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RAPD FINGERPRINTING: USE IN THE ANALYSIS OF MEDITERRANEAN POPULATIONS OF EUROPEAN FALLOW DEER, *DAMA DAMA* LINNAEUS, *1758* (MAMMALIA, ARTIODACTYLA)

MARCO MASSETI, CRISTIANO VERNESI,
BARBARA BRAMANTI AND BRUNETTO CHIARELLI

lstituto di Antropologia dell'Universita di Firenze
Via Del Proconsolo 12, 1 - 50122 Firenze, ltaly

ABSTRACT – The aim of the present paper is to present a preliminary genetic survey of the fallow deer (*Dama dama* L., 1758) population of the island of Rhodes (Greece) in order to verify its genetic variability. Italian population specimens were chosen as a control group because, as can be ascertained from literature, they have a very low level of variability. The analysis was carried out on hair samples obtained from each population. It was performed on a PCR modified method called RAPD which is based on the amplification of genomic **DNA** by using a single oligonucleotide of random sequence as a primer. The results of the analysis of the Rhodian specimens show clearly the presence of polymorphic individuals, absent in the Italian deer sampled.

***Key** words:* European fallow deer, RAPD fingerprinting, Island of Rhodes.

INTRODUCTION

Among the extant species of deer, the European fallow deer, *Dama dama* (L., 1758), is perhaps the taxon whose current distribution has been most influenced and altered by man (Chapman & Chapman, 1980). Paleontological and archaeological evidence confirms that, between Late Pleistocene and Early Holocene, the range of the species was probably restricted to the north-eastern Mediterranean region, from the southern Italian peninsula and south-western Anatolia, where it possibly inhabited flat and open woodland habitats (Heidemann, 1976; Uerpmann, 1981; Masseti & Rustioni, 1988). With the exception of Sicily, the natural occurrence of fallow deer is unknown in the Quaternary biogeography of the larger Mediterranean islands, where its introduction has been documented since ancient times (Masseti, 1996).

Together with the extinct fallow deer of Sardinia, the extant population of the island of Rhodes (Greece) could be considered the oldest population ever to have existed on Mediterranean islands (Masseti, in press). Accounts of the occurrence of the species have been documented in literature at least since Medieval times (Festa, 1914; Bousbouras *et al.*, 1991). At present, the fallow deer of Rhodes occurs also in private enclosures of the island of Crete, where it was imported since the end of the 60s and the beginning of the 70s.

This work aims to present a preliminary analysis of the fallow deer population of the island of Rhodes to verify its genetic variability in the light of the genetic consequences of insularity and domestication.

The analysis was carried out on hair samples. It was performed on a PCR modified method called RAPD (Random Amplified Polimorphic DNA), based on the amplification of genomic DNA by using a single oligonucleotide of random sequence as a primer (Williams *et al.,* 1990; Welsh *et al.,* 1991). The amplification products, resolved on agarose gel, give rise to a complex pattern, specific for a given genome (fingerprinting) (Caetano-Anolles *et al.,* 1991) and worthwhile for taxonomic and phylogenetic reconstruction (Hadrys *et al.,* 1992) and also for assessing genetic variability in natural populations (Huff *et al.,* 1993; Gibbs, *et al.,* 1994). In comparison with the classic biomolecular methods (RFLP, REA, mtDNA, etc.) RAPD does not need previous information regarding the sequence, it uses a smaller quantity of DNA and "last but not least" it is less time consuming and more economically convenient.

MATERIALS AND METHODS

Hair samples were obtained from 9 Rhodian fallow deer of captive populations (4 specimens from enclosures of the island of Crete and 5 from enclosures of the town of Rhodes) (Tab. 1).

Table 1 List of the Rhodian fallow deer specimens examined: all the hair samples have been collected between the beginning of June and the first half of October 1994. The fallow deer of Rhodes displays only specimens of normal colour coat phenotype

LOCALITY	SEX	CLASS OF AGE
G. Gazi (Crete)	m	adult
H. Archanes (Crete)	m	subadult
I. Archanes (Crete)	f	subadult
L. Gazi (Crete)	f	adult
M. Public garden of Rodini (Rhodes)	m	adult
N. Public garden of Rodini (Rhodes)	m	adult
O. Public garden of Rodini (Rhodes)	m	adult
P. Public garden of Rodini (Rhodes)	m	adult
Q. Enclosure of Tafros (Rhodes)	m	adult

From literature, Italian populations are known to have a low genetic variability (Randi & Apollonio, 1988) in line with genetic result obtained from other west European population such as British (Pemberton and Smith, 1985) or German ones (Hartl *et al.,* 1986; Hartl, 1991). Thus, the Italian ones were chosen as a control group. Hair samples were obtained from 6 specimens of free-range and captive populations of Central Italy (Tab. 2). The animal from Monte Ferrato (Prato, Firenze) was killed in a road accident few hours before the time of the sampling. Apart from this, only living animals were sampled.

Table 2 List of the Italian fallow deer specimens

LOCALITY	SEX	CLASS OF AGE	COAT COLOUR
A. Monte Ferrato(Prato, Firenze)	**f**	adult	common
B. Zoological garden of Pistoia	**f**	adult	menil
C. Zoological garden of Pistoia	f	adult	common
D. Zoological garden of Pistoia	m	adult	common
E. Parco faunistico Monte Amiata (Grosseto)	f	subadult	black
F. Parco faunistico Monte Amiata (Grosseto)	m	subadult	common

Chelex 100 (Perkin Elmer) was used as a medium for DNA extraction, following the manufacturer's instructions. For RAPD amplification 'we used three different oligonucleotides, whose characteristics are reported in Tab. 3. Amplification reactions were performed in a 60 µl volume containing $MgCl_2$ 3 mM, primer 2µM, 200 µM of each dNTP, 1.25 U of *Taq* polymerase (Polymed). The reaction mixtures were overlayed with mineral oil, and after incubation at 90" C for 120 s were cycled 45 times through the following temperature sequence: 95" C for 30 s, 36" C for 60 s, and *75"* C for 120 s. The samples were then incubated at 75° C for 10 min, at 60" ***C*** for 10 min in order to avoid heteroduplex artifact formation. We used an MJ (PT 150) thermocycler and the fastest available transitions between each temperature.

Ten microliters of each amplification product were loaded on a 1,5 % (w/v) agarose gel (Boehringer-Mannheim) containing 0.5 mg/ml (w/v) of ethidium bromide and electrophoresis was carried out at 120 V for 2 h.

Table 3 Oligonucleotides used as primers in RAPD-PCR

PRIMER	SEQUENCE	G + C (%)
I	5' ATA GGC GCC G 3'	70
2	5' GTT TCC GCC C 3'	70
3	***5'*** GCG ATC CCC A 3'	70

RESULTS AND DISCUSSION

The amplification patterns (Figs.1 and 2) were analyzed and similarity between each pair of individuals was calculated as the Dice coefficient, S_d (the ratio of twice the number of common fragments to the total number of fragments in the two patterns). These values were used to cluster the representative samples by the **UPGMA** method following the procedures of the **NTSYS** package (Fig. 3). The dendrogram constructed by joining the data of three patterns, concerning the Rhodes population, shows clearly the presence of polymorphic individuals, which are absent in the Italian population sampled. Although this is a preliminary study, it is possible to observe a kind of genetic variability which emerges from the sample considered. This variability leads to the assumption that, in recent times, the fallow deer population of Rhodes might have assimilated new genetic material from abroad, perhaps from the near Turkish mainland, where the species still occurs in what is

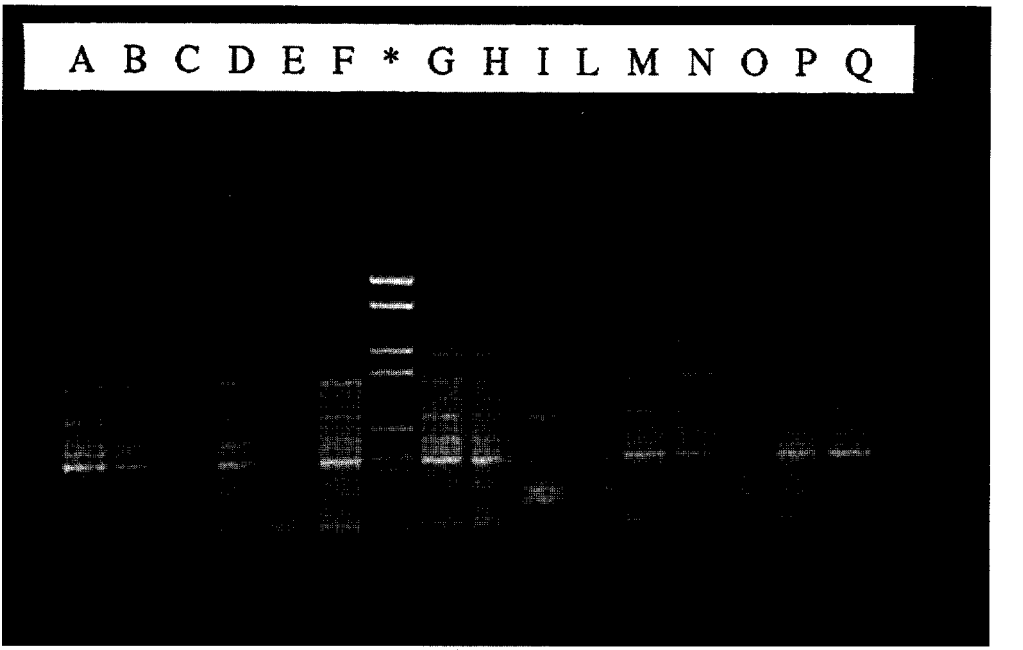

Figure 1: Genomic fingerprint obtained with primer 2. A-F: samples of the Italian population (see Tab. 1). *: DNA molecular weight Marker VI (Boehringer-Mannheim). C-Q: samples of the Rhodian population (see Tab. 2).

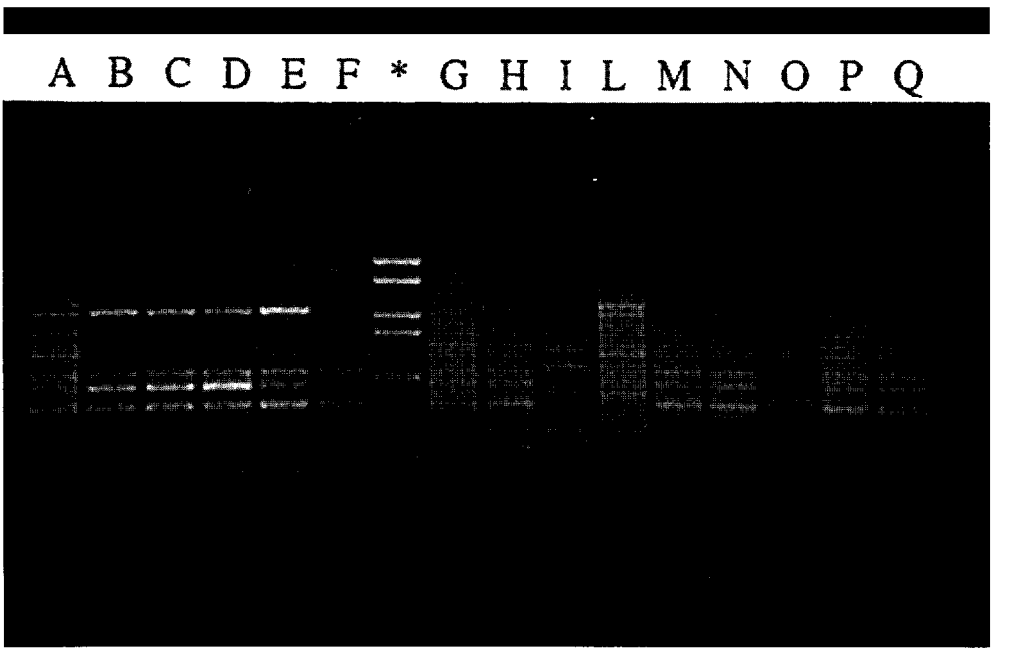

Figure 2: Genomic fingerprint obtained with primer 3. **A-F:** samples of the Italian population (see Tab. 1). *: DNA molecular weight Marker VI (Boehringer-Mannheim). C-Q: samples of the Rhodian population (see Tab. 2).

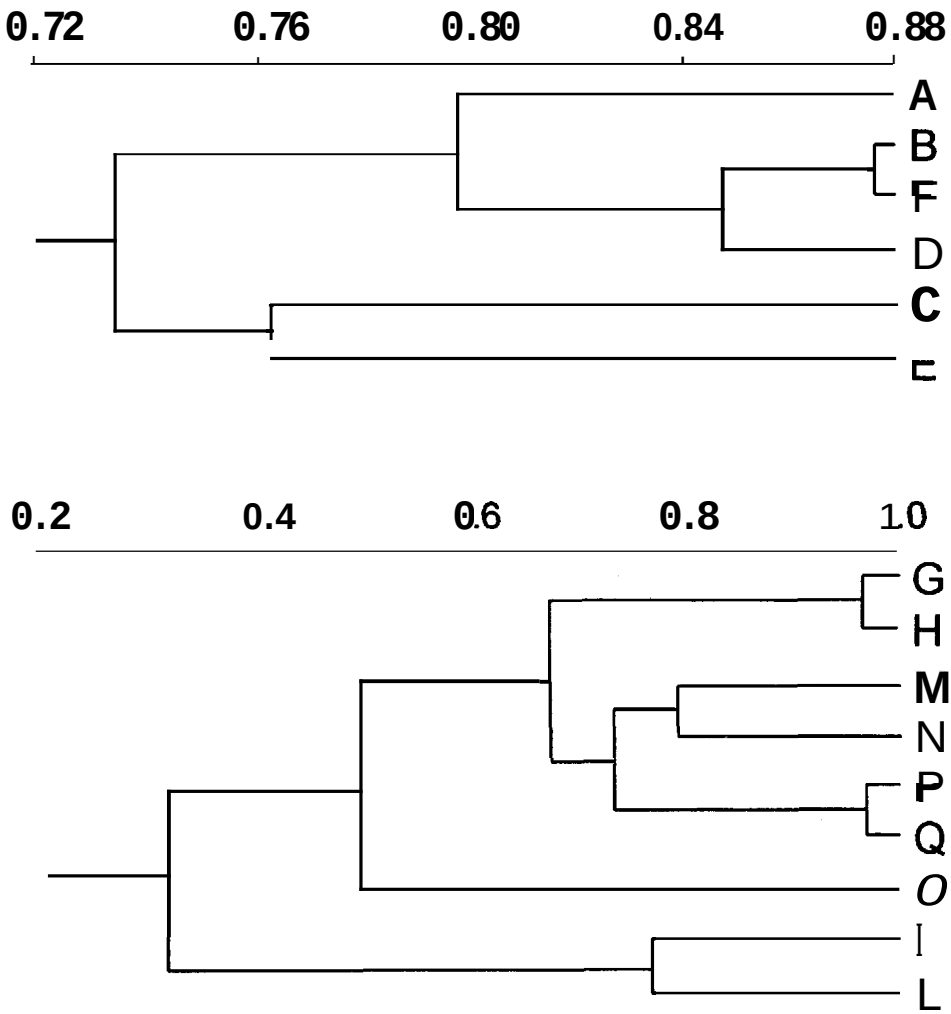

Figure 3: Dendrograms based on UPGMA clustering of the S_D matrix obtained by joining the data from the patterns produced with primers 1, 2, 3. Above: Italian populations. **A-F** (see Tab. 1); below: Rhodian populations, G-Q (see Tab. 2).

supposed to be its last natural stronghold (cf. Heidemann, 1.976; Chapaman & Chapman, 1981). However, according to literature (Ghigi, 1929: Masseti, in press) and to the witnesses of the Forest Department of Rhodes, no importation of fallow deer has taken place from abroad this century. Further investigations are needed to better define the relationship between the Rhodes population and the extant Turkish populations.

Finally, the RAPD technique provides an useful tool for genetic analysis of any mammal population, because it needs a really low amount of template DNA (5-10 ng), thus allowing DNA extraction from few hairs. This is a considerable advantage because the animals are not stressed in anyway. Furthermore RAPD could be used for any kind of genome as it does not need previous knowledge of the sequences studied.

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