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Diet and Effects of Sanford's Brown Lemur (*Eulemur sanfordi*, Archbold 1932) Gut-passage on the Germination of Plant Species in Amber forest, Madagascar

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Kaloantsimo Sarah Chen, Jun Qing Li, Jean Rasoarahona, Fousseni Folega, and Christophe Manjaribe (2016) *Eulemur sanfordi* belongs to a group of endemic lemur species in Amber Mountain National Park, Madagascar. The diet of *E. sanfordi* and the effects of gut-passage on the germination of seeds were studied to determine how the feeding activities of this lemur affect the integrity of this forest ecosystem. A specific group of *E. sanfordi* was observed and monitored during 396 hours from the end of the dry season to the beginning of the rainy season. Field observers recorded the food items taken, plant species consumed, plants organs preferred and the forest layer in which food was harvested by this species. Seeds were sorted from discarded food items left by the group of *E. sanfordi* being followed. Germination tests allowed analysis of the germination potential of the collected seeds. Feeding times for *E. sanfordi* varied significantly (p = 0.01) across the study period (from September to February). Their feeding activities were intense between December and February, peaking in January (90%). They spent more time eating fruits than other organs of plants. Feeding patterns on ripe fruit also varied significantly (p = 0.01) during the study. *E. sanfordi* consumed 34 plant species, with 21% from the family of Moraceae. This group of observed lemurs consumed 9 to 17 plant species per month and preferred trees greater than 10 m tall. Overall, seeds that passed through the gut of these lemurs had significantly higher germination rates than those seeds that did not (t = 5.87, p = 0.02). The average latency period of passed and control seeds ranged from 35 to 83 days and from 52 to 95 days, respectively. *E. sanfordi*'s gut passage provides better germination of seeds species they consumed. This could contribute to the conservation of plant diversity. *E. sanfordi* play an important role in Amber forest ecosystem to preserve some endemic species.

Key words: *Eulemur sanfordi*, Primate food, Seed dispersal, Germination rate, Latency period, Amber forest, Madagascar.

BACKGROUND

Madagascar is home to many exceptional kinds of wildlife (Goodman and Benstead 2003); 90% of the island nation's plants and animals are endemic (Tattersall 2006; Hobbes and Dolan 2008). Madagascar's species richness and endemism is incredible even at the upper taxonomic levels (Mittermeier et al. 2005). Lemurs are among the endemic species of Madagascar. They are a clade of strepsirrhine primates which are characterized by their large brains relative to

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other mammals. They are also characterized by a typically longer snout and wet nose. Madagascar's lemurs serve as a unique symbol of Madagascar's mammalian species, because no lemurs occur elsewhere (Mittermeier et al. 2005). Among the 110 currently described species and subspecies of lemur (Mittermeier et al. 2014), which evolved on Madagascar, 39 of them were described between 2000 and 2008 (Mittermeier et al. 2008).

As a developing country, Madagascar faces critical environmental problems (Mittermeier et al. 2005). The major ecological threats include forest loss caused by slash-and-burn practices, charcoal production, mining exploitation and illegal logging. Historically, forests covered most of Madagascar; however, today only 15 to 20 % of the original primary vegetation remains (Burney 2003). In recent decades, Madagascar's forests have been increasingly harvested and fragmented (Harper et al. 2007). Forest harvest and fragmentation are the greatest threats to the habitats of faunal species; this is especially true for the habitats of primates (Anderson et al. 2007; Marrocoli et al. 2013). Scientists have classified almost all lemurs as rare, vulnerable, or endangered. Since the discovery of Madagascar, at least 17 lemur species (about 25%) have become extinct (Mittermeier et al. 2006; Gommery et al. 2009) with 94% of all lemurs species included in the International Union for the Conservation of Nature (IUCN) red list (Mittermeier et al. 2014) and with *E. sanfordi* treated as an endangered species (IUCN 2011).

Sanford's brown lemur (*E. sanfordi*, Archbold 1932) was previously considered a subspecies of the common brown lemur (*Eulemur fulvus*); however, since 2005, several brown lemurs have been elevated to full species status (Andrainarivo et al. 2008). Sanford's brown is a medium-sized lemur and sexually dichromatic (Terranova and Coffman 1997; Garbutt 1999; Mittermeier et al. 2006). Endemic to the northern tip of Madagascar, they favor areas of sanctuary remaining in the forest ecosystems of Ankarana, Analamerana and Amber mountains. A small population can also found in the Daraina region (Wilson 1995; Mittermeier et al. 2014). This species lives in group with size range from 3 to 15 individuals. Population density ranges from 3.5 to 5.4 individuals/km^2. They live in tropical moist, dry lowland and montane forest up to 1.400 m (Wilson 1989; Wilson 1995; Freed 1996; Mittermeier et al. 2014). *E. sanfordi* diet consists mainly of fruit and includes other plant parts (such as buds, young leaves and flowers) based on seasonal availability (Mittermeier et al. 2014). This species lives sympatrically with *Eulemur coronatus* (Freed 1996).

In addition to the loss of forest and the destruction of their habitat, Sanford's brown lemurs are also exposed to serious problems related to hunting. This species is primarily hunted for illegal exportation, especially for consumption as food (Nicoll and Langrand 1989; Mittermeier et al. 2014).

Primates are thought as principal seed dispersers in many plant communities in tropical forest (Razafindratsima et al. 2014). More than 95 % of tropical seeds are estimated to move by primates (Chapman and Russo 2007). Because many frugivorous primates consume fruits and disperse seeds in their faeces (Corlett 1998; McConkey 2005). Endozoochory is in general increased the seeds germinating power because of the action of acids and mechanical found in frugivores's digestive tract which injury to the seed coats. That phenomenon is very important of some tropical plant species without frugivores the seed dispersals and subsequent recruitment of many plant species may be severely interrupt (Effiom et al. 2013).

Excessive exploitation has caused the forest to lose much of its original cover, causing these lemurs to lose both food and habitat. The conservation and protection of zoochorous species is vital to seed dispersal and forest regeneration in Madagascar (Duncan and Chapman 2002; Agmen et al. 2009). In face of the habitat disturbance related to zoochorous species, a great need exists to devote additional attention to understanding the relationship between fauna such as lemurs and their habitat in an effort to promote management that involves zoochorous species, and especially lemur species.

The goal of this study is to better understand the effects of *E. sanfordi* on natural forest regeneration in Amber Mountain National Park. First, the feeding processes used by *E. sanfordi* were determined; the germination of seeds found in the discarded food of this lemur was tested against controlled seeds. Finally, the paper provides data related to the future planning and designing of a conservation strategy for this species of lemur.

MATERIALS AND METHODS

Study area

The study landscape encompasses tropical mountain rainforest within the Amber Mountain National Park complex and is located between 12°31'16"-12°31'94"S and 49°10'48"-49°10'70"E. The complex of protected areas covers 23,010 ha of the Antsiranana II District, which is an administrative subdivision of one of 22 regions of Madagascar delineated in 2004, the Diana Region. The elevation of the study site varies from 850 to 1475 m with a slope up to 30°. The annual air temperature ranges from 17.60°C to 21°C (Rossi and Rossi 1998). The dry season starts from May to November and the rainy season runs from December to April. The park receives abundant rainfall throughout the year regardless of the season. The mean annual rainfall is about 2978 mm (Humbert and Cours Darne 1965; ANGAP 1998).

Mainly three types of vegetation are dominant in Amber Mountain National Park (Fig. 1): mountain rainforest, herbaceous undergrowth and evergreen seasonal forest. Several plants are frequently encountered across the landscape including *Dalbergia* sp., *Canarium madagascariensis*, *Terminalia mantaly*, *Neodypsis* sp., *Aeranthes* sp. as well as introduced species such as *Abies* sp., *Eucalyptus* sp., *Araucaria* sp., and *Cinchona* sp. (ANGAP 1998). From the perspective of fauna, 24 amphibian, 49 reptile, 77 bird and 19 mammalian species populate the study area, including *E. sanfordi* (*e.g.* Rakotoarimanana and Edmond 1990).

Data Collection

One group of Sanford's brown lemur that had been habituated to human presence was chosen for study and data collection from September 2013 to February 2014. This study period corresponds to two seasons, the end of dry season (September-November) and the beginning of rainy season

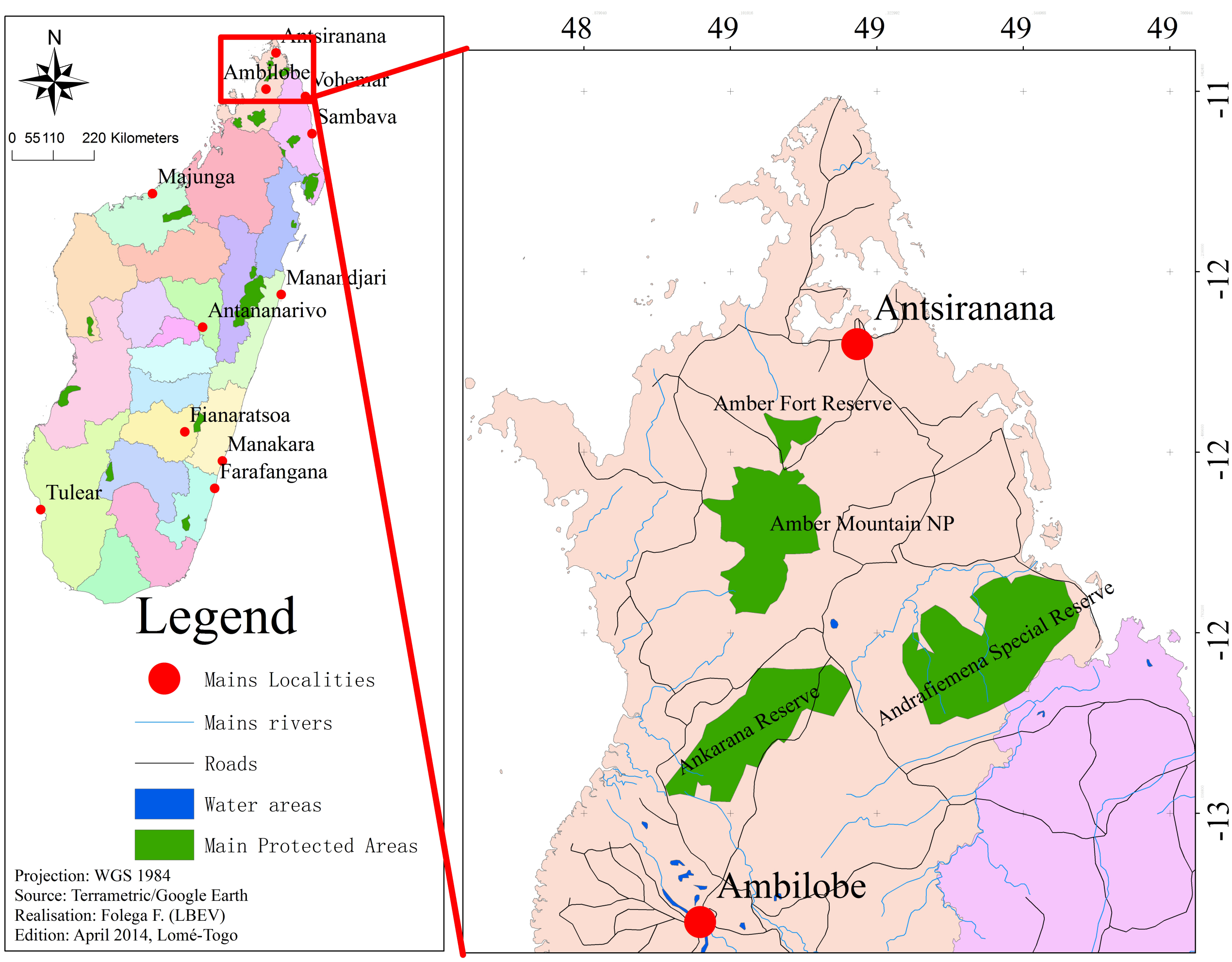

Fig. 1. Study area.

(December-February). The study group included five males and four females.

Diet

The data were obtained using direct observation of a single focal individual following the methods described by Stevenson (2000). The observations were made between 6 a.m. to 6 p.m. by following focal animal on 3 consecutive days per week for 6 hours per day. Each day's observations began and ended at different times but always involved 6 consecutive hours. Different focal individuals were used to ensure representation of all group members in our sample, with an average of 44 hours of observation per individual. The group was formed by six adults and three juveniles.

The observations was recorded every 5 min based on sampling the focal individual (Altmann 1974). Thus, 72 entries were recorded on the focal individual per day. In total, a focal animal was observed for 396 hours with that time distributed over 6 months or 72 h per month; however, in February observations were conducted for only 36 hours.

Each observation was classified into one of three categories of activities, resting (when the lemur is considered to be at rest), feeding (defined as the consumption of solid food items and all activities related to feeding) and moving (all remaining activities).

The time spent by the focal individual feeding on each plant species, the parts of these plant species being consumed and the locations of different feeding sites were recorded. The plant species eaten by the focal lemur were recorded. Food species were also flagged for later collection including for making herbarium specimens. Each plant part eaten by the focal individual was identified and classified as leaves (young or mature), flowers, fruits (unripe, ripe) and others.

The height of each feeding individual in trees was recorded to allow the determination of the feeding strata used by the focal lemur. Five feeding levels in trees were defined (Andriatsarafara 1988): L1, 0 m, ground level; L2, > 0 to 2 m, thick lower layer; L3, > 2 to 5 m, average tree layer; L4, > 5 to 10 m, upper tree layer; L5, > 10 m, emergent layer of large tree tops.

Seed and germination test

During the feeding observations of the focal animal, all fresh fecal samples that fell on the ground from all individuals in the group were collected (Stevenson 2000; Poulsen et al. 2001). In total, 131 fresh fecal samples were recorded. The defecated seeds were extracted from fecal sample by filtering feces through a 1 mm mesh sieve following the methods of Stevenson (2000) and stored in plastic bags prior to identification. Seeds identification was confirmed by comparing these seeds with those of mature trees in the field study. The defecated or passed seeds were counted and measured using calipers. Seed were classified into three size categories according to Traveset and Verdu (2002): large > 10 mm, medium 5-10 mm and small < 5-mm-diameter. Passed seeds were classified in two categories: damaged and intact. Passed seeds are considered damaged if they had visible injuries including bite marks, others scars, or seed damage; all other seeds were categorized as intact following the methods of Razafindratsima and Martinez (2012).

Germination tests were performed using intact large-sized defecated seeds; these were chosen because of the low numbers of small- and medium-sized seeds collected from feces. The control or non-passed seeds were extracted from ripe fruits collected under fruiting trees using nets or on the ground throughout the study site. Intact passed seeds and control seeds were planted in a nursery at the Amber forest. The following methods were used by the conservation agent in the area; the nursery consisted of four 4.90-m^2 seed beds. A sunshade composed of leaves was placed at 1 m above the ground in each seed bed to imitate the closed canopy of the forest. The soil used in the nursery was composed of forest soil to imitate the natural local conditions. We collected soil in several areas within the forest, and mixed all soils before use in the nursery. Seeds were planted in forest soil for the germination trials, and covered with 0.5 to 1 cm of soil. An equal number of seeds was planted for each plant species per treatment (n = 20). In the nursery, 240 seeds (defecated and non-passed) were planted. Germination of seed was defined as the moment the radicle appears (Stevenson et al. 2002). The time between sowing and germination was defined as the latency period. The number of germinated seeds was recorded daily.

Data analysis

The time the focus lemur spent feeding on different food items in a day were estimated using the formula of Gupta and Kumar (1994):

$$Ta = \frac{Na \times 100}{N}$$

where T_a is the percentage of time spent on activity *a*, *Na* is number of records with activity *a*, and *N* is the total number of records for the day.

The mean time spent feeding on a category of food (such as leaves), a food species and the number of food plants of each species eaten per day were used to calculate the monthly mean; in addition, the monthly mean was used to calculate the seasonal mean.

The germination rate for a seed species was obtained by the percentage of dispersed and control seeds that germinated in germination trials (Stevenson 2000).

SPSS 18.0 (IBM, Inc., Armond, NY) was used to perform the statistical analysis. One-way ANOVA was used to compare variations in feeding time across the month, feeding time on food type and number of food plants consumed monthly and seasonally. A paired *t*-test was used to test for differences between the germination rate of passed and non-passed seeds. For each species, Pearson's chi square test was used to analyze the germination rate of six large species the most found in lemur's feces to assess the influence of their gut passage. A Mann-Whitney test was used to compare the latency period between gut-passed and non-passed seeds (for each species and between them).

RESULTS

Diet composition and spatio-temporal feeding

During the study period, the lemur group spent 45% of their time resting, 31% moving and 24% feeding. The monthly percentage of time spent feeding ranged from 16% to 29% across all months. The minimum and maximum percentages of time spent feeding were in November and January, respectively (Fig. 2). The feeding time varied significantly across the months ($F = 4.59$, $p = 0.01$). The feeding time did not vary significantly across the seasons ($F = 0.72$, $p = 0.39$).

The daily feeding time for each food type was combined to present the overall feeding time for each food type. *Eulemur sanfordi* spent 80% of their feeding time eating fruit including ripe fruit (76%) and unripe fruit (4%). The time lemurs spent eating leaves (young and mature) was 6%. Time spent eating young and mature leaves were 5% and 1%, respectively. They spent 13% of their time eating flowers. The lemurs spent 0.26% of feeding time eating soil and 0.34% drinking water.

Feeding time on ripe fruit was high in all months. The amount of time lemurs spent feeding on ripe fruit varied significantly across the months ($F = 8.80$, $p = 0.01$). The lemurs spent more than 40% of each month feeding on ripe fruit. However, they spent more than 90% of their time feeding on ripe fruit from December to February, with a peak of 100% in February. Feeding time spent on eating ripe fruit also varied significantly across the seasons ($F = 36.36$, $p = 0.01$).

The feeding time on unripe fruit ranged from

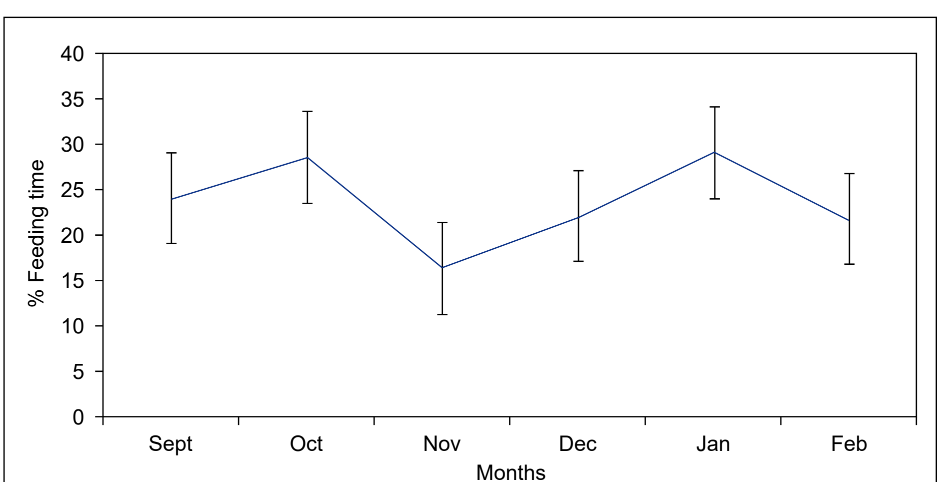

Fig. 2. Monthly variation on feeding time.

0% in February to 11% in September. Feeding time on unripe fruit did not vary significantly across the months ($F = 2.07$, $p = 0.08$) or seasons ($F = 3.30$, $p = 0.07$).

The feeding time on young leaves ranged from 0% in February to 26% in September, and varied significantly across the months ($F = 6.32$, $p = 0.01$) and seasons ($F = 5.24$, $p = 0.03$).

The feeding time on mature leaves ranged from 0% in February to 4% in September, and varied significantly across the months ($F = 2.67$, $p = 0.03$).

The feeding time variation on flowers varied significantly across the months ($F = 27.15$, $p = 0.01$). Sanford's brown lemurs fed on flowers from September to December, with feeding time on flowers peaking at 49% in October; it also varied significantly across the seasons ($F = 19.78$, $p = 0.01$).

Soil was consumed rarely at any time, although the lemurs are observed eating soil in September and December. Figure 3 shows the monthly variation in feeding time on different food types.

Harvested plants species and feeding layer

E. sanfordi individuals consumed 34 plant species belonging to 25 families and 30 genera; of these 64% were trees, 15% shrubs, 15% lianas, 3% herbs, and another 3% were epiphytes. Lemurs consumed seven plant species from Moraceae family and two plant species in Acanthaceae, Lauraceae and Malvaceae. Sanford's brown lemur spent 31% of feeding time on seven Moraceae species including four *Ficus* species (*F. albidula*, *F. barronii*, *F. botryoides*, and *F. pyrifolia*), *Pachytrophe dimepate*, *Streblus dimepate* and *Streblus madagascariensis*; of those only 11% of this 31% was spent feeding on *Streblus dimepate*. Lemur spent 11% of their time feeding on Sapotaceae (*Chrysophyllum boivinianum*) and 8% on two species of Lauraceae (*Cryptocarya ambrensis* (7.5%) and *Raventsara crassifolia* (0.5%).

The lemurs ate more species of *Ficus* than plants of any other genus followed by *Streblus* of which lemurs consumed two species. Of the 34 plant species consumed, 59% were eaten for their fruits, 15% for their fruits and leaves, 12% for their leaves, also 12% for their flowers and 3% for their fruits and flowers (Table 1). Table 1 lists the food plant species, time spent by lemurs on each part of each food plant (%) and their habit.

Among the 34 food plant species used, 71% are endemic to Madagascar, 8% are cosmopolitan and 5% are found only in Madagascar, the Union of the Comoros, the Mascarene Island and Africa. The number of plant species eaten varied from two to 12 each day, but did not vary significantly across the days ($F = 0.83$, $p = 0.61$). The number of plant species eaten each month ranged from nine to

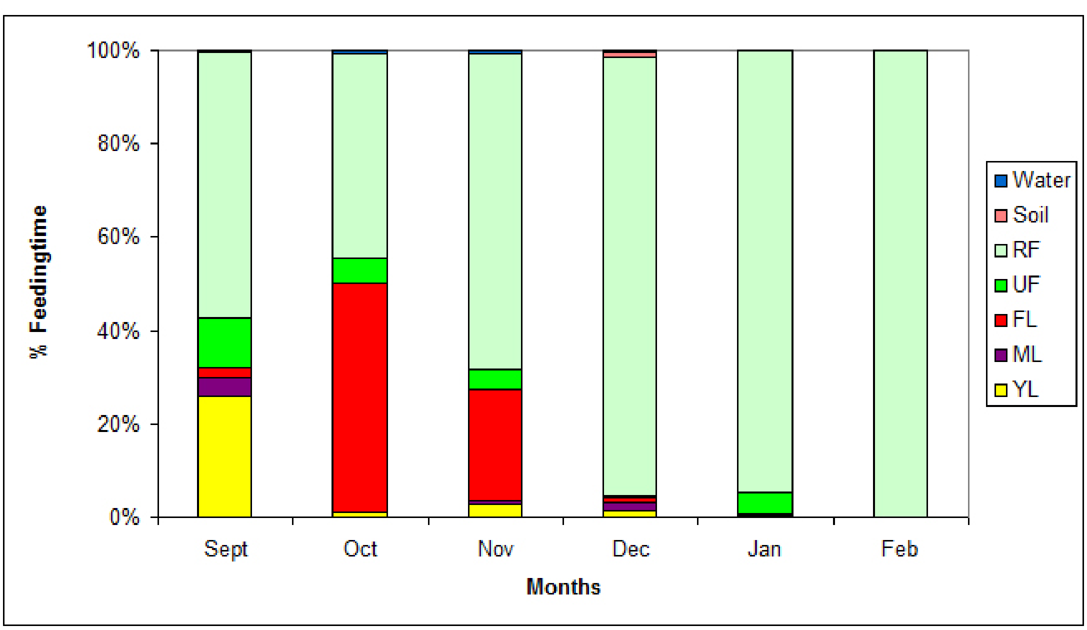

Fig. 3. Monthly feeding time variation on different food types. RF= Ripe Fruit, UF= Unripe Fruit, FL= Flowers, ML= Mature Leaves, YL= Young Leaves.

17 species, with the minimum number eaten in February and the maximum in December. The number of food plant species eaten did not vary significantly across the months ($F = 6.65$, $p = 0.66$).

The number of food plant species used in the dry (late in the dry season in this study) and rainy (early in rainy season in this study) seasons were 24 and 22, respectively, but did not vary significantly across the seasons ($F = 0.51$, $p = 0.48$).

For all plant species eaten, *E. sanfordi* ate *Ficus pyrifolia*, *Streblus dimepate* and *Uapaca ferruginea* across all months of study. *Chrysophyllum boivinianum*, *Ficus barronii*, *Hypoestes angusta*, *Dypsis* sp. and *Mendoncia cowanii* were consumed for 4 months and other plant species were eaten less often (Table 2).

During the study, Sanford's brown lemur spent 60% of their total feeding time in the emergent layer of large tree tops. The monthly variation in feeding time in different feeding layers by lemurs indicates that the time spent feeding in the emergent layer of large tree tops peaked in September (88%). Figure 4 shows the time lemurs spent feeding in different strata during each month.

Germination and the latency periods of seeds

The fecal samples contained fleshy fruit parts, stalks, leaves, flowers, fecal liquid and seeds. Most seeds found in fecal samples were intact with minor scarification and represented

Table 1. Food plants, plant parts eaten and total feeding time (%) on them

Scientific Name	Family	Average of feeding time (%)	Part eaten	Habit
Aphloia theaeformis Var Minima	Flacourtiaceae	2.34	F	Shrub
Bakerella sp.	Loranthaceae	0.94	F,YL,ML	Epiphyte
Canarium madagascariensis Engl	Burseraceae	2.35	F	Tree
Canthium medium A. Rich. Ex Capuron	Rubiaceae	0.21	F	Tree
Cassipourea madagascariensis DC.	Rhizophoraceae	0.73	F	Shrub
Chrysophyllum boivinianum(Pierre) Baehni	Sapotaceae	10.99	F	Tree
Cryptocarya ambrensis van der Werff	Lauraceae	7.50	F	Tree
Dichapetalum bojeri (Tul) Engl	Dichapetalaceae	2.62	F	Climber
Dombeya amplifolia Arènes	Malvaceae	2.92	FL	Tree
Dracaena ensifolia (L.) DC	Asparagaceae	1	F	Tree
Dypsis sp.	Arecaceae	12.46	F	Tree
Eugenia lokohensis H. Perrier	Myrtaceae	0.29	F	Tree
Ficus albidula Baker	Moraceae	9.24	F	Tree
Ficus barronii Baker	Moraceae	0.75	F	Tree
Ficus botryoides Baker	Moraceae	0.80	F	Tree
Ficus pyrifolia Burm	Moraceae	8.56	F,YL	Shrub
Garcinia ambrensis H. Perrier	Clusiaceae	0.09	F	Tree
Grewia antsiranensis Capuron	Malvaceae	2.93	F	Shrub
Harungana madagascariensis Lam.ex Poir	Hypericaceae	0.48	YL,ML	Tree
Hypoestes angusta Benoist	Acanthaceae	0.75	YL,ML	Herbaceous
Landolphia fragrans Pichon	Apocynaceae	0.60	F	Climber
Mendoncia cowanii Benoist	Acanthaceae	5	F,YL	Climber
Olea ambrensis H. Perrier	Oleaceae	1.93	YL	Tree
Oncostemum reflexum Mez	Myrsinaceae	0.08	F	Tree
Pachytrophe dimepate Bureau	Moraceae	0.41	F,FL	Tree
Prunus persica (L.) Batsch	Rosaceae	0.19	F,YL	Tree
Raventsara crassifolia	Lauraceae	0.13	F	Tree
Salacia madagascariensis (Lam) DC	Celastraceae	7.71	FL	Climber
Smilax krussiana Meisn	Smilacaceae	1.34	F	Climber
Streblus dimepate C.C. Berg	Moraceae	10.86	F	Shrub
Streblus madagascariensis Blume	Moraceae	0.48	FL	Tree
Strychnos madagascariensis Poir	Loganiaceae	0.09	FL	Tree
Trema orientalis	Ulmaceae	0.56	YL	Tree
Uapaca ferruginea Baill	Phyllanthaceae	2.68	F,YL,ML	Tree

YL: Young Leaves; ML: Mature Leaves; FL: Flowers; F: Fruit

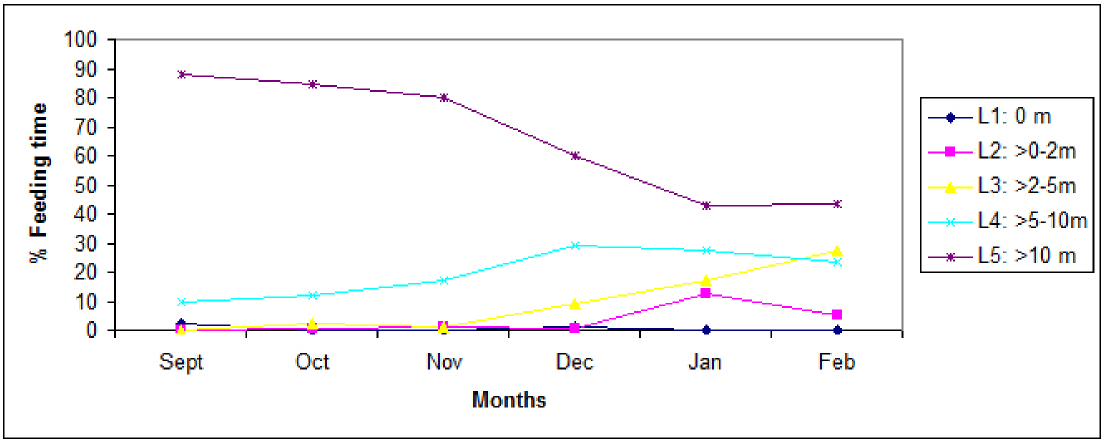

Fig. 4. Monthly variation on feeding time in forest layer used. L1= ground level, L2= thick lower layer, L3= average tree layer, L4= upper tree layer, L5= emergent layer of large tree tops.

Table 2. Monthly variations in feeding time (%) on each plant species

Scientific name	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Aphloia theaeformis	0.48		0.72	12.83		
Bakerella sp.			4.32	0.53	0.80	
Canarium madagascariense			3.60	2.14	8.37	
Canthium medium		1.23				
Cassipourea madagascariensis					1.20	3.19
Chrysophyllum boivinianum	20.29	10.25	29.50	5.88		
Cryptocarya ambrensis				34.22	10.76	
Dichapetalum bojeri	14.49	1.23				
Dombeya amplifolia		5.33	12.23			
Dracaena ensifolia					2.79	3.19
Dypsis sp.		1.23		1.60	31.47	40.43
Eugenia lokohensis	0.50	1.23				
Ficus albidula				3.21	26.69	25.53
Ficus barroni	1.45	1.23	0.72	1.07		
Ficus botryoides				4.82		
Ficus pyrifolia	13.04	16.39	17.27	0.53	1.99	2.13
Garcinia ambrensis				0.53		
Grewia antsiranensis					4.78	12.77
Harungana madagascariensis	2.90					
Hypoestes angusta	2.42	0.82	0.72	0.53		
Landolphia fragrans					0.40	3.19
Mendoncia cowanii	15.46	9.02	5.04	0.53		
Olea ambrensis	11.59					
Oncostemum reflexum	0.48					
Pachytrophe dimepate		2.46				
Prunus persica		0.41	0.72			
Raventsara crassifolia					0.80	
Salacia madagascariensis		37.3	7.91	1.07		
Smilax krussiana	4.35	3.69				
Streblus dimepate	4.35	6.15	15.83	28.34	8.37	2.13
Streblus madagascariensis	2.90					
Strychnos madagascariensis				0.53		
Trema orientalis	3.38					
Uapaca ferruginea	1.93	2.05	1.44	1.60	1.59	7.45

26 different species and 17 families. Based on the collected samples, the most common large-sized seed species found in lemur defecations belonged to six species; these were selected for more detailed analysis in this study (Table 3). For each of these six species, seeds that had been defecated had a higher germination rate than non-passed seeds (Table 3). Passed and non-passed seeds had mean germination rates of 75.83% and 32.50%, respectively. Lemur gut-passed seeds had significantly higher overall germination rates than non-passed seeds (t = 5.87, p = 0.01). For each of these six species, the Pearson's chi square test value was not statistically significant (Table 3).

The average latency period of passed and control seeds ranged from 35 to 83 and from 52 to 95 days, respectively. The difference between these two averages ranged from 12 to 30 days (Table 4).

The Mann-Whitney test analysis was non-significant (U = 11, p = 0.26) between the plant species but had different levels of significant across each plant species (Table 4).

DISCUSSION

The goal of this study was to better understand the relative contribution of forest plants to the food needs of Sanford's brown lemur and to shed light on the role of this species in forest regeneration. This study analysed the dietary habits of a single group of lemurs that had been tolerate the presence of humans. The daily activity of *Eulemur sanfordi* typically involved high proportions of time spent resting and less time for feeding. This activity can be caused by the climatic conditions such as the dry and cold weather at the end of dry season and heavy rain at the beginning of rainy season. Therefore, the lemurs adopt a strategy to protect themselves against the cold and rain by resting in groups. To adapt to the seasonal differences in climate, *Eulemur* taxa may adopt an energy conservation strategy (Curtis 2004; Kerry and Kamilar 2006). In critical environments, these species became less active to allow them to adapt to local conditions (Kerry and Kamilar 2006).

The diet of Sanford's brown lemur includes a variety of food items. The high percentage of fruit consumption confirms this is a frugivorous species (Freed 1996), with ripe fruits dominating its diet. The consumption of ripe fruit increased through the seasons, probably because increased rainfall as the seasons may progress resulted in an increase in the availability of ripe fruit. Changes in diet can also be influenced by seasonal changes in the availability of food (Overdorff 1993). However, the consumption of unripe fruit decreased as the seasons progressed in this study, especially in the second season *i.e.*, the beginning of

Table 3. List of species used to germination trial

Scientific name	Family	Germination rate %		Pearson test	
		Passed seeds	Non-passed seeds	Chi-square	*P*-value
Canarium madagascariense	Burseraceae	100	35	7.93	.09
Chrysophyllum boivinianum	Sapotaceae	80	25	6.00	.20
Cryptocarya ambrensis	Lauraceae	65	40	2.14	.54
Dichapetalum bojeri	Dichapetalaceae	85	65	5.72	.22
Eugenia lokohensis	Myrtaceae	65	10	6.67	.16
Mendoncia cowanii	Acanthaceae	60	20	5.40	.15

Table 4. Average latency period in days

Species	Passed seeds	Non-passed seeds	*U*	*P*
Canarium madagascariense	35	52	11	.01
Chrysophyllum boivinianum	70	85	0.5	.02
Cryptocarya ambrensis	45	57	0.5	.01
Dichapetalum bojeri	60	90	20.5	.13
Eugenia lokohensis	83	95	2.5	.24
Mendoncia cowanii	74	86	1.5	.03

rainy season. The consumption of unripe fruit throughout the season was perhaps caused by the lack of adequate ripe fruit needed during a particular season. Unripe fruit consumption was highest in September during a period when ripe fruit was scarce and was lowest in February during the period with abundant ripe fruit. Leck (1972); Foster (1977); Schaefer and Schmidt (2002) also emphasized that frugivorous animals consume unripe fruits during periods of food scarcity.

Flowers comprise 13% of *E. sanfordi*'s diet. Curtis' study (2004) on the wild-food diet of Mongoose lemurs in northwestern Madagascar revealed that flowers are high-energy foods, and contain the greatest amount of water-soluble carbohydrates when compared with other foods. Most animals consumed greater numbers of flowers during the dry season to satisfy their bodily need for energy and to complement other foods in their diet. Freed (1996) reported *E. sanfordi* consumed more flowers during the dry season than during the rainy season. The consumption of flowers by *E. sanfordi* was very high in October, which corresponds to the period of giving birth (Mittermeier et al. 2014). Perhaps flowers can provide the females with crucial energy needed during that period. Lemurs also seem to prefer flowers and they are readily available during that season. Of all plant species used as food by *E. sanfordi*, five species were eaten for their flowers and three of those were eaten during October and November. This corresponds to the period during which the consumption of flowers was high and with the blooming period of those plant species. Monthly variation in the number of plant species consumed by *E. sanfordi* ranged from 9 to 17 species; in particular, the lemurs consumed 16 and 13 plant species in October and November, respectively.

Consumption of leaves was rare. Dunn et al. (2010) reported that frugivorous animals consumed leaves for many reasons such as to diversify their food sources, to obtain the best complement of nutrients and to avoid an overload of particular toxins. Young leaves contain a high percentage of crude protein; their consumption may provide lemurs with required and essential nutrients and protein (Kumar and Solanki 2004). The consumption of soil helps the lemurs because soils may absorb toxins, treat diarrhea and adjust the pH of the gut (Krishnamani and Mahaney 2000).

Thirty-four plant species, of which 71% are endemic, constituted a diet of *E. sanfordi* in the Amber forest. In its diet, *E. sanfordi* consumed more Moraceae plants than those of other families. Their high level of tannins (Kendrick et al. 2009; Madhavan et al. 2009) and their availability during all periods probably make plants of the Moraceae an important fallback food during periods of resource scarcity (Dunn et al. 2010). In their natural diet, lemurs typically eat plant foods that are rich in tannins (Jolly 1966; Petters et al. 1977; Tattersall 1982; Wood et al. 2003; Bertini et al. 2007) and that could therefore prevent a disease related to the storage of iron (Fowler et al. 1999).

The multiple strata of tropical rainforests have resulted in a vertical stratification of ecological niche opportunities for animals (Pereira et al. 2010). In general, the different food resources of animals are determined by the vertical structure of the forest they used. The vertical exploitation space of Sanford's brown lemur was diverse across the months. *E. sanfordi* exploited the emergent layer of large tree tops largely during the end of the dry season, possibly because of the presence of young leaves and flowers in this layer, which constituted the most important part of the animal's diet in this season. *E. sanfordi* exploited the layers differently during the beginning of the rainy season. During that season, the animals used the various forest layers at nearly equal percentages of their feeding time. The various plant parts most frequently consumed by lemurs were found at different forest levels (Sussman 2002).

Some studies related to the diet of *E. sanfordi* have reported that this lemur occasionally consumed some invertebrates such as spiders and centipedes (Mittermeier et al. 2014). However, the consumption of invertebrates was not observed in the present study, perhaps because of the unique food choices of this particular group of lemurs.

Passed seeds had a mean germination rate of 75.83% and that rate was higher than that of non-passed seeds (32.5%). Dew and Wright (1998) reported nearly equal percentages of germination success in seeds dispersed by *Eulemur rufus*, *Eulemur rubriventer* and *Varecia variegata*. Razafindratsima and Martinez (2012) also reported a 61% germination rate for seeds dispersed by *Varecia rubra*. With a frugivorous diet, the passing of intact seeds and the high germination rate of passed seeds confirm that *E. sanfordi* is a beneficial seed disperser (Bollen et al. 2004). Frugivorous animals are believed to be responsible for seed dispersal in many plant communities in tropical forests (Razafindratsima et al. 2014), and they can help prevent the loss of some types of forest habitat (Traveset et al. 2014). Thus, the presence of *E. sanfordi* can be critical for plant regeneration and forest restoration in Madagascar. For all plant species, the seeds that had passed through a lemur gut had a significantly higher germination rate than non-passed seeds. The Pearson's chi square test value was not statistically significant between passed and non-passed seed for each of these six species. It confirms that there are no relationships between the germination rate of passed and non-passed seed.

Most primates have similar effects on germination parameters (Stevenson 2000). However, the ingestion of seeds by primates can also have negative effects on germination because it can decrease or delay the percentage of germination (Dew and Wright 1998; Traveset 1998; McConkey 2000; Lambert 2001; Poulsen et al. 2001; Stevenson et al. 2002; Chapman and Russo 2007).

The passage of seeds through a primate's gut often improves the germination rate (Poulsen et al. 2001). Some plant species may require a level of chemical scarification inside the lemur gut to stimulate a high level of germination (Dew and Wright 1998; Razafindratsima and Razafimahatratra 2010). When consumed by a frugivore the gut-passage helps to remove the aril of the seed, which helps the seed to germinate (Razafindratsima and Razafimahatratra 2010). Endozoochory is an important strategy for a plant species because it reduces the time a seed spends in the phase of embryogenesis (Dew and Wright 1998; Razafindratsima and Razafimahatratra 2010). This mechanism proved that latency periods of passed seeds were shorter than those of non-passed for all species analysed in that study. Stevenson et al. (2002) emphasized that latency periods of primate passed seeds are shorter than those of control seeds and the dispersal process did not affect germination time. This phenomenon depends on both the seed and the disperser (Traveset et al. 2014). However, the seeds ingested by animals may be classified by their dispersal strategies (Pakeman et al. 2002) in which the plant organ has a mutualistic relationship in that it uses animal dispersal agents (Zhou et al. 2013). Many primate lineages exhibit some anatomical adaptations that simplify the exploitation of particular food types; in addition, many lineages of flowering plants have evolved various characteristics that facilitate seed dispersal (Lambert and Garber 1998).

The wide dispersal of seed by comparatively large dispersal agents can play a potential role in large scale ecological processes such as population diffusion and the colonization of unoccupied habitats (Herrera 1987; Hamilton 1999; Jordano et al. 2007; Zhou et al. 2013). The fact that lemurs feed on certain fruit taxa may have an important ecological effect on entire plant communities (Wright et al. 2011).

The germination rate of the seeds of a species can be affected by the number of seeds used for a germination test if low sample sizes reveal a greater percentage of germination success (Poulsen et al. 2001). The low germination rate of control seeds of *Chrysophyllum boivinianum* may have been caused by their collection from the same piece of fruit because the germination rate of a species may be affected by genetic and developmental histories (Stevenson et al. 2002).

CONCLUSIONS

This study provides valuable information related to the role played by *Eulemur sanfordi* in relation to the regeneration by seeds of various plant species in the Amber forest ecosystem of Madagascar. The daily feeding time of this species is constantly affected by other daily activities; characteristically, these lemurs spend much of their time resting during the period of study (*i.e.* September 2013 to February 2014). *E. sanfordi*'s diet consists almost exclusively of ripe fruit; however, unripe fruit are occasionally consumed.

Clearly, seeds from the lemur's gut had a higher germination rate than that of other seeds; therefore, *E. sanfordi* indirectly generates benefits that help maintain local habitats with high percentage of endemic species and allow a sustainable growth. As occurs in most primate species, the passage of seeds through the gut of *E. sanfordi* improved the seed germination rate and reduces their latency period. The outcomes of this study could be useful for ecosystem managers and land use planners as well as for decision and policy makers who are responsible of the management of fauna and favouring sustainable habitat.

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