ECOTROPICA 18: 15–26, 2012

SEED DISPERSAL BY RED-RUFFED LEMURS: SEED SIZE, VIABILITY, AND BENEFICIAL EFFECT ON SEEDLING GROWTH

Onja H. Razafindratsima[1, 3] & Barbara T. Martinez[2,4]

[1]Department of Animal Biology, University of Antananarivo, Madagascar
[2]Department of Ecology, Evolution and Behavior, University of Minnesota, Minneapolis, MN, USA
[3]Current: Department of Ecology and Evolutionary Biology, Rice University, 6100 Main St. MS 170, Houston, TX 77005, USA
[4]Current: AAAS Science and Technology Policy Fellow, Washington, DC, USA

Abstract. Frugivorous vertebrates play a critical role as seed dispersers of many tropical fruiting plants. In Madagascar, lemurs are the principal dispersers of fruit-bearing plants. This study aims to highlight the potential ecological role of the red-ruffed lemur (*Varecia rubra*) in the regeneration of a degraded forest, based on the quantity and size of passed seeds, their viability and germination, and seedling growth. In Masoala National Park, *V. rubra* groups were followed daily from dawn to dusk to collect fresh fecal samples containing seeds. We determined seed viability by immersion, and created an on-site nursery to compare the germination and growth of emerged seedlings from egested versus non-egested seeds. *V. rubra* dispersed a diverse group of plant species with mainly large-sized seeds, which remained undamaged by gut passage. Defecated seeds were viable and had a higher germination success than control seeds. There was also increased growth among seedlings of defecated seeds, suggesting *V. rubra* may play an important role in the regeneration of a degraded habitat through seed dispersal.

Key words: Masoala National Park, Madagascar, red-ruffed lemur, Varecia rubra, *seed dispersal, seed size, seedling growth*.

INTRODUCTION

Reforestation of degraded tropical rain forest might be accomplished through a combination of planting native trees (Leopold *et al.* 2001, Camargo *et al.* 2002) and zoochory (Duncan & Chapman 2002, Neilan *et al.* 2006), which is the transport and dissemination of plant propagules by animals either through their gut, on their fur, or with their mouth (van der Pijl 1972, Wunderle Jr. 1997). Animal seed dispersal is important for biodiversity maintenance, especially in the tropics where more than 90% of plants rely on dispersers (Jordano 2000, Bascompte & Jordano 2007). Seed passage through animal digestive tracts may enhance the germination probabilities of seeds (Wunderle Jr. 1997, Stevenson *et al.* 2002, Linnebjerg *et al.* 2009, Chapman *et al.* 2010), or it may have a neutral effect on germination (Dew & Wright 1998, Knogge *et al.* 2003). Another benefit of gut passage for seeds might be that they are less likely to be infected by fungi (Spehn & Ganzhorn 2000), and emerged seedlings have an improved competitive ability because of increased growth due to fertilizing effects of feces (Paulsen & Högstedt 2002). Seed dispersal by vertebrates may be important for the regeneration of degraded areas because of an animal's capacity to move seeds of fleshy fruits beyond the canopy of the parent tree or to favorable microhabitats (Murray *et al.* 1988, Gorchov *et al.* 1993, Wunderle Jr. 1997, Duncan & Chapman 2002, Kaplin & Lambert 2002, Culot *et al.* 2010). Seed shadows generated by animal seed dispersal are heterogeneous, creating a more diverse distribution of the plant population, and therefore plant species richness is greater in habitat fragments that receive zoochorously dispersed seeds (Janzen 1988, Redford 1992, Wunderle Jr. 1997, Cramer *et al.* 2007).

In the face of increasing habitat loss and deforestation, a restoration project was established in 1997 in Masoala National Park in Madagascar, near a small village called Ambatoledama, with the goal of attracting vertebrate frugivore seed dispersers into the restoration site (Holloway 2000). This site is characterized as a "corridor" due to the narrow strip of forest, about 1 km x 2.5 km wide, connecting two larger parcels of mature rain forest within the Park. The restoration project aims to transform former agricultural plots in the corridor into forest by planting

* e-mail: onja@rice.edu

fruit-bearing trees that will lure frugivorous vertebrates into the restored areas (Holloway 2000, Dokolahy 2004). The attractiveness to animals of a particular habitat is partially based on food resource availability, and therefore the more generalist frugivores, such as lemurs, could increase the complexity and diversity of a site through seed dispersal (Wunderle Jr. 1997, Berens *et al.* 2008). The plan for the restoration project was based on the knowledge that some frugivorous lemurs are effective seed dispersers (Scharfe & Schlund 1996, Dew & Wright 1998, Overdorff & Strait 1998, Ganzhorn *et al.* 1999, Birkinshaw 2001, Bollen *et al.* 2004, Lahann 2007) and the two largest resident species in Masoala National Park, the red-ruffed lemur *Varecia rubra* and the white-fronted brown lemur *Eulemur albifrons*, are highly frugivorous (Rigamonti 1993, Vasey 2000).

In degraded rain forests, it is important that the contribution of frugivores to the regeneration of primary forest is properly understood. This is especially of interest in the eastern humid evergreen rain forest of Madagascar, where more than half of the pre-colonization forest cover has been converted to agriculture or otherwise degraded (Green & Sussman 1990). The genus *Varecia* is potentially an important seed vector of rain forest plant species based on studies of black-and-white ruffed lemur *V. variegata*, which dispersed mainly large-sized and intact seeds with high germination success in both Ranomafana National Park (Dew & Wright 1998, Overdorff & Strait 1998) and Manombo forest (Moses and Semple 2011). Previous studies of wild populations of *V. rubra* recorded a high amount of time spent feeding on fruits: 86% (Vasey 2000), 73.9% (Rigamonti 1993), and 50-88% (Martinez 2010). This suggests that *V. rubra* is a potential seed disperser of a number of plant species. To understand the seed dispersal potential of an animal species, it is necessary to study both the diversity and viability of passed seeds. A previous publication demonstrated greater germination success of a select number of seed species defecated by *V. rubra* (Razafindratsima & Razafimahatratra 2010). Here, we describe the characteristics of seeds passed by *V. rubra*, the effect of gut passage on seed viability and germination using an expanded sample size, and the effect of gut passage on the growth and survival of seedlings. This is important baseline information to determine whether *V. rubra* contributes to the regeneration of a degraded rain forest at Ambatoledama. We tested the following hypotheses: (1) like *V. variegata*, *V. rubra* is predicted to disperse mainly large-sized seeds; (2) seeds, regardless of size, remain intact and viable after being passed through the gut of *V. rubra*, and (3) lemur-gut passage has a beneficial effect on plant reproductive success by increasing seed germinability and short-term seedling growth.

MATERIAL AND METHODS

Study site and species. The Ambatoledama corridor connects two blocks of forest in Masoala National Park, which is located in northeastern Madagascar (between 15°16’S, 50°0’E and 15°17’S, 50° 01’E; Fig. 1). The vegetation at Ambatoledama is dense evergreen rain forest situated at an elevation of 300 to 700 m, with an annual rainfall of 2200 to7000 mm, relative humidity above 80%, and an average annual temperature range of 21 to 24°C (Hatchwell 1999).

Varecia rubra is a diurnal species of the family Lemuridae, endemic to Masoala Peninsula and classified as “Endangered” (Andrainarivo *et al.* 2008, Mittermeier *et al.* 2010). *V. rubra* is described as living in communities with a fission-fusion social organization, which includes consorting for extended periods of time in small core groups (Vasey 2006). During the study seasons at Ambatoledama, we observed *V. rubra* ranging within core groups of two to six individuals (Table 1). We collected behavioral observations during November 2006 – January 2007 and August – November 2007 on three core groups of *V. rubra*. The core groups of *V. rubra* were habituated from August to October 2006. In order to locate and follow lemurs for daily observations, we fitted four adult females and one adult male with a Telonics brand (Mesa, Arizona, USA) MOD-205 VHF radio-collar. Dr. Edward Louis, DVM (Henry Dorly Zoo, Omaha, Nebraska, USA) and his team conducted the immobilization and capture of the lemurs. Lemurs were immobilized via an injection of 10 mg/kg body weight of Telazol® (tiletamine hydrochloride and zolazepam hydrochloride) using a CO_2-powered Dan Inject dart gun. These procedures were approved by the University of Minnesota’s committee on animal care and use (IACUC), protocol number 0603A83626, and the Malagasy Ministry of the Environment, Water, and Forests.

Fecal sample and seed collection. Radio-collared animals in each core group were followed daily for 3-6 days per week from dawn to dusk. While following the core groups, we attempted to collect all fresh

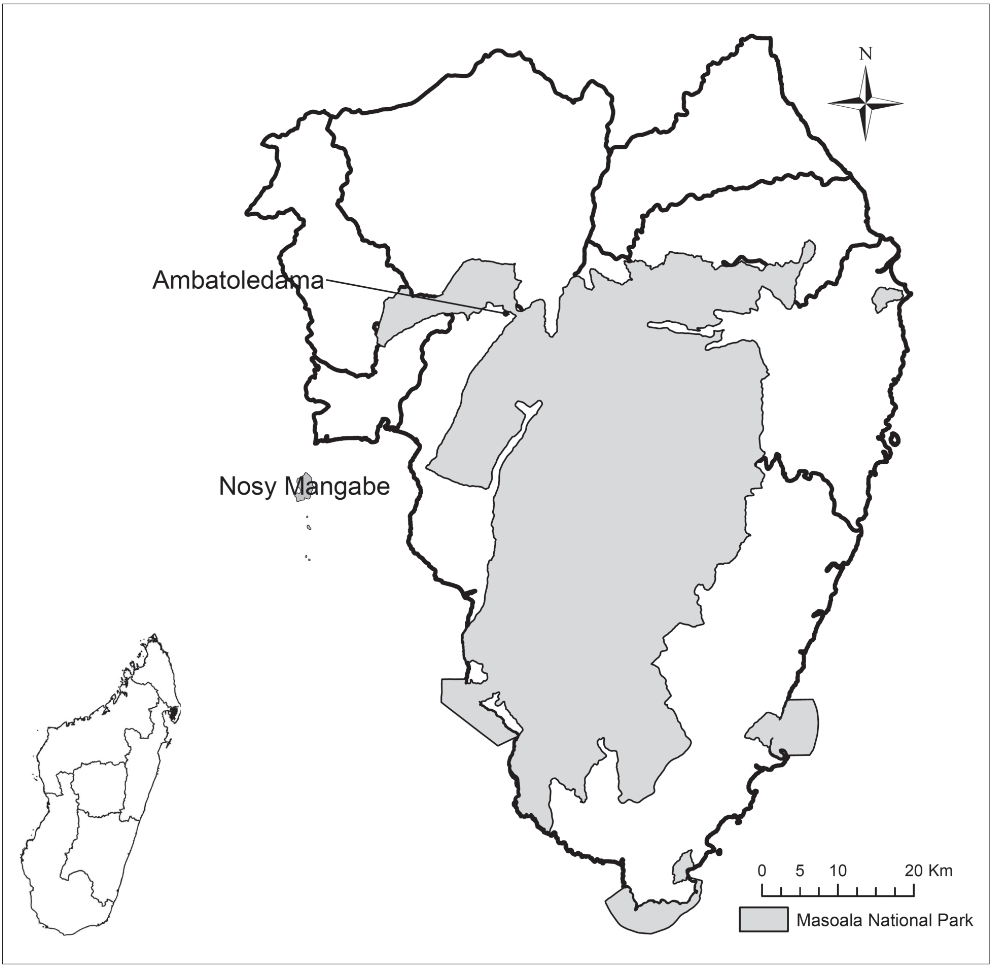

FIG. 1. Study site location, near Ambatoledama in Masoala National Park, Madagascar.

fecal samples that fell to the ground from all individuals in the core group. In the analysis, samples from the same core group that were defecated within a short time period (< 1 min) were grouped as a single sample. We extracted seeds by filtering feces through a 1-mm mesh sieve per Stevenson (2000). Local research guides and a trained parataxonomist familiar with the Masoala flora identified the seeds. We counted the defecated or "passed" seeds, and measured their length and width using calipers. Extracted seeds were classified as either damaged or intact. Seeds were considered "damaged" if there were visible injuries including bite marks, other scars, or seed destruction; all other seeds were classified as "intact." Seeds less than 1 mm could not be retained by the sieve thus were not quantified, but their presence was noted. Sample size information is detailed in Table 2.

Seed viability and seedling growth. Passed seeds were removed from collected feces, and control seeds were extracted manually by removing the pulp from ripe fruits collected throughout the site and from multiple trees. We recognize that there is likely variation in the nutritional content of fruits, and possibly the quality of seeds consumed by lemurs versus fruits we selected to extract seeds for the control in this study differ (Chapman *et al.* 2003, Kunz & Linsenmair 2008), but we assume that detectable differences in

TABLE 1. Composition of groups of V. rubra studied in Ambatoledama.

2006	Adults	Infants	Other
Piste	1♀, 1♂	3	
JJ	1♀, 1♂	4	
JP	1♀, 1♂	2	1 juvenile ♀ born before 2006
2007	**Adults**	**Infants**	**Other**
Piste	1♀, 1♂	0	2 juveniles
JJ	1♀, died in 2007		Not observed
JP	2♀, 1♂	0	2 juveniles

TABLE 2. Summary of fecal and seed sample sizes in this study.

Total fecal samples collected in 2006 & 2007	862	
Fecal samples containing seeds in 2006 & 2007	776	
Fecal samples containing seeds >1mm in length in 2006 & 2007 corrected for samples collected within <1min in a core group	569	
	Passed	Control
Total seeds extracted from 569 fecal samples (>1mm in length)	2110	-
Total seeds used for viability test	905	923
Total seeds used in germination trials	417	428

germination are due to the seed being passed or not. We only used seeds collected from feces and trees from November 2006 to January 2007 for the seed viability and growth trials (Table 2). We employed two methods to determine seed viability after passing though the digestive tract of *V. rubra*. The first method tested seed viability by flotation in water; seeds that sank in a bucket of water were considered "viable" since they have a specific density heavier than water when viable, while empty, dead-filled or insect-damaged seeds usually float (Simak 1973, Demelash *et al.* 2003, Dokolahy 2004). Seeds that were determined "viable" in this first method were planted in the nursery for the second method, which consisted of planting both passed and control seeds in an outdoor nursery located about 50m from the edge of the forest corridor at Ambatoledama. The nursery was situated in secondary forest under an open canopy adjacent to the restoration project nursery. We planted nearly the same number of seeds for the passed and control seed treatments per plant species.

Following the nursery protocol of the restoration project, seeds were placed on top of the soil and covered with a 1-mm layer of river sand to avoid insect predation and maintain a constant temperature. We monitored the following parameters once a month until the end of fieldwork in September 2007: germination, seedling growth in height (cm) measured as the length of the stem from the ground to apical sprout, and seedling survival (noted as dead, alive, or stressed). All observable signs of seedling damage were classified as "stressed". We define germination success in this study as a binomial variable: seeds either germinated over the entire study period or not. The analysis of seedling growth included only those species with emerging seedlings in both treatments and nearly equal sample sizes for statistical comparison.

Statistical analysis. We performed our statistical analysis using JMP 9 (SAS Institute Inc.). We tested the differences in seed size between taxa using a one-way ANOVA. In order to assess whether there is a relationship between seed size and physiological treatment (seeds damaged or intact), we performed a Mann-Whitney U-test. Contingency tables with Chi-squared tests were used to test seed viability and germination, and seedling mortality between passed and control seeds and between taxa (Chaves *et al.* 2011). We acknowledge that the passed seeds are not independent observations, thus violating an assumption of a Chi-squared test and our results might not hold in a truly random sample of seeds. However, we assume that variation between the digestive tracts of individual lemurs is minimal compared with the effect of passing through a digestive tract versus not. We used univariate repeated measures to compare the performance of seedling growth between the treatments with respect to height over time. Finally, a Student's t-test tested individual pairwise comparisons (within species) of least-square means of seedling height.

RESULTS

Characteristics of dispersed seeds. From the three groups we collected 776 fecal samples containing seeds over 108 observation days. On average, four seed-containing fecal samples per day per core group were collected, ranging from one to 18 defecations. We extracted 2110 seeds with a length greater than 1 mm and an average of four individual seeds (greater than 1 mm) per sample. Based on the fecal samples we managed to collect, we estimated that *V. rubra* core groups dispersed a mean of 16 seeds per day (four seeds per feces multiplied by four defecations per day). A fraction of the collected feces did not contain seeds but instead contained only fleshy fruit, leaves, or fecal liquid (See Table 2).

Disseminated seeds belonged to 38 taxa in 15 families, but eight passed seed species could not be identified while two were only identified by their vernacular names. One to four different seed taxa were extracted per feces. In the two study seasons, the most abundant large-seeded species found in the fecal samples were both from the family Clusiaceae: in 2007 *Calophyllum* sp. and in 2006 *Garcinia verrucosa* (Table 3).

Excluding seeds less than 1 mm in length, *V. rubra* dispersed seeds with a mean length of 21.5 mm (sd = 9.2, range = 1.3 to 54.6, n = 2110) and a mean width of 13.7 mm (sd = 4.5, range = 0.7 to 29.8, n = 2110). The largest defecated seed taxon was represented by a liana in the family Cucurbitaceae, *Ampelosicyos humblotii*, which measured on average 48.0 mm in length and 24.7 in width. Results demonstrated that 85% of collected seeds had a length greater than 10 mm (n = 2110) and 76% had a width greater than 10 mm (n = 2110). Seed size varied significantly across seed species (width: F = 203.08, $p < 0.0001$; length: F = 366.16, $p < 0.0001$).

Quality of dispersed seeds. Ninety-eight percent of seeds dispersed by *V. rubra* remained intact after gut passage, sometimes with fruit pulp still attached, and 2% of passed seeds were slightly damaged. Only one passed *G. verrucosa* seed was observed partially bitten. There were few scars on the defecated seeds. Seed size did not influence the physiological treatment (where status = damaged or intact) through the gut passage (Mann-Whitney U-test; length vs. status: Z = -1.288, p = n.s.; width vs. status: Z = -0.221, p = n.s.). Immersion tests demonstrated that passed seeds were significantly more viable (95%) than control seeds (78%) ($\chi^2 = 104.65$, $p < 0.0001$). In the nursery, the germinability of passed seeds (68%) was higher than that of control seeds (50%) ($\chi^2 = 28.02$, $p < 0.001$). For detailed accounts of germination success for a sample of the plant species see Razafindratsima & Razafimahatratra (2010). Seed germination differed significantly between plant taxa ($\chi^2 = 154.03$, $p < 0.001$).

Seedling growth and survival. Emerged seedlings from defecated seeds showed better growth performance over time than control seeds with regard to seedling height (F = 12.93, $p < 0.0001$; Table 4). Likewise, the mortality (the percentage of dead seedlings relative to the quantity of emerged seedlings) was significantly higher in the control group than in the passed seeds ($\chi^2 = 8.661$, $p < 0.001$). Few seedlings within either treatment showed signs of stress, which was characterized by withered leaves, stained leaves or herbivory (6% for passed seeds and 4% for control seeds, Figure 2).

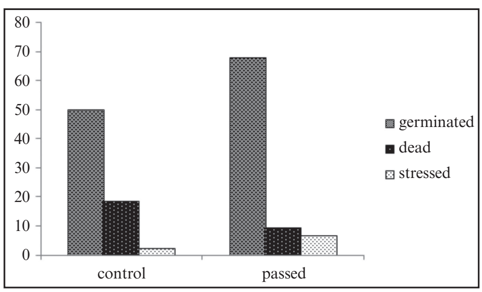

FIG. 2. Proportion of dead and stressed seedlings that emerged from passed and control seeds relative to the percentage of germinated seeds in either treatment.

TABLE 3. Seeds defecated by *V. rubra* in Ambatoledama forest corridor, Masoala National Park, 2006 and 2007. Seeds identified only by vernacular names are in parentheses; the asterisk (*) signifies that hundreds of seeds less than 1 mm in length were extracted but were not measured.

Family	**Genus and species**	**#seeds extracted**	**mean width (mm)**	**mean length (mm)**
Annonaceae	*Ambavia capuronii*	4	18.5	23.4
	Polyalthia sp.	4	11.6	17.9
Arecaceae	*Dypsis sp.1*	3	13.9	24.4
	Dypsis sp.2	20	7.9	11.6
Burseraceae	*Canarium madagascariensis*	1	21.0	33.3
	Protium madagascariensis or Canarium boivinianum	2	16.7	12.5
Clusiaceae	*Callophyllum milvum*	663	13.4	21.8
	Garcinia aphanophlebia	40	6.7	10.3
	Garcinia verrucosa	414	19.3	32.0
Combretaceae	*Terminalia ombrophila*	1	18.0	27.2
Cucurbitaceae	*Ampelosicyos humblotii*	18	24.7	48.0
Euphorbiaceae	*Tannodia sp.*	1	24.2	20.2
	Uapaca silvestris	84	11.8	20.7
	Uapaca sp.	9	8.6	15.8
	(Vahegny)	5	5.3	12.7
Lauraceae	*Aspidostemon sp. or Cryptocarya sp.*	26	19.7	30.3
	Cryptrocarya sp.	188	12.2	18.2
	Cryptocarya peirvillei or Ravensara acuminate	4	16.7	29.4
	Ocotea sp.	5	12.0	20.6
	Ocotea sp. or Potameia velutina	18	15.3	25.0
	Potameia sp.	73	13.7	21.5
Moraceae	*Ficus sp.*	*		
Myrtaceae	*Eugenia cloiselii*	356	8.2	9.7
	Syzygium parkeri	34	12.3	10.5
Oleaceae	*Noronhia grandifolia*	11	16.8	29.5
	Noronhia ovalifolia	2	18.4	30.0
Pandanaceae	*Pandanus sp.*	30	14.6	38.6
	Pandanus odoratissimus	24	16.5	32.2
Rubiaceae	*Canthium boivinianum*	13	15.4	26.0
	Gaertnera sp.	5	9.1	17.4
	Pyrostria sp. or Hyperacanthus sp.	11	5.0	6.0
Sapindaceae	*Allophyllus cobbe*	3	19.1	22.1
	Macphersonia madagascariensis	3	19.0	18.2
Sapotaceae	*Faucherea glutinosa*	2	13.2	30.3
	Faucherea parvifolia	4	13.6	20.4
	Mimusops lecontei	13	15.6	27.0
	Sideroxylon sp.	10	17.9	12.9
N/A	(Ramangitrika)	2	16.4	23.6
	Unidentified species (08)	11		

TABLE 4. Mean seedling height after seven months of monitoring.

		Mean height (cm)							
		Months after sowing							
Seed Species	**Treatment**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**p-value**
Cryptocarya sp.	Control		3.87	5.47	5.86	7.14	7.94	7.72	< **0.0001**
	Passed	7.34	8.12	8.37	8.59	9.08	9.10	9.60	
Eugenia cloiselii	Control	2.27	3.99	4.32	4.68	5.14	6.15	8.98	< **0.0001**
	Passed		8.64	8.90	9.29	9.53	9.99	10.50	
Gaertnera sp.	Control		3.70	5.70	5.90	6.86	7.60	8.38	0.85
	Passed		0.00	0.00	0.00	0.00	0.00	8.00	
Garcinia verrucosa	Control	0.27	4.13	6.67	7.23	7.81	8.38	7.75	**0.06**
	Passed	2.59	4.53	6.13	6.31	6.70	7.29	8.39	
Macphersonia madagascariensis	Control		3.88	4.18	4.55	4.50	7.00	8.00	0.18
	Passed		3.67	3.67	3.73	3.83	3.93	4.00	
Noronhia grandifolia	Control	10.92	14.74	15.67	16.60	17.79	18.41	19.25	0.62
	Passed	12.82	15.49	15.96	16.31	16.89	17.57	19.85	
Potameia sp.	Control	0.00	4.65	8.67	8.79	9.04	10.09	10.64	< **0.0001**
	Passed	6.75	9.06	11.74	11.88	12.11	12.49	13.06	
Sideroxylon sp.	Control		7.33	7.87	8.27	9.35	11.08	11.73	0.25
	Passed		7.67	8.28	9.01	9.56	10.63	11.63	
Uapaca silvestris	Control	0.61	2.69	3.95	4.69	5.61	6.29	6.35	0.70
	Passed	2.70	3.16	3.44	4.22	5.43	5.38	7.73	

DISCUSSION

Vertebrate seed dispersal effectiveness is determined by quantity and quality (Schupp 1993, Kaplin & Moermond 1998, Kaplin & Lambert 2002). Dispersal quantity is defined by the amount of seeds defecated, the diversity of taxa dispersed, the proportion of large seeds, the dispersal distance, and the retention time of seeds (Schupp 1993). Dispersal quality refers to the physiological treatment of seeds while in dispersers' mouths and digestive tracts, and the growth conditions at deposition sites. In this study, dispersal quality was determined by seed viability after being processed in the gut of *V. rubra* and germination success compared with control seeds planted in a nursery that mimics forest-gap deposition sites under an open canopy. A mean of 16 seeds per day was dispersed by *V. rubra* core groups and 76% of the seed taxa dispersed during this study season were large-sized, which included seeds over 10 mm in length (per size classes in Traveset & Verdú 2002). It is noteworthy that *V. rubra* aids in the dispersal of large-sized seeds since they are less likely to be carried by wind away from the parent tree (Wunderle Jr. 1997). Other rain forest lemur species also disperse large-sized and intact seeds (Table 5).

The majority of seed taxa passed by *V. rubra* remained intact and viable. The immersion test may provide a rough measure of viability, but the germination experiment shows whether seeds were viable or not. This analysis using a larger sample size supports the earlier publication by Razafindratsima & Razafimahatratra (2010) that defecated seeds had a higher germination success than control seeds. Emerged seedlings from passed seeds show better growth performance than seedlings from control seeds. Growth can have important consequences for early seedling

TABLE 5. Published accounts of seed dispersal by lemur species. The categories of seed sizes follow Traveset & Verdú (2002): large > 10 mm, medium 5-10 mm, small < 5 mm.

Species	Percent fruit in diet	Mean # of seeds passed per day	# Seed species defecated	Majority seed size	Intact seeds	Germination success	References
E. fulvus	80.6%	-	9	large	81.8%	60%	Dew & Wright (1998)
E. macaco	-	-	57	large	-	37%	Birkinshaw (2001)
E. rubriventer	66.8%	-	7	large	63.6%	80%	Dew & Wright (1998)
P. diadema	30%	-	2	small	15.4%	0%	Dew & Wright (1998)
V. rubra	50-88%†	20	46	large	98%	61%	This study
V. variegata	70.8%*	9	14 & 40‡	large	77.8%	66.6%	Dew & Wright (1998)

†Martinez (2010), *White (1991), ‡Moses and Semple (2011)

competition. For example, increased seedling growth is advantageous for plant reproductive success because of the improved competitive ability among seedlings (Paulsen and Högstedt 2002). Similar to some other vertebrate-dispersed plants, species in our study may require or benefit from a chemical scarring process inside the lemur gut for rapid and successful germination (McKey 1975).

The persistence of lemurs at Ambatoledama depends on a forest that contains the necessary resources and, conversely, forest regeneration depends on seed dispersers. Thus the conservation of *V. rubra* is of key importance for an effective regeneration program at Ambatoledama. *V. rubra* is an endemic and endangered species in Masoala National Park, and potentially an important actor in the corridor regeneration. *V. rubra* is an obligate frugivore (Vasey 1997a) and the genus *Varecia* is susceptible to forest disturbance (Balko & Underwood 2005). However, *V. rubra* was observed spending time feeding and resting in the regenerating forest patches at Ambatoledama, which are characterized by an open canopy, a mixture of pioneer species, and young interior forest trees (Martinez 2010). Like the majority of Malagasy vertebrates, *V. rubra* is threatened by anthropogenic pressures such as the destruction of their habitat for fuel-wood harvesting and local slash-and-burn agriculture known as "*tavy*" (Vasey 1997b, Hekkala *et al.* 2007, Dunham *et al.* 2008). Decrease in the population size or extinction of this species would affect the diversity and demography of the vegetation in the Masoala rain forest, as observed in other forests where frugivorous vertebrates have gone extinct (Redford 1992, Chapman & Onderdonk 1998, Ganzhorn *et al.* 1999, Bascompte & Jordano 2007, Babweteera & Brown 2009). As *V. rubra* is the largest-bodied frugivore in Masoala (Vasey 2004), seed dispersal by this species is important because they are highly mobile and can therefore contribute to the connecting of rain forest fragments (Bascompte & Jordano 2007).

The effectiveness of seed dispersal can be evaluated not only by the diversity and viability of dispersed seeds but also by the seed fate after deposition, which is influenced by many factors, including: the physiological and chemical treatment in the digestive tract (Dew & Wright 1998, Stevenson *et al.* 2002, Knogge *et al.* 2003, Linnebjerg *et al.* 2009, Chapman *et al.* 2010), the microhabitat at the site of deposition (Schupp 1988, Wehncke & Dalling 2005), and secondary seed dispersers (Bleher & Böhning-Gaese 2001). Although we found improved seed germination and seedling growth with passed seeds, recruitment and survival of seeds in their natural habitat may be limited by competition with faster-growing exotic species (Hooper *et al.* 2005, Styger *et al.* 2007) or other existing native vegetation (Teegalapalli *et al.* 2010), but more detailed studies are needed in the rain forests of Madagascar. Abiotic factors like slope, aspect, and soil type will also influence germination and recruitment (Holl 1999, Duncan & Chapman 2002). Ambatoledama is characterized by a rough and rocky terrain with steep slopes averaging 37° (range: 21°–58°) (Razafindratsima, unpublished data), and the steep slopes probably affect the successful recruitment of seed and seedling establishment since some seeds can roll downhill. In addition, frequent soil erosion is possible because of the steep terrain. Ambatoledama receives 2200–7000 mm of rainfall per year and is subject to violent cyclones (Hatchwell 1999, Ratsisetraina 2006). Run-off during heavy rainfall can cause seeds to wash away from deposition sites. Seed predation by insects and rodents is another factor that affects the fate of passed seeds (Wehncke & Dalling 2005). There are presumably a large number of natural predators of seeds in Masoala National Park, but much research is still needed. Finally, in order to better understand the role of fruit-eating Malagasy vertebrates in maintaining the rain forest ecosystem, we encourage further research on all aspects of seed dispersal.

ACKNOWLEDGMENTS

We wish to express our thanks to the Ministère des Eaux et Forêts, the University of Antananarivo, and the Wildlife Conservation Society in Madagascar, and all MNP Maroantsetra staff for logistical support. We thank E. Louis and his team for help with capturing animals. We also thank J. Kloppenburg, J. Schmitt, C. Catania and the local guides in Ambatoledama, especially Paul and Jao Aridy for assistance in the field. We are very grateful to Leon, MNP Conservation Agent, for taking care of the nursery during our absence. Thanks are also due to C. Birkinshaw and E. Razafimahatratra for their advice. We are grateful to A.E. Dunham, N. Farwig, and anonymous reviewers for their useful suggestions in improving an earlier draft of this manuscript. Funding was provided by a Fulbright Fellowship, the Margot Marsh Biodiversity Foundation, the Minnesota Zoo Ulysses S. Seal Conservation Fund, the Primate Action Fund, the University of Minnesota Graduate School, the University of Minnesota Graduate Program in Conservation Biology, the University of Minnesota Interdisciplinary Center for the Study of Global Change, and the IdeaWild Foundation. O.H.R. was supported by Philanthropic Educational Organization, The Leakey Foundation and Schlumberger Foundation fellowships during writing and analysis.

REFERENCES

Andrainarivo, C., Andriaholinirina, V.N., Feistner, A.T.C., Felix, T., Ganzhorn, J.U., Garbutt, N., Golden, C., Konstant, B., Louis, E.E. & D. Meyers. 2008. *Varecia rubra*. *In* IUCN 2009. IUCN Red List of Threatened Species. Version 2009.2. www.iucnredlist.org.

Babweteera, F. & N. Brown. 2009. Can remnant frugivore species effectively disperse tree seeds in secondary tropical rain forests? Biodiversity and Conservation 18: 1611-1627.

Balko, E.A. & H.B. Underwood. 2005. Effects of forest structure and composition on food availability for *Varecia variegata* at Ranomafana National Park, Madagascar. American Journal of Primatology 66: 45-70.

Bascompte, J. & P. Jordano. 2007. Plant-animal mutualistic networks: the architecture of biodiversity. Annual Review of Ecology and Systematics 38: 567-593.

Berens, G.D., Farwig, N., Schaab, G. & K. Böhning-Gaese. 2008. Exotic guavas are foci of forest regeneration in Kenyan farmland. Biotropica 40: 104-112.

Birkinshaw, C. 2001. Fruit characteristics of species dispersed by the black lemur (*Eulemur macaco*) in the Lokobe Forest, Madagascar. Biotropica: 478-486.

Bleher, B. & K. Böhning-Gaese. 2001. Consequences of frugivore diversity for seed dispersal, seedling establishment and the spatial pattern of seedlings and trees. Oecologia 129: 385-394.

Bollen, A., Elsacker, L.V., & J.U. Ganzhorn. 2004. Relations between fruits and disperser assemblages in a Malagasy littoral forest: a community-level approach. Journal of Tropical Ecology 20: 599-612.

Camargo, J.L.C., Ferraz, I.D.K. & A.M. Imakawa. 2002. Rehabilitation of degraded areas of Central Amazonia using direct sowing of forest tree seeds. Restoration Ecology 10: 636-644.

Chapman, C.A. & D.A. Onderdonk. 1998. Forests without primates: primate/plant codependency. American Journal of Primatology 45: 127-141.

Chapman, C.A., Chapman, L.J., Rode, K.D., Hauck, E.M. & L.R. McDowell. 2003. Variation in the nutritional value of primate foods: among trees, time periods, and areas. International Journal of Primatology 24: 317-333.

Chapman, H.M., Goldson, S.L. & J. Beck. 2010. Postdispersal removal and germination of seed dispersed by *Cercopithecus nictitans* in a West African montane forest. Folia Primatologia 81: 41-50.

Chaves, O.M., Stoner, K.E., Arroyo-Rodriguez, V. & A. Estrada. 2011. Effectiveness of spider monkeys (*Ateles geoffroyi vellerosus*) as seed dispersers in continuous and fragmented rain forests in southern Mexico. International Journal of Primatology 32: 177-192.

Cramer, J.M., Mesquita R.C.G. & G.B. Williamson. 2007. Forest fragmentation differentially affects seed dispersal of large and small-seeded tropical trees. Biological Conservation 137: 415-423.

Culot, L., Munoz Lazo, F.J.J., Huynen, M., Poncin, P. & E.W. Heymann. 2010. Seasonal variation in seed dispersal by tamarins alters seed rain in a secondary rain forest. International Journal of Primatology 31: 553-569.

Demelash, L., Tigabu, M. & P.C. Oden. 2003. Enhancing germinability of *Schinus molle* L. seed lot from Ethiopia with specific gravity and IDS techniques. New Forests 26: 33-41.

Dew, J.L. & P.C.Wright. 1998. Frugivory and seed dispersal by four species of primates in Madagascar's eastern rain forest. Biotropica 30: 425-437.

Dokolahy, R.J. 2004. Etude de la régénération naturelle des savoka du pont forestier d'Ambatoledama en vue d'une restauration forestière. DEA Dissertation, University of Antananarivo.

Duncan, R.S. & C.A. Chapman. 2002. Limitations of animal seed dispersal for enhancing forest succession on degraded lands. Pp. 437–450 *in* Levey, D.J., Silva, W.R. & M. Galetti (eds.). Seed dispersal and frugivory: ecology, evolution and conservation. CAB International, New York.

Dunham, A.E., Erhart, E.M., Overdorff, D.J. & P.C. Wright. 2008. Evaluating effects of deforestation, hunting, and El Niño events on a threatened lemur. Biological Conservation 141: 287-297.

Ganzhorn, J.U., Fietz, J., Rakotovao, E., Schwab, D. & D. Zinner. 1999. Lemurs and the regeneration of dry deciduous forest in Madagascar. Conservation Biology 13: 794-804.

Gorchov, D.L., Cornejo, F., Ascorra, C. & M. Jaramillo. 1993. The role of seed dispersal in the natural regeneration of rain forest after strip-cutting in the Peruvian Amazon. Vegetatio 107/108: 339-349.

Green, G.M. & R.W. Sussman. 1990. Deforestation history of the eastern rain forests of Madagascar from satellite images. Science 248: 212-215.

Hatchwell, M. 1999. Plan de gestion de complexe des Aires Protegees de Masoala. Wildlife Conservation Society report (unpublished).

Hekkala, E.R., Rakotondratsima, M. & N. Vasey. 2007. Habitat and distribution of the ruffed lemur, *Varecia rubra*, North of the Bay of Antongil in Northeastern Madagascar. Primate Conservation 22: 89-95.

Holl, K.D. 1999. Factors limiting tropical rain forest regeneration in abandoned pasture: seed rain, seed germination, microclimate, and soil. Biotropica 31: 229-242.

Holloway, L. 2000. Catalyzing rainforest restoration in Madagascar. Pp. 115-124 *in* Lourenço, W.R. & S.M. Goodman (eds.). Diversité et endemisme à Madagascar. Memoires de la Societe de Biogeographie, Paris.

Hooper, E., Legendre, P. & R. Condit. 2005. Barriers to forest regeneration of deforested and abandoned land in Panama. Journal of Applied Ecology 42: 1165-1174.

Janzen, D.H. 1988. Management of Habitat Fragments in a Tropical Dry Forest: Growth. Annals of the Missouri Botanical Garden 75: 105-116.

Jordano, P. 2000. Fruits and frugivory. Pp. 125-166 *in* Fenner, M. (ed.). Seeds: The ecology of Regeneration in Natural Plant Communities. CABI International, UK.

Kaplin, B.A. & T.C. Moermond. 1998. Variation in seed handling by two species of forest monkeys in Rwanda. American Journal of Primatology 45: 83-101.

Kaplin, B.A. & J.E. Lambert. 2002. Effectiveness of Seed Dispersal by *Cercopithecus* Monkeys: Implications for Seed Input into Degraded Areas. Pp. 351-364 *in* Levey, D.J., Silva, W.R. & M. Galetti (eds.). Seed dispersal and frugivory: ecology, evolution and conservation. CABI International, UK.

Knogge, C., Herrera, E.R.T. & E.W. Heymann. 2003. Effects of passage through tamarin guts on the germination potential of dispersed seeds. International Journal of Primatology 24: 1121-1128.

Kunz, B.K. & K.E. Linsenmair. 2008. Seed size selection by olive baboons. Primates 49(4): 239-245.

Lahann, P. 2007. Feeding ecology and seed dispersal of sympatric cheirogaleid lemurs (*Microcebus murinus*, *Cheirogaleus medius*, *Cheirogaleus major*) in the littoral rainforest of south-east Madagascar. Journal of Zoology 271: 88-98.

Leopold, A.C., Andrus, R., Finkeldey, A. & D. Knowles. 2001. Attempting restoration of wet tropical forests in Costa Rica. Forest Ecology and Management 142: 243-249.

Linnebjerg, J.F., Hansen, D.M. & J.M. Olesen. 2009. Gut passage effect of the introduced red-whiskered bulbul (*Pycnonotus jocosus*) on germination of invasive plant species in Mauritius. Austral Ecology 34: 272-277.

Martinez, B.T. 2010. Forest restoration in Masoala National Park, Madagascar: the contribution of the red-ruffed lemur (*Varecia rubra*) and the livelihoods of the subsistence farmers at Ambatoladama. Ph.D. Dissertation, University of Minnesota, Saint Paul.

McKey, D. 1975. The ecology of coevolved seed dispersal systems. Pp. 159-191 *in* Gilbert, L.E. & P. Raven (eds.). Coevolution of animals and plants: Symposium V, First International Congress of Systematic and Evolutionary Biology, Boulder, Colorado, August 1973.

Mittermeier, R.A., Louis Jr., E.E., Richardson, M., Schwitzer, C., Langrand, O., Rylands, A.B., Hawkins, F., Rajaobelina, S., Ratsimbazafy, J., Rasoloarison, R., Roos, C., Kappeler, P.M. & J. Mackinnon. 2010. Conservation International Tropical Field Guide Series: Lemurs of Madagascar. 3rd edition. Conservation International, Arlington, VA.

Moses, K.L. & Semple, S. 2011. Primary seed dispersal by the black-and-white ruffed lemur (*Varecia variegata)* in the Manombo forest, south-east Madagascar. Journal of Tropical Ecology 27: 529-538.

Murray, K.G. 1988. Avian seed dispersal of three neotropical gap-dependent plants. Ecological Monographs 58: 271-298.

Neilan, W., Catterall, C.P., Kanowski, J. & S. McKenna. 2006. Do frugivorous birds assist rainforest succession in weed dominated oldfield regrowth of subtropical Australia? Biological Conservation 129: 393-407.

Overdorff, D.J. & S.G. Strait. 1998. Seed handling by three prosimian primates in southeastern Madagascar: implications for seed dispersal. American Journal of Primatology 45: 69-82.

Paulsen, T.R. & G. Högstedt. 2002. Passage through bird guts increases germination rate and seedling growth in *Sorbus aucuparia*. Functional Ecology 16: 608-616.

Ratsisetraina, I.R. 2006. Etude du recouvrement de population de *Varecia variegata rubra* et d' *Eulemur fulvus albifrons* à la suite d'une perturbation cyclonique dans le Parc National de Masoala, Madagascar. Mémoire de DEA. DEA Dissertation, University of Antananarivo.

Razafindratsima, O.H. & E. Razafimahatratra. 2010. Effect of red ruffed lemur gut passage on the germination of native rainforest plant species. Lemur News 15: 39-42.

Redford, K. 1992. The empty forest. BioScience 42: 412-423.

Rigamonti, M.M. 1993. Home range and diet in red ruffed lemurs (*Varecia variegata rubra*) on the Masoala Peninsula, Madagascar. Pp. 25-40 *in* Kappeler, P.M. & J.U. Ganzhorn (eds.). Lemur social systems and their ecological basis. Plenum Press, New York.

Scharfe, F. & W. Schlund. 1996. Seed removal by lemurs in a dry deciduous forest of western Madagascar. Pp. 295-304 *in* Ganzhorn, J.U. & J.-P. Sorg (eds.). Ecology and economy of a tropical dry forest in Madagascar. Primate Report Special Issue 46-1.

Schupp, E.W. 1988. Factors affecting post-dispersal seed survival in a tropical forest. Oecologia 76: 525-530.

Schupp, E.W. 1993. Quantity, quality and the effectiveness of seed dispersal by animals. Plant Ecology 107: 15-29.

Simak, M. 1973. Separation of forest seed through flotation. *In* Seed Processing. Proceedings IUFRO Working Group on Seed Problems, IUFRO, Bergen Vol. I, Paper 16.

Spehn, S.E. & J.U. Ganzhorn. 2000. Influence of seed dispersal by brown lemurs on removal rates of three *Grewia* species (Tiliaceae) in the dry deciduous forest of Madagascar. Ecotropica 6: 13-21.

Stevenson, P.R. 2000. Seed dispersal by woolly monkeys (*Lagothrix lagothricha*) at Tinigua National Park, Colombia: dispersal distance, germination rates, and dispersal quantity. American Journal of Primatology 50: 275-289.

Stevenson, P.R., Castellanos, M.C., Pizarro, J.C. & M. Garavito. 2002. Effects of seed dispersal by three ateline monkey species on seed germination at Tinigua National Park, Colombia. International Journal of Primatology 23: 1187-1204.

Styger, E., Rakotondramasy, H.M., Pfeffer, M.J., Fernandes, E.C.M. & D.M. Bates. 2007. Influence of slash-and-burn farming practices on fallow succession and land degradation in the rainforest region of Madagascar. Agriculture, Ecosystems and Environment 119: 257-269.

Teegalapalli, K., Hiremath, A.J. & D. Jathanna. 2010. Patterns of seed rain and seedling regeneration in abandoned agricultural clearings in a seasonally dry tropical forest in India. Journal of Tropical Ecology 26: 25-33.

Traveset, A., & M. Verdú 2002. A meta-analysis of the effect of gut treatment on seed germination. Pp. 339-350 *in* Levey, D.J., Silva, W.R. & M. Galetti (eds.). Seed dispersal and frugivory: ecology, evolution and conservation. CABI International, UK.

van der Pijl, L. 1972. Principles of dispersal in higher plants, 2nd edition. Springer-Verlag, Berlin.

Vasey, N. 1997a. Community ecology and behavior of *Varecia variegata rubra* and *Lemur fulvus albifrons* on the Masoala Peninsula, Madagascar. Ph.D. Dissertation, Washington University, St. Louis.

Vasey, N. 1997b. How many red ruffed lemurs are left? International Journal of Primatology 18: 207-216.

Vasey, N. 2000. Niche separation in *Varecia variegata rubra* and *Eulemur fulvus albifrons*: I. Interspecific patterns. American Journal of Physical Anthropology 112: 411-431.

Vasey N. 2004. *Varecia*, Ruffed Lemurs. Pp. 1332-1336 *in* Goodman, S.M. & J.P. Benstead (eds.). The Natural History of Madagascar. The University of Chicago Press, Chicago.

Vasey, N. 2006. Impact of seasonality and reproduction on social structure, ranging patterns, and fission-fusion social organization in red ruffed lemurs. Pp. 275-304 *in* Gould, L. & M.L. Sauther (eds.). Lemurs: Ecology and Adaptation. Springer, New York.

Wehncke, E.V. & J.W. Dalling. 2005. Post-dispersal seed removal and germination-selected tree species dispersed by *Cebus capucinus* on Barro Colorado Island, Panama. Biotropica 37: 73-80.

White, F.J. 1991. Social organization, feeding ecology and reproductive strategy of Red-ruffed lemurs, *Varecia variegata*. Pp. 81-84 *in* Ehara A., Kimura, T., Takenka, O. & M. Iwamoto (eds.). Primatology Today: Proceedings of the XIII Congress of the International Primatological Society, Nagoya and Kyoto 18-24 July 1990. Elsevier Science Publishers, Amsterdam.

Wunderle Jr., J.M. 1997. The role of animal seed dispersal in accelerating native forest regeneration on degraded tropical lands. Forestry Ecology and Management 99: 223-235.