

graphic position of the reserve, coupled with its isolation from the continuous rainforest corridor to the east, means that such studies may well find new species and unique communities. Effective management plans should be implemented, and demographic and socio-economic changes in and around the reserve should be monitored carefully, in order to maintain the sanctity of this protected area.

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Indications for Hybridisation between Red-fronted Lemurs (*Eulemur fulvus rufus*) and Mongoose Lemurs (*E. mongoz*) in Northwest Madagascar

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Eulemur is the most diverse and widespread genus of the family Lemuridae, with five currently recognised species. The mongoose lemur, *Eulemur mongoz*, occurs in three geographically isolated populations; on two Comorian islands (Anjouan and Mohéli) and in northwest Madagascar. The brown lemur, *E. fulvus*, has the largest distribution of the five *Eulemur* species and contains at least six subspecies. Brown lemurs are found on the Comorian island Mayotte, in all forested areas of Madagascar except the South and. *E. fulvus* is found in sympatry with all four other *Eulemur* species, including *E. mongoz* in northwestern Madagascar (for review see Tattersall 1982; Harcourt and Thornback 1990; Mittermeier *et al.* 1994).

One of the authors (AZ) collaborated on a 10-month study on mongoose lemurs at Anjamena in northwestern Madagascar (Fig. 1) carried out by D.J. Curtis (Curtis and Zaramody 1998, 1999; Curtis *et al.* 1999). During the field work from September 1994 to September 1995, animals were observed which presented pelage coloration intermediate between *E. mongoz* and *E. f. rufus*. This phenotypic variation led to the suspicion that interspecific hybridisation might be occurring at this site (see Table 1 in Curtis and Zaramody 1998). However, apart from this anecdotal observation, no one has reported hybrids among *Eulemur* species in the wild and no genetic evidence has been published supporting that claim. Mongoose lemurs and brown lemurs represent well accepted discrete species which are phenotypically distinct.

Within the scope of a comprehensive study on the systematics of lemurs (Pastorini 2000; Pastorini *et al.* 2000, 2001, 2001) a mitochondrial DNA (mtDNA) fragment for several representatives of *E. mongoz* and *E. fulvus* from Anjamena was sequenced. The small subset of samples thus far analysed hardens the suspicion of a hybrid zone between mongoose lemurs and red-fronted lemurs in northwestern Madagascar. The goal of this report is to present the available preliminary data which indicate that *E. mongoz* and *E. f. rufus* may hybridise in the wild.

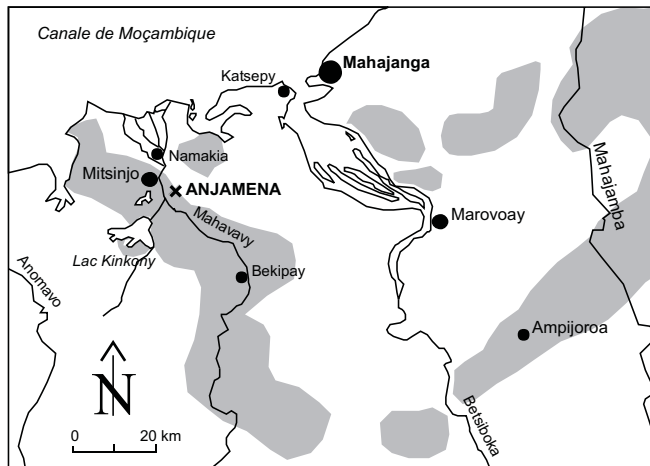


Fig. 1: Map of Madagascar showing the study site (adapted from Curtis and Zaramody 1999). Primary and secondary forests are shaded.

Methods

Field work

We conducted the field study from July to September 1997 on several neighbouring groups of *E. mongoz* and *E. fulvus* in northwestern Madagascar. The study site was close to the village Anjamena (45°55'E, 16°03'S) in the riverine forests to the East of the Mahavavy river (Fig. 1). The village Anadabomandry is located on the other side of the river (west) and is near our additional capture location. Samples were analysed from five *Eulemur mongoz* and six *E. fulvus rufus* (Table 1). For comparison, one *E. coronatus*, two *E. macaco* (2ssp.), one *E. rubriventer* and four *E. fulvus* (4 ssp.) were included in the dataset (GenBank Accession Numbers AF2244524, AF224530, AF224531, AF224525, AF224536, AF224558, AF224560, AF224568). One *Lemur catta* (AF053684) was used as the outgroup.

Table 1: Sample information for the mongoose lemurs and brown lemurs sequenced from the hybrid zone.

Taxon	Origin	ID	GB
<i>E. mongoz</i> 1	Anjamena	JP169	AF224514
<i>E. mongoz</i> 2	Anjamena	JP178	AF224516
<i>E. mongoz</i> 3	Anjamena	JP196	AF224517
<i>E. mongoz</i> 4	Anadabomandry	JP177	AF224515
<i>E. mongoz</i> 5	Anadabomandry	JP211	AF224518
<i>E. fulvus rufus</i> 1	Anjamena	JP161	AF224545
<i>E. fulvus rufus</i> 2*	Anjamena	JP162	AF224546
<i>E. fulvus rufus</i> 3	Anjamena	JP171	AF224547
<i>E. fulvus rufus</i> 4	Anjamena	JP206	AF224550
<i>E. fulvus rufus</i> 5	Anadabomandry	JP176	AF224548
<i>E. fulvus rufus</i> 6	Anadabomandry	JP181	AF224549

ID = identification number, GB = GenBank accession number; * = potential hybrid

Genetic analysis

MtDNA analysis has become established as a powerful tool for the study of molecular evolution. DNA sequence data can also be used to determine the taxonomic affiliation of indi-

viduals of unknown origin. In this study we present the phylogenetic relationships among mongoose lemurs and red-fronted lemurs from Anjamena, based on the sequences of a large fragment of mtDNA. Close attention is given to the relative positions within the genus *Eulemur* of each individual taken from this site. This approach allows detection of individuals whose genotype (mtDNA) is not the same as their phenotype (fur coloration).

DNA was extracted from hair, blood or tissue samples. The segment of the mtDNA amplified and sequenced includes a fragment of the COIII gene, complete sequences for three NADH-dehydrogenase subunits (ND3, ND4L, ND4), as well as five tRNA genes (Gly, Arg, His, Ser, partial Leu). Amplification and internal sequencing primers are provided in Pastorini *et al.* (2000). The sequencing reactions were electrophoresed and analysed on an automated sequencer (ABI 377). All templates were sequenced in their entirety for both strands. The procedures are detailed in Pastorini *et al.* (2000).

Aligned sequences were analysed using maximum parsimony, neighbor-joining, and maximum likelihood methods in PAUP* 4.0b4 (Swofford 1999). Branch-and-bound searches were utilised in parsimony analyses and Kimura 2-parameter distance corrections were used for neighbor-joining analyses. Maximum likelihood trees were calculated via heuristic search using the equivalent of the HKY model. Bootstrap analyses of 2500 replicates were performed to estimate statistical support for each clade.

Results

The mtDNA sequences for the taxa examined have been deposited in GenBank (Table 1). The nucleotide sequences span a total of 2393 base positions (bp) and yielded 337 parsimony-informative characters. The maximum parsimony search with all characters weighted equally results in four trees of 919 steps in length with a consistency index of 0.69 and a retention index of 0.79. The final maximum likelihood tree ($-\ln$ likelihood = 5646.85) was obtained with a previously estimated transition/transversion ratio of 13.61 and gamma shape parameter of 0.05.

The relationships among the clades remain consistent in all analyses. Generally, there is very high bootstrap and jackknife support in maximum parsimony or neighbor-joining analyses for the branching order of the different taxa (Fig. 2). All analyses group five *E. f. rufus* into one clade and five *E. mongoz* into another clade. However, the latter clade additionally includes one *E. f. rufus* (No. 2). In maximum parsimony or neighbor-joining analyses, the mongoose lemur clade, which includes this single *E. f. rufus* individual, is supported with bootstrap values of 100%, as is the clade containing the remaining five *E. f. rufus* individuals. The maximum likelihood phylogram presented in Figure 2c maintains branch lengths proportional to the number of changes. Long branches separate the clade including five red-fronted lemurs from the clade formed by five mongoose lemurs and one red-fronted lemur.

Absolute pairwise distances range from a maximum of 318 to 341 bp between *L. catta* and the ingroup to between 0 and 90 bp within the genus *Eulemur*. Examination of absolute pairwise distances within the genus *Eulemur* reveals three levels of differentiation: The divergences between *Eulemur* species are higher (142-198 bp) than between *Eulemur* subspecies (31-89 bp). Pairwise comparisons of the individuals within the *E. mongoz* clade (including *E. f. rufus* No. 2) and the *E. f. rufus* clade give values in the range of 0 to 10 bp.

Discussion

Animals which exhibited an intermediate pelage pattern of *E. f. rufus* and *E. mongoz* were observed in the area of sympatry at Anjamena in northwestern Madagascar. The suspi-

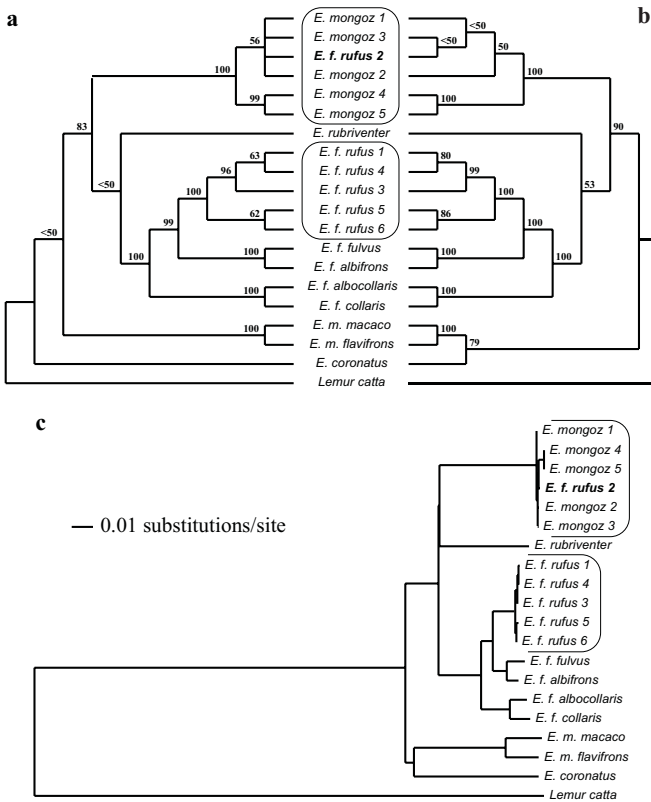


Fig. 2: (a): Maximum parsimony tree with bootstrap values (as percentages, above nodes). (b): Neighbor-joining tree with bootstrap values. (c): Maximum likelihood phylogram with proportional branch lengths. The potential hybrid is highlighted with bold letters.

cion of this potential hybrid zone has been substantiated by a red-fronted lemur which showed the mtDNA genotype of a mongoose lemur. All genetic analyses unambiguously group five *E. f. rufus* individuals into one clade and link one *E. f. rufus* individual with *E. mongoz* into another clade. Both clades are strongly supported by bootstrap analyses and in the maximum likelihood phylogram they are separated from each other by long branches. Such paraphyly strongly indicates that this *E. f. rufus* individual No. 2 has the mtDNA genotype of a mongoose lemur. Pairwise distance comparisons confirm this conclusion: Genetic distances between that single *E. f. rufus* individual and *E. mongoz* (3-9 bp) are of the same order as among *E. mongoz* individuals (0-10 bp). In contrast, the genetic distances between *E. f. rufus* individual No. 2 and the other five *E. f. rufus* individuals (151-159 bp) are fairly high and clearly lie in the range of comparisons among *Eulemur* species (142-198 bp).

The 11 lemurs sequenced for this study all had the correct and unmistakable fur coloration of either *E. mongoz* or *E. fulvus* and lived in social groups including only animals of their own taxon. However, one individual which phenotypically looks like a *E. f. rufus* groups genetically within the *E. mongoz* clade. A mistake in identifying the correct taxon is not very likely, because it is quite easy to distinguish among male as well as among female *E. f. rufus* and *E. mongoz* (for illustrations see Mittermeier *et al.* 1994). The fact, that the *E. f. rufus* individual No. 2 which had the mtDNA genotype of *E. mongoz* was phenotypically a normal red-fronted lemur female could indicate that the hybrid cross occurred generations ago. This would imply that crosses between *E. f. rufus* and *E. mongoz* give rise to fertile hybrids. However, as no artificial hybrids are known in captivity we cannot be certain of the F1 phenotypic results from

such a mating. It is possible that a simple F1 mating between these species could result in a phenotype indistinguishable from either parental species. Furthermore, because mtDNA is inherited through matriline it follows that the hybrid cross included a female mongoose lemur and a male red-fronted lemur. Interestingly, during our field work in 1997 we observed two mongoose lemur groups with females which had a pelage coloration intermediate between *E. mongoz* and *E. f. rufus*. Both groups consisted of one adult female (phenotypic hybrid), one adult male (*E. mongoz*) and a juvenile. If we assume the females to be the mothers of those juveniles, this would support the hypothesis, that the hybrids are fertile.

The small sample presented here provides support that hybridisation might occur between *E. mongoz* and *E. f. rufus*. So far there are no reports of hybridisation among *E. fulvus* and another sympatric *Eulemur* species in the wild. As *E. fulvus* and *E. mongoz* appear to fail to meet the widely specified criterion, namely reproductive isolation from each other, recognition of two species might be considered as questionable. However, there is no doubt that *E. mongoz* and *E. fulvus* should continue to be considered separate species. There is strong morphological evidence supporting the distinction between species of *E. mongoz* and *E. fulvus*. The genetic sequence data presented here unambiguously depict high levels of differentiation between *E. mongoz* and *E. fulvus* scaled relative to other members of the genus *Eulemur*, which further supports their species status. However, *E. mongoz* and some of the *E. fulvus* subspecies have the same karyotype (2N=60), which might explain the successful interbreeding resulting in fertile hybrids. In contrast, *E. coronatus* (2N=46), *E. macaco* (2N=44), *E. rubriventer* (2N=50), as well as the two subspecies *E. f. albocollaris* (2N=48) and *E. f. collaris* (2N=52) have different karyotypes (Rumpler 1975). Despite the identical karyotype and case for sympatric hybridisation presented here, the two taxa are distinguishable both phenotypically and genetically for their range.

The genetic results presented here are only preliminary and were not specifically designed to test the hybrid zone. The sample size is very small and no individuals with the observed intermediate pelage pattern of *E. f. rufus* and *E. mongoz* were available for analyses. The use of mtDNA sequence data exclusively tends to underestimate the number of potential hybrids because only the genetic contribution through the matriline is considered. Additional samples from the site are currently being analysed using mtDNA sequence data as well as microsatellites. The addition of nuclear data will provide a more accurate picture of the potential genetic exchange among brown lemurs and mongoose lemurs at Anjamena. The processing of phenotypically intermediate individuals for genetical characterisation should assist with determining the value of fur coloration to predict a hybrid.

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Suivi écologique de deux espèces de lémuriers diurnes *Varecia variegata rubra* et *Eulemur fulvus albifrons* dans la presqu'île de Masoala (1993-1998)

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Depuis l'établissement du Programme de Conservation et de Développement Intégré de Masoala, *Varecia variegata rubra* et *Eulemur fulvus albifrons* ont été choisis comme indicateurs biologiques compte tenu de l'importance de leur rôle écologique au sein de ce massif forestier en l'occurrence dans la dispersion des graines. Comme ces animaux occupent un large domaine vital, ils sont considérés comme espèces "parapluie". Si bien que les lémuriers seraient non seulement

des espèces porte-fanion, mais aussi des espèces-clés appropriées pour aider à suivre et évaluer la réussite des efforts de préservation de l'écosystème tout entier et de la gestion des ressources forestières à travers de l'établissement d'un PCDI et du Parc National Masoala (Kremen *et al.* 1994; Merenlender *et al.* 1998).

Peu d'études ont été effectuées sur la dynamique de populations des lémuriers Malagasy et sur leur réponse à la destruction et la perturbation de l'habitat (e.g., Ganzhorn and Schmid 1998). En outre, les lémuriers subissent aussi la pression de la chasse (Mittermeier *et al.* 1994). A Masoala, la destruction forestière se manifeste par la transformation progressive des forêts en zones d'agriculture et la perturbation est provoquée par les exploitations forestières telles que l'extraction d'espèces d'arbre. *V. variegata rubra* apparaît l'espèce la plus touchée par ces deux formes de menace. Elle est connue comme une espèce strictement arboricole et forestière, montrant une exigence plus élevée vis-à-vis de la typologie de la forêt (Rigamonti 1993). Par ailleurs, les lémuriers jouent des rôles écologiques importantes. Une altération de la structure et de la composition de l'habitat pourrait modifier la distribution et réduire l'abondance des lémuriers, et vice versa (Merenlender *et al.* 1998). Comme la chair de ces deux espèces est très appréciée et qu'elles figurent parmi les plus grands mammifères de la forêt, elles sont fortement chassées. Les pièges sont plus fréquents surtout dans les forêts près des zones d'agriculture. La chasse au fusil est également rapportée par les villageois.

Cet article montrera les résultats de suivi de populations des deux espèces de lémuriers depuis 1993. L'étude a été conduite chaque année afin de suivre l'évolution et la tendance de la dynamique de population de ces deux espèces de lémuriers à l'intérieur du noyau dur du Parc et à l'extérieur dans les zones tampons et zones périphériques.

Méthodologie

Choix des sites

Les six sites d'étude sont localisés principalement dans le versant ouest de la presqu'île entre le village d'Amбанизана et la pointe de Tampolo. Trois de ces sites, Andranobe, Antrafonaomby et Bedinta, sont choisis dans des zones de forêt non ou peu perturbées. Alors que les trois autres, Amбанизана, Antambakoanorona et Ambodiforaha, sont dans des zones perturbées (Merenlender 1995; Rakoton-

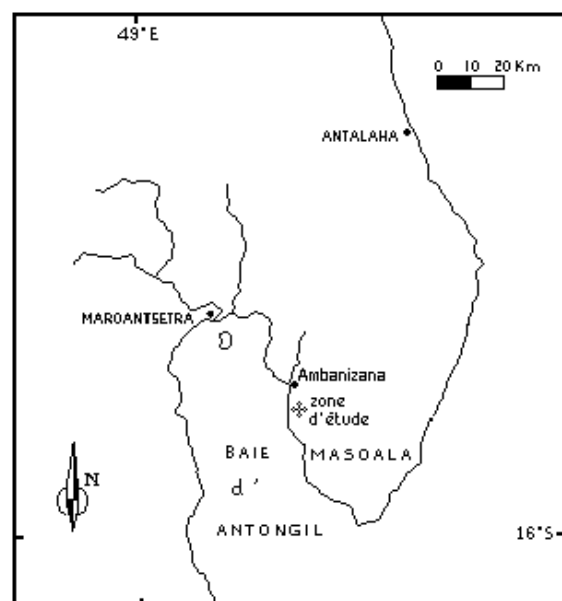


Fig. 1: Carte de localisation de la presqu'île de Masoala et la zone d'étude.