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Effect of red ruffed lemur gut passage on the germination of native rainforest plant species

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Abstract
Like much of Madagascar's remaining rainforest, the forest of Masoala National Park is facing severe threats from deforestation and fragmentation. The remaining fragmented areas are connected by degraded corridors which are important for biological exchange. Frugivorous animals such as lemurs may have an important role in the restoration of such degraded areas through seed dispersal. Unfortunately, no studies have been carried out before concerning the role lemurs play in the restoration of the largest corridor in Masoala, Ambatoledama. This study explores the effect of seed passage inside the gut of the frugivorous red-ruffed lemur (*Varecia rubra*) on the germination of some native tropical plants with the aim to understand the capacity of *V. rubra* to help in the restoration of the Ambatoledama corridor. We planted seeds of nine plant species that we collected from *V. rubra*'s fresh feces in a nursery to compare with seeds that we extracted manually from corresponding fruits. The germination of seeds was monitored each month after planting them. Results showed that defecated seeds had overall a significantly higher germination rate than non-passed seeds. Thus, lemur ingestion of seeds has the capacity to improve seed germination of several species and some plants require the physiological treatment inside the gut to germinate. Results suggested that restoration projects in the area including the Ambatoledama corridor should take into account the important role *Varecia rubra* plays in the regeneration of the forest and corridor. Management actions that increase movement and protection of animals moving into and out of the corridor will be important for the long term success of the project.

Introduction
The rainforest of the Masoala Peninsula suffers greatly from loss and fragmentation caused by the human population living around the area. The forest is subdivided into different fragments, connected by corridors of degraded habitat which are Ambatoledama, Analambolo and Ilampy (Holloway, 1997). Corridors are vital for enabling gene flow and dispersal of wildlife among habitat fragments (Mech and Hallett, 2001). The largest of these is the Ambatoledama corridor, which connects two large parcels of the forest (Fig. 1). The restoration of this corridor is critical for safeguarding wildlife populations in the fragments and for preserving gene flow between fragments (Mech and Hallett, 2001; Haddad *et al.*, 2003). To restore this degraded corridor, it is necessary to plant native trees or to encourage zoochory (biological dispersal of seeds through animal defecation) (Duncan and Chapman, 2002; Neilan *et al.*, 2006). Since 1997, Madagascar National Parks (MNP) and the Wildlife Conservation Society (WCS) have established a restoration project in the Ambatoledama corridor by planting native fruiting trees (Holloway, 1997) with the aim of attracting frugivorous vertebrates which will in turn carry seeds into the degraded parts of the forest and into forest clearings. Unfortunately, no studies have previously been carried out to shed light on the importance of frugivorous animals, especially lemurs, in the reforestation of the Ambatoledama corridor. Unlike the majority of tropical forests, the diversity of the frugivorous bird community in Madagascar is impoverished, and therefore primates are the principal dispersers of its tropical trees (Goodman, 1997; Dew and Wright, 1998; Ganzhorn *et al.*, 1999; Bleher and Böhning-Gaese, 2001). Ten lemur species are indentified as living in the Masoala Forest (Mittermeier *et al.,* 2006); one of which (*Varecia rubra*) is endemic to this region and has Endangered status (IUCN, 2008), and can be found in both the corridor habitat and adjacent forest fragments (Razakamaharavo *et al.,* 2010). Previous studies demonstrated that *Varecia variegata* is an effective disperser in the southeastern rainforests (Dew and Wright, 1998). However, we know very little about the potential role of *V. rubra* for regeneration and restoration of the corridor habitat in Ambatoledama.
In this study, we explored the germination success of seeds defecated by *Varecia rubra* in order to understand their capacity for seed dispersal and potential impact on the restoration of the degraded rainforest corridor at Ambatoledama. Our objective was to shed light on the role of this species in forest regeneration. Understanding their influence on tree germination is particularly important given the threatened status of this species. This paper tested the hypothesis that gut passage of seeds by *Varecia rubra* facilitates seed germination. Our prediction was that lemur-gut-passed seeds have a higher germination rate than non-passed seeds because of the physiological treatment affecting the seed coat inside the gut.

Materials and methods

Field site

This study was carried out at the Ambatoledama corridor (S15°27' E050°01') on the north-eastern part of the Masoala Peninsula. Ambatoledama connects Masoala National Park with Makira National Forest to the West. Its forest has undergone significant deforestation but restoration projects have augmented Ambatoledama such that it now forms a 1 km wide corridor of secondary forest (Hekkala *et al*., 2007; Razakamaharavo *et al*., 2010). It consists of a dense evergreen rainforest with an altitude ranging from 300 to 700 m. The forest is mostly characterized by the presence of tree species of the Pandanacea, Ebenaceae, Clusiaceae, Euphorbiaceae, Sapotaceae and Rubiaceae families (Martinez, unpublished).

Study species

Varecia rubra belongs to the family Lemuridae (Gray, 1821) and is one of two species recognized within the genus (Mittermeier *et al*., 2006). *V. rubra* is only found on the Masoala peninsula and it is classified by the World Conservation Union (IUCN) as Endangered (IUCN, 2008). *V. rubra* is a large-sized diurnal species with a body length ranging from 43 to 57 cm (Vasey, 2003) and has a typically frugivorous diet (Rigamonti, 1993; Vasey, 1997). They currently inhabit both the corridor habitat and the adjacent protected areas (Razakamaharavo *et al*., 2010) and are thus potentially important for regeneration of the corridor habitat.

Field experiment

Focal animals were followed for 3-5 days per week from dawn to dusk (from 0600 hours to 1800 hours) to collect fresh fecal samples (Dew and Wright, 1998; Kaplin and Moermond, 1998; Stevenson, 2000; Poulsen *et al*., 2001; Link and Di Fiore, 2006). Each fecal sample was washed and filtered through a 1-mm sieve (Stevenson, 2000). Seeds were extracted and then identified with the help of local research guides and an expert local botanist familiar with the Masoala flora.

We planted gut-passed seeds and control seeds that were extracted manually from fruits in an outdoor nursery adjacent to the corridor at Ambatoledama. The nursery consisted of two "flower beds" of 11.2 m^2: one for defecated seeds and the other one for non-passed seeds. Following methods used by the conservation agents of MNP in Ambatoledama, a sunshade of 80 cm height, composed of Longoza leaves (*Afromomum angustifolium*) was placed above each flower bed to imitate the closed canopy of the forest. Also, the soil of the nursery was mixed with fertile soil from cultivated field. Seeds were placed in the soil mixture and covered by 1 mm-thick river sand to keep a constant temperature.

An equal number of seeds were planted within each species per treatment. However, the numbers varied between species depending on how many seeds were collected from lemur feces. The germination of seeds was assessed each month after planting.

Data analysis

We performed a paired t-test to test for differences between the germination rate of lemur-gut-passed and non-passed seeds, an ANOVA analysis to test if the two factors (seed species and treatment) had effects on the germination rate of the seeds and to determine whether there was interaction between these factors. We analyzed the germination of each species in order to assess the influence by lemur gut passage, with Pearson test using contingency tables, which was adjusted with Bonferroni correction for multiple comparisons (Sokal and Rohlf, 1995).

Results

In total, 268 fresh fecal samples from three individuals of red-ruffed lemur were collected during 58 days of observation. The fecal samples contained fleshy fruit parts, stalks, leaves, soil and fecal liquid. 95.52 % of these contained seeds, to some of which fleshy fruit parts were still attached. 906 seeds of more than 1mm size were extracted. A majority of them were intact with minor scarification. They represented 34 different plant species that belong to 15 Families. Based on our collected sample, the most common seed species found in lemur defecations were the nine species we chose to study here (Tab. 1). In the nursery, we planted a total of 390 defecated seeds and compared them with 398 non-passed seeds.

Lemur-gut-passed seeds had significantly higher germination rates overall than non-passed seeds ($t=3.284$, $df=8$, $p=0.011$). Passed seeds had a germination rate of 64.61 %, whereas non-passed seeds had a rate of 39.69 %. For each species, seeds that had been defecated had a higher germination rate than non-passed seeds, except for Tsilaitra (Tab. 1). This pattern was driven primarily by four species, including Antaivaratra, Matahobaratra, Tsilaitra, and Vongobe species.

In a two factor analysis of variance for seed germination, there was a significant interaction between the species of seeds and their treatment (passed or non-passed) ($F= 4.2004$, $p<0.0001$). When the analysis was repeated excluding the interaction, seed germination differed significantly both between seed species ($F=23.268$, $p<0.0001$) and between their origins ($F= 58.706$, $p<0.0001$). For some species, gut passage might only be important for dispersal away

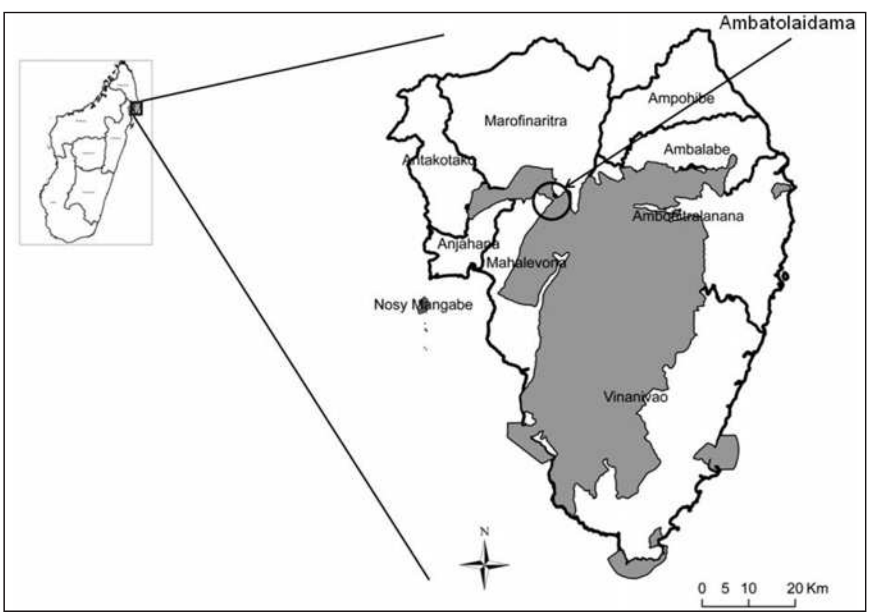

Fig. 1: Location of the Ambatoledama corridor.

Tab. 1: List of species studied with their germination rate. Sample sizes are represented in brackets. The star on p-values corresponds to their significance (Pearson test) after a Bonferroni correction for multiple tests.

#	Malagasy name	Scientific name	Family	Germination rate		Pearson test	
				gut-passed seeds	non-passed seeds	Chi-square	P-value
1	Antaivaratra	*Potameia* sp.	Lauraceae	41.67 (n = 48)	17.86 (n = 56)	7.139	0.0075*
2	Hazondronono	*Sideroxylon*	Sapotaceae	80.00 (n = 10)	60.00 (n = 10)	0.952	0.3291
3	Karaka	*Pandanus*	Pandanaceae	40.00 (n = 20)	15.00 (n = 20)	3.135	0.0766
4	Matahobaratra	*Garcinia* sp.	Clusiaceae	58.06 (n = 31)	00.00 (n = 31)	25.364	<0.0001*
5	Rotro Beravina	*Eugenia* sp.	Myrtaceae	60.61 (n = 33)	42.42 (n = 33)	2.184	0.1394
6	Tavolo madinidravina	*Cryptocarya* sp.	Lauraceae	51.25 (n = 80)	26.25 (n = 80)	1.043	0.307
7	Tsilaitra	*Norhonia* sp.	Oleaceae	91.67 (n = 12)	100.0 (n = 12)	10.533	0.0012*
8	Vapakafotsy	*Uapaca silvestris*	Euphorbiaceae	75.00 (n = 56)	25.00 (n = 56)	1.17	0.2795
9	Vongobe	*Garcinia verrucosa*	Clusiaceae	84.00 (n = 100)	78.00 (n = 100)	28	<0.0001*

from the parent tree; for others, it is also important for their germination success. The difference of germination rate within each species showed that for four species, the germination rate of defecated-seeds was higher than for non-passed seeds (Tab. 1).

Discussion

As we predicted, our results showed that lemur-gut-passed seeds had a higher germination rate than non-passed seeds. Based on our collected fecal samples, our study confirmed that *V. rubra* has a mainly frugivorous diet. The nine species (Tab. 1) studied here represented the most common species in *V. rubra*'s diet during the humid hot season. Its frugivorous diet and passing of intact seeds suggest that *V. rubra* is predisposed to be a beneficial seed disperser. Frugivorous animals are, in general, categorized into three classes (Kaplin and Moermond, 1998; Bollen *et al.*, 2004; Gosper *et al.*, 2005): (1) seed dispersers which have the capacity to carry seeds from one place to another, (2) those who drop seeds under the parent tree without ingesting them, and (3) seed predators which digest seeds. Our results showed that *V. rubra* may be an effective seed disperser of several tropical rainforest plants in the Ambadoledama corridor through endozoochory. The passage of seeds in *V. rubra*'s gut improved the germination of several species in this study (Chapman and Chapman, 1996; Poulsen *et al.*, 2001). It appears that some plant taxa in particular, require a chemical scarification process inside the lemur gut to acquire a high level of germination capacity, like the majority of vertebrate-dispersed plants (McKey, 1975; Dew and Wright, 1998), as they may not be able to germinate without the removal of their aril by a frugivore (Howe, 1986). Moreover, the plant species making up the majority of the diet appeared to be primarily large-sized seeds (10-30 mm; Razafindratsima and Martinez, unpublished data), and are therefore difficult to swallow by frugivorous birds.

As a seed vector, *V. rubra* may play an important role in maintaining forest diversity by affecting the spatial distribution and dynamics of plants (Bleher and Böhning-Gaese, 2001; Clark *et al.*, 2001; Brodie *et al.*, 2009). Endozoochory by this species is an important strategy for the plant to increase its fitness because seeds can minimize the time they spend in the embryogenesis phase (Dew and Wright, 1998), which is likely to reduce the rate of seed predation by rodents and other granivores (Wehncke and Dalling, 2005). Also, it helps plants to avoid disproportionate seed and seedling mortality near the parent, and to be deposited in a microhabitat suitable for their establishment and growth (Howe and Smallwood, 1982).

The results presented here suggest that *V. rubra* may be vital to corridor restoration, which is important for maintaining the biotic exchange between the forested blocks of the Masoala Peninsula. Ambatoledama is vulnerable and currently facing significant fragmentation (Dokolahy, 2005; Razakamaharavo *et al.*, 2010). Thus, the existence of such dispersers in this site is likely to be very important for quickly facilitating seed dissemination. A potential loss of the floral diversity will occur if this lemur species goes extinct or moves into other forest blocks. Recent increases in both bushmeat hunting for lemurs and tree poaching in the area (Hatchwell, 1999; Barrett and Ratsimbazafy, 2009; Golden, 2009; Into, 2009; Schuurman and Lowry, 2009) may have dire consequences for forest regeneration and future habitat restoration efforts. Loss of the floral diversity and change in community structure of the vegetation is expected to occur in the absence of these lemurs which may be critical for dispersal of many larger-seeded species. Decline or loss of this species may also limit successful forest regeneration and habitat restoration of the corridor. Thus the conservation of *V. rubra* is likely to be key for an effective restoration program at Ambatoledama.

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