Population Genetic Study of the Red-Collared Brown Lemur (*Eulemur collaris* É. Geoffroy) in Southeastern Madagascar

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Abstract: The red-collared brown lemur, *Eulemur collaris* (É. Geoffroy), is relatively isolated geographically from other *Eulemur* species in southeastern Madagascar. Like many other lemur species, the red-collared brown lemur is particularly threatened due to habitat loss and human activities. To evaluate population structure and genetic diversity of this species, we utilized ten informative microsatellite loci derived from the *Eulemur rubriventer* (I. Geoffroy) genome. Significant genetic differentiation among populations was detected and that differentiation was found to be correlated to geographic isolation. Moreover, we found evidence to support recent weak to moderate reductions in effective population sizes in two of the perturbed populations.

Keywords: *Eulemur collaris*, microsatellites, population genetics, conservation, madagascar.

INTRODUCTION

Considered a conservation priority due to its high levels of endemic biodiversity, Madagascar has experienced in recent history extensive loss of its eastern rainforest [1-3]. With fourteen recognized species, the brown lemurs (genus *Eulemur*) are among the most widespread prosimians in Madagascar [4-6]. This genus is represented in the tropical moist lowland and montane forests in southeastern Madagascar by the red-collared brown lemur, *Eulemur collaris* (É. Geoffroy) (Fig. **1**; [7]).

Long-term studies have defined the geographic distributions within the known range [8-12]. *Eulemur collaris* (É. Geoffroy) ranges from Tolagnaro (Fort-Dauphin) in the south to the Mananara River in the north to the Mandrare River in the west, delineating this species from the neighboring gray-headed lemur, *Eulemur cinereiceps* (A. Grandidier and Milne-Edwards), in the northern Farafangana region [13]. Once considered a subspecies of *Eulemur fulvus* (É. Geoffroy), cytogenetic and molecular genetic evidence has recently supported the elevation to full species [11, 14]. Of the 94 lemurs listed, six are classified Critically Endangered, twelve are classified Endangered, three are classified Near Threatened and *Eulemur collaris* (É. Geoffroy) is one of the twelve lemur species classified as Vulnerable A2cd according to the most recent IUCN Red List assessment [15]. The species populations have been studied in Andohahela and Midongy du Sud National Parks, Kalambatritra Special Reserve, and Saint Luce Private Reserve [2, 16].

Anthropogenic disturbance influenced by an ever expanding human population continues to exert pressure on the remaining forest [8, 17, 18] and has resulted in the presently discontinuous range. The combination of fragmentation and geographic barriers has affected the distribution patterns of lemurs [19-24]. Such habitat fragmentation and population isolation can be factors increasing the extinction risks of some species [25]. This paper presents population genetic parameter baselines for *Eulemur collaris* (É. Geoffroy). Using nuclear DNA microsatellite loci, we explore how habitat fragmentation and isolation of the populations may be reducing gene flow, thus accelerating the genetic differentiation of the populations.

MATERIALS AND METHODOLOGY

Field research and data collection were conducted within three protected areas, Kalambatritra Special Reserve (Sahalava site), Midongy du Sud National Park (Beharena Sagnira and Ampasy sites), and Andohahela National Park (Manangotry site) (Fig. **2**) between 2003 and 2007. A total of 40 wild *Eulemur collaris* (É. Geoffroy) individuals (Table **1**), ten from each site, were immobilized and sampled as described in Andriantompohavana *et al*. [26] and Louis *et al*. [27]. Tissue samples were taken as 2.0 mm biopsy punches from the ear pinnae and a whole blood sample was drawn from the femoral vein at a ratio 1ml/kg body weight. All samples were deposited into 1.8 ml Nunc® tubes of room temperature tissue or blood storage solution [28]. Global Positioning System (GPS) locations were recorded within six meters accuracy for each of the immobilized lemurs using a Garmin eTrex® Summit (Olathe, Kansas, USA). A

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(**A**) (**B**)

Fig. (1). Male (**A**) and female (**B**) red-collared brown lemur (*Eulemur collaris* [É. Geoffroy]). Photo credit Edward E. Louis, Jr.

Fig. (2). Sample locations of red-collared brown lemur (*Eulemur collaris* [É. Geoffroy]). Green areas indicate forest cover as of 1999.

HomeAgain® microchip (Schering-Plough; Kenilworth, New Jersey, USA) was placed subcutaneously between the scapulae of each lemur for permanent identification in the event of recapture during any future immobilizations. Each lemur was safely released upon complete recovery in familiar territory where it was initially immobilized.

Genomic DNA was extracted from *Eulemur collaris* (É. Geoffroy) samples in accordance with Sambrook *et al*. [29]. DNA was amplified with 19 microsatellite loci described by Andriantompohavana *et al.* [30] to generate genotype data. Fragment lengths were assigned with Genescan-500 (ROX) and allele scoring was conducted with Genescan software (Applied Biosystems; Foster City, California, USA).

Genotypes were checked with both Micro-Checker [31] and Microsatellite Analyzer [32] for scoring errors [33]. Null allele frequencies and polymorphic information content

Table 1. Free-Ranging Red-Collared Brown Lemur Samples Utilized in the Present Genetic Study

Animal ID	Site Names	Gender	GPS Location		Microchips ID
KALA5.2	Sahalava (Kalambatritra SR)	Female	S23°32'24.1"	E046°32'28.8"	45705C1057
KALA5.3	Sahalava (Kalambatritra SR)	Male	S23°32'24.1"	E046°32'28.8"	456F53430C
KALA5.4	Sahalava (Kalambatritra SR)	Male	S23°32'24.1"	E046°32'28.8"	456C515306
KALA5.5	Sahalava (Kalambatritra SR)	Female	S23°32'24.1"	E046°32'28.8"	457A147F2D
KALA5.8	Sahalava (Kalambatritra SR)	Female	S23°32'46.4"	E046°32'23.8"	4565735D70
KALA5.9	Sahalava (Kalambatritra SR)	Male	S23°32'46.4"	E046°32'23.8"	456F634060
KALA5.10	Sahalava (Kalambatritra SR)	Male	S23°32'46.4"	E046°32'23.8"	4570421629
KALA5.11	Sahalava (Kalambatritra SR)	Male	S23°32'46.4"	E046°32'23.8"	456E2C0B65
KAL7.10	Sahalava (Kalambatritra SR)	Male	S23°32'20.5"	E046°32'12.7"	475C10666B
KAL7.11	Sahalava (Kalambatritra SR)	Female	S23°32'20.5"	E046°32'12.7"	485E187B0A
DOG1	Beharena Sagnira (Midongy du Sud NP)	Male	S23°31'11.5"	E047°05'40.7"	43146D7371
DOG2	Beharena Sagnira (Midongy du Sud NP)	Female	S23°31'11.5"	E047°05'40.7"	433C513272
DOG3	Beharena Sagnira (Midongy du Sud NP)	Female	S23°31'11.5"	E047°05'40.7"	4317443676
DOG4	Beharena Sagnira (Midongy du Sud NP)	Female	S23°31'11.5"	E047°05'40.7"	4331033404
DOG5	Beharena Sagnira (Midongy du Sud NP)	Male	S23°31'11.5"	E047°05'40.7"	4332273B47
DOG6	Beharena Sagnira (Midongy du Sud NP)	Male	S23°31'11.5"	E047°05'40.7"	431917083C
DOG7	Beharena Sagnira (Midongy du Sud NP)	Female	S23°31'14.6"	E047°05'33.2"	4314593472
DOG8	Beharena Sagnira (Midongy du Sud NP)	Female	S23°31'14.6"	E047°05'33.2"	433A147554
DOG9	Beharena Sagnira (Midongy du Sud NP)	Male	S23°31'14.6"	E047°05'33.2"	4332277762
DOG10	Beharena Sagnira (Midongy du Sud NP)	Female	S23°31'14.6"	E047°05'33.2"	433B20153D
DONGY5.1	Ampasy (Midongy du Sud NP)	Female	S23°44'27.4"	E047°01'30.7"	464D5C6673
DONGY5.2	Ampasy (Midongy du Sud NP)	Male	S23°44'27.4"	E047°01'30.7"	46533F0A57
DONGY5.3	Ampasy (Midongy du Sud NP)	Male	S23°44'27.4"	E047°01'30.7"	464F082448
DONGY5.4	Ampasy (Midongy du Sud NP)	Female	S23°44'27.4"	E047°01'30.7"	464E686B68
DONGY5.5	Ampasy (Midongy du Sud NP)	Female	S23°44'27.4"	E047°01'30.7"	464E4B5C7C
DONGY5.6	Ampasy (Midongy du Sud NP)	Female	S23°44'33.1"	E047°01'35.3"	4653394D48
DONGY5.7	Ampasy (Midongy du Sud NP)	Male	S23°44'33.1"	E047°01'35.3"	4651194932
DONGY5.8	Ampasy (Midongy du Sud NP)	Female	S23°44'33.1"	E047°01'35.3"	4633321424
DONGY5.9	Ampasy (Midongy du Sud NP)	Female	S23°44'33.1"	E047°01'35.3"	463368162D
DONGY5.10	Ampasy (Midongy du Sud NP)	Male	S23°44'33.1"	E047°01'35.3"	46340B7A41
AND22	Manangotry (Andohahela NP)	Male	S24°45'49.8"	E046°51'58.1"	43381C2169
AND23	Manangotry (Andohahela NP)	Male	S24°45'49.8"	E046°51'58.1"	4332697C6E
AND24	Manangotry (Andohahela NP)	Female	S24°45'49.8"	E046°51'58.1"	4330230D6F
AND25	Manangotry (Andohahela NP)	Female	S24°45'44.3"	E046°52'02.2"	433B515B22
AND26	Manangotry (Andohahela NP)	Female	S24°45'49.8"	E046°51'58.1"	433C093B4D
AND27	Manangotry (Andohahela NP)	Female	S24°45'50.9"	E046°52'05.2"	433149073F
AND35	Manangotry (Andohahela NP)	Female	S24°45'35.1"	E046°51'31.5"	4331412648
AND52	Manangotry (Andohahela NP)	Female	S24°45'21.1"	E046°51'28.3"	433C3E576F
AND54	Manangotry (Andohahela NP)	Male	S24°45'21.1"	E046°51'28.3"	44232D5E44
AND5.2	Manangotry (Andohahela NP)	Male	S24°46'03.5"	E046°51'43.6"	46571C522E

(PIC) were estimated for all polymorphic loci with CERVUS 2.0 [34]. FSTAT [35, 36] was used to test for linkage equilibrium, i.e. marker independence, for the accepted loci. The web-based Genepop3.4 [37] was used to test globally and by population for deviations from Hardy-Weinberg equilibrium (HWE) using Fisher's exact probability test. FSTAT was used to estimate gene diversity (*Hs*), rarefacted allelic richness (*AR*), Wright`s *FIS* (within population *f* statistic), and Wright`s *FST* (among population *f* statistic) the latter two as by Weir and Cockerham [38]. Both *FIS* and *FST* were tested as departure from HWE with 10,000 permutations. Genic (allelic) and genotypic differentiation were performed in Genepop3.4. Observed (*Ho*) and unbiased expected (*He*) heterozygosities and gene flow as the number of effective migrants (*NM*) per generation using the Private Allele's method were estimated in Genepop3.4. The Mantel test, regressing the transformed *FST*/(1-*FST*) on the Euclidean distance (km) between the sampling locations, was performed to assess the effect of isolation by distance between the populations, also in GenePop3.4.

The number of effective breeders (*Neb*) was estimated in NeEstimator [39] to establish a baseline value for an estimate of the effective population sizes for each population. Both the linkage disequilibrium (LD; [40]) and heterozygosity excess (HEx; [41]) methods were used as they are applicable to single-sampled populations. Both Pudovkin *et al*. [41] and Luikart and Cornuet [42] found that the HEx method often overestimates *Neb* while Waples [43] found flaws in the development of the model. Balloux [44], on the other hand determined that in spite of the limitations, this method was excellent for single-sampled population datasets. Therefore, both methods were employed and are reported as suggested by Waples [43].

The populations were tested for evidence of recent reductions in effective population size using Bottleneck 2.0. The population bottlenecks are detected when the observed heterozygosity across all loci exceeds the expected heterozygosity assuming mutation drift equilibrium [45]. Three available models are presented, the Infinite Allele Model (IAM), the Stepwise Mutation Model (SMM), and the Two Phase Model (TPM; [45-47]). The TPM allows the user to vary the proportion and variance of single step mutations in the presence of multi step mutation events [48]. We held the variance constant at the default value (30%) and varied the proportion of single step mutations to determine the TPM model that best described the data.

The populations were tested for cryptic substructure using the Bayesian clustering methodology in the program STRUCTURE 2.0 [49]. We used the Admixture Model with an inferred α having an initial value of 1.0 for all populations, a maximum value of 10.0 and a standard deviation for updating the proposal of 0.025. We assumed that the allele frequencies were correlated among the populations and different values for *FST* in each subpopulation. We gave the prior mean *FST* to initiate at 0.01 with a prior standard deviation of 0.05 for each population. We used the allele frequency parameter, lambda, at a constant value of 1.0. We initiated a burn in period of 10^5 MCMC (Markov Chain Monte Carlo) repetitions and 10^6 MCMC repetitions following each burn in period. We produced 20 runs for each *K* value which were used to estimate the most likely *K* suggested by Pritchard *et al*. [49]. The *ad hoc* test statistic Δ*K* [50] was also used to elucidate the most likely number of genetic clusters, and then compared with the Pritchard *et al*. [49] model with the highest posterior probability.

RESULTS

Nineteen loci were amplified and genotyped (Table **2**). One locus was found to be monomorphic and eight loci harbored null allele frequencies estimated above a moderate threshold (*nf* > 0.1) which were eliminated from the analysis [51-53]. Locus characteristics, number of alleles (k) within all populations, allelic size ranges of the loci, and the relative quality of the loci as PIC values are presented as cross amplified in *Eulemur collaris* (É. Geoffroy) (Table **2**). Within population parameter estimates (number of alleles, allelic richness, *FIS*, expected and observed heterozygosities) are reported in Table **3**. The locus 44HDZ11 harbored the highest number of alleles (k = 8) although neither the average number of alleles nor the estimates of allelic richness were significantly different among populations. Estimates for *Ho* (0.586 – 0.681) and estimates for *He* (0.559 – 0.609) did not vary significantly among the four populations nor did they vary from each other ($P > 0.10$). While the Sahalava population has the lowest *FIS* estimate (*FIS* = -0.225; $P < 0.05$), none of the *FIS* estimates for the other populations (-0.115 – 0.008) were significant (Table **3**). Genic and genotypic differentiation were significant among the Sahalava, Beharena Sagnira, and Ampasy populations ($0.05 > P > 0.001$) and were highly significant between the Manangotry population and the Sahalava, Beharena Sagnira, and Ampasy populations ($P < 0.001$). Gene flow measured as the number of effective migrants per generation (*NM*) exceeded 1 per generation among the Kalambatritra and Midongy du Sud populations. The isolation of the Manangotry population from the Kalambatritra and Midongy du Sud populations was revealed by the estimate of genetic exchange of one individual every four to five generations (Table **4**). Genetic differentiation (*FST*; Table **4**) was significant at $P < 0.01$ in all comparisons except between Beharena Sagnira and Ampasy ($P < 0.05$), the two neighboring populations in Midongy du Sud. The isolation by distance (Fig. **3**) was strongly correlated to the transformed *FST*/(1 – *FST*) regressed on the Euclidean distance between sampling locations. The Bayesian approach implemented in STRUCTURE 2.0 suggested no substructure within any of the populations or any significant genetic clustering among the four populations (data not shown) by either the posterior likelihood or Δ*K* [50].

The numbers of effective breeders estimated for each population by the LD and HEx methods are presented in Table **3**. No evidence of a recent reduction in population size was detected by any of the three models implemented in Bottleneck ($P > 0.05$) for the Ampasy and Sahalava populations. Weak evidence supporting a recent bottleneck was detected assuming the IAM ($P < 0.05$) but not the SMM or the TPM for the Beharena Sagnira population. Stronger evidence for a genetic bottleneck was detected using both full likelihood models, the IAM ($P < 0.01$) and SMM ($P < 0.05$) in the Manangotry population.

Table 2. Characterization of the 19 Loci (Andriantompohavana *et al*. [30]) Utilized in this Study

Locus	Annealing Temperature (˚C)	Size Range	k	n	PIC
44HDZ001 (EUL1)	52	100	Monomorphic		
44HDZ005 (EUL5)*	60	160-172	6	34	0.673
44HDZ009 (EUL9)*	54	144-148	4	40	0.524
44HDZ011 (EUL11)	55	160-174	8	38	0.629
44HDZ014 (EUL14)*	56	(152)138-174	5	36	0.646
44HDZ016 (EUL16)	54	232(213-223)	4	40	0.698
44HDZ035 (EUL35)	54	158(146-174)	5	40	0.664
44HDZ040 (EUL40)	62	235-241	4	39	0.513
44HDZ041 (EUL41)*	58	80-98	7	40	0.469
44HDZ042 (EUL42)	54	150(140-156)	5	40	0.67
44HDZ083A (EUL83A)*	54	157- 165	4	40	0.597
44HDZ083B (EUL83B)*	54	162	4	40	0.39
44HDZ091 (EUL91)	52	150-160	5	40	0.622
44HDZ119 (EUL119)	60	151-161	4	38	0.52
44HDZ124 (EUL124)	54	138(116-140)	3	39	0.173
44HDZ193 (EUL193)	54	172-190(168-184)	4	40	0.569
44HDZ287 (EUL287)	52	162-178	4	40	0.59
44HDZ475 (EUL475)*	56	287(276-294)	7	40	0.742
44HDZ480 (EUL480)*	56	174(170-180)	5	40	0.673

k: total number of alleles.
n: total number of individuals genotyped.
PIC: polymorphic information content.
* *nf*: null allele frequency (*nf* > 0.10; subsequently removed from further analysis).

Table 3. Locus Information by Population (n: sample area, k: number of alleles, *AR*: allelic richness, *FIS*: Wright's within population *f*-statistic, *He* and *Ho*: expected and observed heterozygosities, respectively) and *Neb*: number of effective breeders estimated by LD (linkage disequilibrium) with 95% confidence interval (CI) and HEx (Heterozygosity excess) methods.

	Sahalava									Beharena Sagnira								
							Neb									*Neb*		
Locus	n	k	*AR*	*FIS*	*He*	*Ho*	LD	95% CI	HEx	n	k	*AR*	*FIS*	*He*	*Ho*	LD	95% CI	HEx
EUL11	16	4	4.0	0.138	0.575	0.500				20	3	2.8	0.118	0.563	0.500			
EUL16	20	4	3.8	-0.421	0.647	0.900				20	4	4.0	-0.134	0.711	0.800			
EUL35	20	4	4.0	-0.231	0.658	0.800				20	5	4.7	-0.395	0.732	1.000			
EUL40	20	3	3.0	-0.252	0.647	0.800				18	4	4.0	0.216	0.699	0.556			
EUL42	20	4	3.6	-0.023	0.489	0.500				20	4	3.7	0.325	0.437	0.300			
EUL91	20	3	3.0	-0.019	0.589	0.600				20	4	3.8	-0.263	0.642	0.800			
EUL119	16	2	2.0	-0.750	0.525	0.875				20	2	2.0	0.633	0.526	0.200			
EUL124	18	3	2.9	-0.091	0.307	0.333				20	2	2.0	-0.125	0.268	0.300			
EUL193	20	3	3.0	-0.190	0.679	0.800				20	3	2.8	-0.235	0.574	0.700			
EUL287	20	3	2.8	-0.400	0.511	0.700				20	4	3.8	-0.068	0.658	0.700			
All		3.3	3.2	-0.225	0.563	0.681	6.2	4.4-9.6	3.5 - ∞		3.5	3.4	-0.008	0.581	0.586	45.7	15.5-∞	26.1-∞

(Table 3). Contd…..

	Ampasy									Manangotry								
							Neb									*Neb*		
Locus	**n**	**k**	***AR***	***FIS***	***He***	***Ho***	**LD**	**95% CI**	**HEx**	**n**	**k**	***AR***	***FIS***	***He***	***Ho***	**LD**	**95% CI**	**HEx**
EUL11	20	5	4.6	-0.241	0.653	0.800				20	5	4.8	0.131	0.800	0.700			
EUL16	20	3	3.0	-0.252	0.647	0.800				20	4	4.0	-0.171	0.689	0.800			
EUL35	20	4	3.8	-0.210	0.668	0.800				20	4	4.0	0.074	0.753	0.700			
EUL40	20	3	2.8	-0.301	0.468	0.600				20	2	2.0	0.217	0.505	0.400			
EUL42	20	3	3.0	-0.190	0.679	0.800				20	3	3.0	-0.241	0.653	0.800			
EUL91	20	3	3.0	-0.050	0.668	0.700				20	3	3.0	0.357	0.611	0.400			
EUL119	20	3	3.0	-0.125	0.358	0.400				20	4	4.0	-0.083	0.742	0.800			
EUL124	20	2	1.8	0.000	0.100	0.100				20	2	1.8	0.000	0.100	0.100			
EUL193	20	4	3.8	0.107	0.668	0.600				20	3	3.0	0.211	0.626	0.500			
EUL287	20	4	3.8	0.129	0.684	0.600				20	3	3.0	0.018	0.611	0.600			
All		3.4	3.2	-0.115	0.559	0.62	6.1	4.4-8.9	7.5 - ∞		3.3	3.2	0.050	0.609	0.580	10.4	6.6-19.7	nd

Table 4. Geographic Distances Between Collection Sites, Wright's Fixation Index (*FST*) and Significance (* *P* < 0.05, ** *P* < 0.01), *NM*: Number of Effective Migrants Per Generation

Location/Population	Geographic Distance (km)	*FST* Estimates	*P* Values	*NM*
Beharena Sagnira - Ampasy	24.5	0.0475	*	1.5
Beharena Sagnira - Sahalava	62.0	0.0384	**	1.1
Beharena Sagnira - Manangotry	145.0	0.1572	**	0.3
Ampasy - Sahalava	55.1	0.0630	**	1.6
Ampasy - Manangotry	131.0	0.1352	**	0.2
Manangotry - Sahalava	142.5	0.1431	**	0.2

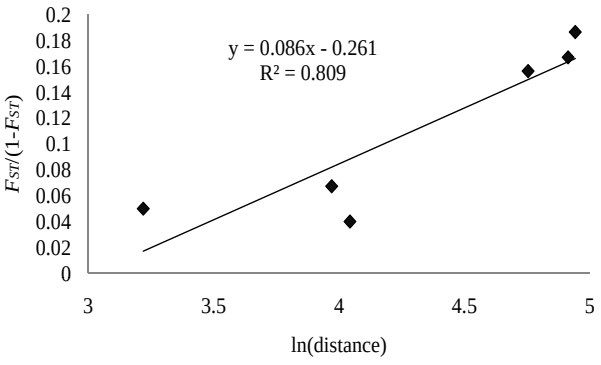

Fig. (3). Isolation by distance derived from the transformed *FST*/(1 – *FST*) regressed on the Euclidean distance between the sample locations.

DISCUSSION AND CONCLUSION

The remaining brown lemur habitats are confined to the periphery of Madagascar [6, 54]. These habitats have undergone extensive fragmentation resulting in populations that are becoming isolated from each other. Such fragmentation has occurred in Kalambatritra Special Reserve, within which, the largest continuous forest block is Ambalabe [2]. The forest areas now defined as Midongy du Sud National Park and Kalambatritra Special Reserve were historically connected and only in recent times have become fragmented or disrupted by slash-and-burn cultural agriculture practices (*tavy*), fuel wood harvest and charcoal production. Sparse forest corridors still exist as a thin mosaic between the two protected areas (e.g. Beakora forest; [55]) facilitating minimal gene flow between these relatively close populations.

Initial population genetic parameters estimated for four populations of *Eulemur collaris* (É. Geoffroy) reflect elements of the genetic health of the species in three protected areas in the southern region of Madagascar. The effects of habitat fragmentation may be inferred as disruption of gene flow and appears to occur between the two northern locations (Kalambatritra Special Reserve and Midongy du Sud National Park) and the southern location (Andohahela National Park). With habitat fragmentation and the disruption of gene flow comes genetic differentiation fueled by genetic drift which we observe in the elevated fixation

indices and in significant allelic and genotypic differentiation. Encouraging though, is that this differentiation does not reflect significant losses in alleles or differences in levels of genetic diversity.

Two populations could be subjected to the anthropogenic challenges supported by weak evidence of a recent bottleneck event in the Beharena Sagnira population and more likely in the Manangotry population. Both of these sites are in close proximity to human populations and foot traffic. The Beharena Sagnira site is surrounded by three villages along the national road (RN52) connecting the Vangaindrano and Midongy du Sud districts. Andohahela National Park is divided by the heavily travelled RN61, connecting Tolagnaro (Fort-Dauphin) and Ranomafana village, which passes alongside the Manangotry site. Gene flow, however, between the Kalambatritra and Midongy du Sud populations does not appear to be inhibited by the Itomampy or Ionaivo Rivers (Fig. **2**).

Eulemur collaris (É. Geoffroy) has the ability and propensity to travel across open ground short distances between close forest fragments which present more favorable opportunities to maintain some degree of gene flow than what are experienced by the primarily arboreal species. These observations support the maintenance of *Ho* which in three of the populations is not significantly different from the heterozygosity levels expected in Hardy-Weinberg equilibrium. Thus, the suggestion can be made that at least the genetic signal of recent gene flow, between the Kalambatritra and Midongy du Sud populations, has maintained some level of genetic mixing between the two areas. This information establishes baseline population genetic parameter estimates which may provide insight to the conservation status of *Eulemur collaris* (É. Geoffroy). They provide reference points for future monitoring studies within these populations and for comparison to other lemur populations.

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