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Population Genetics of *Eulemur macaco macaco* (Primates: Lemuridae) on the Islands of Nosy-Be and Nosy-Komba and the Peninsula of Ambato (Madagascar)

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ABSTRACT. Analysis for genetic variation of insular and mainland populations of *Eulemur macaco* has revealed: (1) a different degree of genetic variation between populations; and (2) the phylogenetic relationships between groups, on the islands of Nosy-Be and Nosy-Komba, and in the Peninsula of Ambato (Madagascar). Eleven systems of blood proteins from 157 animals were used as genetic markers. The genetic variation was lower on the island of Nosy-Komba than in the mainland of Ambato. This is consistent with the expectation that genetic variation is lower on islands than on mainlands. In contrast, the genetic variation on the island of Nosy-Be was the highest of the three populations. This finding can best be explained by assuming that the sample of Nosy-Be consists of individuals of several small isolated groups, where genetic drift computation showed the population of Nosy-Be to be distinct, and the populations of Nosy-Komba and Ambato to be close within the same branch of the dendrogram. These findings give an insight into the population history of the island of Nosy-Komba, which might have been populated by mainland groups from Ambato.

Key Words: *Eulemur macaco*; Island populations; Protein markers; Founder effect; Genetic variation; Genetic drift.

INTRODUCTION

Studies on natural populations including plants (GLOVER & BARRET, 1987) as well as animals (GOODMAN, 1987; STEWART & BAKER, 1991; WAYNE et al., 1991) demonstrated that insular populations generally possess a reduced genetic variation compared with continental ones. This fact is of considerable relevance for island and reserve ecology. In the case of primates, blood markers were used to observe that three insular populations of *Macaca fascicularis* were less polymorphic than a continental reference population of the same species (KAWAMOTO et al., 1991). However, there are exceptions to this general phenomenon. For instance, SHOTAKE and SANTIAPILLAI (1982) found that the genetic variation of *Macaca sinica* on the island of Sri Lanka is higher compared to continental groups. A comparative study of populations of Malagasy prosimians, based on the same method, revealed that an insular population of *Eulemur macaco* did not show all the alleles existing in the population of a nearby peninsula (ARNAUD et al., 1992).

In order to try to relate the amount of genetic variation to the size of the area occupied, we have extended our study to an *E. macaco* population of a larger island, Nosy-Be, located nearby (Fig. 1).

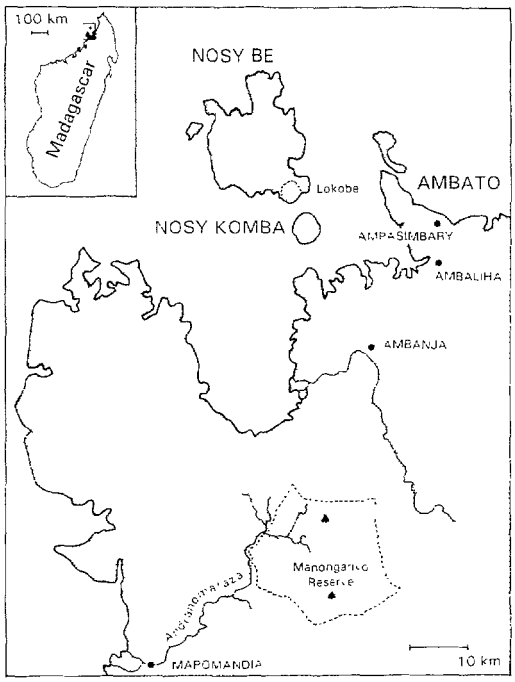

Fig. 1. Map indicating the location of the *Eulemur macaco* area: Nosy-Be, Nosy-Komba, and Ambato peninsula.

MATERIALS AND METHODS

Sixty-seven *E. macaco* were captured in traps baited with bananas on the island of

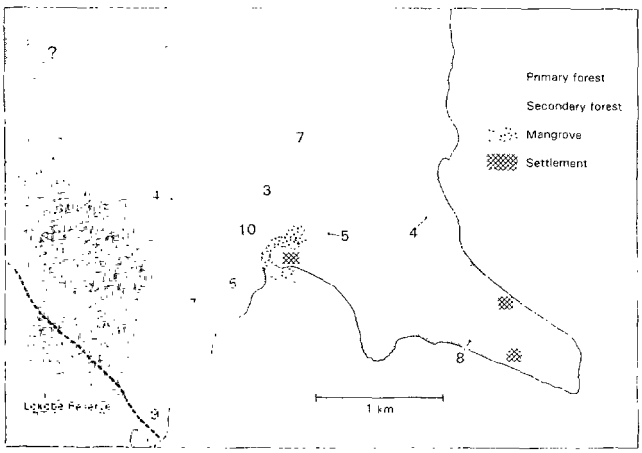

Fig. 2. Geographical drawing of groups of *Eulemur macaco* captured on Nosy-Be. Figures localise the capture areas and indicate the numbers of animals captured for the study. ?: Uncertain limit of the primary forest.

Nosy-Be, northwest Madagascar in a secondary forest close to Ambalahonko, a village located in the proximity of the Lokobe reserve. Ten traps were approximately equally distributed (Fig. 2) in an area of about 450 ha. The traps were spaced at an average distance of 500 m from each other. The distribution pattern of the groups in this area is not yet known, and it seems possible that animals from different groups were caught in the same trap (since animals of the same group were caught in different traps). It is also possible that groups originating from the primary forests close to the Lokobe reserve have migrated into the study area.

The traps were constructed similarly to basketwork fishing traps, so that the animals could enter easily and without injury. Thus, it was not uncommon to observe the same animal in the same trap repeatedly. The animals were captured at nine different sites, ranging in numbers from 4–15 animals each (Fig. 2). To compute allele frequencies of larger groups of almost the same size, the localities were pooled into three groups on the basis of their preanalyzed relatedness:

Group	Locality	Original number
1	Antafondro	1
	Rano Ankatafa	3
2	Rano Lemby	9
	Rano Doanilahy	10
	Rano Madirotelo	11
3	Rano Ankoraka	5
	Rano Antorivario	6
	Ala-be kely kely	7
	Rano Andokobe	8.

Groups and sample size are given in Table 1. To avoid repeated sampling, the animals were conspicuously marked before release by cutting some of their tail hairs. Anaesthesia was performed with an aqueous solution of 0.5 ml of Ketamine (1 mg/ml). Three to 5 ml of blood were frozen in liquid nitrogen and sent to Strasbourg for analysis. The same blood protein markers were analyzed as in the previous study on the populations of Nosy-Komba ($N=46$), and of Ambato peninsula ($N=44$) (ARNAUD et al., 1992) which include: AK (adenylate kinase), ADA (adenosin deaminase), CA2 (carbonic anhydrase 2), GLO1 (glyoxolase 1), DIA1 (NADH diaphorase 1), 6-PGD (6-phosphogluconate dehydrogenase), AcP (acid phosphatase), and EstD (esterase D). In addition, we studied the following plasma proteins: ALB (albumin), TF (transferrin), and C3 (complement factor C3). These plasma protein systems were investigated not only in the new samples from Nosy-Be, but also in the plasma samples which were previously obtained from animals caught on Nosy-Komba and on the Ambato peninsula and have been stored deep frozen. The overall genetic marker systems applied revealed 23 alleles.

Horizontal electrophoresis was performed for all red cell systems on an 1% agarose gel (SEAKEM LE), at 8–10°C for short runs (1.5 hrs) at constant voltage (400 V). Conventional enzyme staining procedures were used (HARRIS & HOPKINSON, 1976) with minor modifications. The plasma proteins ALB, TF, and C3 were analyzed by applying TEISBERG's method (TEISBERG, 1970), in a 1% agarose (Litex HSA) gel; all the methods used are described in detail elsewhere (SCHEFFRAHN, 1992). DTT (2 μl of a 77 mg/10 ml solution) has been given to freshly prepared hemolysates to strengthen or reconstitute the peptide bonds; this procedure was especially important for the DIA1. For reasons of

Table 1. Blood genetic markers, sample size, genotypes, • allele frequencies (X 0.001), CHI^2-test showing significant deviation from the Hardy-Weinberg equilibrium of three subpopulations of Nosy-Be.

	Genotypes/alleles	Subpopulation 1	Subpopulation 2	Subpopulation 3
ALB		$N=16$	$N=27$	$N=24$
	11	16	27	24
	•1	1000	1000	1000
TF		$N=16$	$N=27$	$N=24$
	11	6	15	5
	12	8	7	12
	22	2	5	7
	•1	625	685	458
	•2	375	315	542
C3		$N=16$	$N=27$	$N=24$
	11	16	27	24
	•1	1000	1000	1000
CA2		$N=13$	$N=22$	$N=22$
	11	7	13	17
	12	0	4	1
	13	5	4	4
	22	0	0	0
	23	0	1	0
	33	1	0	0
	•1	731	773	886
	•2	0	114	023
	•3	269	114	091
AcP		$N=14$	$N=26$	$N=23$
	11	2	8	7
	12	4	7	6
	22	8	11	10
	•1	286	442	435
	•2	714	558	565
			$CHI^2 1 = 5.365$*	$CHI^2 1 = 5.064$*
DIA1		$N=13$	$N=23$	$N=22$
	11	1	3	2
	12	2	0	4
	13	2	3	5
	14	0	5	5
	15	0	0	0
	22	0	0	0
	23	4	0	1
	24	0	0	0
	25	0	0	0
	33	1	2	0
	34	0	9	1
	35	0	0	2
	44	0	0	0
	45	3	1	2
	55	0	0	0
	•1	231	304	409
	•2	231	0	114
	•3	308	348	205
	•4	115	326	182
	•5	115	22	91
ADA		$N=15$	$N=26$	$N=23$
	11	14	25	22
	12	1	1	1
	22	0	0	0
	•1	967	981	978
	•2	33	19	22

(continued)

Table 1. (continued)

	Genotypes/alleles	Subpopulation 1	Subpopulation 2	Subpopulation 3
AK		$N=15$	$N=26$	$N=23$
	11	15	26	23
	•1	1000	1000	1000
6-PGD		$N=15$	$N=26$	$N=23$
	11	15	26	23
	•1	0	0	0
	•2	1000	1000	1000
EstD		$N=15$	$N=24$	$N=23$
	11	7	9	11
	12	5	5	7
	22	3	10	5
	•1	633	479	630
	•2	367	521	370
			$CHI^2 1 = 8.146^{**}$	
GLO1		$N=15$	$N=25$	$N=23$
	11	11	21	18
	12	4	3	5
	22	0	1	0
	•1	876	900	891
	•2	133	100	109

*Significant at $p<0.05$; **significant at $p<0.01$.

accuracy, some of the systems have been analyzed at least twice (AcP, EstD, and DIA1) (Figs. 3 & 4).

The comparison of protein genetic data between Nosy-Be, Nosy-Komba, and the

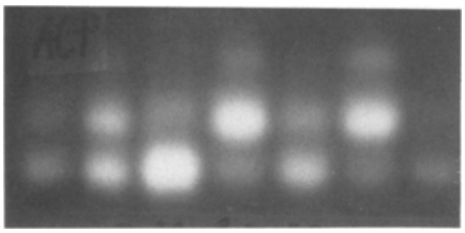

Fig. 3. Electrophoretic phenotypes of the enzyme AcP. From left to right: Lane 1: AcP1; Lane 2: AcP12; Lane 3: AcP1; Lane 4: AcP2; Lane 5: AcP1; Lane 6: AcP2; Lane 7: AcP1.

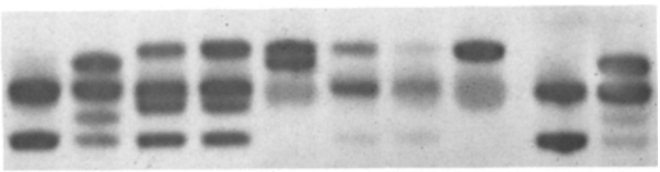

Fig. 4. Electrophoretic phenotypes of the enzyme DIA1. From left to right: Lane 1: DIA1 1 3; Lane 2: DIA1 3 5; Lane 3: DIA1 1 4; Lane 4: DIA1 1 4; Lane 5: DIA1 4 5; Lane 6: DIA1 3; Lane 7: DIA1 3; Lane 8: DIA1 4; Lane 9: DIA1 1 3; Lane 10: DIA1 3 5.

peninsula of Ambato has been performed with the help of a variety of statistical procedures for the following analyses: allele frequencies and genetic variability measures (rate of polymorphism, mean heterozygosity as unbiased estimate), using corrections for small sample size (NEI, 1978), chi-square goodness-of-fit test of Hardy-Weinberg equilibrium (HWE), genetic similarity (ROGERS, 1972) and distance (NEI, 1972, 1978, unweighted pair group method), F-statistics (NEI, 1977), and significance probabilities etc., all of them available in the computer package of Biosys-1 (SWOFFORD & SELANDER, 1989). Polymorphism is defined as the situation where the most common allele does not exceed 0.99 (FORD, 1940).

RESULTS

The data summarized in Tables 1 and 2 widely confirm previously results of starch gel electrophoresis given by ARNAUD et al. (1992). However, the present study enlarges the number of systems applied (with the three plasma proteins ALB, TF, and C3) and the number of population studied (with the island of Nosy-Be).

In addition to previous data, the plasma protein TF and the erythrocytic enzymes ADA, AcP, and EstD were also encountered as being polymorphic in *E. macaco*. This leads to the conclusion that at least 8 systems out of 14 (with the inclusion of PGM1, PGM2, and 6-PGD, see ARNAUD et al., 1992), studied so far, are polymorphic in the species *E. macaco*.

The rate of polymorphism and the mean number of alleles per locus are higher on Nosy-Be, thus indicating a more elevated degree of genetic variation. It is interesting to note

Table 2. Number of alleles per locus (rate of polymorphism), size, mean number of alleles per locus, number of alleles, and average heterozygosity (*H*) on Nosy-Be, Nosy-Komba, and Ambato and in the *Eulemur macaco* species.

	Nosy-Be $N=67$	Nosy-Komba $N=46$	Ambato $N=44$	*Eulemur macaco* $N=157$
ALB	1	1	1	1
TF	2	2	2	2
C3	1	1	1	1
CA2	3	2*	2*	3
AcP	2	1*	1*	2
DIA1	5	2*	2*	5
ADA	2	1*	1*	2
AK	1	1*	1*	1
6-PGD	1	1*	2*	2
EstD	2	1*	1*	2
GLO1	2	1*	2*	2
PGM1	—	1*	1*	1
PGM2	—	1*	1*	1
6-PGD	—	1*	1*	1
No. of alleles	22	17	19	26
Polymorphic systems (0.99 criterion)	7	3	5	8
Rate of polymorphism (11 systems)	0.6363	0.2727	0.4545	0.7272
Mean number of alleles per locus (11 loci)	2.0±0.4	1.3±0.1	1.5±0.2	2.09±0.3
H	0.254±0.081	0.074±0.046	0.113±0.052	0.194±0.063

N=No. of specimens studied. *ARNAUD et al., 1992.

that almost all alleles which are common on the mainland also occur on Nosy-Be, with the exception of the 6-PGD 1. Beyond this, on Nosy-Be prevail some alleles (CA2 3, AcP 2, DIA 2, DIA 3, DIA 5, ADA 2, and EstD 2) which do not exist in the gene pool neither of Nosy-Komba nor of the mainland.

In comparison with the mainland, Nosy-Komba exhibits less genetic variation indicated by a lower rate of polymorphism and a lower average number of alleles per locus. There is no significant deviation from HWE for all the systems of the population of Nosy-Komba (RABARIVOLA, 1993). In most of the systems analyzed, Nosy-Komba is genetically very close to the mainland.

Table 1 lists genotypes, allele frequencies and Hardy-Weinberg conditions for three subpopulations of Nosy-Be (see also Fig. 2). All the genotypes of the subpopulations "1" are in HWE; this is also the case for "2" (exception AcP and EstD), and "3" (exception AcP). The allele differences between these neighbouring subpopulations are clearly noticeable. The subpopulation 1 is distinct from the other two subpopulations, most notably by the absence of the allele CA2 2. The genetic variation within the population of Nosy-Be is rather remarkable; 7 out of 11 genetic marker systems are polymorphic (0.99 criterion) indicating a far greater level of variability than on Nosy-Komba and on the mainland. Furthermore, a considerably higher number of alleles is found at some of the loci (CA2 and DIA1). The higher degree of variation of Nosy-Be is also recognizable by the balanced distribution of the alleles (TF1, CA2, DIA1, AcP, and EstD). As a consequence of all these findings, the average heterozygosity (H) per locus (unbiased estimate) within the population of Nosy-Be comes close to 0.254 (0.081). The H values indicate that the three subpopu-

Table 3. Average heterozygosity (H) per locus (unbiased estimate, HW expected) and inbreeding ($F = H_O - H_F/H_O$) within the subpopulations of *Eulemur macaco* of Nosy-Be.

	H	F
Antafondro	0.264 ± 0.083	0.1535
Doanilahy	0.250 ± 0.079	0.0135
Antorivario	0.245 ± 0.082	0.0490

Table 4. ROGERS's genetic similarity (WRIGHT, 1978) and NEI's genetic distance (NEI, 1978) coefficients of three subpopulations of *Eulemur macaco* of Nosy-Be.

	Antafondro	Doanilahy	Antorivario
Antafondro	—	0.928	0.937
Doanilahy	0.006	—	0.937
Antorivario	0.003	0.008	—

Table 5. Average heterozygosity (H) per locus (unbiased estimate, HW expected) for the populations of Nosy-Be, Nosy-Komba, and Ambato and all the *E. macaco*. Genetic differentiation (F_{ST}) between (*) the three subpopulations and the population of Nosy-Be, and (**) between the populations of Nosy-Be, Nosy-Komba, and Ambato and all the *E. macaco*.

	H	F_{ST}
Nosy-Be	0.254 ± 0.083	0.029*
Nosy-Komba	0.074 ± 0.046	
Ambato	0.113 ± 0.052	
All the *E. macaco*	0.194 ± 0.063	0.190**

Table 6. ROGERS's genetic similarity (WRIGHT, 1978) and NEI's genetic distance (NEI, 1978) coefficients of the *Eulemur macaco* populations of Nosy-Be, Nosy-Komba, and Ambato.

	Nosy-Be	Nosy-Komba	Ambato
Nosy-Be	—	0.819	0.839
Nosy-Komba	0.093	—	0.956
Ambato	0.074	0.011	—

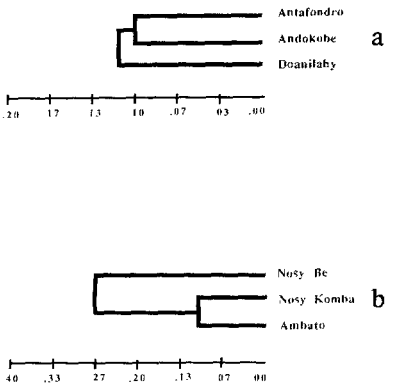

Fig. 5. Relatedness dendrograms (UWPG after ROGERS; WRIGHT, 1978) a) of *Eulemur macaco* groups on Nosy-Be; b) of *Eulemur macaco* on Nosy-Be, Nosy-Komba, and Ambato. The values are on the scale NEI's genetic distances.

lations of Nosy-Be do not differ very much in the degree of genetic variation, the computed values are ranging from 0.245 – 0.264 (Table 3).

The computation of the genetic similarity between the subpopulations of Nosy-Be shows that Antafondro is genetically closer to Antorivario than to Doanilahy (Table 4).

The average heterozygosity values of the overall population of Nosy-Be, Nosy-Komba, and Ambato are to be seen in Tables 5 and 6. Nosy-Komba exhibits the lowest *H* value and stands in this respect very close to Ambato. The two populations of Nosy-Komba and Ambato resemble each other also in the genetic similarity and genetic distance which can also be seen in the dendrogram (Fig. 5a). The F-statistics value of 0.190 underlines that the genetic differentiation between these three populations of *E. macaco* is rather expressed.

DISCUSSION

As expected, all the measures of genetic variation applied in the present study (average heterozygosity (*H*), rate of polymorphism (*P*), and mean number of alleles per locus) indicate that the population of the island Nosy-Komba is less polymorphic than that of the larger island Nosy-Be and the peninsula of Ambato.

For many species, genetic variation of island populations has been shown to be reduced in comparison to continental populations (GOODMAN & POULIK, 1961; SELANDER, 1978). Thus, KAWAMOTO et al. (1991) outlined that the polymorphism of the plasma proteins

of the crab-eating macaque is higher in the Malaysian peninsula than on the islands of Sumatra, Java, and Bali, and that the level of polymorphism decreases with the size of the islands (P=0.484 for Malaysia, 0.387 for Sumatra, 0.258 for Java, and 0.226 for Bali). Therefore, the extremely reduced values of H and P of the population on the island of Nosy-Komba could be seen as a consequence of the very small island size (23 km^2), small group size, founder effect and genetic drift. It seems highly likely that the population *E. macaco* on Nosy-Komba has been recently (re)introduced by humans with only a few founders, apparently from Ambato. And indeed, no other mammal species have been introduced into the island besides *Rattus rattus*, although the island is only separated from the Malagasy mainland by a 12 m-deep, 4 km-wide channel. The lack of other lemurs and competitors on Nosy-Komba may have played a role during the process of populating of *E. macaco*. Whatsoever, there are good arguments for the explanation of the low genetic variation on this relatively small island.

On the peninsula of Ambato (a part of the Malagasy mainland, but ecologically separated) on the contrary, six species are found (*E. macaco, Lepilemur dorsalis, Hapalemur griseus, Mirza coquereli, Microcebus murinus*, and recently also *Cheirogaleus*). Even *Daubentonia madagascariensis* has been found near Ambanja in 1967 (LEHMAN, pers. comm.), and again in 1988 by one of us (B. M.). On Nosy-Be, three species (*E. m. macaco, L. dorsalis*, and *M. murinus*) are known. It cannot yet be ruled out that the extinction of all the Ambato land mammal species on Nosy-Komba (even that of the ubiquitous *Microcebus*) is due to a severe former deforestation event. However, the strong presumption exists that the extinction process was similar to that on other Pleistocene landbridge islands. If virtually all but our land mammal species were wiped out by such processes, it is hard to believe that one of the biggest species, *E. macaco*, would alone have survived.

Comparison of the rate of genetic variation between the population of Nosy-Be and Ambato gave an unexpected result. That is, that the polymorphism of the Nosy-Be island is higher than on the mainland of the Ambato peninsula. Furthermore, some alleles, present in Nosy-Be are absent in Ambato (Table 2). One explanation for this striking event is that the forest of the Ambato peninsula has been largely destroyed during past decades, leaving a small forested fragment along the coast, completely separated by large cultivated areas from the other forested areas of the Sambirano. The *E. macaco* from this small area are also hunted. Thus, the population may well be bottle-necked in several ways. Among the 50 animals captured and earmarked in 1987, only few were still living in 1992. The former population of this small area (more than 80 animals) was reduced to less than 30. According to the Hardy-Weinberg equilibrium this threatened population will not be as balanced as it might appear. This is indeed indicated by the highly significant difference between the expected and the observed frequency of genotypes of the systems AcP and EstD. The phenomenon can best be explained by the fact that the samples come from small and widely dispersed social groups.

The calculation of the genetic distances between the populations of Nosy-Be, Nosy-Komba, and Ambato (Table 6) reveals the ancient isolation of the population of Nosy-Be. The island has been separated since 8,000 years from the mainland (BATTISTINI, 1960) and the population of *E. macaco* thus being isolated from those of the mainland has progressively genetically differentiated. The relatively high genetic distance of Nosy-Be against Ambato fits well this scenario. On the other hand, the degree of genetic differentiation is very much smaller than that of well-separated species such as *Galago senegalensis*

and *Galago moholi* (0.532) or *Galago senegalensis* and *Galago crassicaudatus* (0.988) (SCHEFFRAHN et al., submit.). It is noteworthy that the genetic distances of these species correspond very well with anatomical and cytogenetic differences. Cluster analysis reveals that the populations of Nosy-Komba and Ambato are genetically very close, while that of Nosy-Be is markedly different (Fig. 3). It can thus be deduced that the population of Nosy-Komba has probably been introduced from the mainland, and not from Nosy-Be, even though both islands are very physically close. It could even be possible that the population of Nosy-Komba originally came from the population of the peninsula of Ambato. Another clear result is that the population of the Ambato peninsula has also become a fragmented isolate, and is therefore not a good reference. A better reference population should be studied in an area not disturbed by humans, such as the Manongarivo or Tsaratanana reserves.

As far as conservation genetics is concerned, it seems to be clear on the basis of the evidence presented in this study that research on the population genetics and general biology of natural populations should always preceed a captive breeding program in order to prevent the selection of founder animals from areas where the genetic diversity is low. Secondly, breeding programs which have the aim to release bred animals in the wild, have to orientate themselves along the values of genetic variation of wild populations and subpopulations which can drastically differ from area to area, as noticed in the case of *E. macaco* on the islands of Nosy-Be and Nosy-Komba and on the mainland around Ambato, to avoid lowering of heightening of the genetic variation of natural groups.

CONCLUSIONS

The genetic comparison of three populations of *E. macaco* by the use of phenotypic blood markers shows that the isolated population of the Nosy-Be island is strongly differentiated from the others. It confirms previous results which showed that the population of Nosy-Komba derives from a small number of founders. It also gives strong arguments in favour of the settlement of Nosy-Komba by animals originating from the mainland population and not from that of Nosy-Be.

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