JBA, -JBB, Beza Mahafaly-P1, -P2, Ranomafana and Tsinjoarivo. Before combining data from different years, we confirmed that the seasonal and inter-annual isotopic differences for both plants and animals at single sites are minimal (Appendix S3). We combined samples of different plant tissues into two categories: leaves (young and mature, shoots, and stems) and fruit (both ripe and unripe). The isotopic difference between these categories at single sites is negligible (Appendix S1). For statistical analyses, we combined fruit and leaf data at Ampijoroa-JBA, Ranomafana and Tsinjoarivo. We analysed plants using CAM or C₃ photosynthetic pathways separately; we did not examine any plants using C₄ photosynthesis.

We assessed the assumptions of normality and homoscedasticity of variance for all analyses. We tested for homogeneity of variances using Bartlett tests. For those cases for which these assumptions were not violated, we used Student's *t*-tests or one-way analysis of variance (ANOVA) coupled with Tukey's post hoc honestly significant difference (HSD) tests to test whether isotopic differences exist among populations of mouse lemurs and plant communities. In cases for which the assumption of homogeneity of variance was violated, we report Welch ANOVA results. We used nonparametric pairwise Spearman's rho correlations to test whether average δ¹³C and δ¹⁵N values and the variance around these means were correlated across localities. To correct for the increased probability of committing Type I errors when making pairwise comparisons, we adjusted our criteria of significance downward using the sequential Bonferroni technique (Sokal & Rohlf, 1995).

Isotopic differences between mouse lemurs and plants

To test our prediction that isotopic values in mouse lemurs mirror those of plants, we compared the mean isotopic difference between fur and C₃ plants at four of the localities at which we collected both fur and plant samples (Ampijoroa-JBA and -JBB, Ranomafana and Tsinjoarivo). The prevalence of CAM plants has the potential to complicate calculations for mean mouse lemur–plant isotope differences at Beza Mahafaly-P1, given the possibility that mouse lemurs consume CAM plants or insects that eat CAM plants at this site. We therefore used a simple mass balance model (equations 1–3) to estimate the relative consumption of C₃ versus CAM foods in the diets of *M. griseorufus* at Beza Mahafaly-P1 and -P2. In these equations, plant δ¹³C values are shifted to account for the isotopic difference between fur and plants (see Results for estimation of these differences):

$$\delta^{13}C_{\text{mouselemurs}} = \text{Fraction}_{\text{C}_3} \times \delta^{13}C_{\text{C}_3} + \text{Fraction}_{\text{CAM}} \times \delta^{13}C_{\text{CAM}} \quad (1)$$

$$1 = \text{Fraction}_{\text{C}_3} + \text{Fraction}_{\text{CAM}}, \quad (2)$$

and after substitution and solving for Fraction_{CAM}

$$\text{Fraction}_{\text{CAM}} = (\delta^{13}C_{\text{mouse lemurs}} - \delta^{13}C_{\text{C}_3}) / (\delta^{13}C_{\text{CAM}} - \delta^{13}C_{\text{C}_3}) \quad (3)$$

Explaining spatial variation in mouse lemur isotope values

We used multiple linear regression models to examine the degree to which six abiotic habitat variables (latitude, longitude, elevation, mean annual rainfall, mean daily maximum and minimum ambient air temperature) and mean δ¹³C and δ¹⁵N values of C₃ plants (which integrate both biotic and abiotic habitat variables) explain mean mouse lemur isotope values at each locality. We assessed the assumption of independence of independent variables. Our independent variables were not correlated (Appendix S3). We used Akaike information criterion (AIC) values to select the best-fit regression model for mouse lemur δ¹³C values and mouse lemur δ¹⁵N values (Burnham & Anderson, 2002). We verified that the AIC-selected models explained mouse lemur isotopic variation better than other combinations of variables (Appendix S3).

When more than one explanatory variable was selected for our regression models, we used the partial sum of squares (SS) for each explanatory variable divided by the total SS from all variables to estimate the amount of variation explained by each variable in each model. Mean rainfall was calculated from the annual totals for all complete years with available data. Mean daily temperature maxima and minima were based on the average daily values recorded across all complete annual periods for which data were available (see Table 1). We natural log-transformed data for elevation and climate and verified that the assumptions of normality were not violated. We combined data for *M. murinus* and *M. ravelobensis* at Ampijoroa-JBA, and we combined data from males and females for each species (Appendix S3). We ran all analyses in JMP v.8.0.2 (SAS Institute, Cary, NC, USA) and R v.2.10.1 (R Development Core Team 2009). All probability levels are two-tailed, and the significance for all tests was set at $\alpha < 0.05$.

We acknowledge the potential for pseudoreplication in our models given that the rainfall and temperature data are identical for Beza Mahafaly-P1 and -P2 and for Ampijoroa-JBA and -JBB. Although we do not have unique rainfall data for these two localities, comparable patterns are expected. Beza Mahafaly-P1 and -P2 are c. 3 km apart (Loudon *et al.*, 2007), and Ampijoroa-JBA and -JBB are only 1.5 km apart (Rendigs *et al.*, 2003). Despite the geographical proximity of the localities at each of these sites, and their presumed similarity in rainfall and temperature, other factors (e.g. soil composition, distance from a lake or river) make these habitats very different; thus pooling the data from these localities is inadvisable.

RESULTS

Observed isotopic variation in C₃ plants and mouse lemurs

Among C₃ plants, we observed significant isotopic differences among localities (Fig. 2b, Table 2). Carbon and nitrogen isotope values were lowest at the wettest, coolest localities (Ranomafana and Tsinjoarivo). Carbon isotope values were highest for Beza Mahafaly-P2, the driest locality, and δ¹⁵N values were highest for Ampijoroa-JBA (Fig. 2b, Table 3). Average δ¹³C and δ¹⁵N values in plants were uncorrelated across localities (Spearman’s rho = 0.49, P = 0.33, n = 6). Mean δ¹⁵N values for leguminous taxa were significantly lower than those in non-nitrogen-fixing sympatric C₃ taxa at Beza Mahafaly-P1, but not at Ampijoroa-JBA or Ranomafana (Table 4). The variances for plant δ¹³C and δ¹⁵N values were uncorrelated with

mean isotope values, sample size, temperature, rainfall or elevation across localities (P > 0.05 for all comparisons).

As with plants, we observed significant isotopic differences for mouse lemurs among localities (Fig. 2a, Table 2). Mouse lemurs from the wettest localities (Tsinjoarivo, Betampona and Ranomafana) evinced distinctly low δ¹³C and δ¹⁵N values, and the highest δ¹³C and δ¹⁵N values belonged to *M. griseorufus* at Beza Mahafaly-P2, and *M. murinus* at Ampijoroa-JBA, respectively (Table 5). Mean δ¹³C and δ¹⁵N values in mouse lemurs were uncorrelated across localities (Spearman’s rho = 0.42, P = 0.30, n = 8). The variances for mouse lemur δ¹³C and δ¹⁵N values were uncorrelated with mean isotope values, sample size, temperature, rainfall or elevation across localities (P > 0.05 for all comparisons).

Isotopic differences between mouse lemurs and C₃ plants

In general, the isotopic values of plants and mouse lemurs varied in tandem (Fig. 2b). However, the δ¹³C and δ¹⁵N differences between mouse lemur fur and C₃ plants were uncorrelated (Spearman’s rho = 0.30, P = 0.62, n = 5). The difference between mouse lemur fur and C₃ plants averaged 5.0‰ and 5.9‰ for δ¹³C and δ¹⁵N values, respectively (Table 6). We used the mean δ¹³C difference between mouse lemurs and C₃ plants (5.0‰) to account for trophic fractionation in our mass balance models (as described in Materials and Methods). Mass balance model results suggest that mouse lemurs at Beza Mahafaly-P1 and -P2 consumed an average of 7.6% and 0.0% CAM-derived foods, respectively.

Geographical variation in mouse lemur isotope values

Mean annual rainfall was the only explanatory variable included in the AIC-selected model for mouse lemur δ¹³C values (Table 7). Mean C₃ plant δ¹⁵N values, mean C₃ plant δ¹³C values, and longitude were included as explanatory variables in the AIC-selected model for mouse lemur δ¹⁵N values. However, mean plant δ¹⁵N values alone explained most (99.7%) of the variation in mouse lemur δ¹⁵N values (Table 7). Because consumption of CAM resources might affect mouse lemur isotope values at Beza Mahafaly-P1, we ran all models including all localities except Beza Mahafaly-P1.

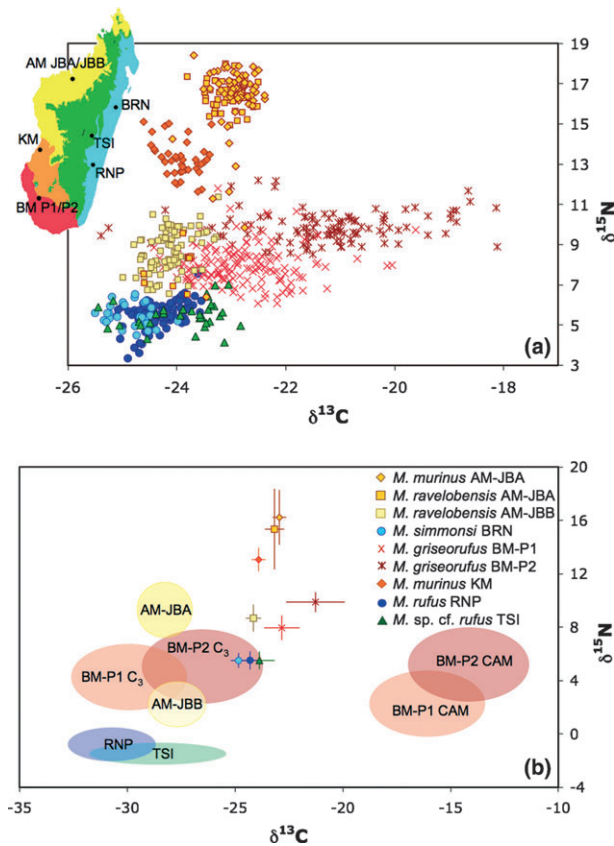


Figure 2 Stable isotope data for Madagascar. (a) Individual mouse lemur (*Microcebus* spp.) δ¹³C and δ¹⁵N values. (b) Mean isotope values (± 1σ) for mouse lemur species and plants (coloured bubbles) at each locality. Isotope data for crassulacean acid metabolism (CAM) plants from Beza Mahafaly-P1 and -P2 are presented separately from data for C₃ plants. Locality abbreviations: AM-JBA, Ampijoroa Jardin Botanique A; AM-JBB, Ampijoroa Jardin Botanique B; BRN, Betampona Réserve Naturelle; BM-P1, Beza Mahafaly-P1; BM-P2, Beza Mahafaly-P2; KM, Kirindy Mitea; RNP, Ranomafana National Park; TSI, Tsinjoarivo.

Table 2 ANOVA and Welch ANOVA results for locality comparisons. Plant and mouse lemur (*Microcebus* spp.) comparisons include six and eight localities in Madagascar, respectively.

	<i>n</i>	<i>F</i>	d.f.	<i>P</i>
C ₃ plants				
δ ¹³ C	417	29.17	5, 44.3	< 0.0001
δ ¹⁵ N	417	194.5	5, 35.8	< 0.0001
Mouse lemurs				
δ ¹³ C	661	210.92	7, 187.3	< 0.0001
δ ¹⁵ N	661	748	7, 185.1	< 0.0001

Table 3 Descriptive statistics [mean, standard deviation, standard error of the mean (SEM), and range] for plant samples in Madagascar. Localities in bold that share the same superscript letter are statistically indistinguishable [Tukey's honestly significant difference (HSD) test; $\alpha = 0.05$]. Locality abbreviations follow those in Table 1.

Site and locality	Tissue	<i>n</i>	Mean $\delta^{13}\text{C} \pm 1\sigma$	$\delta^{13}\text{C}$ SEM	$\delta^{13}\text{C}$ range	Mean $\delta^{15}\text{N} \pm 1\sigma$	$\delta^{15}\text{N}$ SEM	$\delta^{15}\text{N}$ range
AM-JBA	Leaves	21	-28.2 ± 1.4	0.3	-31.4, -25.8	9.0 ± 2.1	0.5	2.7, 11.5
	Fruit	16	-28.1 ± 1.5	0.4	-30.7, -25.6	10.1 ± 3.0	0.8	2.8, 16.1
	Total	37	-28.1 ± 1.4^B	0.2	-31.4, -25.6	9.5 ± 2.5^A	0.4	2.8, 16.1
AM-JBB	Fruit	5	-27.6 ± 0.5 ^{ABC}	0.2	-27.9, -26.7	2.4 ± 1.6 ^B	0.7	0.2, 4.4
BM-P1	C₃ leaves	216	-29.8 ± 2.1^C	0.1	-37.3, -23.5	4.4 ± 2.6^B	0.2	-1.5, 12.2
	CAM leaves	31	-15.3 ± 0.9	0.2	-17.0, -13.2	2.9 ± 1.9	0.5	0.8, 2.5
BM-P2	C₃ leaves	24	-26.4 ± 2.8^A	0.6	-30.9, -22.8	5.2 ± 2.1^B	0.4	1.4, 9.7
	CAM leaves	35	-14.7 ± 1.4	0.2	-17.4, -11.4	5.4 ± 3.3	0.6	0.2, 12.9
RNP	Leaves	63	-31.6 ± 1.1	0.1	-34.5, -29.1	-0.6 ± 1.3	0.2	-5.8, 2.1
	Fruit	23	-27.8 ± 2.0	0.4	-32.7, -25.1	-0.8 ± 1.7	0.4	-5.8, 2.1
	Total	86	-30.6 ± 2.2^D	0.2	-34.5, -25.1	-0.6 ± 1.4^C	0.1	-5.8, 2.2
TSI	Leaves	36	-28.5 ± 1.9	0.3	-32.1, -24.7	-1.1 ± 1.9	0.3	-6.2, 3.0
	Fruit	13	-28.1 ± 1.8	0.5	-31.7, -26.1	-2.5 ± 1.9	0.5	-5.4, 0.8
	Total	49	-28.4 ± 1.8^B	0.3	-32.1, -24.7	-1.5 ± 2.0^C	0.3	-6.2, 3.0

CAM, crassulacean acid metabolism.

Complete plant isotope data are available in Appendix S1.

Table 4 Intra-locality comparisons of $\delta^{15}\text{N}$ values for Fabaceae and non-Fabaceae. Locality abbreviations follow those in Table 1.

Locality	Mean $\delta^{15}\text{N} \pm 1\sigma$ for Fabaceae (<i>n</i>)	Mean $\delta^{15}\text{N} \pm 1\sigma$ for non-Fabaceae (<i>n</i>)	<i>t</i> -value	<i>P</i> -value
BM-P1	3.2 ± 2.0 (68)	5.0 ± 2.6 (149)	4.89	< 0.0001
AM-JBA	7.4 ± 3.5 (4)	10.1 ± 1.6 (22)	-1.54	0.21
RNP	0.6 ± 1.1 (2)	-0.6 ± 1.4 (80)	1.56	0.35

Excluding mouse lemurs from Beza Mahafaly-P1 did not affect which variables were included in the AIC-selected models. It did, however, change the significance of the AIC-selected models, increasing the explanatory power of the AIC-selected model for mouse lemur $\delta^{15}\text{N}$ values (Appendix S3).

DISCUSSION

Isotopic variation in plant isotope values

Carbon

The average $\delta^{13}\text{C}$ values of C₃ plants ranged from -30.6‰ to -26.4‰ across six localities. This range resembles the $\delta^{13}\text{C}$ values reported from large areas of Australia and South Africa, where mean annual rainfall ranges from 450 to 1800 and 200 to 1000 mm, respectively (Schulze *et al.*, 1998; Swap *et al.*, 2004). In agreement with our expectations, the $\delta^{13}\text{C}$ values of C₃ plants and the variance around these means were lowest in the coolest, wettest and most densely forested localities (Betampona, Ranomafana and Tsinjoarivo) and highest in the drier, warmer, more seasonal localities (Figs 3a & 4a). Indeed, the $\delta^{13}\text{C}$ values of C₃ plants at Beza Mahafaly-P2 (the driest

locality in our study) were the highest and most variable compared with other localities. This pattern in $\delta^{13}\text{C}$ values probably reflects differences in water availability among localities, whereas the increased variability reflects the greater potential for microhabitat differences in water availability (and plant response to water stress) in drier sites.

Nitrogen

The average $\delta^{15}\text{N}$ values of C₃ plants ranged from -1.5‰ to 9.5‰. This range is comparable to ranges reported for plants from other regions with similar rainfall and temperature gradients (Shearer *et al.*, 1983; Heaton, 1987; Ambrose, 1991; Swap *et al.*, 2004). As for $\delta^{13}\text{C}$ values, the mean $\delta^{15}\text{N}$ values of C₃ plants, and the variances around these means, were lowest in the coolest, wettest localities and highest in the drier, warmer and more seasonal localities (Figs 3b & 4b). Such a pattern is most likely related to increased rates of ammonia volatilization and denitrification in hot, dry localities (Ambrose, 1991; Amundson *et al.*, 2003). Increased microhabitat variation in soil chemistry and the degree to which nitrate and ammonium are taken up by different plant species could be responsible for the increased variance in plant $\delta^{15}\text{N}$ values at drier localities (Amundson *et al.*, 2003).

Explaining geographical variation in mouse lemur isotope values

Carbon

The average $\delta^{13}\text{C}$ values of *Microcebus* ranged from -24.8‰ to -21.3‰. This spread of 3.5‰ resembled that found among C₃ plants across Madagascar. *Microcebus griseorufus* from Beza Mahafaly-P2, the driest site, evinced the highest $\delta^{13}\text{C}$ values,

Table 5 Descriptive statistics [mean, standard deviation, standard error of the mean (SEM), and range] for mouse lemurs (*Microcebus* spp.) in Madagascar. Localities in bold that share the same superscript letter are statistically indistinguishable [Tukey's honestly significant difference (HSD) test; $\alpha = 0.05$]. Locality abbreviations follow those in Table 1.

Site and locality	Species	Sex	<i>n</i>	Mean $\delta^{13}\text{C} \pm 1\sigma$	$\delta^{13}\text{C}$ SEM	$\delta^{13}\text{C}$ range	Mean $\delta^{15}\text{N} \pm 1\sigma$	$\delta^{15}\text{N}$ SEM	$\delta^{15}\text{N}$ range
AM-JBA	<i>M. murinus</i>	M	29	-23.1 \pm 0.3	0.06	-24.1, -22.6	15.8 \pm 2.7	0.5	6.4, 18.4
		F	31	-22.8 \pm 0.3	0.05	-23.5, -22.4	16.6 \pm 1.1	0.2	11.6, 17.9
		All	60	-22.9 \pm 0.3	0.04	-24.1, -22.4	16.2 \pm 2.1	0.3	6.4, 18.4
AM-JBA	<i>M. ravelobensis</i>	M	30	-23.3 \pm 0.5	0.09	-24.6, -22.7	15.0 \pm 3.5	0.6	16.5, 17.9
		F	31	-23.1 \pm 0.4	0.07	-23.8, -22.5	15.7 \pm 2.5	0.4	8.3, 18.0
		All	61	-23.2 \pm 0.5	0.06	-24.6, -22.5	15.3 \pm 3.0	0.4	6.5, 18.0
AM-JBA	All AM-JBA	All	121	-23.1 \pm 0.4 ^{BC}	0.04	-24.6, -22.4	15.8 \pm 2.6 ^A	0.2	6.4, 18.4
AM-JBB	<i>M. ravelobensis</i>	M	38	-24.2 \pm 0.4	0.06	-24.6, -23.4	8.7 \pm 1.1	0.2	6.8, 11.4
		F	51	-24.1 \pm 0.4	0.06	-25.0, -23.3	8.6 \pm 1.0	0.1	6.6, 10.5
		All	89	-24.1 \pm 0.4 ^D	0.04	-25.0, -23.2	8.7 \pm 1.0 ^D	0.1	6.6, 11.4
BRN	<i>M. simmonsii</i>	M	23	-24.8 \pm 0.4	0.08	-25.5, -24.0	5.5 \pm 0.6	0.1	4.5, 6.5
		F	5	-25.0 \pm 0.3	0.1	-25.3, -24.5	5.7 \pm 0.5	0.2	5.2, 6.4
		All	28	-24.8 \pm 0.4 ^E	0.07	-25.5, -24.0	5.5 \pm 0.6 ^F	0.1	4.5, 6.5
BM-P1	<i>M. griseorufus</i>	M	53	-23.0 \pm 0.7	0.1	-24.2, -21.2	8.2 \pm 1.0	0.1	6.4, 11.8
		F	37	-23.1 \pm 0.7	0.1	-24.0, -20.0	8.0 \pm 0.7	0.1	6.1, 9.7
		All	157	-22.8 \pm 0.8 ^B	0.07	-24.4, -19.6	8.0 \pm 0.9 ^E	0.07	6.1, 11.8
BM-P2	<i>M. griseorufus</i>	M	22	-21.5 \pm 1.3	0.3	-23.0, -18.6	10.2 \pm 0.9	0.2	8.6, 12.2
		F	20	-21.3 \pm 1.2	0.3	-23.2, -18.7	10.0 \pm 0.8	0.2	8.8, 11.9
		All	111	-21.3 \pm 1.4 ^A	0.1	-25.4, -18.1	9.9 \pm 0.8 ^C	0.07	8.5, 12.2
KM	<i>M. murinus</i>	M	27	-23.9 \pm 0.4	0.08	-24.6, -23.4	13.5 \pm 0.9	0.2	11.7, 15.1
		F	13	-23.9 \pm 0.2	0.06	-24.3, -23.6	12.8 \pm 0.6	0.2	11.6, 13.4
		All	40	-23.9 \pm 0.3 ^D	0.05	-24.6, -23.4	13.3 \pm 0.9 ^B	0.1	11.6, 15.1
RNP	<i>M. rufus</i>	M	55	-22.3 \pm 0.4	0.05	-25.1, -25.6	5.5 \pm 0.7	0.09	3.6, 7.6
		F	27	-24.4 \pm 0.4	0.08	-25.1, -25.6	5.6 \pm 0.7	0.1	3.4, 6.6
		All	82	-24.3 \pm 0.4 ^D	0.04	-25.1, -23.6	5.5 \pm 0.7 ^F	0.08	3.4, 7.6
TSI	<i>Microcebus</i> sp. cf.	M	11	-23.4 \pm 0.4	0.1	-24.2, -22.7	5.5 \pm 0.7	0.2	4.4, 7.0
		F	19	-23.4 \pm 0.3	0.07	-23.9, -22.9	5.4 \pm 0.7	0.2	4.1, 7.0
		All	33	-23.4 \pm 0.3 ^C	0.06	-24.2, -22.7	5.4 \pm 0.7 ^F	0.1	4.1, 7.0

Complete mouse lemur isotope data are available in Appendix S2. M, male; F, female.

whereas *M. simmonsii* from Betampona, the wettest site, had the lowest values (Fig. 3a). There is no discernible trend between mouse lemur $\delta^{13}\text{C}$ values and elevation or temperature (Fig. 4a, Appendix S3).

In our AIC-selected model, rainfall emerged as the sole explanatory variable for mouse lemur $\delta^{13}\text{C}$ values (Table 7). A negative relationship between mean annual rainfall and mouse lemur $\delta^{13}\text{C}$ values supports our predictions that stable isotope values in mouse lemurs can be used to distinguish populations from different habitats. This trend in mouse lemurs very likely reflects isotopic differences in plants among localities that cascade up the food web. Yet, despite a positive correlation, the mean $\delta^{13}\text{C}$ values in plants did not explain the $\delta^{13}\text{C}$ values of mouse lemurs (Spearman's $\rho = 0.4$, $P = 0.33$, $n = 6$). Excluding Beza Mahafaly-P1 from our model did not strengthen this relationship (Appendix S3). One factor contributing to this unexpected result is that all eight localities were included when we compared climate and mouse lemur $\delta^{13}\text{C}$ values. Because we have no plant data for Betampona or Kirindy Mitea, only six localities were included when we compared C_3 plant and mouse lemur $\delta^{13}\text{C}$ values. With just six

localities the substantial within-locality variability in plant $\delta^{13}\text{C}$ values and the relatively small among-locality variability in $\delta^{13}\text{C}$ values may have contributed to the insignificant relationship between C_3 plant and mouse lemur $\delta^{13}\text{C}$ values.

Temporal and spatial averaging in mouse lemurs may also help explain why plants did not emerge as a significant explanatory variable for the $\delta^{13}\text{C}$ values of mouse lemurs (Bump *et al.*, 2007). Plant isotope values can be strongly affected by microhabitat variability. Yet at any given locality, mouse lemurs consume a tremendous number of plants as well as insects (which also consume numerous plants). Because the among-habitat spread in $\delta^{13}\text{C}$ values in both plants and mouse lemurs is not large, small differences in the amount of ^{13}C -enriched arthropods consumed at each locality or a subtle mismatch between the vegetation consumed by mouse lemurs (and their prey) and the vegetation sampled for this study could weaken the correlation between mouse lemurs and plants. One source of such mismatch is that the majority of our plant samples came from the upper canopy, whereas mouse lemurs (and their prey) may have ingested vegetation from all canopy levels, including understorey plant tissues that were

Table 6 The mean isotopic difference between mouse lemur (*Microcebus* spp.) fur and C₃ plants at each locality in Madagascar. Standard error of the mean (SEM) values were propagated from the SEM values for mouse lemurs and C₃ plants at each locality. Locality abbreviations follow those in Table 1.

Locality	Species	Lemur $\delta^{13}\text{C}$	Plant $\delta^{13}\text{C}$	$\delta^{13}\text{C}$ difference	$\delta^{13}\text{C}$ SEM	Lemur $\delta^{15}\text{N}$	Plant $\delta^{15}\text{N}$	$\delta^{15}\text{N}$ difference	$\delta^{15}\text{N}$ SEM
AM-JBA	<i>M. murinus</i>	-22.9	-28.1	5.2	0.2	16.2	9.5	6.7	0.5
AM-JBA	<i>M. ravelobensis</i>	-23.2	-28.1	4.9	0.2	15.3	9.5	5.8	0.6
AM-JBB	<i>M. ravelobensis</i>	-24.1	-27.6	3.5	0.2	8.7	3.4	5.3	0.7
RNP	<i>M. rufus</i>	-24.3	-30.6	6.3	0.2	5.5	-0.6	6.1	0.2
TSI	<i>Microcebus</i> sp. cf. <i>M. rufus</i>	-23.4	-28.4	5.0	0.3	5.4	-1.5	6.9	0.3
BM-P1*	<i>M. griseorufus</i>					8.0	4.4	3.6	0.2
BM-P2	<i>M. griseorufus</i>					9.9	5.2	4.7	0.4
Mean $\pm 1\sigma$				5.0 \pm 1.0	0.5			5.9 \pm 0.8	1.2

*Beza Mahafaly-P1 was not included in the mean $\delta^{15}\text{N}$ difference or the mean $\delta^{15}\text{N}$ SEM calculations. *Microcebus griseorufus* consumes c. 7.6% crassulacean acid metabolism (CAM) foods, and CAM plants have lower $\delta^{15}\text{N}$ values than C₃ plants at this locality (Table 3).

Table 7 Results from Akaike information criterion (AIC) selected multiple regression models for mean mouse lemur (*Microcebus* spp.) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values at all localities except Beza Mahafaly-P1. See Appendix S3 for AIC values and statistical results from other models.

Dependent variable	Explanatory variables	r^2	F	d.f.*	P	Partial SS	% Explained†
Mean <i>Microcebus</i> $\delta^{13}\text{C}$ values	Ln rainfall	0.58	6.57	1, 6	0.051		
Mean <i>Microcebus</i> $\delta^{15}\text{N}$ values	Whole model‡	0.99§	330,811.4	3, 4	0.0013		
	Mean C ₃ plant $\delta^{15}\text{N}$		805,406.4	1	0.0007	58.7	99.7
	Mean C ₃ plant $\delta^{13}\text{C}$		2348.39	1	0.0131	0.17	0.2
	Longitude		26,509.72	1	0.0039	0.004	0.01

*Model and total degrees of freedom are both reported.

†% Explained indicates the amount of the explained variation (adjusted r^2) that is accounted for by each explanatory variable. These values were calculated by dividing each variable's sum of squares (SS) by the total sum of squares.

‡Whole model includes the following variables: Mean C₃ plant $\delta^{15}\text{N}$ values, Mean C₃ plant $\delta^{13}\text{C}$ values, Longitude.

§The adjusted r^2 value is reported for the mouse lemur $\delta^{15}\text{N}$ model.

^{13}C -depleted relative to the upper canopy (van der Merwe & Medina, 1989).

Nitrogen

The average $\delta^{15}\text{N}$ values of *Microcebus* ranged from 5.4‰ to 16.2‰. This spread of nearly 10‰ is similar to that found among C₃ plants and is consistent with regional variation reported for herbivores in Africa, Australia and North America (Ambrose & DeNiro, 1986; Sealy *et al.*, 1987; Cormie & Schwarcz, 1994; Murphy & Bowman, 2006). Although our AIC-selected model included mean $\delta^{15}\text{N}$ values of C₃ plants, mean $\delta^{13}\text{C}$ value of C₃ plants and longitude, the mean $\delta^{15}\text{N}$ value of C₃ plants was by far the most essential variable, explaining 99.7% of the variation in mouse lemur $\delta^{15}\text{N}$ values.

The large, and very similar, overall range in $\delta^{15}\text{N}$ values for both mouse lemurs and C₃ plants accounts for the very strong correlation between these two variables. Although a negative relationship exists between rainfall and mouse lemur $\delta^{15}\text{N}$ values (Fig. 3), it would appear that this trend is mediated entirely by plants (Murphy & Bowman, 2006;

Hartman, 2011). Previous research has suggested that biome type might explain more of the isotopic variation in plants than climate because biome type encompasses more unmeasured variables than either temperature or precipitation (e.g. Diefendorf *et al.*, 2010). In a similar manner, mean C₃ plant $\delta^{15}\text{N}$ values also incorporate a number of unmeasured variables, enabling the strong relationship between mouse lemur and plant $\delta^{15}\text{N}$ values. These results demonstrate the central importance of vegetation in driving the isotopic composition of mouse lemurs. They also indicate that isotope values in *Microcebus* may serve as an acceptable proxy for habitat $\delta^{15}\text{N}$ values.

Behavioural and dietary implications

The mean differences between mouse lemur and plant isotope values (5.0‰ and 5.9‰, for carbon and nitrogen, respectively) resemble reported isotopic differences between faunivorous bats and leaves in a Malaysian forest (c. 5.4‰ and 6.4‰, for carbon and nitrogen, respectively), and are substantially larger than those reported for herbivorous and omnivorous bats and rodents (Hyodo *et al.*, 2010). Despite this overall similarity,

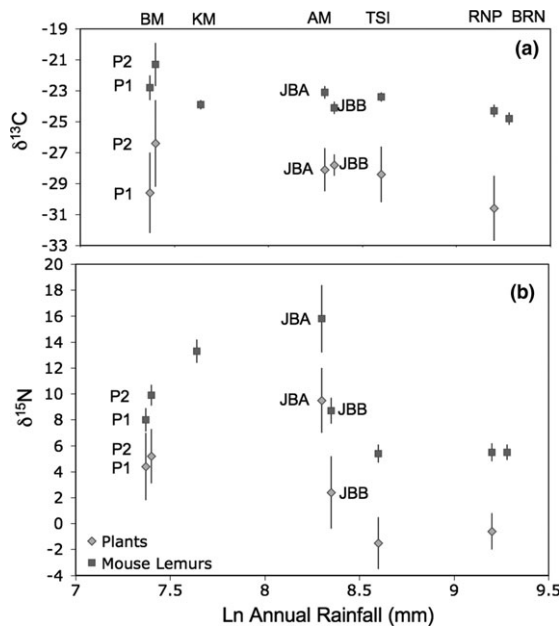


Figure 3 Relationship between mean annual rainfall and (a) mean $\delta^{13}\text{C} \pm 1\sigma$ and (b) mean $\delta^{15}\text{N} \pm 1\sigma$ for C_3 plants and mouse lemurs (*Microcebus* spp.) at each locality in Madagascar. Rainfall values for Ampijoroa (AM) and Beza Mahafaly (BM) have been shifted slightly to avoid overlap in error bars. Locality abbreviations: BRN, Betampona; KM, Kirindy Mitea; RNP, Ranomafana; TSI, Tsinjoarivo; P1, BM-P1; P2, BM-P2; JBA, AM-Jardin Botanique A; JBB, AM-Jardin Botanique B.

there is some variability in the isotopic differences between mouse lemurs and C_3 plants among localities (Table 6).

This variability could reflect differences in the relative degree of faunivory among populations. In particular, it is possible that the mouse lemurs at Ampijoroa-JBB and Beza Mahafaly consume less animal matter than mouse lemurs at other localities. Observed arthropod consumption is, indeed, lower at Beza Mahafaly-P1 than -P2, but arthropods still comprise > 20% of mouse lemur diet at Beza Mahafaly-P1 (Rasoazanabary, 2011). No direct feeding data are available for *M. murinus* at Ampijoroa-JBB; however, arthropod content in mouse lemur faeces was similar in Ampijoroa-JBA and Ampijoroa-JBB (Meusel, 2009).

Given the fact that CAM plants at Beza Mahafaly-P1 have lower $\delta^{15}\text{N}$ values than C_3 plants, and that *c.* 7.6% of the *M. griseorufus* diet at this locality is CAM-derived, it is, perhaps, unsurprising that the $\delta^{15}\text{N}$ difference at Beza Mahafaly-P1 is smaller than at other localities. The relatively small $\delta^{15}\text{N}$ difference at both Beza Mahafaly-P1 and -P2 may also reflect consumption of N-fixing legumes such as *Acacia bellula* and *Albizia* sp., although these tree species are favoured for nitrogen-poor plant exudates rather than protein-rich leaves (Rasoazanabary, 2011). Moreover, both *M. murinus* and *M. ravelobensis* retain large mouse lemur- C_3 plant $\delta^{15}\text{N}$ differences at Ampijoroa-JBA despite high rates of intake of leguminous plant exudates (Radespiel *et al.*, 2006). Despite

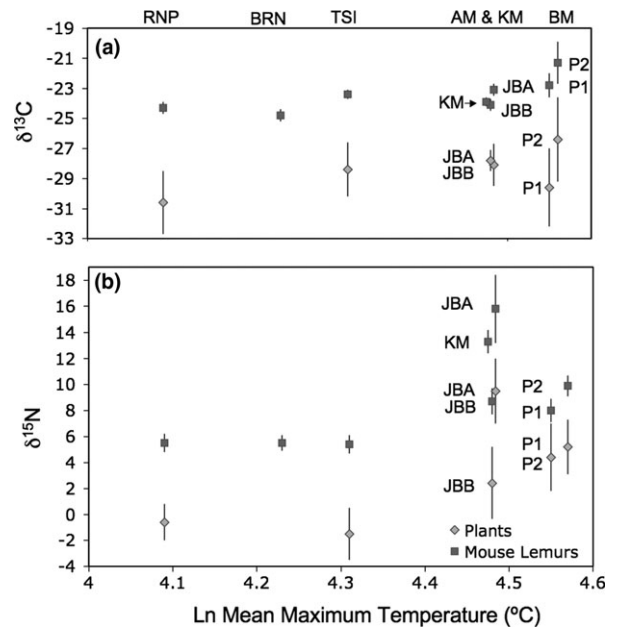


Figure 4 Relationship between mean maximum daily temperature and (a) mean $\delta^{13}\text{C} \pm 1\sigma$ and (b) mean $\delta^{15}\text{N} \pm 1\sigma$ for C_3 plants and mouse lemurs (*Microcebus* spp.) at each locality in Madagascar. Temperature values for Ampijoroa (AM) and Beza Mahafaly (BM) have been shifted slightly to avoid overlap in error bars. Locality abbreviations: BRN, Betampona Réserve Naturelle; KM, Kirindy Mitea; RNP, Ranomafana National Park; TSI, Tsinjoarivo; P1, BM-P1; P2, BM-P2; JBA, AM-Jardin Botanique A; JBB, AM-Jardin Botanique B.

some variability, the isotopic differences between mouse lemurs and C_3 plants are similar enough among localities to suggest that all populations obtain the majority of their protein from animal sources, such as arthropods. This finding is especially useful for localities where diets are poorly documented. For instance, the consumption of arthropods by *Microcebus* sp. cf. *M. rufus* at Tsinjoarivo is expected to resemble that of other populations based on a comparable mouse lemur-plant isotopic difference.

Perhaps surprisingly, such dietary similarity apparently extends to sympatric species. At Ampijoroa-JBA, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *M. murinus* and *M. ravelobensis* were very similar, implying substantial trophic overlap. Our results suggest that if competitive interactions among mouse lemurs shaped their behaviour, then space may have been a more limiting resource than food, although we acknowledge that isotopic data provide a relatively coarse measure of dietary specialization. This finding is in agreement with observations that sympatric cheirogaleids tend to have overlapping food resources (Radespiel *et al.*, 2006; Lahann, 2007; Dammhahn & Kappeler, 2008, 2010; Thorén *et al.*, 2011).

To test our conclusion that all mouse lemurs obtain the majority of their protein from animal sources, we compared mouse lemur isotope data with the available data for sympatric predominantly herbivorous *Propithecus edwardsi* from

Table 8 Isotope data for fur from sympatric lemur species (*Microcebus* spp.) at Beza Mahafaly-P1 (BM-P1) and Ranomafana (RNP) in Madagascar.

Locality	Species	<i>n</i> *	Lemur $\delta^{13}\text{C}$	Lemur $\delta^{15}\text{N}$	Source
BM-P1	<i>Lemur catta</i>	10	-24.9 ± 0.5	5.9 ± 0.2	Loudon <i>et al.</i> (2007)
BM-P1	<i>Lepilemur petteri</i>	9	-21.3 ± 0.8	5.5 ± 1.0	Schoeninger <i>et al.</i> (1998)
BM-P1	<i>Microcebus griseorufus</i>	157	-22.8 ± 0.8	8.0 ± 0.9	This paper
RNP	<i>Microcebus rufus</i>	82	-24.3 ± 0.4	5.5 ± 0.7	This paper
RNP	<i>Propithecus edwardsi</i>	–	-23.4 ± 0.2	2.1 ± 0.2	McGee & Vaughn (2003)

**n* was not reported for *P. edwardsi*.

Ranomafana and *Lemur catta* and *Lepilemur petteri*² from Beza Mahafaly-P1 (Table 8). The mean difference in $\delta^{15}\text{N}$ values between *P. edwardsi* and C_3 plants is 3.6‰ , half that calculated for *M. rufus* (7‰). However, the mean difference in $\delta^{13}\text{C}$ values between *P. edwardsi* and C_3 plants at RNP is 7.2‰ , slightly higher than the $\delta^{13}\text{C}$ difference between *M. rufus* and C_3 plants at Ranomafana (6.3‰). It is somewhat surprising that the $\delta^{13}\text{C}$ difference is higher for herbivorous *P. edwardsi* than for *M. rufus*. Based on the weak relationship between plant and mouse lemur $\delta^{13}\text{C}$ values, the strong correlation between plant and mouse lemur $\delta^{15}\text{N}$ values and the small overall spread in $\delta^{13}\text{C}$ values across habitats, we believe that the $\delta^{15}\text{N}$ difference is more accurately reflecting trophic dietary differences between these two species.

At Beza Mahafaly-P1, the presence of CAM plants makes it difficult to interpret mean $\delta^{13}\text{C}$ differences between *Lemur catta* or *Lepilemur petteri* and C_3 plants. The mean lemur–plant $\delta^{15}\text{N}$ differences are 1.5‰ and 1.1‰ for *Lemur catta* and *Lepilemur petteri*, respectively. As with Ranomafana, these differences are substantially smaller than that calculated for *M. griseorufus* (3.6‰). Because CAM plants have lower $\delta^{15}\text{N}$ values than C_3 plants at Beza Mahafaly-P1, consumption of CAM foods might be reflected in the lemur $\delta^{15}\text{N}$ values at this locality. The considerably smaller lemur–plant $\delta^{15}\text{N}$ differences for all three taxa at Beza Mahafaly-P1 compared with Ranomafana may reflect some CAM consumption (discussed below). Smaller $\delta^{15}\text{N}$ differences between lemurs and C_3 plants could also reflect some consumption of N-fixing leguminous taxa. Regardless, these results support our conclusion that mouse lemurs obtain the majority of their protein from arthropods. Despite variation in the $\delta^{15}\text{N}$ difference between mouse lemurs and C_3 plants, the difference for mouse lemurs is at least twice as large as the difference for sympatric herbivorous lemurs at both Ranomafana and Beza Mahafaly-P1.

Despite the prevalence of CAM plants at Beza Mahafaly, our mass balance models indicate that CAM-derived resources account for just 7.6% of the diet of *M. griseorufus* at Beza Mahafaly-P1, and that they are not used by *M. griseorufus* at

Beza Mahafaly-P2. Importantly, this model only considers the $\delta^{13}\text{C}$ values for CAM and C_3 plants. It does not take into account the $\delta^{13}\text{C}$ values for arthropods that may have been consumed by mouse lemurs. Because arthropods will be slightly enriched in ^{13}C relative to plants, CAM contributions calculated by this model should be taken as maximum estimates. Nevertheless, the $\delta^{13}\text{C}$ difference between arthropods and C_3 plants ($2\text{--}5\text{‰}$; Hyodo *et al.*, 2010) is much smaller than the $\delta^{13}\text{C}$ difference between CAM and C_3 plants (12‰ or more; Table 3). Thus the contribution of arthropods should not cause large overestimates of CAM-derived resources for mouse lemurs at Beza Mahafaly-P1.

In order to validate our %CAM estimates for mouse lemurs, we estimated the relative contribution of CAM foods to the diets of sympatric *Lemur catta* and *Lepilemur petteri* from Beza Mahafaly-P1. As in *M. griseorufus*, CAM foods are of minor importance to *Lemur catta* at this locality (Yamashita, 2002). Conversely, observations suggest that *Lepilemur petteri* consumes a fair amount of foliage from CAM plants (Schoeninger *et al.*, 1998). The $\delta^{13}\text{C}$ difference between keratin and C_3 plants is not documented for either of these species. If we use trophic fractionation values for herbivorous bats (2.4‰ ; Hyodo *et al.*, 2010), we estimate that CAM foods contribute 15.9% and 41.4% to the diets of *Lemur catta* and *Lepilemur petteri*, respectively. Although these values can only be considered rough estimates, they help authenticate our estimates for mouse lemurs; compared with *L. petteri*, CAM resources are of relatively minor importance for both *M. griseorufus* and *Lemur catta* at Beza Mahafaly. These calculations also demonstrate the degree to which small shifts in the $\delta^{13}\text{C}$ difference between lemurs and C_3 plants can affect estimates of %CAM.

As more isotope data for other lemurs become available, geographical patterns in mouse lemur isotope values will be useful for quantifying modern or historic dietary and habitat preferences of other extant taxa. They can also be used to infer dietary or habitat preferences of Late Pleistocene and early Holocene lemur communities.

CONCLUSIONS

Here we present the first analyses of spatial isotopic variation in Madagascar, and isotopic covariation between plants and primates on a large spatial scale. Relatively consistent $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ differences between mouse lemurs and plants agree well

²As reviewed by Mittermeier *et al.* (2010), the *Lepilemur* at Beza Mahafaly, once classified as *Lepilemur leucopus*, is now called *Lepilemur petteri*; the *Propithecus* at Ranomafana, once classified as *Propithecus diadema edwardsi*, is now *Propithecus edwardsi*.

with our anticipation of trophic similarity among mouse lemur populations. We found significant differences among localities in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for both plants and mouse lemurs. In agreement with our expectations, cooler, moist localities tended to have lower $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than warmer, drier localities. Rainfall influenced the $\delta^{13}\text{C}$ values of mouse lemurs, but surprisingly we did not find a significant correlation between lemur and plant $\delta^{13}\text{C}$ values, as expected if the effect of rainfall on lemur $\delta^{13}\text{C}$ values was mediated by food. In contrast, the $\delta^{15}\text{N}$ values of mouse lemurs were most strongly influenced by the mean $\delta^{15}\text{N}$ values of C_3 vegetation in each locality. We suspect that this difference between results for carbon and nitrogen is largely an artefact of the much greater among-habitat spread in $\delta^{15}\text{N}$ than $\delta^{13}\text{C}$ values. Within-habitat variability in both lemurs and plants is larger for $\delta^{15}\text{N}$ values than $\delta^{13}\text{C}$ values (Tables 3 & 6), yet the plant-to-lemur correlation is significant for nitrogen isotopes (which exhibit large among-habitat variability) but not significant for carbon isotopes (which exhibit smaller among-habitat variability). Given these observations, we suspect that if we had a larger number of sites with lemur–plant comparisons, that a significant relationship between lemur and plant $\delta^{13}\text{C}$ values would emerge. These results highlight the importance of incorporating isotope data from primary producers into spatial studies. More importantly, however, they indicate that *Microcebus*, which is both widespread and abundant, is an excellent indicator taxon for tracking baseline isotopic differences among habitats, particularly for $\delta^{15}\text{N}$ values. Given the very wide range in $\delta^{15}\text{N}$ values among different regions of Madagascar, or even different types of habitat in the same region, developing isotopic baselines will be crucial for identifying niche partitioning in both modern and ancient communities. It will also simplify large-scale comparisons of communities. Using variation in *Microcebus* to establish such a baseline will facilitate future research on modern mammal communities in Madagascar as well as ecological interpretations of Holocene communities.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Raw isotope data for plant samples.

Appendix S2 Raw isotope data for lemur samples.

Appendix S3 Additional results.

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BIOSKETCH

Brooke E. Crowley is a post-doctoral fellow in the Department of Anthropology at the University of Toronto. She received her PhD in ecology and evolutionary biology in 2009, an MA in anthropology in 2008, and an MS in earth science in 2005. Her research focuses on the application of stable isotope biogeochemistry to geological, ecological and palaeontological questions. Most recently, she has explored the causes and effects of Holocene extinction events in Madagascar.

Author contributions: B.E.C. and P.L.K. conceived the ideas; M.A.B., M.B.B., K.C.M., S.A.N., E.R., M.T.I., S.T. and S.Z. collected the data; L.R.G., U.R. and P.C.W. facilitated data collection; B.E.C., L.R.G. and E.R.V. analysed the data; and B.E.C., N.J.D., L.R.G. and P.L.K. did the writing. All authors contributed with draft revisions.

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