

**RESULTS CONCERNING GENETIC
CHARACTERIZATION OF MANGALITA BREED
USING MICROSATELLITE MARKERS**

**REZULTATE PRIVIND CARACTERIZAREA
GENETICĂ A RASEI MANGALIȚA PE BAZA
MARKERILOR MICROSATELIȚI**

M. ZĂHAN¹, P. RAICA¹, V. MICLEA¹, ILEANA MICLEA¹, R. RENAUVILLE²,
O. DUTERME², M. MIHĂILESCU³, AL. NAGY⁴

1. *University of Agricultural Sciences and Veterinary Medicine, Faculty of Animal Sciences and Biotechnology, Cluj-Napoca, Romania, e-mail: mzahan@usamvcluj.ro*
2. *Progenus S.A., 13 Avenue Marechal Juin, 5030 Gembloux, Belgium*
3. *S.C. Suinprod S.A. Roman, 336 km Ștefan cel Mare Street, 611410 Roman, Romania*
4. *Agricultural Research and Development Station, Turda, Romania*

The proper use, further development, testing and deployment of animal genetic resources is essential to enhancement of food security and sustainable intensification of food production. For Red Mangalita conservation, we studied four microsatellite markers (SO228, SW72, SW911 and SW936) in order to genetic characterization of two populations. The results showed that both populations are in genetic imbalance, but also indicate high population variability, without the risk of genetic drift.

Key words: microsatellite markers, genetic characterization, Mangalita, population

Introduction

Mangalita is one of the old type breeds, originating several centuries ago as a result of crossing between European and Asian primitive pigs. Mangalita was introduced into Romania from Serbia in the 19th century. Red Mangalita is one of the varieties, established by crosses between the Blond Mangalitsa and the Salonta pig (Ciobanu et al., 2001).

Conservation is considered to be a very important tool to avoid irrecoverable loss of breeds or genes, to re-establish a breed, to support breeding in small populations and to conserve genetic variation (genes, traits or breeds) in selection program (Hiemstra, 2004). Ruane and Sonino (2006) provided a set of criteria to be considered when choosing a specific breed for a conservation program. Using these programs, at the early nineties in Hungary there were less than 200 registered Mangalita sows and at the moment there are almost 10,000 (Ratky et al., 2007). Beside Romania and Hungary there are mentionable populations of Mangalita in

Austria, Germany, Switzerland and in area of the formed Yugoslavia (Zsolnai et al. 2006).

For genetic distance characterization of animal breed Food and Agriculture Organization (FAO) and International Society of Animal Genetics (ISAG) recommend the use of microsatellite markers. Molecular markers are an indispensable tool to understand the genetic structure of populations. For the sampling of germoplasm to create an animal gene bank, they are necessary to make adequate decisions (Ruane and Sonino, 2006). A lot of pig breeds were genetic characterization using microsatellite markers (Laval et al., 2000; Groenen et al., 2003; Li et al., 2004). Another important application of microsatellite markers is the identification of individuals and parentage control (Putnova et al., 2003).

The aim of this paper is to genetic characterization of two population of Red Mangalita from Romania in order to identified representative individuals for germoplasm cryopreservation.

Material and Methods

A total number of 107 individual hair samples from Red Mangalita were collected from 69 females and 33 males from the population of Roman (S.C. Suinprod S.A. Roman) and 3 females and 2 males from the population of Turda (Agricultural Research and Development Station Turda). The selection of microsatellite markers for DNA fingerprinting was performed at ISAG-FAO recommendation, 4 from the 15 microsatellites selected being analyzed in the present study (table 1).

Table 1. Characteristics of microsatellite marker

Locus	Chromosome arm	Size (bp)	Sequence of primers (5' → 3')*
SO228	6q	20 24	F: GGC ATA GGC TGG CAG CAA CA R: AGC CCA CCT CAT CTT ATC TAC ACT
SW72	3p	18 20	F: ATC AGA ACA GTG CGC CGT R: TTT GAA AAT GGG GTG TTT CC
SW911	9p	22 21	F: CTC AGT TCT TTG GGA CTG AAC C R: CAT CTG TGG AAA AAA AAA GCC
SW936	15q	21 20	F: TCT GGA GCT AGC ATA AGT GCC R: GTG CAA GTA CAC ATG CAG GG

* F – forward; R – reverse

Porcine genomic DNA was extracted from pylorus bulb, while PCR conditions included denaturation step of 10 min at 95°C, 30 cycles of 30 s at 95°C, 30 s at 50-60°C, 1 min at 72°C and a final extension of 60 min at 72°C. Genotyping was done with ABI Genetic Analyzer by fluorescent fragment analysis.

Results were analyzed by **Genepop 1.2** software (Raymond and Rousset, 1995), in order to determine the number of alleles per locus, genotype distribution (expected and observed number of heterozygote using Levene's correction), allele frequencies and inbreeding coefficients (according to Weir and Cockerham, 1984 and also Robertson and Hill, 1984). The dendrogram were obtained by **Population 1.2.30** software, using the standard Nei's distances (Ds) and unweighted pair-group method with arithmetic mean (UPGMA).

Results and Discussion

All four markers generated amplification products in both populations. Microsatellite markers generated a number between 3 (**SW72**) and 6 (**SW911**) alleles per locus, with a total number of 19 alleles. Concerning the size of alleles, these are very much alike the data from bibliography (table 2). At the Red Mangalita we found a number of 18 alleles, with an average of 4.5 alleles at the population of Roman, respectively 10 alleles at the population of Turda, with an average of 2.5.

Table 2. The number and size of alleles

Locus	no. of alleles		size of alleles (pb)	
	references*	observed	references**	observed
SO228	12	5	222-266	250-272
SW72	9	3	90-120	97-107
SW911	9	6	149-179	151-165
SW936	13	5	80-124	93-109

* - Laval G., et al. (2000)

** - FAO (MoDAD); Yang S-L. et al. (2003)

The number of alleles observed was lower than that observed by other authors at different breeds (European and Chinese commercial and indigenous pig breeds) and unfortunately, there are a few research papers on Mangalita breed. It is interesting to know if the allele frequencies are different, but the most articles do not published them, so that a comparative study was impossible.

The average values of the observed heterozygosity were of 0.688 (population of Roman), respectively 0.750 (population of Turda) higher than the expected heterozygosity (0.591 – Roman and 0.583 – Turda). This level of polymorphism is similar to the values so far reported for microsatellites in European pig breeds, where the average heterozygosity observed is around 0.5 (Laval et al., 2000). The individual values of the observed and expected heterozygosity per locus and population are shown in table 3. In both studied populations the observed heterozygosity for each locus was higher than the expected one.

Table data indicates that both populations are in genetic imbalance, the number of observed heterozygote being higher than the expected one. In the case of Roman population this is due to the way that Mangalita population was made: the

incoming of individuals from foreign population (import from Hungary and Austria).

Table 3. Frequency of heterozygosis and inbreeding coefficient (F_{IS}) in Mangalita population

Locus	Heterozygote		F_{IS} (total)		
	expected	observed	W&C	R&H	no. individuals
Population of Roman					
SO228	37.9548	44	-0.1602	-0.0715	100
SW72	65.3645	77	-0.1790	-0.1673	102
SW911	69.5222	84	-0.2095	-0.0963	102
SW936	68.6847	76	-0.1071	+0.0405	102
Population of Turda					
SO228	3.0000	4	-0.3913	-0.2500	5
SW72	3.4444	4	-0.1852	-0.2125	5
SW911	2.5556	3	-0.2000	-0.1161	5
SW936	2.6667	4	-0.6000	-0.6250	5

In the same time we can observe that the values of inbreeding coefficients are almost “zero” that indicates high population variability, without the risk of genetic drift. For the Turda population, even though the number of individuals is low, the results show a similar situation to that from Roman. It is possible that certain individuals from this population to have in their pedigree crosses with another breeds.

Conclusions

The large number of individuals from the population of Roman allowed the selection of a representative Mangalita trail -based on morphological characters and pedigree- for genetic characterization needed for *in vitro* conservation program. Due to the higher number of observed heterozygote than the expected one, both populations are in genetic imbalance. Inbreeding coefficients have values that indicate high population variability, without the risk of genetic drift.

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