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Re-introduction of diademed sifaka (*Propithecus diadema*) and black and white ruffed lemurs (*Varecia variegata editorum*) at Analamazaotra Special Reserve, eastern Madagascar

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Deforestation, mainly as a result of slash-and-burn agriculture (tavy) and selective logging, has occurred continuously since the arrival of humans to Madagascar over 2000 years ago (Jolly, 1989; Harcourt and Thornback, 1990). This has resulted in rising pressure on native wildlife as the island has lost 80-90 % of its original forest habitat (Mittermeier *et al.*, 2005). One group experiencing the greatest impact from this biodiversity loss are lemurs, a diverse assembly of over 100 primate species found no where else in the world (Green and Sussman, 1990; Du Puy and Moat, 1998; Mittermeier *et al.*, 2006). In addition to disappearing habitat, lemurs are also under continuous pressure from illegal poaching as a food source (Lehman and Wright, 2000; Mutschler *et al.*, 2001; Goodman and Raselimanana, 2003), especially the larger species such as sifakas and ruffed lemurs (Mittermeier *et al.*, 2006). This hunting pressure ultimately led to the local extinction of the endangered Diademed sifaka (DS, *Propithecus diadema*) and the critically endangered black and white ruffed lemur (BWRL, *Varecia variegata editorum*; IUCN, 2009; Mittermeier *et al.*, 2008) from Analamazaotra Special Reserve (ASR) in 1973 and 1976, respectively (pers. comm., B. M. Ratsisakanana, P. Rabearisoa, Joseph).

Analamazaotra Special Reserve (810 ha), also referred to as Andasibe after the neighboring local village or as Perinet by European tourists, was initially part of a continuous forest with Mantadia National Park (15,500 ha) to the north, Maromizaha Classified Forest (1,600 ha) to the southeast, and Anosibe an'ala (1,500 ha) to the south (ANGAP, 2005). Although all four are now relatively isolated forest fragments, Andasibe-Mantadia National Park and ASR remain one of the most popular tourist destinations in Madagascar due to the opportunity to view the indri (*Indri indri*), Madagascar's largest remaining extant lemur. These four rain forest habitats are home to vast biodiversity encompassing many endemic rare and endangered plants and animals, including 13 lemur species. Although hunted to extinction in ASR, *P. diadema* and *V. v. editorum* continued to exist in the forests of Mantadia, Maromizaha, and Anosibe an'ala.

Beginning in January 2006, after more than four years of planning, the Madagascar Biodiversity and Biogeography Project of Henry Doorly Zoo (MBP-HDZ), in collaboration with Madagascar National Parks (MNP; formerly known as Association Nationale pour la Gestion des Aires Protégées, or ANGAP) and the Ministère de l'Environnement, des Forêts et de Tourisme (MEFT), initiated the first ever attempt to recover a species' former distribution in Madagascar. Following the recommendations of the *IUCN / SSC Re-introduction Specialist Group: Guidelines for Nonhuman Primate Re-introductions* (2002), the MBP-HDZ initiated the Analamazaotra Re-introduction/Translocation (ART) Project. This paper provides a summary to date of the project's efforts to re-establish the Diademed sifaka and the black and white ruffed lemur back into their historical habitat range within Analamazaotra Special Reserve.

Methods

Institutional Animal Care and Use Committee approval for the project was obtained through Omaha's Henry Doorly Zoo. Permits to work at forests in Madagascar were obtained from and with the collaboration of the Tripartite Committee (CAFF/CORE), MNP, MEFT, and the University of Antananarivo.

Translocation sites: Four forests with significant, ongoing habitat deterioration due to mining or agricultural activities were chosen as sites from which to translocate animals (Fig. 1). These included Mantadia National Park (S18º48'57.6", E048º25'19.9"), Sahanody Classified Forest (S18º51'24.3", E048º25'15.8"), Ambatovy Classified Forest (S18º51'04.3", E048º17'58.5"), and Anosibe an'ala Classified Forest (S19º14'05.0", E048º16'18.9"). In the case of Mantadia, Sahanody, and Anosibe an'ala, human encroachment for hunting, crop production and/or firewood collection has had a detrimental impact on forest integrity while Ambatovy Classified Forest has been altered due to activity from the Ambatovy nickel mining project.

Re-introduction Site: Analamazaotra Special Reserve (S18º48'56.1", E048º25'11.2") is an evergreen rain forest located approximately 150 km east of the capital city of Antananarivo. The 810 ha reserve consists of rain forest with altitudes ranging from 850 m to 1100 m above sea level (Tattersall and Sussman, 1975); mean annual rainfall in the region is 1700 mm with an average temperature of 18° C (MNP, 2006).

Animals: A total of 27 Diademed sifakas (Fig. 2) and 7 black and white ruffed lemurs (Fig. 3) were translocated between January 2006 and July 2007; all animals were free ranging juveniles or adults. Animals were moved with their entire social or family group and were extensively evaluated prior to the translocation event. In the case of the DS and BWRL from Mantadia, complete genetic and biomedical information had already been determined for these animals prior to the initiation of the ART Project. Groves (2001) reported that BWRL has three distinct subspecies: *Varecia variegata subsincta, Varecia variegata editorum*, and *Varecia variegata variegata*. The later was the subject subspecies according to geographic distribution (Mittermeier *et al*., 2008) and pelage (Vasey and Tattersall, 2002).

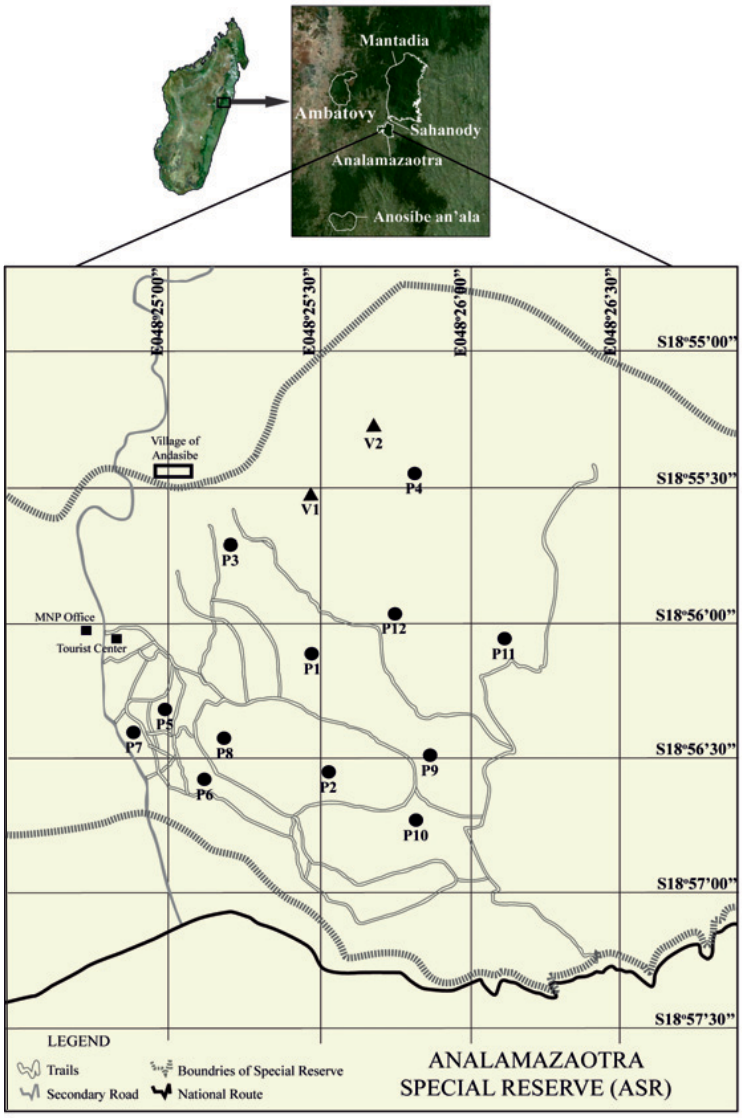

Fig. 1: Analamazaotra Special Reserve (ASR) and the four translocation sites. Approximate home range locations of the 12 Diademed sifaka (P#; *P. diadema*) and 2 black and white ruffed lemur (V#; *V. v. editorum*) groups are shown within ASR.

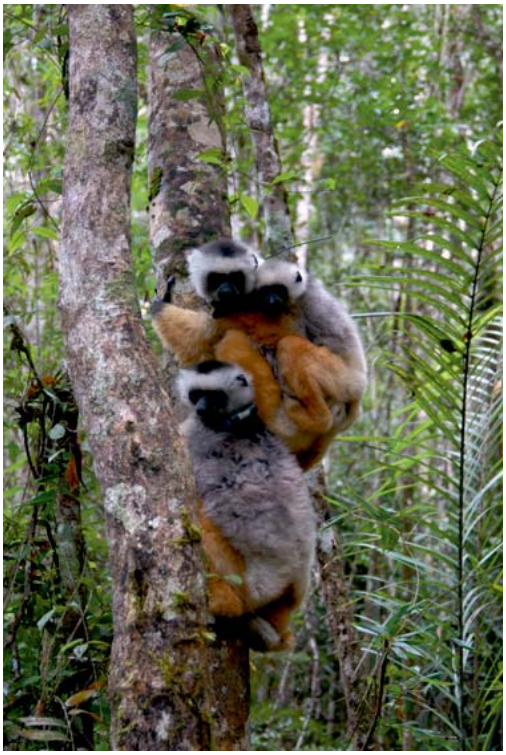

Fig. 2: Three re-introduced diademed sifaka females (2 adult, 1 juvenile) from Sahanody Classified Forest. (Photo E. E. Louis, Jr., February 2006)

Capture, Evaluation and Sampling: The detail of capture method and evaluation protocol has been previously described in Junge *et al.* (2008). Briefly, 2-8 months before a translocation was carried out, each lemur to be re-introduced was immobilized using a CO_2 projection rifle (Dan-Inject MJ model, Dan-Inject, Knoxville, TN) and dart (Type C Disposable Dart, Pneu-Dart, Williamsport, PA) with 10 mg/kg of Telazol® (Fort Dodge Animal Health, Fort Dodge, IA) and given a complete physical examination. To generate genetic data later, four 2.0 mm biopsies were collected from each sedated animal and immediately stored in a room temperature storage buffer (Longmire *et al.*, 1992). Additionally, 1.0 cc whole blood per kg body weight was also collected for later biomedical analysis. The location of each captured lemur was recorded using a Garmin eTrex Summit global positioning system (GPS; Garmin International, Inc., Olathe, KS) and a HomeAgain® microchip (Schering-Plough HomeAgain LLC, Kenilworth, NJ) was implanted subcutaneously between the scapulae of each animal. This procedure was used to field catalog each animal with a unique recognition code to provide the capability to positively re-identify individuals during future immobilizations. When the date for translocation arrived, each animal was again immobilized and given a follow-up physical. During this second physical a radiocollar (Advanced Telemetry Systems, Inc., Isanti, MN) was also placed on each individual adult to support the monitoring and habituation phases of the re-introduction (Williams and Feistner, 2003).

Hard Release: ASR is divided between a tourist area accessible to the public and a re-

Fig. 3: Black and white ruffed lemur offspring (one of two F2 twins) born in November 2008 to the F1 female and a male translocated from Anosibe an'ala Classified Forest. (Photo E. E. Louis, Jr., March 2009)

serve area that is off-limits to all but authorized personnel. The first groups of re-introduced animals were initially released inside the reserve area where habitat assessments had demonstrated desirable forest cover. Later releases were more centrally located near the boundary between the tourist and reserve areas.

Habituation and Behavioral Observations: Habituation of the study animals typically lasted three to eight weeks using radiocollars to track and monitor the animals. In contrast to solitary males, who were highly sensitive to the movements of those monitoring them, habituation of male-female pairs was achieved more quickly. Collection of behavioral data began in 2006 with the first translocations and continues today. Animals are monitored five days a week from dusk to dawn using focal animal sampling and continuous recording (Altmann, 1974) to collect behaviors such as group interaction, predator response, scent marking and reproductive activity.

Territorial Home Range Determination: GPS locations are taken during daily monitoring in order to determine home range, daily range, sleeps sites and food trees for all re-introduced groups. In some instances, animals have been fitted with GPS collars (e-obs GmbH, Grünwald, Germany) to allow remote monitoring of movement within the reserve. Animal territories and ranges are determined using either GPS Utility, ver 4.0 (GPS Utility Limited, Southampton, United Kingdom) or polygon area determination in Google Earth Pro (Google, Inc., Mountain View, CA).

Reproductive Monitoring: Fecal collections for both lemur species have been ongoing since the initiation of the ART Project. For each translocation, collection began following a two month habituation period, during which time the animals were tracked but no samples were collected. Fecals have since been used to determine reproductive hormone profiles for both species (unpublished data).

Botanical Survey: After determining approximate home ranges for each group of DS and BWRL, five plots (100 m by 20 m) were laid out inside the animals' territory. Within the plot, the following parameters were recorded: all trees with diameter at breast height (DBH) > 5 cm, tree height, canopy height, canopy size, canopy cover and ground cover. For lignin plants with DBH # 5 cm, species name, number and diameters were recorded for one 10 m by 10 m subset plot within the original, larger plot. Food plants that were observed to be eaten by the animals were identified by species; DBH, height, and canopy volume were recorded. Samples of each type of fruit were then collected in order to record size, shape, color, weight, and seed type.

Results

Group Size and Territorial Home Range: Immediately after release individuals typically took to the trees quickly and rested for a few hours. Over the next few days many of the animals were observed to change their behavior in the presence of field guides, spending more time traveling, resting and sending out alarm calls. Approximately one month after release, DS groups began to establish their ranges and were observed using their scent glands to mark or define their territories. Territories for multiple male-female DS subgroups were established and became relatively stable, whereas the territories of solitary males initially remained more fluid, perhaps in attempts to join established groups or to opportunistically breed. Eventually these lone individuals became settled in their new habitat and their activity budget and behavioral ecology appeared to be the same as *P. diadema* in their original forest habitat.

In the 30 months since the first translocations, both species have spread throughout ASR to occupy the reserve and tourist areas. BWRL have formed 2 groups while DS have divided into 12 stable units. Group size varies between either 3 or 4 for BWRL and 1 to 4 for DS. GPS collar data from one BWRL group has shown a home range of 150 ha while territory size for the GPS-collared DS group is smaller at 95 ha.

Behavior: Similar to their behavior prior to translocation, relocated *P. diadema* and *V. v. editorum* individuals were found to spend the majority of daylight hours actively feeding, socializing, and resting. During the cold season both species start their daily activity later in the morning, but during the warmer months are active earlier in the day.

Botanical Survey and Diet: Between the two lemur species, more than 33 families of food plants have been recorded, with the most important being *Euphorbiacea, Lauraceae*, *Apocynaceae*, *Myrtaceae*, *Clusiaceae*, *Erythroxylaceae*, *Pittosporaceae*, *Moraceae*, and *Flacourtaceae*. DS have been documented feeding on > 53 species (Table 1) while BWRL have been shown to consume approximately 20 species (11 listed in Table 2). In addition to leaves (buds, immature, mature) and fruit, *P. diadema* routinely consumes a variety of plant materials such as herbs, lianas, bark, ferns, flowers, and parasitic plants, as well as soil. The primary dietary component for BWRL is ripe fruit (> 80 %) from trees with an average DBH of 40 cm (range 15 to 100 cm, 120 feeding trees).

Reproductive Success: To date, multiple births have occurred within the original social groups and within newly established founder groups formed with animals mixing from different translocation sites. Two sets of BWRL twins have been born, with the female from the first pair (F1 generation) recently giving birth to her own set of twins (F2 generation; Fig. 3). Seven individual DS offspring have also been born over the last 30 months.

Survival: Shortly after the initial BWRL release in March and April 2006, the animals moved to the northern portion of the reserve. Following a weekend when the animals were not monitored, two juvenile offspring disappeared from radio contact; no remains or radiocollars were found. Given the history of Analamazaotra Special Reserve, reports of local hunting activity in the region, and the fact that the animals were still too young to have willingly left their parents, MNP officials and the MBP-HDZ team suspected both individuals had been poached due to their proximity to the forest edge and the two surrounding villages. In addition, another female and her offspring moved southwest from the release site in the direction of Mitsinjo Reserve, a small forested area adjacent to ASR. It is believed that these two animals crossed the road and settled in this area, with its own small population of *Varecia*, as it is the only forest accessible in that direction.

Of the 27 re-introduced DS, one appeared to die of natural causes (remains found) while another animal left the area and returned to Mantadia National Park; the remaining animals dispersed throughout ASR. Out of the

Table 1: Food plants (53 species) consumed by Diademed sifaka in ASR between October 1, 2008 and March 31, 2009.

Plant name	**Family name**	***Scientific name***	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**
Ambavy	Annonaceae	*Polyathia ghesquiereana*	LF			LF		LF
Ambora	Monimiaceae	*Tambourissa* sp.						LF
Ampody	Pittosporaceae	*Pittosporum* sp.	LF	LF				LF
Cabucala	Apocynaceae	*Cabucala* sp.				FR		
Camellia	Theacea	*Camellia thea*	FR					
Dipaty	Moraceae	*Pachytrophe* sp.				FR	LF	LF
Ditimena	Anacardiaceae	*Protorhus ditimena*	LF					LF
Famelona	Sapotaceae	*Gambeya boiviniana*	LF					
Famohalambo	Mimosaceae	*Desmanthus tenuifolius*		LF	LF	LF	LF	LF
Fandramanana	Flacourtiaceae	*Aphloia theaeformis*				LF		
Fanjavala	Euphorbiaceae	*Blotia oblongifolia*	LF	LF				
Fotona	Chlaenaceae	*Leptolaena pauciflora*			FL			
Goavitsinahy	Myrtaceae	*Psidium cattleyanum*						FR
Havoha	Moraceae	*Bosqueia boiviana*		FR				
Hazoambo	Flacourtiaceae	*Homalium albiflorum*	LF	LF				
Hazolahy	Apocynaceae	*Carissa edulis*						FR
Hazombary	Pittosporaceae	*Pittosporum ochrosiaefolium*	LF					LF
Hazomboahangy	Moraceae	*Maillardia montana*						FR
Hazondomohina	Euphorbiaceae	*Domohinea perrieri*	LF / FL	FL				
Hazontoho madinidravina	Myrsynaceae	*Oncostemum* sp.	LF	LF				
Hoditrovy	Erythroxylaceae	*Erythroxylum corymbosum*	LF			FR		
Karakaratoloha	Schizaeaceae	*Lygodium lanceolatum*	LF		LF			LF
Karambito	Sapindaceae	*Allophylus cobbe*		FR				
Kijy	Clusiaceae	*Symphonia* sp.	LF	LF / FR / FL	LF / FR / FL		LF	LF
Kijy boalavo	Clusiaceae	*Symphonia tanalensis*		LF / FR / FL	LF / FR / FL		LF	
Kijy bonaka	Clusiaceae	*Symphonia macrocarpa*	LF	LF / FR / FL	LF / FR / FL	LF	LF	LF
Kijy fotsy	Clusiaceae	*Mammea* sp.		LF / FR / FL	LF / FR / FL	FR	LF	
Kijy masina	Clusiaceae	*Mammea bongo*	LF	LF / FR / FL	LF / FR / FL	LF/ FR	LF	
Kijy rano	Clusiaceae	*Symphonia fasciculata*		LF / FR / FL	LF / FR / FL		LF	
Kijy vongo	Clusiaceae	*Symphonia* sp.		LF / FR / FL	LF / FR / FL	FR	LF	
Lianas (ambiguous)			LF	LF / FL		FR	LF / FR	LF
Maitsoririnina	Olacaceae	*Olax glabriflora*	LF	LF		LF		LF
Malambovony	Ochnaceae	*Campylospermum lanceolatum*						LF/ FR
Menahihy	Erythroxylaceae	*Erythroxylum* sp.	LF / FL	LF / FL	LF / FR / FL	LF / FR		
Menahihy be ravina	Erythroxylaceae	*Erythroxylum* sp.			LF / FR / FL			
Menahihy fotsy	Erythroxylaceae	*Erythroxylum* sp.			LF / FR / FL			
Meanahihy madinidravina	Erythroxylaceae	*Erythroxylum* sp.			LF / FR / FL			
Mistletoe	Lorantaceae	*Bakerella clavata*	FL	LF			LF	LF
Nonoka	Moraceae	*Ficus pyrifolia*	LF	LF				
Pitsikahitra	Rubiaceae	*Schismatoclada* sp.	LF	LF				
Robary	Myrtaceae	*Eugenia emirnense*	LF		LF/ FR	LF	LF	LF
Ropadirana	Myrtaceae	*Eugenia* sp.		LF				LF
Rotra be ravina	Myrtaceae	*Eugenia jambolana*	LF	LF / FR			LF	FR
Tavolo lava ravina	Lauraceae	*Ravensara acuminata*	LF	LF		LF		
Tsiramiramy	Anacardiaceae	*Micronychia tsiramiramy*				FR		FL
Vahinkorofoka	Apocynaceae	*Secamone* sp.	LF	LF		LF	LF	LF
Varongy mainty	Lauraceae	*Ocotea cymosa*	LF	LF		LF / FR	FR	LF / FR
Varongy fotsy	Lauraceae	*Ocotea macrocarpa*	LF	LF		LF / FR	FR	
Voamboana	Papilionaceae	*Dalbergia baroni*	LF			LF		LF
Voantsilana	Araliaceae	*Schefflera vantsilana*	LF	LF				
Voapaka	Euphorbiaceae	*Uapaca thouarsii*	LF					
Vongo	Clusiaceae	*Garcinia verrucosa*	LF	FR		FR		
Zamborizano	Myrtaceae	*Eugenia jambosa*	LF	LF				

Table 2: Food plants (11 species) consumed by black and white ruffed lemurs in ASR between October 1, 2008 and March 31, 2009.

Malagasy name	**Family**	**Species**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**
Dipaty	Moraceae	*Pachytrophe* sp.				FR	LF/FR	
Ditimena	Anacardiaceae	*Protorhus ditimena*		LF				
Gavoala	Myrtaceae	*Eugenia gavoala*			FR			
Kijy	Clusiaceae	*Symphonia* sp.		FL				
Nanto	Sapotaceae	*Mimusops* sp.			FR			
Rotra be ravina	Myrtaceae	*Eugenia jambolana*	FR	FR	FR			FR
Tavolo lava ravina	Lauraceae	*Ravensara acuminata*	FR	FR				
Tikitiky	Palmaceae	*Dypsis* sp.					FR	
Tsikafekafe	Rubiaceae	*Tricalysia* sp.					FR	
Vagnana	Elaeocarpaceae	*Elaeocarpus* sp.		LF				
Vongo	Clusiaceae	*Garcinia verrucosa*	FL					
LF = leaves; FR = fruits; FL = flowers								

seven *P. diadema* offspring born, three have fallen victim to infanticide from competing adult females. Two others were lost to natural aerial predation, leaving two surviving offspring.

Competing Fauna: Several lemur species are sympatric with *P. diadema* and *V. v. editorum* in ASR, including *Eulemur fulvus*, *Hapalemur griseus*, *Indri indri*, and *Eulemur rubriventer*. Despite regular intra-species encounters, no persistent, aggressive behavior was observed between DS or BWRL and these other species. In their original forests, all relocated animals had previously encountered these species and did not appear to be disturbed by their

presence. These other diurnal and cathemeral species at Analamazaotra, however, had not experienced DS or BWRL in their territory since the early 1970's. As a result they were found to react by sending out long alarm calls and other territorial defining behaviors. Indri were occasionally observed to be aggressive to *P. diadema* by running after the groups, but this aggression coincided with indri mating season. Aerial predators, such as the Madagascar kestrel and Peregrine falcon, are found in ASR and caused re-introduced DS and BWRL to send out loud alarm calls. Although not abundant, blue pigeons also fed frequently on fruits with BWRL.

Discussion

The Diademed sifaka, *Propithecus diadema*, inhabits Madagascar's eastern rain forests and is one of the largest, most colorful of all lemurs (Mittermeier *et al.*, 2006). It is described as a diurnal, folivorous/frugivorous indridae (Powzyk, 1997; Powzyk and Mowry, 2003) weighting 5.5-8.5 kg (Glander *et al.*, 1992; Smith and Jungers, 1997; Lehman *et al.*, 2005). Living in groups of two to eight individuals with multiple males and females, DS defend home ranges of 20 to 50 ha using perimeter scent territorial markings by both males and females (Powzyk, 1997; Powzyk and Mowry, 2003).

The black and white ruffed lemur (*V. variegata*) also inhabits the eastern humid forests of Madagascar (Ratsimbazafy, 2002; Vasey, 2005). These critically endangered animals are the most frugivorous lemurs (Mittermeier *et al.*, 2006) and have a body mass of 2.6-4.1 kg with 73 % of their diet composed of fruit (Ratsimbazafy *et al*., 2002; Vasey and Tattersall, 2002). Due to the large amount of fruit required in their diet, and the associated territory size necessary to provide it, BWRL can be viewed as an indicator species as to the health of the forest and its related biota (Vasey, 2005). BWRL also play an important role as pollinators and seed dispersers (Wright, 1998).

It was precisely for these reasons, as indicator species and as potential dynamic ambassadors to the existing local ecotourist industry, that MNP and MEFT approached MBP-HDZ with a request to return these remarkable animals to Analamazaotra Special Reserve. As a result, the collaborative, multi-disciplinary ART Project translocated 27 Diademed sifakas and 7 black and white ruffed lemurs from four forests experiencing significant, ongoing habitat deterioration. Currently, the project has successfully evaluated the biomedical, genetic, habitat, nutritional, and reproductive parameters of these animals while monitoring their daily activity via radiocollar tracking.

Varecia reproduction is strictly seasonal and dependent upon food availability (Ratsimbazafy, 2002; Vasey and Tattersall, 2002). In October 2006, one set of twins (a male and female) was born to a translocated pair of BWRL. This F1 female offspring subsequently gave birth in 2008 to her own set of twins sired by a translocated male from Anosibe an'ala. Additionally, seven Diademed sifakas have been born since the first translocations. Although five of the DS offspring have been lost to natural causes (2 to raptors, 3 to infanticide), as of March 2009, two offspring from the June 2008 births are thriving and doing quite well. In the two and a half years since the first translocations, both species of re-introduced animals have adjusted well as evidenced by these 11 successful births and 6 surviving offspring.

While the ART Project is the first to successfully translocate wild black and white ruffed lemurs within Madagascar, the Madagascar Fauna Group has previously re-introduced captive-born BWRL (*Varecia variegata variegata*) into a protected low altitude rain forest in eastern Madagascar (Britt *et al*., 2004, 2008). Between November 1997 and January 2001, in an attempt to reinforce a small, isolated resident wild population, 13 captive-born BWRL underwent a soft release into Betampona Reserve (2,228 ha). Although this project initially suffered from high *Cryptoprocta ferox* predation, most likely due to animal naiveté (Britt *et al*., 2001), and utilized supplemental food provisioning (Britt *et al*., 2004), parallels can still be drawn between the two re-introduction efforts. Data show that ten of the eleven plant families consumed by lemurs within ASR were also consumed by Betampona BWRL (Britt and Iambana, 2003). Additionally, the ART Project and the Betampona re-introduction effort have both led to a strengthening or restoration of indigenous lemur populations in their respective reserves. Although only 3 of the 13 original Betampona animals still survive, five of the lemurs reproduced post-release, with three of those believed to have bred with the resident wild population. And like the work within ASR, a number of offspring have survived, contributing much needed genetic diversity to Betampona Reserve's BWRL population (Britt *et al*., 2008).

Overall, the MBP-HDZ ART Project has succeeded in 1) re-establishing healthy, viable wild populations of *P. diadema* and *V. v. editorum* into ASR; 2) confirming a natural expansion of each species as evidenced by births of multiple offspring; 3) training two doctorate students from the University of Antananarivo and a revolving contingent of six MNP and two local field guides to collect data on activity budgets, social behavior, feeding habits, and response to predation and competition; 4) training another doctorate student to collect nutritional data for both species; 5) collecting and preserving fecal samples for hormone analysis to better understand reproductive physiology and behavior; 6) increasing protection of ASR and habituating the re-introduced groups to human presence in order to enhance ASR's established eco-tourism industry; and 7) educating the local communities about the biodiversity of their local ecoregion.

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