Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Gianfranco Biondi, Olga Rickards, Roberta Priori

Electrophoretic analysis of some genetic markers in the troop of Macaca fuscata fuscata of the Zoo of Rome

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **82** (1988), n.4, p. 787–796.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1988_8_82_4_787_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1988.

SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Atti Acc. Lincei Rend. fis.
(8), LXXXII (1988), pp. 787-796

Antropologia. — *Electrophoretic analysis of some genetic markers in the troop of Macaca fuscata fuscata of the Zoo of Rome.* Nota di GIANFRANCO BIONDI (*), OLGA RICKARDS (**) e ROBERTA PRIORI (*), presentata (***) dal Socio G. MONTALENTI.

ABSTRACT. — In the present paper the results are reported of an electrophoretic survey of ALB, PI, TF, ADA. ACP1, AK1, ESD, PGD and PGM1, in a troop of *Macaca fuscata fuscata*. PI was the only polymorphic marker found (PI*D = 0.098). *Macaca fuscata* turned out to have a lower level of genetic heterogeneity than other animal species (range of heterozygosity per individual: 0.1-3.3% vs 5-15%). However, this species shows values peculiar to several animal species inhabiting narrow and close environments.

KEY WORDS: Primates; Macaca fuscata; Genetic markers.

RIASSUNTO. — *Analisi elettroforetica di alcuni marcatori genetici in una colonia di Macaca fuscata fuscata dello Zoo di Roma.* In questa nota sono riportati i risultati di uno studio sulla variabilità elettroforetica di una colonia di *Macaca fuscata fuscata*. Tra i marcatori studiati, ALB, PI, TF, ADA, ACP1, AK1, ESD, PGD and PGM1, solo il PI è risultato polimorfico (PI*D = 0.098). Questa specie presenta un basso valore di eterogeneità genetica rispetto ad altre specie animali (intervallo di eterozigosità per individuo: 0.1-3.3% vs 5-15%). Tuttavia il valore riscontrato in *Macaca fuscata* è simile a quelli noti per numerose altre specie animali che vivono in ambienti ristretti.

INTRODUCTION

The Japanese macaque (*Macaca fuscata*) is subdivided into two subspecies, the mainland macaque, *Macaca fuscata fuscata* and *Macaca fuscata yakui* living in the Yaku Island, a small Southern island of the Japanese archipelago. The whole population (20,000-70,000 individuals) is composed of several troops, and each troop comprises one or more adult males, adult females, and young and infants of both sexes. The number of adult females in each troop is generally more than that of adult males because of emigration of young males which become «solitary males», and don't integrate themselves with any colony. The adult members of each troop are socially organized in a rank system. From the reproductive point of view, each troop is a partially open system because some solitary males come into contact with the adult females in the breeding season [1, 2].

(*) Dipartimento di Biologia Animale e dell'Uomo, Università degli Studi di Roma «La Sapienza», Piazzale Aldo Moro 5, 00185 Roma, Italia.

(**) Dipartimento di Biologia, Università degli Studi di Roma «Tor Vergata», Via Orazio Raimondo, 00173 Roma, Italia.

(***) Nella seduta del 22 giugno 1988.

The present paper adds information on the electrophoretic variability of some genetic markers in *Macaca fuscata fuscata.*

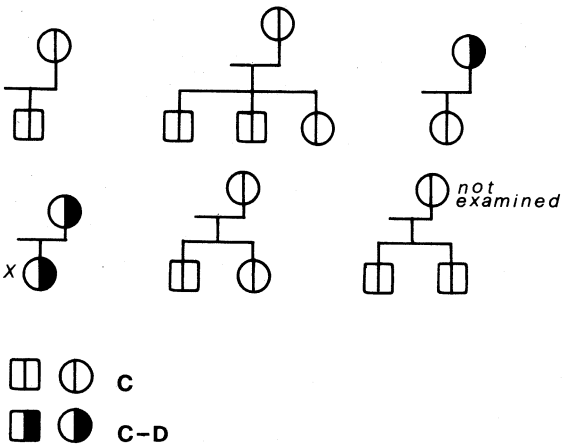

Fig. 1. – Pedigrees of the related subjects with their PI phenotype.

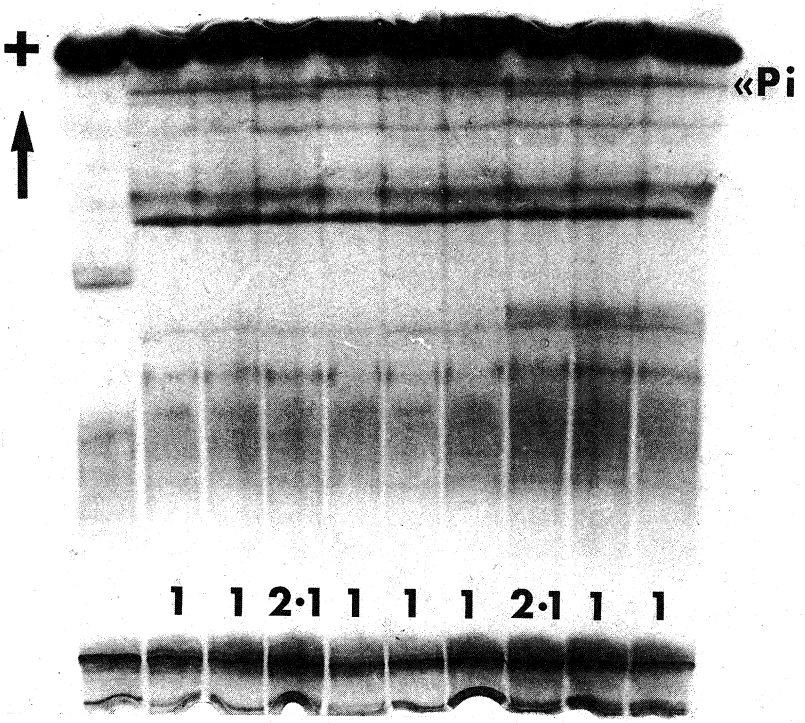

Fig. 2. – Electophoretic pattern of Protease inhibitor (PI) (2-1 phenotype according to [5 and 10] or C-D phenotype according to [2, 8 and 10]).

MATERIALS AND METHODS

The investigated troop was living in the Zoo of Rome coming directly from Japan (see for details [3 and 4]). It consisted of 34 subjects (20 females and 14 males), of which 11 females and 10 males were unrelated (see Table 1 and Fig. 1). The blood samples were drawn by venipuncture and collected in EDTA tubes. Plasma and eyrthrocyte aliquotes were then kept frozen at −20 degree Centigrade until the analyses.

Polyacrylamide gel electrophoresis (PAGE) of Protease inhibitor (PI) was performed according to [5]. Electrophoretic separation of Adenosine deaminase (ADA), Acid phosphatase (ACP1), Adenylate kinase (AK1), Esterase D (ESD), Phosphogluconate dehydrogenease (PGD) and Phosphoglucomutase 1 (PGM1) was performed on cellulose acetate strips (Cellogel, Chemetron Labometrics, Milan, Italy) according to [6] modified by Chemetron Labometrics.

TABLE 1

Serum and red cell electrophoretic phenotypes in the troop of Macaca fuscata fuscata of the Zoo of Rome ($#)

	ALB (*)	PI		TF (*)	ADA	ACP1	AK1	ESD	PGD	PGM1
	A	C	CD	F	1	A	1	1	A	1
Unrelated subjects										
Males	8	6	2	8	8	8	8	8	8	8
Females	11	10	1	11	11	11	11	11	11	11
Related subjects (**)										
Males	6	6	—	6	6	6	6	6	6	6
Females	9	6	3	9	9	9	9	9	9	9

($) Symbols of phenotypes according to [2], [8] and [9].
(#) As our sample includes some related subjects, the gene frequencies were computed as follows: we counted two genes for all unrelated subjects and for the first member of each related group (mother), while the second member of each related group (son) was counted as one gene. The third and fourth members of the related groups (sons) were excluded. For example, the X individual (Fig. 1), heterozygote at the PI locus, was counted as 1 independent gene, of which 0.5 PI*C and 0.5 PI*D.
(*) Albumin and Transferrin, reported in [7].
(**) The pedigrees are reported in Fig. 1.

RESULTS AND DISCUSSION

No electrophoretic polymorphism was found for ADA, ACP1, AK1, ESD, PGD, PGM1, ALB and TF (the last two already turned out to be monomorphic [7]). The observed common patterns were named according to [2, 7, 8 and 9]: 1, A, 1, 1, A, 1, A and F respectively (Table 1). The ADA, AK1 and ESD common patterns can also be named 5′, 3 and 2 according to [5 and 10].

In 33 troops of *Macaca fuscata fuscata* living in Japan, ALB, ADA and PGD loci

TABLE 2

Allels frequencies at 9 loci in 34 troops of Macaca fuscata fuscata

Alleles	Troops (*)							
	1	2	3	4	5	6	7	8
ALB*A								
B	1.	1.	1.	1.	1.	1.	1.	1.
PI*B	.016							
C	.984	1.	1.	1.	1.	1.	1.	1.
D								
TF*F	.968	1.	.869	.807	.778	.917	1.	1.
G^-	.032		.123	.070	.111			
G			.008	.123	.111	.083		
E								
H'								
ADA*1	1.	1.	1.	1.	1.	1.	1.	1.
ACP1*A	1.	1.	1.	1.	1.	1.	1.	1.
C								
AK1*1	1.	1.	1.	.965	1.	1.	1.	1.
2				.035				
ESD*1	1.	1.	.951	1.	1.	1.	1.	1.
3			.049					
PGD*A	1.	1.	1.	1.	1.	1.	1.	1.
PGM1*1	1.	1.	.869	.965	1.	1.	1.	.978
2								.022
3			.131	.035				
Total	31	22	61	57	10	12	74	45

turned out to be monomorphic [2 and 8], while TF was found polymorphic within 13 troops, ACP1 and PGM1 within 4, AK1 in 8 and ESD in 1 [2]. The frequency of the less common alleles of these polymorphic loci ranges from 0.03 ± 0.022 to 0.123 ± 0.030 for TF*G^- and from 0.008 ± 0.008 to 0.197 ± 0.026 for TF*G, TF*E and TF*H' alleles were only found polymorphic in the 9th and 13th troops respec-

TABLE 2 (*Continued*)

Alleles	Troops (*)							
	9	10	11	12	13	14	15	16
ALB*A	1.	1.	1.	1.	1.	1.	1.	1.
B								
PI*B	.004					.016		
C	.991	1.	1.	1.	1.	.988	1.	1.
D	.004							
TF*F	.772	.923	.935	.929	.952	.953	.947	1.
G⁻								
G	.197	.077	.065	.071	.020	.047	.053	
E	.031							
H'					.027			
ADA*1	1.	1.	1.	1.	1.	1.	1.	1.
ACP1*A	.987	1.	.986	1.	1.	1.	.971	.960
C	.013		.014				.029	.040
AK1*1	.955	1.	1.	1.	1.	1.	1.	1.
2	.045							
ESD*1	1.	1.	1.	1.	1.	1.	1.	1.
3								
PGD*A	1.	1.	1.	1.	1.	1.	1.	1.
PGM1*1	1.	1.	1.	.959	1.	1.	1.	1.
2				.041				
3								
Total	114	13	46	49	147	32	19	26

tively (0.031 ± 0.011; 0.027 ± 0.009); from 0.013 ± 0.007 to 0.040 ± 0.027 for ACP1*C; from 0.014 ± 0.006 to 0.163 ± 0.037 for AK1*2; from 0.022 ± 0.015 to 0.041 ± 0.020 for PGM1*2 and from 0.035 ± 0.017 to 0.131 ± 0.031 for PGM1*3, and is equal to 0.049 ± 0.020 for ESD*3 in the unique troop in which this locus was found to be polymorphic (Table 2).

The only genetic marker found polymorphic in the present study is the Protease inhibitor (PI) (Fig. 2). As reported in Table 1, six subjects showed the heterozygote

TABLE 2 (*Continued*)

Alleles	Troops (*)							
	17	18	19	20	21	22	23	24
ALB*A		1.	1.	1.	1.	1.	1.	1.
B								
PI*B			.015					
C	1.	.982	.985	1.	1.	1.	1.	1.
D		.018						
TF*F	1.	1.	.941	1.	1.	1.	1.	1.
G⁻			.059					
G								
E								
H'								
ADA*1	1.	1.	1.	1.	1.	1.	1.	1.
ACP1*A	1.	1.	1.	1.	1.	1.	1.	1.
C								
AK1*1	1.	1.	1.	.837	.908	.971	1.	1.
2				.163	.092	.029		
ESD*1	1.	1.	1.	1.	1.	1.	1.	1.
3								
PGD*A	1.	1.	1.	1.	1.	1.	1.	1.
PGM1*1	1.	1.	1.	1.	1.	1.	1.	1.
2								
3								
Total	25	28	34	49	38	73	23	17

C-D phenotype, according to [2, 8 and 10] or the 2-1 phenotype, according to [5 and 10], and the frequency of the PI*D (or PI*2) allele was 0.098 ± 0.040 (5.5 PI*D alleles on a total of 56 independent genes; see Table 1 for details). This allele was found in 11 troops among the 33 studied in Japan, and its frequency ranged from 0.004 ± 0.004 to 0.106 ± 0.030 [2]. The second rare allele (PI*B) was found to be polymorphic in 7 troops (range of frequency from 0.004 ± 0.004 to 0.016 ± 0.016 [2]).

TABLE 2 (*Continued*)

Alleles	Troops (*)							
	25	26	27	28	29	30	31	32
ALB*A	1.	1.	1.	.998	1.	1.	1.	1.
B				.002				
PI*B				.005	.013	.004		
C	1.	1.	.894	.955	.907	.961	.967	.974
D			.106	.040	.080	.035	.033	.026
TF*F	1.	1.	1.	1.	1.	1.	1.	1.
G⁻								
G								
E								
H'								
ADA*1	1.	1.	1.	1.	1.	1.	1.	1.
ACP1*A	1.	1.	1.	1.	1.	1.	1.	1.
C								
AK1*1	1.	1.	.962	.986	1.	1.	1.	.969
2			.038	.014				.031
ESD*1	1.	1.	1.	1.	1.	1.	1.	1.
3								
PGD*A	1.	1.	1.	1.	1.	1.	1.	1.
PGM1*1	1.	1.	1.	1.	1.	1.	1.	1.
2								
3								
Total	26	37	52	224	75	116	30	20

Several studies on Japanese macaque showed very low genetic variability [5, 7, 8, 9, 11, 12, 13, 14, 15, 16 and 17]. In fact, in 32 structural genes [2], the average proportion of electrophoretically polymorphic structural genes was estimated to be 9.1% with a range among the various troops between 3.1% and 21.8%, and an average heterozygosity per individual of 1.3% with a range between 0.1% and 3.3% [2]. Such a result suggests that the structural genes' variability in *Macaca fuscata* is much lower than that of several other animal species, as referred by [18], who reported that the

TABLE 2 (*Continued*)

Alleles	Troops (*)							
	33	34						
ALB*A	1.	1.						
B								
PI*B								
C	1.	.902						
D		.098						
TF*F	1.	1.						
G$^-$								
G								
E								
H'								
ADA*1	1.	1.						
ACP1*A	1.	1.						
C								
AK1*1	1.	1.						
2								
ESD*1	1.	1.						
3								
PGD*A	1.	1.						
PGM1*1	1.	1.						
2								
3								
Total	21	34						

(*) Numbers from 1 to 33: troops of *Macaca fuscata* living in Japan [2]; 34: *Macaca fuscata* of the Zoo of Rome (present paper).

proportion of electrophoretically polymorphic structural genes ranged, with very few exceptions, between 25% and 40%, with an average (electrophoretic) heterozigosity per individual between 5% and 15%. A low level of genetic heterogeneity, quite similar to that described in *Macaca fuscata*, was found in several animal species inhabiting «narrow» and «close» environments [19, 20].

It is worth noting that, as stated above, the troops of *Macaca fuscata* living in Japan are not completely close units from the genetic point of view since quite a number of genes flow from one troop to another, as supported by the fact that in several occasions close-by troops share the same rare alleles [2].

ACKNOWLEDGEMENTS

This paper is part of a long-term project on non-human primates' biology organized by Prof. B. Chiarelli. The Authors thank Dr. F. Baschieri-Salvadori and Dr. G. Mangili of the Zoo of Rome for supplying us with the blood specimens.

REFERENCES

[1] KURLAND J. A. (1977) – *Kin selection in the Japanese monkey*, «Contributions to Primatology N. 12», S. Karger, Basel-Munchen-Paris-London-New York-Sydney.

[2] NOZAWA K., SHOTAKE T., KAWAMOTO Y. and TANABE Y. (1982) – *Population genetics of Japanese monkeys: II. Blood protein polymorphisms and population structure*, «Primates», 23, 252-271.

[3] CHIARELLI B. (1981) – *Ricerche su un gruppo di Macaca fuscata presente nel Giardino Zoologico di Roma*, «Antropologia Contemporanea», 4, 1-2.

[4] TROISI A., D'AMATO F. R., FUCCILLO R. and SCUCCHI S. (1981) – *A general overview of the confined group of Japanese Macaques in the Rome Zoo*, «Antropologia Contemporanea», 4, 3-9.

[5] SCHEFFRAHN W. and ZIGGIOTTI E. (1981) – *Risultati dello studio di alcune proteine sieriche, delle emoglobine e di alcuni enzimi eritrocitari di Macaca fuscata, Macaca arctoides, Macaca maura e Macaca silenus*, «Antropologia Contemporanea», 4, 109-115.

[6] HARRIS H. and HOPKINSON D. A. (1976) – *Handbook of enzyme electrophoresis in human genetics*, North Holland, Amsterdam.

[7] BIONDI G., RICKARDS O. and SANTOLAMAZZA P. (1981) – *Serum transferrin and albumin in a troop of Macaca fuscata living in the Zoo of Rome*, «Antropologia Contemporanea», 4, 105-107.

[8] NOZAWA K., SHOTAKE T., OHKURA Y. and TANABE Y. (1977) – *Genetic variations within and between species of Asian macaques*, «Japan. J. Genetics.», 52, 15-20.

[9] NOZAWA K., SHOTAKE T., OHKURA Y., KITAJIMA M. and TANABE Y. (1975) – *Genetic variations within and betqween troops of Macaca fuscata fuscata.* In: *Contemporary primatology*, (Kondo S., Kawai M. and Ehara A., eds.), S. Karger AG, Basel.

[10] SCHEFFRAHN W. (1978) – *Classification of blood proteins variants in Primates*, «J. Hum. Evol.», 7, 437-438.

[11] SHOTAKE T. and NOZAWA K. (1974) – *Genetic polymorphism in blood proteins in the troops of Japanese macaques, Macaca fuscata: I. Cytoplasmic malate dehydrogenase polymorphism in Macaca fuscata and other non-human Primates*, «Primates», 15, 219-226.

[12] SHOTAKE T. (1974) – *Genetic polymorphism of blood proteins in the troops of Japanese macaques, Macaca fuscata: II. Erythrocyte lactate dehydrogenase polymorphism in Macaca fuscata*, «Primates», 15, 297-303.

[13] SHOTAKE T. and OHKURA Y. (1975) – *Genetic polymorphisms of blood proteins in the troops of Japanese macaques, Macaca fuscata: III. Erythrocyte carbonic anhydrase polymorphism in Macaca fuscata*, «Primates», 16, 17-22.

[14] KITAJIMA M., OHKURA Y., SHOTAKE T. and NOZAWA K. (1975) – *Genetic polymorphisms of blood proteins in the troops of Japanese macaques, Macaca fuscata: IV. Erythrocyte esterase polymorphism in Macaca fuscata*, «Primates», 16, 339-404.

[15] SHOTAKE T., OHKURA Y. and ISHIMOTO G. (1977) – *Genetic polymorphisms of blood proteins in the troops of Japanese macaques, Macaca fuscata: V. Erythrocyte phosphohexose isomerase polymorphism*, «Primates», 18, 285-290.

[16] SHOTAKE T., OHKURA Y. and NOZAWA K. (1977) – *Genetic polymorphisms of blood proteins*

in the troops of Japanese macaques, Macaca fuscata: VI. Serum transferrin polymorphism, «Primates», 18, 291-297.

[17] Spedini G,. Fuciarelli M. and Rickards O. (1981) – *The determination of the CP and HP polymorphysms*, «Antropologia Contemporanea», 4, 99-101.

[18] Selander R. K., Yang S. Y., Lewontin R. C. and Johnson W. E. (1970) – *Genetic variation in the horsehoe crab (Limulus polyphemus), a phylogenetic «relic»*, «Evolution», 24, 402-414.

[19] Avise J. C. and Selander R. K. (1972) – *Evolutionary genetics of cave-dwelling fishes of the genus Astyanax*, «Evolution», 26, 1-9.

[20] Nevo E., Kim Y. J., Shaw C. R. and Thaeler C. S. (1974) – *Genetic variation, relation and speciation in Thomomys talpoides pocket gophers*, «Evolution», 28, 1-23.