RESEARCH ARTICLE

Unpredictable Environments, Opportunistic Responses: Reproduction and Population Turnover in Two Wild Mouse Lemur Species (*Microcebus Rufus* and *M. Griseorufus*) From Eastern and Western Madagascar

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Small-bodied, nocturnal mouse lemurs (*Microcebus*) are widespread across diverse forest habitats in Madagascar. They are strict seasonal breeders and can, depending on the habitat and species, undergo daily or prolonged torpor to minimize energy expenditure during periods of food and water scarcity. Duration of reproduction, number of litters per season and timing of births vary across individuals and species. The "polyestry-seasonality" hypothesis proposes that the duration of reproduction and number of litters per year are positively correlated with rainfall but negatively correlated with longevity, whereas the "hypervariability" hypothesis suggests that the duration of reproduction is negatively correlated with the degree of predictability of food resources. We test these hypotheses in two mouse lemur species inhabiting contrasting habitats, the brown mouse lemurs, *Microcebus rufus*, from Ranomafana (a less seasonal and more climatically predictable habitat) and the gray-brown mouse lemurs, *M. griseorufus*, from Beza Mahafaly (a more seasonal and less climatically predictable environment). We use capture/mark/recapture techniques and records of female reproductive status. We found evidence of polyestry at both study sites but faster population turnover and longer duration of the reproductive season at Beza Mahafaly. The "polyestry-seasonality" hypothesis is not supported but the "hypervariability" hypothesis could not be rejected. We conclude that reproductive output cannot be tied to climatic factors in a simple manner. Paradoxically, polyestry can be expressed in contrasting habitats: less seasonal forests where females can sustain multiple reproductive events, but also highly seasonal environments where females may not fatten sufficiently to sustain prolonged torpor but instead remain active throughout the year by relying on fallback resources. Am. J. Primatol. 77:936–947, 2015. © 2015 Wiley Periodicals, Inc.

Key words: brown mouse lemur; gray-brown mouse lemur; Ranomafana; Beza Mahafaly; reproduction; population

INTRODUCTION

The mouse lemurs of Madagascar (genus *Microcebus*) are a speciose group of small-bodied (30–80 g) nocturnal lemurs. They are omnipresent in Madagascar's habitats from rainforests, deciduous dry and spiny forests to degraded forest fragments where no other lemurs can survive [Kobbe and Dausmann, 2009; Rasoloarison et al., 2013]. They display ecological flexibility in that they can employ daily and/or seasonal torpor (i.e., heterothermy) as an energy saving strategy and practice dietary opportunism by exploiting a variety of food resources [Kobbe et al., 2011; Schmid and Ganzhorn, 2009; Wright, 1999]. They are, however, strict seasonal breeders [Blanco, 2008, 2010].

Mouse lemurs have been intensively studied in the laboratory thanks to their small body size, fast generation times, adaptability and resilience to environmental stress [e.g., Génin, 2007; Génin et al., 2005; Génin and Perret, 2003; Perret, 1992; Perret and Aujard, 2001]. Gray (*M. murinus*) and Goodman's (*M. lehilahytsara*) mouse lemurs maintain reproductive seasonality under laboratory

conditions. Females undergo 1–4 estrous cycles per "season" and can raise up to two litters per year [Perret, 1992, 1994; Radespiel and Zimmermann, 2001; Wrogemann and Zimmermann, 2001; Wrogemann et al., 2001].

In the wild, mouse lemurs begin reproduction prior to the rainy season. Yet, the timing and duration of reproduction, including the number of litters females have per year, vary among mouse lemur species and between populations of the same species occupying different forests [Blanco, 2011; Lahann et al., 2006; Randrianambinina et al., 2003; Schmelting et al., 2000]. For example, gray mouse lemurs have one litter per year at Kirindy, a dry deciduous forest in western Madagascar [Eberle and Kappeler, 2004] but at least two litters at Mandena and Ampijoroa, a littoral southeastern forest and a northern dry deciduous forest, respectively [Lahann et al., 2006; Schmelting et al., 2000]. Differences in timing and duration of the reproductive season have been also reported in populations of brown mouse lemurs at Ranomafana [Atsalis, 2008; Blanco, 2005, 2010] and golden-brown mouse lemurs (*M. ravelobensis*) at Ampijoroa [Randrianambinina et al., 2003; Schmelting et al., 2000; Weidt et al., 2004].

A survey of the literature on wild mouse lemur reproduction, however, indicates that evidence of regular polyestry, i.e., renewed estrus after successful infant survival, has been reported primarily in mouse lemur populations from northwestern Madagascar, which have been extensively studied [Eberle and Kappeler, 2004; Schmelting et al., 2000]. Regular polyestry may be underreported in other species, however, due to logistic difficulties in capturing or tracking individuals during the rainy season when females are likely lactating or weaning their first offspring before or around the time they may be undergoing a second estrus. Indeed, indirect evidence of polyestry, i.e., females observed gestating or lactating well after presumed parturition, suggests that this may be the case [Blanco, 2010]. Multiple reproductive opportunities per season may also occur when a female loses her offspring and resumes estrus soon afterwards (i.e., rebound polyestry). The distinction between rebound and regular polyestry in the field is difficult without direct evidence of infant survival and these types of polyestry are generally inferred on the basis of inter-estrous intervals [Blanco, 2008, 2010].

Environmental factors have been posited as primary determinants of lemur reproductive schedules. For instance, Rasmussen [1985] compared the timing of reproduction in several diurnal lemur species in captivity and showed that photoperiodic cues (including a species-specific light-dark threshold sensitivity) and a period of physiological and "social" preparation could explain variation in reproductive onset of lemurs bred at different latitudes. A link between photoperiod and the timing of reproduction had also been proposed for captive nocturnal lemurs [Petter-Rousseaux, 1980; Radespiel and Zimmermann, 2001; Wrogemann et al., 2001]. In his pioneering field study, Martin [1973] observed differences between western and southeastern gray mouse lemurs in the timing of reproduction. Martin suggested that a later onset of rains in the dry forests could explain the month-long delay in reproductive activity of western mouse lemurs. Although his work preceded the finding of photoperiodic regulation in lemurs, latitude alone (a ~5 min difference in day length between locations) would appear to be insufficient to explain the observed month-long difference in reproductive onset. More recently, field studies from eastern Madagascar support the argument for strong photoperiodic regulation of reproduction. Brown mouse lemur females begin reproducing at Ranomafana about a month after the vernal equinox and undergo estrus within 3–4 weeks of each other. Individual females, whose reproductive status was monitored over multiple years, showed inter-annual estrous periodicities of around 365 days, regardless of their body condition [Blanco, 2011]. Additional factors may account for differences in reproductive onset as well as the duration of reproduction in mouse lemurs. Climatic factors, such as rainfall and temperature could correlate with the duration of the reproductive season and the expression of polyestry in mouse lemurs because these factors can affect resource availability [Lahann et al., 2006].

Using comparative analyses of wild populations, Lahann et al. [2006] linked the duration of reproduction and expression of polyestry with habitat seasonality in gray mouse lemurs, stating that extended periods of food availability in habitats with high rainfall per year (and low seasonality) would provide energy for pregnant and lactating females later in the reproductive season and ultimately favor an increase in reproductive rates, i.e., more litters. However, mouse lemurs inhabiting less seasonal environments would not live very long, trading longevity for higher reproductive rates. Among the possible factors affecting survival rates in less seasonal habitats (by virtue of individuals being more active throughout the year) are higher exposure to predators, higher parasitic loads (with detrimental effects on the immune system) and faster aging due to physiological strain. We call this the "polyestry-seasonality hypothesis."

An alternative hypothesis was presented by Génin [2008] in his study of *M. griseorufus* (gray-brown mouse lemur) at Berenty in southern Madagascar. He suggested that the duration of reproduction should be positively correlated with climate unpredictability rather than seasonality. Berenty is a dry and seasonal forest that is highly unpredictable due to sea current oscillations such as El Niño/La Niña. At Berenty, gray-brown mouse lemur females

lactate repeatedly, from the beginning of December to the end of April. Génin observed rebound (renewed estrus after loss of offspring) polyestry and considered that mouse lemur females at Berenty had comparatively longer reproductive seasons than mouse lemurs inhabiting more climatically predictable habitats. Harsh conditions at this forest would increase mortality rates as a result of predation and produce food shortages under extreme aridity. Such conditions might be absent at other dry forests (such as Kirindy) that experience higher climate predictability. We call this the "hypervariability hypothesis."

Although Madagascar is characterized by hyper-variable environments, eastern regions are less seasonal, albeit with high intra-annual variation in rainfall, whereas the central, northern, western, and southern regions are highly seasonal with high inter-annual variation in total rainfall and in the length of the dry season [Dewar and Richard, 2007]. In eastern Madagascar, some precipitation falls predictably in almost every month of the year. Temperature also

expectedly varies throughout the year in Madagascar's forests, particularly at high elevation eastern sites where values can decrease below 0°C occasionally during the dry season; intra-daily temperature variation is particularly marked in western dry deciduous forests where there may be more than 30°C difference between the coldest and hottest time of day [Blanco et al., 2013; Dausmann et al., 2004]. To date, relationships between seasonality (or climate unpredictability) and reproductive parameters such as polyestry have not been directly investigated in mouse lemurs. In this study, we present population and reproductive data from two mouse lemur species living under very different environmental conditions: brown mouse lemurs (*M. rufus*) from Ranomafana, a southeastern rainforest, and gray-brown mouse lemurs (*M. griseorufus*) from Beza Mahafaly, which includes mesic (riparian), dry deciduous and spiny forests. Skeletal remains of mouse lemurs from Beza Mahafaly were also included in our study; by including these, we could test for polyestry using
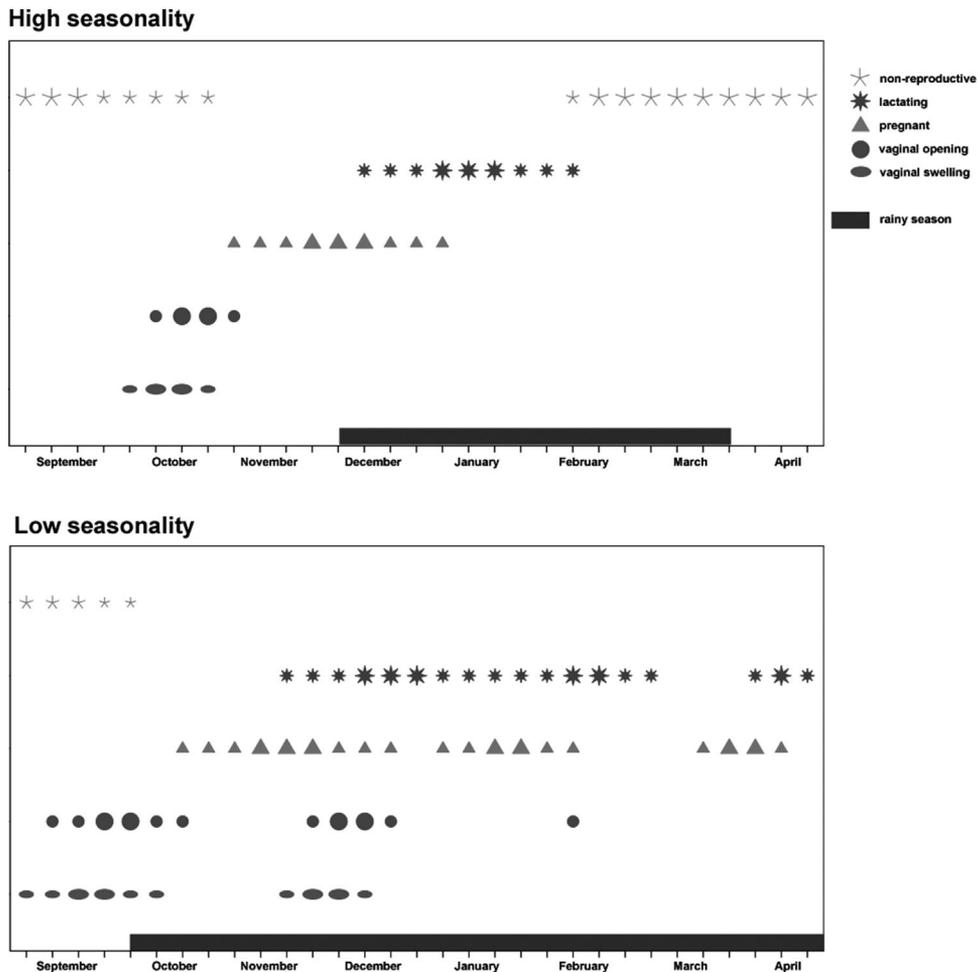

Fig. 1. Predictions of the Polyestry-Seasonality Hypothesis for gray mouse lemurs as represented by a dry deciduous forest characterized by high seasonality (top) and a less seasonal forest (bottom). Symbol size increases with frequency of observations.

birth date estimates for immature skeletons collected from owl pellets at different times of the year. Under Génin's [2008] habitat ranking, which was a modification of Dewar and Richard's [2007] classification, the climate of Ranomafana is highly predictable (with a score of 1 on a scale of 1–4, i.e., the lowest score for "unpredictability") whereas that of southern habitats such as Beza Mahafaly is not (not ranked by Génin, but habitats with similar characteristics are given scores of 4) [Fig. 1].

We compare the reproductive profiles, e.g., duration of reproduction, expression of polyestry and population parameters such as density and recapture success (i.e., index of population turnover) of the two species to test the predictions of the "polyestry-seasonality" and "hypervariability" hypotheses.

The "polyestry-seasonality" hypothesis predicts:

1. Brown mouse lemur females from Ranomafana (less seasonal) should express a high degree of polyestry (i.e., have more than one litter per season) whereas gray-brown mouse lemur females from Beza Mahafaly (more seasonal) should have a single litter per year.
2. Annual recapture should be lower in brown mouse lemurs at Ranomafana (if reproductive rates are positively correlated with population turnover) and higher in gray-brown mouse lemurs at Beza Mahafaly.
3. Life span of mouse lemurs at Ranomafana (less seasonal habitat) should be shorter than at Beza Mahafaly (more seasonal habitat).

The "hypervariability" hypothesis predicts:

1. The mouse lemur reproductive season at Beza Mahafaly (with its less predictable climate) should be longer than the reproductive season at Ranomafana (with its more predictable climate).
2. Life span of mouse lemurs at Beza Mahafaly should be shorter than at Ranomafana.

Alternatively, if environmental variables and reproductive parameters are not correlated in a simple manner, the expression of polyestry should be unrelated to the degree of habitat seasonality or climate unpredictability.

METHODS

This research was conducted under permission of institutional and governmental agencies that regulate animal research in Madagascar and adhered to the American Society of Primatologists Principles for the Ethical Treatment of Nonhuman Primates. Research protocols complied with those approved by the University of Massachusetts Animal Care and Use Committee.

Captures at Ranomafana

We used capture/mark/recapture techniques at Ranomafana National Park (47° 18′ E and 21° 02′ S, 1000 m), a southeastern rainforest. The Park includes 43,500 hectares from lowland to montane rain forest [Atsalis, 2000; Wright and Andriamihaja, 2002]. We conducted the trapping within a portion of the Talatakely Trail System, a section of the Park that was selectively logged in 1986 and 1987 and includes an area of 5 km² mapped trail system. This area of the Park is heavily visited by tourists [Wright and Andriamihaja, 2002].

We sampled brown mouse lemurs at Ranomafana between 2004 and 2008 (three months, October to December in 2004 and 2005; four months, January, October to December in 2006; three months, January, October, and December in 2007; and three months, January, February, and October in 2008). For more details on trapping schedules see Blanco [2011]. The total number of sampling months was 16, most of which fell in the rainy season (December to March) but some of which fell at the beginning of the reproductive season (October and November).

We placed up to fifty Sherman live traps along selected trails at about 25–50 m intervals, covering an area of approximately 30 hectares. Traps were baited with a small piece of fresh banana and set ∼17:00 h at heights of about 1–2 m. Traps were checked between 19:30 and 20:30 h and all captured mouse lemurs were brought to the Centre ValBio Research Station, where we identified or marked them with Avid microchips, weighed them, and checked their reproductive status. Captured mouse lemurs were released at around midnight on the day of capture. We set an average of 25 traps per night during 236 nights resulting in a total of 5,930 trap nights.

Captures at Beza Mahafaly

Beza Mahafaly Special Reserve, located in southwestern Madagascar (44° 37′ E and 23° 39′ S, 150 m), is ecologically diverse with 80 hectares of riparian and 520 hectares of spiny thicket forests [Axel and Maurer, 2011]. The two are separated by a transitional forest which is not a part of the reserve. A dry deciduous forest (also not part of the reserve) lies just southeast of the riparian forest; it is separated from the riparian forest by the Sakamena River which is dry most of the year.

We captured *M. griseorufus* at Beza Mahafaly between 2003 and 2007 (five months, April to August, in 2003; four months, September to December, in 2004; three months, February, April, and July, in 2005; three months, October to December, in 2006; and nine months, January to September, in 2007). The total number of months during which sampling occurred over the research period was 24, and every month of the year was sampled at least once.

Trapping schedules in the three forests varied from year to year. In 2003, 2004, and 2005, trapping occurred in successive weeks, one forest at a time. In 2006 and 2007, three teams trapped simultaneously at each forest. Traps were set at varying heights (0–2 m above ground) at distances of 25 m, although some were set at smaller distances in small "random" sampling areas (i.e., reduced sampling areas were selected randomly and periodically to supplement data collection in the main sampling areas at each forest). The total number of traps set in the three forests also varied from year to year. From September 2004 to July 2005, 4,320 traps were set in the riparian forest, 3,240 in the dry deciduous forest, and 4,560 in the spiny forest. From October 2006 to September 2007, 33,120 traps were set in each forest.

We set Sherman traps, baited with banana, at dusk (between 17:00 and 18:00 h). Traps were checked at dawn (between 5:00 and 7:00 h). All traps containing captured animals were brought to the campsites, where animals were checked for reproductive status, marked, microchipped, measured, weighed, and monitored before being released at their capture sites in the afternoon of the same day.

Evaluating Reproductive Status

Reproductive condition of mouse lemur females was assessed by examining vaginal and nipple morphology. Females display vaginal openings during estrous cycles, preceded by a period of progressive vaginal swelling. Vaginal openings can be observed also soon after parturition but can be visually distinguished from openings during estrus. Female reproductive status was scored as follows: (1) female displayed a vaginal swelling, (2) female displayed a vaginal opening, (3) female was pregnant, (4) female was lactating, (5) female did not show evidence of reproductive activity. A score of six denoted unrecorded reproductive status. Especially during its second half, pregnancy could be determined by palpation and by assessing weight gain profiles. Early pregnancies could be inferred *a posteriori* if females were recaptured in advanced stages of gestation or during lactation. Lactation could be assessed by enlarged and well-developed nipples. Reproductive observations were clustered by month and week. We use an inclusive definition of polyestry, i.e., any evidence of more than one reproductive event for single individuals within a season. This definition thus includes rebound as well as regular polyestry.

Evaluating Population Parameters, Recapture Success, and First-to-Last Capture Periods

We estimated density and population sizes using a maximum likelihood-based spatially explicit capture-recapture model (ML SECR) in the program Density 5.0 [Efford, 2012]. This model assumes closed populations with home ranges that are invariable and comparable in size between individuals. Although assumptions may not be fully met by our study populations, estimates may be justified given the high number of capture/recaptures obtained from our samples (between 66 and 400 recaptures per site; individuals were captured ~7 and ~5 times on average at Ranomafana and Beza Mahafaly, respectively). GPS locations were used to estimate effective sampling areas. A "buffer" area of 50 m was selected from the outermost trap locations in all cardinal directions to account for home range areas not included in the trapping area. Buffer width was calculated assuming a home range of 1 ha, and that outermost traps were in the core of the range of the mouse lemurs trapped at that location. Population size refers to the number of individuals predicted to occupy the effective sampling areas and it is therefore highly sensitive to areas devoted to trapping. Effective trapping areas are delineated only by "successful" locations, i.e., those traps in which at least one individual was captured. Traps that were set but remained empty during the selected sampling period were not entered in the computation. Due to differences in trapping effort and schedules between sites, we selected similar months and years for comparison. We pooled capture data from October 2006 and 2007 at Ranomafana and combined data from October 2006 and September 2007 for each study site (riparian, spiny and dry deciduous forests) at Beza Mahafaly.

Recapture success was determined by the proportion of total individuals captured in a given year that had also been captured in at least one previous year. Trapping success refers to the percentage of captured mouse lemurs over total number of set traps. Recapture success at Ranomafana was estimated from individuals trapped in October–November, i.e., at the beginning of the reproductive season when trapping success is the highest, between 2004 and 2007 (i.e., data recorded in October for 4 years and in November for 3 years). At Beza Mahafaly, trapping success was higher in the dry season than in the wet season, and sampling schedules changed from year to year, so that the interval between capture dates in consecutive years varied from only a few months to almost two years.

Additionally, we calculated the period (in days) between the first and last capture for each individual and compared these values for sexes within and between sites using independent *t*-tests in SPSS 22.0.

Skeletal Sample Collection From Near Beza Mahafaly

Skeletons were found in owl pellets that were collected by Steve Goodman between November 1990 and November 1991 at a forest near the village of Ambinda [Goodman et al., 1993a, b], close to the

TABLE I. Recapture Success at Ranomafana Based on Trapping Conducted Between 2005 and 2007

Year	Total # mouse lemur individuals	First captures	Recaptures	Percentage of total individuals captured in given year that had also been captured in at least one previous year
2005	51 (28 ♀ 23 ♂)	36 (19 ♀ 17 ♂)	15 (9 ♀ 6 ♂)	29.4 (17.6 ♀ 11.8 ♂)
2006	58 (28 ♀ 30 ♂)	29 (11 ♀ 18 ♂)	29 (17 ♀ 12 ♂)	50 (29.3 ♀ 20.7 ♂)
2007	61 (30 ♀ 31 ♂)	29 (17 ♀ 12 ♂)	32 (13 ♀ 19 ♂)	52.5 (21.3 ♀ 31.2 ♂)

reserve at Beza Mahafaly. The skeletal remains of individuals killed by owls and preserved in owl pellets [Goodman et al., 1993a, b] were collected and donated to the Field Museum of Natural History in Chicago (FMNH). These specimens were measured and aged on the basis of developmental markers recorded for known-aged individuals. For more on age estimation methods used in this study see Godfrey et al. [2001]. Captive data on known-aged skeletons supplemented wild data in age determination.

RESULTS

Ranomafana: Captures

A total of 133 individual mouse lemurs (65 females, 68 males) was trapped 1,474 times between 2004 and 2008. Overall annual recapture success ranged between 29 and 52% (Table I). Over 29% of mouse lemurs captured in 2005 were marked in 2004; 15.5% of individuals captured in 2006 were marked in 2004 and 32.8% were marked in 2005; 6.6% of mouse lemurs captured in 2007 were marked in 2004, 18% were marked in 2005 and 27.9% in 2006. Trapping success was high in October (30–45%) but decreased during the following months, November (23–34%), December (10–15%), January (12% in 2007, but 0.6% in 2008), February (1%). If trapping success is plotted by year, percentages were 23.8% in 2004, 20.5% in 2005, 24% in 2006, 22.7% in 2007, and 16.3% in 2008.

Beza Mahafaly: Captures

A total of 435 individual mouse lemurs (200 females, 235 males) was marked with Avid microchips and trapped 2055 times between 2003 and 2007

in all combined study sites. Overall annual recapture success ranged between 8 and 29% (Table II). Over 19% of mouse lemurs captured in 2004 were marked in 2003; 6.4% of individuals captured in 2005 were marked in 2003 and 22.2% were marked in 2004; 2.9% of mouse lemurs captured in 2006 were marked in 2003, 5.7% were marked in 2004 and 14.3% in 2005. Of mouse lemurs captured in 2007, 1.7% were marked in 2004, 1.3% in 2005, and 4.7% in 2006. Trapping success was very low at Beza Mahafaly although there were differences across forest types and years. In 2003, trapping success was 1.4% in the riparian forest, 2.5% in the dry deciduous forest, and 4% in the spiny forest site. For the 2004–2005 seasons, percentages reported at the three study sites are as follows: 2.7% in riparian forest, 2.1% in deciduous forest, and 2.7% in spiny forest. For the 2006–2007 seasons there was a decrease in trapping success across sites: 2.1% in the riparian forest, 1% in the deciduous forest, and 1.4% in the spiny forest.

Ranomafana: Reproduction

A total of 280 reproductive observations from 50 mouse lemur females at Ranomafana were included in this analysis. Most females showed swollen or open vaginas (proxy for estrus) in October, were gestating in November and December and were lactating in late December and January (Table III). The percentages of females displaying vaginal swelling or openings varied by month and year: 43% (may be underestimated because of lack of experience by researcher during first year of data collection), 85, 94, and 89% of females displayed vaginal swelling or openings in October 2004, 2005, 2006, and 2007, respectively. During the month of

TABLE II. Recapture Success at Beza Mahafaly Based on Trapping Conducted Between 2004 and 2007

Year	Total # mouse lemur individuals	First captures	Recaptures	Percentage of total individuals captured in given year that had also been captured in at least one previous year
2004	76 (41♀ 35♂)	61 (34 ♀ 27♂)	15 (7♀ 8♂)	19.7 (9.2 ♀ 10.5♂)
2005	63 (35♀ 28♂)	46 (22♀ 24♂)	17 (13 ♀ 4♂)	27.0 (20.6 ♀ 6.3♂)
2006	35 (21♀ 14♂)	27 (16♀ 11♂)	8 (5♀ 3♂)	22.9 (14.3 ♀ 8.6♂)
2007	232 (110♀ 122♂)	214 (95 ♀ 119♂)	18 (15♀ 3♂)	7.8 (6.5♀ 1.3♂)

TABLE III. Reproductive Observations From Brown Mouse Lemur Females at Ranomafana; Years (2004, 2005, 2006, 2007) are Combined; n = Total Number of Reproductive Observations Within a Week. Numbers of Females on Which These Observations Were Based are in Parentheses. Reproductive Codes are as Follows: 1 = Vaginal Swelling, 2 = Vaginal Opening, 3 = Gestating, 4 = Lactating, 5 = No Reproductive Activity, 6 = Unknown Reproductive Status

Month	Week	n	1	2	3	4	5	6
September	2	1 (1)					1	
	3	1 (1)					1	
October	4	5 (5)	2	1			2	
	5	20 (18)	8	6			5	1
	6	29 (23)	7	17	1		3	1
	7	41 (30)	2	17	6		7	9
November	8	21 (18)	1	2	8		4	6
	9	22 (19)	1	1	13		3	4
	10	9 (9)		2	5		2	
	11	19 (17)			11		6	2
December	12	15 (14)			7	1	3	4
	13	29 (20)	1		14	7	5	2
	14	14 (12)	1		2	10		1
	15	13 (11)	1			11	1	
January	16	12 (10)				11	1	
	17	8 (8)				8		
	18	19 (12)	6		1	12		
February	21	2 (1)			1	1		

TABLE IV. Reproductive Observations From Gray-brown Mouse Lemur Females at Beza Mahafaly (Years 2004–2005, 2006–2007 are Combined); n = Total Number of Reproductive Observations Within a Week. Numbers of Females on Which These Observations Were Based are in Parentheses. Reproductive Codes are as Follows: 1 = Vaginal Swelling, 2 = Vaginal Opening, 3 = Gestating, 4 = Lactating, 5 = No Reproductive Activity, 6 = Unknown Reproductive Status

Month	Week	n	1	2	3	4	5	6
September	0	33 (33)	10	2			21	
	1	22 (22)	8				14	
	2	25 (25)	6	2	4		13	
	3	32 (31)	9	5	8		10	
October	4	11 (11)		4	5			2
	5	5 (5)		1	4			
	6	11 (11)	7	3			1	
	7	11 (11)	6	5				
November	9	1 (1)		1				
	10	10 (10)		1	7	1	1	
	11	6 (6)			4	1		1
December	12	2 (2)			2			
	13	1 (1)			1			
	14	3 (3)			1	2		
	15	1 (1)					1	
January	16	7 (7)			1	5	1	
	17	3 (3)			3			
	18	4 (4)			1	3		
	19	2 (2)				1	1	
February	20	11 (11)	1		5		5	
	21	5 (5)		1	3		1	
March	24	1 (1)					1	
April	28	11 (11)			6		5	
	29	23 (23)			8		13	2
	30	4 (4)			3		1	
May	32	1 (1)					1	
	33	4 (4)					4	
	34	6 (6)					6	
	35	8 (8)					8	
June	37	2 (2)					2	
	38	1 (1)					1	
July	40	1 (1)					1	
	41	6 (6)					6	

November, 40% of females were pregnant in 2004, 69% in 2005, and 70% in 2006. With the exception of 2004, where only 20% (2 out of 10) showed signs of lactation, the majority of females lactated at some time in December: 67% in 2005, 100% in 2006, and 100% in 2007. Finally, in January, 87% (2005), 92% (2006), and 100% of females showed signs of lactation. These percentages include only observations for which reproductive status could be assessed (i.e., scores between 1 and 5 as shown in Table III). Indirect evidence of polyestry was found in 11 females: three brown mouse lemur females were observed with vaginal swelling in mid-December and six exhibited swollen vaginas in mid-January. These females (with the exception of one female captured in mid-December for the first time) had been observed pregnant earlier the same year. One individual was likely pregnant in mid-January, whereas another female appeared to be pregnant in February (Table III).

Beza Mahafaly: Reproduction

The reproductive status of 112 females was recorded during the study period: 62 females captured in 2004–2005 and 57 in 2006–2007 (seven females were trapped during both sampling periods) (Table IV). Twenty one percent (12 out of 57) of females trapped in September were pregnant. Sixty-three percent of females captured in October (19 of 30) showed vaginal swelling or vaginal opening indicative of estrus whereas 30% were pregnant ($n = 9$). Lactation started in late November, but only two of the 18 females captured during that month showed signs of lactation. Twelve females (66%) were still pregnant. In December, seven females were captured of which three were pregnant and three were lactating. In February, seven of 14 captured females were gestating, five were not pregnant, and two showed vaginal activity consistent with estrus. In April, 36 females were captured, 17 of which showed signs of being pregnant. Only during the driest months (May through July) were all captured females "non-reproductive." It should be

noted, however, that despite the lack of observations, a portion of the female population was likely lactating in May, as individuals were caught pregnant during the previous month. Individuals for whom direct evidence of polyestry existed included an individual found pregnant on September 16, 2004, February 17, 2005, and April 4, 2005. Another individual was found to be pregnant on February 2, 2005 and April 21, 2005. Additional evidence of polyestry was found in females captured in 2006–2007.

In sum, there was evidence of polyestry at both sites. At Ranomafana, at least 11 females (out of 50 total captures all years, 22%) likely experienced a multi-estrous season. It should be noted that 54% of those females (6 of 11) were captured during the 2006 season. Adjusted for the number of females captured that particular year ($n = 28$), the percentage of likely polyestrous females amounts to 21.4%. At Beza Mahafaly, 12 females (out of 112 total, 10.7%) were actually observed undergoing multiple pregnancies within a season. Although the duration of the reproductive season cannot be directly compared between sites due to differences in the trapping schedules, it appears that females at Beza Mahafaly

begin reproduction at least a month earlier than at Ranomafana (i.e., in September as opposed to October) and as early as two months before (i.e., some females were observed pregnant in September), and may remain reproductively active until later (April), extending the reproductive period to more than 9 months per year, unlike brown mouse lemurs at Ranomafana, who have a reproductive season of ~7 months [Fig. 2].

Skeletal Sample Collection: Reproduction

Table V shows evidence of polyestry in *M. griseorufus* at Beza Mahafaly in skeletons of mouse lemurs found in owl pellets that were collected by Steven Goodman and made available for the study at the Chicago Field Museum. Here too is evidence of the existence of two mouse lemur birth periods, with very young individuals (judged to be around one month in skeletal age) appearing in the population in December and equally young individuals appearing in the months of April and May when older immature individuals (also found in owl pellets) are also in the population.
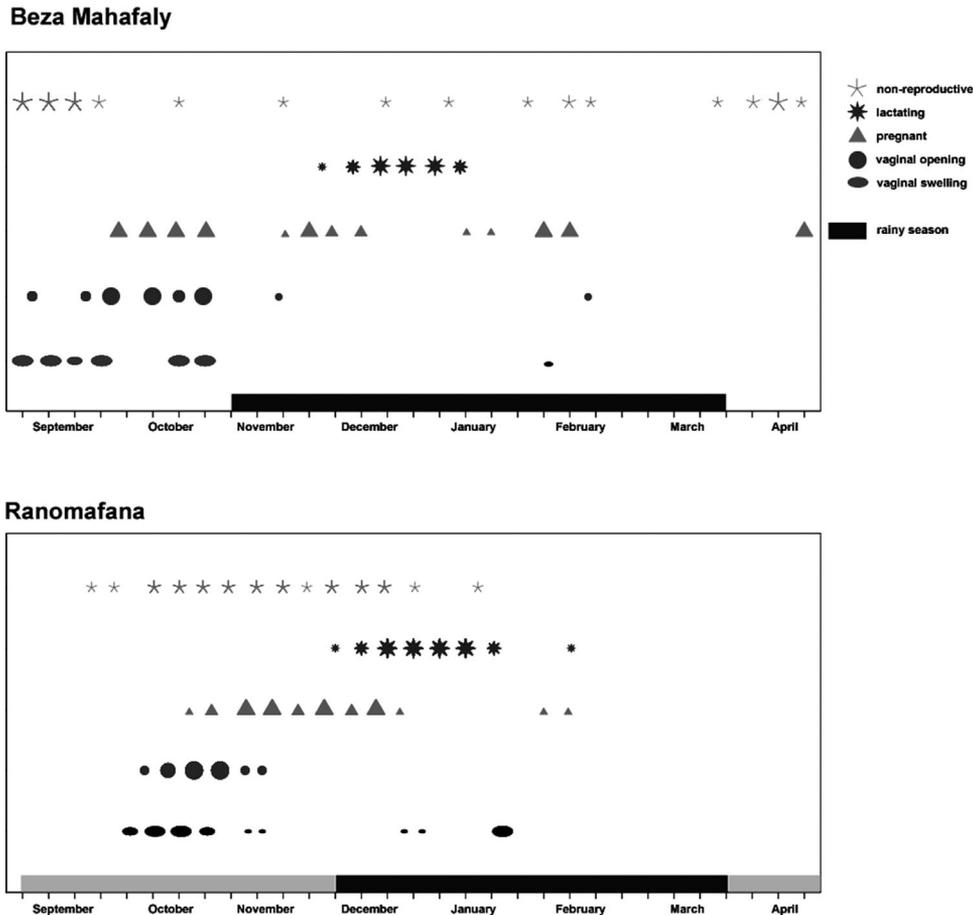

Fig. 2. Reproductive observations from gray-brown mouse lemur females at Beza Mahafaly (highly seasonal, low rainfall levels) and brown mouse lemur females at Ranomafana (less seasonal, high rainfall levels). Symbol size increases with frequency of observations.

TABLE V. Very Young *M. griseorufus* Individuals Found in Owl Pellets at Ambinda, and Now Housed at the Field Museum of Natural History in Chicago (FMNH)

Date owl pellet or specimen was collected	Skeletons of very young wild individuals from Ambinda (with inferred ages of ~two months or younger)	Notes
December 6, 1990	FMNH 1557a, b, e, f, Humeral diaphyses short (12.5–13.7 mm), deciduous dentition present but adult toothcomb erupted, M1 and m1 erupted, M2 erupted, M3 and m3 in crypt, p2 erupting, distal humerus unfused or fusing, no other humeral or femoral epiphyses fused.	Four individuals, no older infants or juveniles in pellet.
December 20, 1990	FMNH1569, Skull and mandible with milk teeth, permanent incisors and toothcomb erupting, upper and lower first and second molars erupted; humerus and femur with unfused epiphyses; diaphysial lengths 17.3 mm (femur) and 13.4 mm (humerus).	One individual, ~one or two months.
March, 1990	FMNH 1574 a, b, c, No postcrania, milk dentition, M1 erupted, M2 erupting, toothcomb erupting, m1 and m2 erupted. Third molars in crypt.	Three individuals, 1–2 months.
April 6, 1991	FMNH1571a, Humeral diaphysis12.2 mm in length with only the distal epiphysis beginning to fuse.	One individual ~1 month.
May 20, 1991	FMNH1559a, Humerus and femur with unfused epiphyses; diaphysial lengths 17.6 mm (femur) and 12.1 mm (humerus). FMNH1572, Partial skull (no mandible) with milk teeth, upper and lower first and second molars erupted.	Two individuals ~1 month.

Population Turnover Between Sites

Density estimates are highest in Ranomafana followed by the riparian forest, then the dry deciduous forest and spiny forest at Beza Mahafaly, the most disturbed and unprotected habitats, respectively, where density is considerably lower (Table VI). Population size was higher at Ranomafana (but so was the effective sampling area) and lowest at the dry and spiny forests (Table VI).

Recapture success was higher at Ranomafana than Beza Mahafaly. Overall recapture success – when all years were combined – was twice as high at Ranomafana as at Beza Mahafaly (44 vs. 20%). At Ranomafana, there were no significant differences between sexes in recorded first-to-last day of trapping (females, $n = 47$, mean = 378.3 days, $SD = 401.8$; males, $n = 53$, mean = 375.6 days, $SD = 343.6$) ($t = 0.037$, df = 98, P = NS). Only individuals captured at least twice prior to our last year (2008) were considered. In contrast, at Beza Mahafaly (with all three sites pooled and considering only individuals captured at least twice between 2003 and 2007), there were significant differences between sexes in the recorded first-to-last day of trapping (females, $n = 88$, mean = 233.2 days, $SD = 244.1$; males, $n = 81$, mean = 153.4 days, $SD = 214.4$) ($t = 2.25$, df = 167, $P = 0.026$). There were also significant differences between sites (pooling males and females for each site, Beza Mahafaly, $n = 169$, mean = 194.9 days, $SD = 233.09$; Ranomafana, $n = 100$, mean = 376.9, $SD = 370.1$) ($t = -4.42$, df = 146.17, $P < 0.001$; equal variances not assumed, they are significantly different).

DISCUSSION

Evidence of Polyestry and Duration of the Reproductive Season

We noted evidence of polyestry in some but not all captured females in our study populations. This may be an indication that factors other than temperature and rainfall (used as proxy for seasonality) are

TABLE VI. Maximum Likelihood-Based Spatially Explicit Capture–Recapture Model

Site	Trapping days	Number individuals	Number captures	Est. density	Sampling area	Est. pop. size
Ranomafana	48	69	466	3.9	17.6 ha	69.1
Beza-riparian	40	38	158	3.4	11.1 ha	39.4
Beza-dry deciduous	31	20	86	2.5	8.0 ha	21.6
Beza-spiny	39	24	176	2.2	10.9 ha	24.5

modulators for the fine tuning of reproductive schedules. If nutritional balance ultimately affects adult survival and reproductive success, differential access to limited high quality food resources may render some females more likely to sustain multiple successful reproductive events within a season. Those females that are able to gain weight consistently during pregnancy may be at an advantage over those with an irregular pattern of body mass gain during gestation [Blanco, 2008].

Do Observations From This Study Support "Polyestry-seasonality" or "Hypervariability" Hypotheses?

Accurate assessments of population turnover and mortality rates are difficult to obtain in open, dynamic populations studied over short periods, particularly if trapping areas are selected opportunistically and trapping efforts (i.e., number of traps and frequency of trapping) vary across seasons. Because data were not collected consistently between sites, i.e., because trapping efforts differed greatly, we had to rely on general population estimates. Values, however, were clearly distinct between sites and showed markedly different trends. Although density estimates were comparable across sites, population turnover (as crudely measured by recapture rates) was considerably higher at Beza Mahafaly than at Ranomafana. For our study populations, we found no evidence supporting the "polyestry-seasonality" hypothesis, i.e., that higher reproductive rates and shorter life spans should occur in mouse lemurs from less seasonal habitats. In contrast, Beza Mahafaly does seem to have a longer reproductive season than Ranomafana; thus we cannot reject the "hypervariability" hypothesis. Indeed, if the much higher apparent population turnover rates at Beza Mahafaly do signal shorter life spans at this site, then the hypervariability hypothesis would gain more support. There is one caveat, however: Females were not sampled between March and August at Ranomafana, which means that the end of the reproductive season at this location cannot be firmly established.

Lahann et al. [2006] acknowledged that a simple relationship between seasonality, reproductive rates and life span did not fully explain their data. At Ampijoroa (1,200 mm/year), a site with annual rainfall between that at Kirindy (800 mm) and Mandena (1600 mm), gray mouse lemurs had two litters per season (as at Mandena). However, recapture rates were as high at Ampijoroa as at Kirindy. Thus, the relationship between reproductive rates and longevity is evidently more complex than previously assumed [Lahann et al., 2006]. Furthermore, reproductive data from habitats with rainfall profiles higher than Mandena have provided little evidence of regular polyestry for most females.

As shown by this study, polyestry can occur under very different environmental circumstances. It occurs at Ranomafana where fruit availability may be extended during a relatively long rainy season [Atsalis, 2008]. It also occurs at Beza Mahafaly where high quality resources are limited to the period following first reproduction, thus allowing little opportunity for females to undergo opportunistic fattening. During the dry season, when both females and males tend to remain active, individuals rely on fallback food such as gums, whose consumption – favored by females – significantly increases during this period [Rasoazanabary, 2011]. In addition to rainfall, temperature minima influence habitat resource productivity and may impose thermoregulatory constraints to reproduction. Indirectly, lower ambient temperature minima would, in turn, facilitate energy storage because torpid mouse lemurs adjust their body temperature to approximate ambient temperature, thus lowering their energetic needs. The use of prolonged torpor has been correlated with high survival rates attributed to protection from predation [Ruf et al., 2012]; thus females who remain active during the dry season may be subjected to higher mortality rates. Different metabolic trajectories, i.e., with individuals trying to secure fat storages for prolonged torpor or to devote energy to sustain reproduction may be expressed by members of the same population for reasons that are not completely understood. Physiologically, expression of daily torpor or prolonged torpor (determined by degree of fattening) in mouse lemurs may be indicative of the ability of individuals to recruit and store extra calories.

The expression of polyestry varies among individuals and populations. On the basis of captive data, we know that female mouse lemurs can have 1–4 estrous cycles per season and give birth to two surviving litters within a season. These reproductive activities last approximately six months, which is consistent with the duration of reproductive seasons reported in wild populations [Atsalis, 2008; Perret and Aujard, 2001; Wrogemann and Zimmermann, 2001]. The "reproductive potential" (of multiple litters) may not be realized in the wild and likely depends on a set of local conditions that will ultimately affect the amount and quality of energy that females can acquire and allocate to reproduction. Low energy levels, however, do not preclude females from undergoing estrus, as the reproductive season for mouse lemurs generally begins when females are at their lowest body mass.

CONCLUSIONS

Mouse lemur females are polyestrous in captivity and there is evidence of polyestry in the wild. This is not surprising as small-bodied species tend to have relatively high reproductive rates compared to larger species to compensate for their shorter life spans.

Females with low energetic restrictions should maximize their reproductive potential and have multiple litters. However, if individuals are subjected to nutritional constraints after a period of food abundance (and be exposed to low ambient temperature), "fattening" and prolonged torpor may be favored as a strategy. If this is the case further reproductive efforts will be turned off because reproduction is not compatible with prolonged torpor [but see Canale et al., 2012]. The ability for individual mouse lemurs to "fatten" will depend to a large extent to their ability to secure high-energy foods, but other variables such as ambient temperature will affect the capacity to save energy by adjusting body temperature to ambient temperature. In habitats with low ambient temperature but less marked seasonality (food sources may be available during an extended period), thermoregulatory constraints on reproduction may be overcome by a higher caloric intake (as it may be the case in Ranomafana). Finally, in highly seasonal/unpredictable environments with uneven availability of food resources year round, "fattening" may be unlikely, but polyestry could be attained by females able to rely on fallback resources to sustain reproduction. This may be the case for females at Beza Mahafaly. This fact, in addition to other evidence from feeding behavior and morphometrics, suggests that females do have priority to access limited high quality food resources [Crowley et al., 2014; Génin, 2010; Rasoazanabary, 2011; Rasoazanabary and Godfrey, 2015]. We suggest that individual variation in the expression of polyestry and seasonal torpor should then be studied in the light of social structure (i.e., degree of female dominance to secure scarce resources) in addition to climatic factors such as rainfall and temperature. We found no support for the "polyestry-seasonality" hypothesis but could not reject the "hypervariability" hypothesis. The relationship between polyestry, number of litters, and habitat seasonality in mouse lemurs may be explained by the physiological adjustments mouse lemurs make to meet energy demands: e.g., the ability of females to access high quality or fallback food resources throughout the reproductive season or the ability of females to sustain prolonged torpor during the austral winter. Although polyestry may exist under contrasting environmental conditions, reproductive success and infant survival may differ between sites.

ACKNOWLEDGEMENTS

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