

*Full Length Research Paper*

# Genetic characterization of the Mascaruna goat, a Sicilian autochthonous population, using molecular markers

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Accepted 13 May, 2013

The aim of this work was to characterize a Sicilian autochthonous goat population using microsatellite markers and genetic polymorphisms at the casein genes. In order to investigate the genetic structure of the Mascaruna goat, a total of 60 (20 Girgentana, 20 mixed populations, and 20 Mascaruna) individuals were analyzed, using a panel of 18 microsatellite markers. Moreover, the Mascaruna goats were genotyped at casein loci using several molecular techniques. Based on the genetic structure at casein genes, the Mascaruna goat was similar to most goat breeds from the Mediterranean area, which are characterized by the predominance of strong alleles. The low value of genetic differentiation among populations ( $F_{st}=0.027$ ) could indicate that these populations were differentiated little probably due to gene flow and breeding practices. The analysis of genetic distances between groups indicated that the Mascaruna goat was the most distanced group, and this result was confirmed by the unrooted neighbor-joining dendrogram, the factorial correspondence analysis, the presence of several private alleles and the Bayesian assignment test. However, the Mascaruna group, despite the influences from other populations, presents a certain degree of uniqueness and could be considered as a population with particular genetic background.

**Key words:** Local goat population, genetic characterization, microsatellite markers, casein gene cluster.

## INTRODUCTION

The global decline in genetic diversity is mainly the result of the use of increased numbers of livestock from a small number of selected breeds. However, in recent years, there has been great interest in recovering and preserving local breeds and/or populations (Glowatzki-Mullis et al., 2008; Bruno-de-Sousa et al., 2011). The Sicilian goats are characterized by a strong population admixture and structure caused by geographical location of the farms, influences of natural mating and traditional breeding systems where flock represents an important

breeding unit (Siwek et al., 2010). Nowadays, several local populations are reared in Sicily, some of which do not have a recognized or defined genetic structure, having taken origin from several crosses between animals of different breeds sharing the same environment. These particular groups of animals are reared for their peculiar characteristics in some parts of the Island and are quite referred to as populations.

However, at present it is not possible to assess whether they can actually be intended as populations rather

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than just mixtures of different breeds, as they have never been genetically characterized. Breed or population characterization requires basic knowledge of genetic variations that can be effectively measured within and between breeds/populations (Ramamoorthi et al., 2009). Molecular genetic information may be used to correctly assign individuals to a breed or population, especially when the phenotypic differentiation between populations is difficult and pedigree information are not available (Baumung et al., 2006). Among the currently used molecular markers for genetic characterization in goats, microsatellites are the most preferred ones because of their extremely informative polymorphic nature, abundance in the genome, and neutrality with respect to selection.

The Mascaruna goat is an autochthonous small population; it was estimated to be approximately 100 heads, reared in the provinces of Palermo and Agrigento for milk production. Semi-extensive farming is practiced in all the farms. From a morphological point of view, the animals are relatively homogeneous especially in terms of coat color, shape of horns and size. This population presents medium-high size. Animals present long hair type coat, cream color with a dark brown line on the back. The abdomen and the legs are also dark brown. In both sexes the head is light and presents long horns turned backward, small ears and dark brown elongated spots around the eyes. This population is not subject either to breeding programs or milk recording scheme in order to improve production traits, and is still not officially recognized as a differentiated breed. However, the interest for this population seems to be increasing, given its good milk production.

In milk of ruminants, more than 95% of proteins are synthesized by six structural genes (Martin et al., 2002). The two main whey proteins,  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin, are encoded by the LALBA and BLG genes, respectively (Ng-Kwai-Hang and Grosclaude, 2003). Caseins are encoded by four genes (CSN1S1, CSN2, CSN1S2, and CSN3), which cluster on a DNA fragment of about 250 kb, mapped on caprine chromosome 6, in the following order:  $\alpha$ S<sub>1</sub>-,  $\beta$ -,  $\alpha$ S<sub>2</sub>-, and  $\kappa$ -casein (Martin et al., 2002). Goat casein genes show high polymorphisms that affect milk quality and composition; indeed goat milk protein polymorphisms play an essential role in the quantity, composition and technological properties of milk (Marletta et al., 2007). The primary structures of each casein vary considerably due to point mutations (Single Nucleotide Polymorphisms, SNPs), insertion/deletion and differential splicing patterns and post-translation modifications (Marletta et al., 2007).

The aim of this study was to characterize a Sicilian autochthonous goat population, named Mascaruna, using: i) microsatellite markers in order to verify if Mascaruna goat could be defined as a population or it should just be considered as a group of mixed animals belonging to different breeds/populations and ii) Genetic polymorphisms of casein genes due to direct relationship between

between casein variants and milk composition.

In order to achieve this goal, other two groups of animals reared in the same area have been included in the analysis, that are, Girgentana goat breed individuals with a defined genetic structure and individuals of mixed population with a not defined genetic structure.

## MATERIALS AND METHODS

### Sampling and DNA extraction

A total of 60 randomly chosen unrelated animals from four farms of Girgentana and mixed goats and three farms of Mascaruna goat were used for the analysis. The sample consisted of 20 Girgentana (GIR), 20 Mixed (MIX), and 20 Mascaruna (MAS) goats, chosen on the basis of phenotypic profiles and information supplied by the farmers. About 10 ml of blood was collected from jugular vein using vacutainers tube containing EDTA as anticoagulant. Genomic DNA was extracted from buffy coats of nucleated cells using a salting out method (Miller et al., 1988). The concentration of extracted DNA was checked using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

### Genotyping data and analysis of caseins

The four casein loci, CSN1S1, CSN2, CSN1S2, and CSN3 were genotyped in MAS goat individuals. The CSN1S1 A/01, B/E, F and N alleles were simultaneously identified by PCR and PCR-RFLP protocols (Ramunno et al., 2000a). Allele-specific (AS)-PCR was used for the detection of the CSN1S1 E (Dettori et al., 2009) and CSN1S1 01 alleles (Cosenza et al., 2001). The CSN2 A, A1, C, C1, E, and 0' alleles were identified using PCR protocols of Chessa et al. (2005, 2008a) followed by sequencing of amplified fragments with ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). At CSN1S2 locus alleles, B and C/E were detected by multiplex AS-PCR (Ramunno et al., 2000b). The allele E was identified by amplification of part of 16th exon using primers of Chessa et al. (2008b) and PCR-RFLP protocol of Lagonigro et al. (2001). The alleles D, 0, and F were genotyped by PCR-RFLP (Ramunno et al., 2001a, b). The allele A at this locus was assigned when all the other alleles were not present. The several alleles at CSN3 locus were identified by PCR protocol described by Prinzenberg et al. (2005), followed by sequencing of amplified fragment. GENEPOP software v4.0.11 (Rousset, 2008) was used to calculate allele and genotype frequencies and to test deviations from Hardy-Weinberg equilibrium (Table 1).

### Microsatellites amplifications

A total of 18 microsatellite markers (Table 2) were selected as suggested by ISAG ([http://www.isag.org.uk/Docs/2005\\_PanelsMarkersSheepGoats.pdf](http://www.isag.org.uk/Docs/2005_PanelsMarkersSheepGoats.pdf)) and FAO (<http://www.fao.org/dad-is/>). Microsatellite analyses were performed on the 60 individuals belonging to the three groups. Genotypes for all 18 microsatellite markers were determined by means of three multiplex fluorescent PCR reactions and fragment lengths determined in a single semi-automated multiplex electrophoresis run by using an AB3130 Genetic Analyzer and Gene Mapper version 4.0 following recommended protocols (Applied Biosystems). Each reaction was performed in a total volume of 20  $\mu$ L containing 50 ng of template DNA, 1X Qiagen Multiplex PCR Master Mix, 1X PCR Master Mix, primers mix, and nuclease-free water. For the three multiplex reactions, the PCR program was ini-

**Table 1.** Allele and genotype frequencies, Hardy-Weinberg equilibrium (HWE P-value  $\pm$  Standard Error, SE) at casein genes in Mascaruna goat population (n=20).

Locus	Genotype	Frequency	Allele	Frequency	HWE P-value $\pm$ SE
CSN1S1	AB	0.350	A	0.275	0.4696 $\pm$ 0.0017
	BB	0.250	B	0.525	
	AF	0.200	F	0.200	
	BF	0.200			
CSN2	AA	0.100	A	0.175	0.0171 $\pm$ 0.0005
	AC	0.050	C	0.300	
	CC1	0.550	C1	0.525	
	AC1	0.100			
	C1C1	0.200			
CSN1S2	AA	0.100	A	0.300	0.0907 $\pm$ 0.0010
	AF	0.400	C	0.225	
	CC	0.100	F	0.475	
	CF	0.250			
	FF	0.150			
CSN3	AB	0.500	A	0.275	0.2104 $\pm$ 0.0029
	BB	0.400	B	0.675	
	BG	0.050	G	0.025	
	BM	0.050	M	0.025	

**Table 2.** Total number of alleles per locus (TNA), polymorphism information content (PIC), Hardy-Weinberg equilibrium (HW), observed and expected heterozygosity ( $H_o$  and  $H_s$ ) and Wright's fixation index of each microsatellite considering the whole sample (n=60).

Locus	TNA	PIC	HW	$H_o$	$H_s$	$F_{is}$	$F_{it}$	$F_{st}$
BM321	11	0.76	**	0.465	0.778	0.068	0.093	0.027
FCB48	7	0.78	NS	0.822	0.804	0.096	0.121	0.028
OLADRB	16	0.86	NS	0.844	0.854	0.092	0.116	0.026
DU323541	10	0.65	NS	0.667	0.687	0.089	0.115	0.028
INRABERN172	9	0.76	NS	0.762	0.786	0.090	0.116	0.029
MCM54B	8	0.80	NS	0.579	0.818	0.072	0.098	0.028
INRA063	5	0.60	NS	0.600	0.655	0.087	0.114	0.029
MAF64	7	0.75	NS	0.772	0.760	0.091	0.115	0.026
ADCYC	6	0.54	NS	0.639	0.608	0.094	0.118	0.027
SRCRSP024	8	0.67	NS	0.557	0.697	0.082	0.105	0.025
BRN	6	0.67	NS	0.622	0.676	0.086	0.109	0.025
SRCRSP009	11	0.72	NS	0.629	0.725	0.084	0.109	0.027
MB056	5	0.57	NS	0.510	0.629	0.081	0.107	0.029
TCRB	8	0.81	NS	0.779	0.812	0.090	0.114	0.026
SRCRSP008	7	0.65	NS	0.672	0.709	0.090	0.116	0.029
APPO10	7	0.79	NS	0.783	0.824	0.091	0.117	0.029
INRABERN185	6	0.36	NS	0.378	0.395	0.088	0.113	0.028
BM1329	11	0.76	NS	0.839	0.785	0.098	0.122	0.027
Mean	8.22	0.69		0.662	0.722	0.087	0.112	0.027

HW, significance of deviation from Hardy-Weinberg equilibrium; \*\*Significant at the 0.05 level; NS, not significant.

initial denaturation at 95°C for 15 min, 32 cycles of 95°C for 45 s, 58°C for 1 min 50 s, and 72°C for 1 min 20 s, and final extension at

60°C for 30 min. Amplification was carried out using GeneAmp PCR system 9700. A total of 3.7  $\mu$ L of PCR product of each multiplex

was mixed with 0.3  $\mu$ L of LIZ 600 Size Standard and 6.0  $\mu$ L of Hi-Di Formamide.

### Statistical analysis

FSTAT ver. 1.2 software (Goudet, 1995) was used to estimate total number of alleles (TNA), observed and expected heterozygosity ( $H_o$  and  $H_e$ ) per locus, mean number of alleles (MNA), observed heterozygosity ( $H_o$ ) and gene diversity ( $H_e$ ) per population. Moreover, FSTAT was used to estimate the Wright's fixation indexes ( $F_{is}$ ,  $F_{it}$ , and  $F_{st}$ ) (Weir and Cockerham, 1984). Polymorphism information content (PIC) and Hardy-Weinberg equilibrium analysis per locus were estimated with Cervus ver. 2.0 software (Marshall et al., 1998). Nei minimum (Nei, 1987) and Reynolds (Reynolds et al., 1983) distances were used to estimate pair-wise genetic distances among populations. The neighbour-joining consensus tree was obtained with PHYLIP ver. 3.69 (Felsenstein, 2009), whereas TREEVIEW software ver. 1.6.6 (Page, 1996) was used to visualize the dendrogram. Tree robustness was evaluated by bootstrapping over loci (1,000 replicates). A factorial correspondence analysis (FCA) was further performed as an alternative approach to understand the genetic relationship among the three groups of individuals, using the GENETIX ver. 4.05 (Belkhir et al., 1996). STRUCTURE software ver. 2.3.3 (Pritchard et al., 2000) was used to analyze the genetic structure of the populations considered. The program estimates the natural logarithm of the probability that a given genotype (G) is part of a given population (K). The model used was based on an assumption of admixture and correlated allele frequencies (Pritchard et al., 2000). To choose the appropriate number of inferred clusters, the Ln Pr (G|K) was calculated for K ranging from 2 to 6, with 50 independent runs for each K. All runs consisted of a burn-in period of 100,000 steps, followed by 100,000 Markov Chain Monte Carlo (MCMC) iterations.

## RESULTS AND DISCUSSION

### Genetic polymorphism of caseins

Genotype, allele frequencies and P-value for Hardy-Weinberg equilibrium for each casein gene are showed in Table 1. In goat,  $\alpha$ S<sub>1</sub>-casein is the most extensively investigated locus among the four casein genes. The P-value reported in the Table 1 evidenced that the MAS population was in equilibrium at CSN1S1 locus (P-value>0.05). Three alleles were identified in MAS goat population: two strong alleles (A and B) and one weak allele (F). Null (01, N) and intermediate (E) alleles were not observed. These results are in agreement with those of previous studies that described the presence of strong alleles in the autochthonous goat breeds/populations of Southern Italy, whereas weak and intermediate alleles showed higher frequencies in the Northern breeds/populations (Marletta et al., 2005; Sacchi et al., 2005). Alleles at this locus are associated with different level of protein synthesis: strong alleles producing almost 3.5 g/L each; intermediate alleles 1.1 g/L; weak alleles 0.45 g/L and null alleles are associated with a non-detectable amount of this protein in milk. In MAS goat, the most frequent allele was CSN1S1 B (0.525), followed by A and F (0.275 and 0.200, respectively). Genotypes AB and BB showed higher frequency values compared with AF and BF (0.350, 0.250

and 0.200, respectively) (Table 1). The homozygous weak genotype FF was not found. It is well known that  $\beta$ -casein is the major casein fraction in goat milk (Chessa et al., 2005; 2008a). The MAS population was not in Hardy-Weinberg equilibrium at this locus (P-value<0.05) (Table 1). The most frequent allele was C1 (0.525) followed by C and A (0.300 and 0.175, respectively). Our results are in agreement with those reported in previous studies for several goat breeds reared in Southern Italy (Marletta et al., 2005; Sacchi et al., 2005; Gigli et al., 2008), in which strong alleles at  $\beta$ -casein locus were present with frequency values higher than 0.900. The A, C and C1 alleles are associated with a normal content of  $\beta$ -casein (5 g/L). The CSN2 01 allele was not found in MAS goat. This allele is defined "null" and is associated with the absence of this casein in milk. Generally, null alleles are present in the Southern Italian goat breeds with a low frequency (Marletta et al., 2005; Sacchi et al., 2005; Gigli et al., 2008), and absent in Alpine and Northern Italian goat breeds (Chessa et al., 2005). The most frequent genotype at CSN2 locus was CC1 (0.550) followed by C1C1 (0.200) (Table 1).

Only three alleles were identified at  $\alpha$ S<sub>2</sub>-casein locus in MAS goat population. The most frequent allele was F (0.475), followed by A and C (0.300 and 0.225, respectively) (Table 1). The MAS population was in Hardy-Weinberg equilibrium at CSN1S2 locus. These "strong" alleles have been associated with a normal synthesis level (2.5 g/L content per allele of  $\alpha$ S<sub>2</sub>-casein). The B, E, D and 0 alleles were not identified in this population. The absence of the defective alleles is not surprising because the intermediate D allele is rare and the null 0 allele was found only in some Italian breeds (Vacca et al., 2009). Genotypes AF and CF were the most common and showed the highest frequencies (0.400 and 0.250, respectively). The allele frequencies recorded for A, C, and F were similar to those reported by Sacchi et al. (2005) in Mediterranean goat breeds, and by Vacca et al. (2009) in Sarda goat breed, whereas differ from other autochthonous goat breeds reared in Sicily (Marletta et al., 2005; Gigli et al., 2008). The presence of A, C, and F alleles is associated with a normal content of  $\alpha$ S<sub>2</sub>-casein in milk.

As reported in previous studies, the high frequencies of A and B strong alleles at CSN1S1 locus (Martin et al., 2002; Marletta et al., 2007) in MAS population, as well as the strong alleles found at CSN2 and CSN1S2 loci, could be associated with milk production and with optimal milk technological properties.

The CSN3 is the most polymorphic gene in goat but in MAS population, alleles A and B showed the highest frequencies (0.275 and 0.675, respectively). Alleles M and G were observed only in two animals and in heterozygous condition. The population was in Hardy-Weinberg equilibrium at this locus (Table 1). The higher frequency of B (0.675) that we found in MAS population is in agreement with previous results reported by other authors for

**Table 3.** Mean number of alleles (MNA), observed heterozygosity ( $H_o$ ) and gene diversity ( $H_e$ ), standard deviation (SD) and  $F_{is}$  values in three goat populations. GIR=Girgentana; MAS=Mascaruna; MIX=Mixed population.

Breed	MNA $\pm$ SD	$H_o$ $\pm$ SD	$H_e$ $\pm$ SD	$F_{is}$
GIR (n=20)	5.89 $\pm$ 1.74	0.590 $\pm$ 0.200	0.666 $\pm$ 0.128	0.140
MAS (n=20)	5.94 $\pm$ 1.95	0.697 $\pm$ 0.131	0.703 $\pm$ 0.116	0.045
MIX (n=20)	7.17 $\pm$ 1.98	0.700 $\pm$ 0.170	0.731 $\pm$ 0.111	0.068

Italian (Sacchi et al., 2005), Sicilian (Gigli et al., 2008), European, and African (Prinzenberg et al., 2005) goat breeds. The most frequent genotype was AB (0.500), followed by BB (0.400) (Table 1). A and B alleles belong to the A<sup>IEF</sup> group described by Prinzenberg et al. (2005), which is the less favorable  $\kappa$ -casein variant in terms of milk composition and technological properties (Chiatti et al., 2007).

Based on the genetic structure of casein genes, the MAS goat population showed similarity with most goat breeds from the Mediterranean area, including Southern Italy (Marletta et al., 2005; Gigli et al., 2008)

### Microsatellite markers

A total of 148 alleles were observed considering the 18 loci analyzed, 106 of which in GIR, 107 in MAS, and 129 in MIX. The TNA per locus ranged from 5 for INRA063 and MB056 to 16 for OLADRB, with an average of 8.22. The PIC considering all loci was equal to 0.69. INRABERN185 was found to be the least informative marker (0.36), whereas OLADRB the most informative one (0.86) (Table 2). The average  $H_o$  and  $H_s$  for the 18 microsatellites are given in Table 2. Considering the whole sample,  $H_o$  ranged from 0.378 to 0.844 and  $H_s$  from 0.395 to 0.854. INRABERN185 marker showed the lowest  $H_s$ , whereas OLADRB the highest one. The microsatellite OLADRB is within the major histocompatibility complex (MHC), therefore, like other loci located in this region, is characterized by high allelic diversity and high level of polymorphism that can be the result of balancing selection (Hoda et al., 2010). Estimates of observed heterozygosity over all loci confirmed the remarkable level of genetic variability in these populations.

Hardy-Weinberg equilibrium was rejected for BM321 (Table 2) that showed the largest difference between  $H_o$  and  $H_s$ , and this marker was not further considered for genetic characterization analysis. These results reflect a notably high variability, characteristic of microsatellites derived from a greater mutation level than other genetic markers, which makes them a valuable tool for genetic characterization analyses (Arranz et al., 2001).

Table 2 also reports the population differentiation examined by fixation indexes for each locus and across loci. The mean estimates of  $F$ -statistics obtained were:  $F_{it}$ =0.112 (total inbreeding estimate),  $F_{is}$ =0.087 (within population inbreeding estimate) and  $F_{st}$ =0.027 (measure-

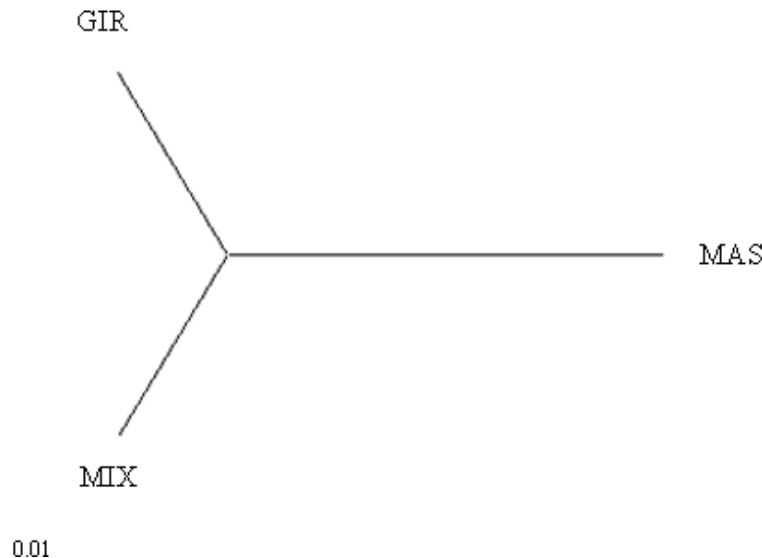
ment of population differentiation). The  $F_{st}$  value may indicate that these populations are not differentiated enough and that they may have common history and breeding practices. Thus, a large part of the total genetic diversity corresponded to differences among individuals (0.973) and, to a smaller extent, among populations (0.027). These estimates of genetic differentiation are comparable to those reported by other authors for Southern Italian ( $F_{st}$ =0.030) (Iamartino et al., 2005) and Portuguese ( $F_{st}$ =0.031) (Bruno-de-Sousa et al., 2011) goat breeds and/or populations, but lower than those reported by Glowatzki-Mullis et al. (2008) in Swiss goat breeds. The  $F_{it}$  value in the overall population could be due to the low genetic differentiation among populations. This level of differentiation was expected, as these populations have a geographical proximity, similarities in environment and breeding practices, but most likely due to past gene flow among them.

Crossbreeding is a common practice among breeders and it is used to modify the characteristics of breeds according to the needs and also to expand the size of a population when it is becoming too small. This practice is useful in a sense to prevent excessive inbreeding, but it is also deleterious when small-endangered populations are concerned. The genetic diversity parameters for each population are reported in Table 3. The highest MNA,  $H_o$ , and  $H_e$  values were found in MIX (MNA=7.17,  $H_o$ =0.700, and  $H_e$ =0.731) followed by MAS (MNA=5.94,  $H_o$ =0.697 and  $H_e$ =0.703) and GIR (MNA=5.89,  $H_o$ =0.590 and  $H_e$ =0.666). The average values for the genetic diversity parameters suggested that the MIX group had the highest variability, which was expected given the genetic structure of the population, whereas the GIR breed had the lowest values. In a study on Italian goat breeds, Negrini et al. (2012) reported high values of genetic diversity for Argentata dell'Etna population, as shown in the MIX group, and similar values for GIR breed. The MAS group also showed good levels of genetic variability. This is in agreement with the high variability observed in phenotypic traits, for example coat colour within MIX and MAS goat groups.

A positive  $F_{is}$  value was observed for all groups. The GIR breed showed the highest coefficient of inbreeding ( $F_{is}$ =0.140), whereas the MAS the lowest one ( $F_{is}$ =0.045). The  $F_{is}$  value for GIR breed is similar to those reported in previous studies (Canón et al., 2006; Negrini et al., 2012) but higher than those reported by Pariset et al. (2009).

**Table 4.** Reynolds (above the diagonal) and Nei-minimum (below the diagonal) genetic distances per pair of breeds. GIR, Girgentana; MAS, Mascaruna; MIX, Mixed population.

Breed	GIR	MAS	MIX
GIR (n=20)	-	0.044	0.018
MAS (n=20)	0.055	-	0.020
MIX (n=20)	0.034	0.041	-



**Figure 1.** Unrooted Neighbor-Joining dendrogram among goat populations using Reynolds genetic distances. GIR=Girgentana, MAS, mascaruna; MIX, mixed population.

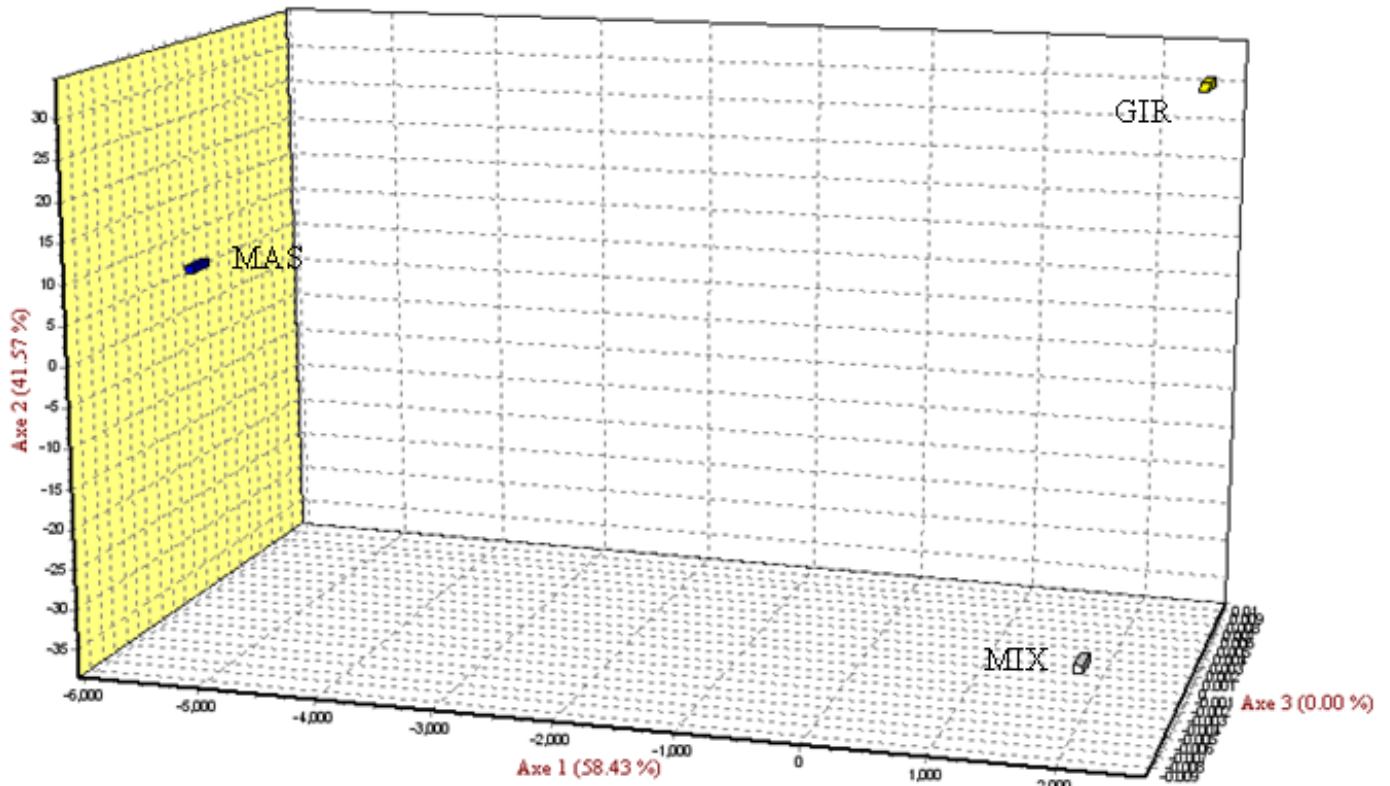
The high level of inbreeding and the relatively high expected heterozygosity in GIR breed is probably due to the presence of local bottlenecks. This breed is, indeed, reared in a restricted area of Sicily and nowadays about 650 animals are enrolled in the herd book (ASSONAPA, 2012); moreover, it is listed by FAO with endangered risk status (Canón et al., 2006). The MIX group, not being a defined breed, showed the highest genetic diversity, because it takes origin from the mixture of other populations. The gene flow among populations may indeed explain the relative high heterozygosity observed.

Private alleles, that are alleles unique for a single population, were evidenced in all groups. In general, the frequency of unique alleles was low (<5%) in all populations. However, the MAS goat presented one allele with frequency of 13.3% (269 bp allele) in the OLADRB. As mentioned above, it should be considered that this marker is located within the MHC region. There are several reasons for the existence of private alleles, like multi-origin of the breeds/populations, little subsequent genetic exchange between them or genetic drift (Agha et al., 2008). It is likely that the MAS goat is derived from crosses of different breeds and populations. The

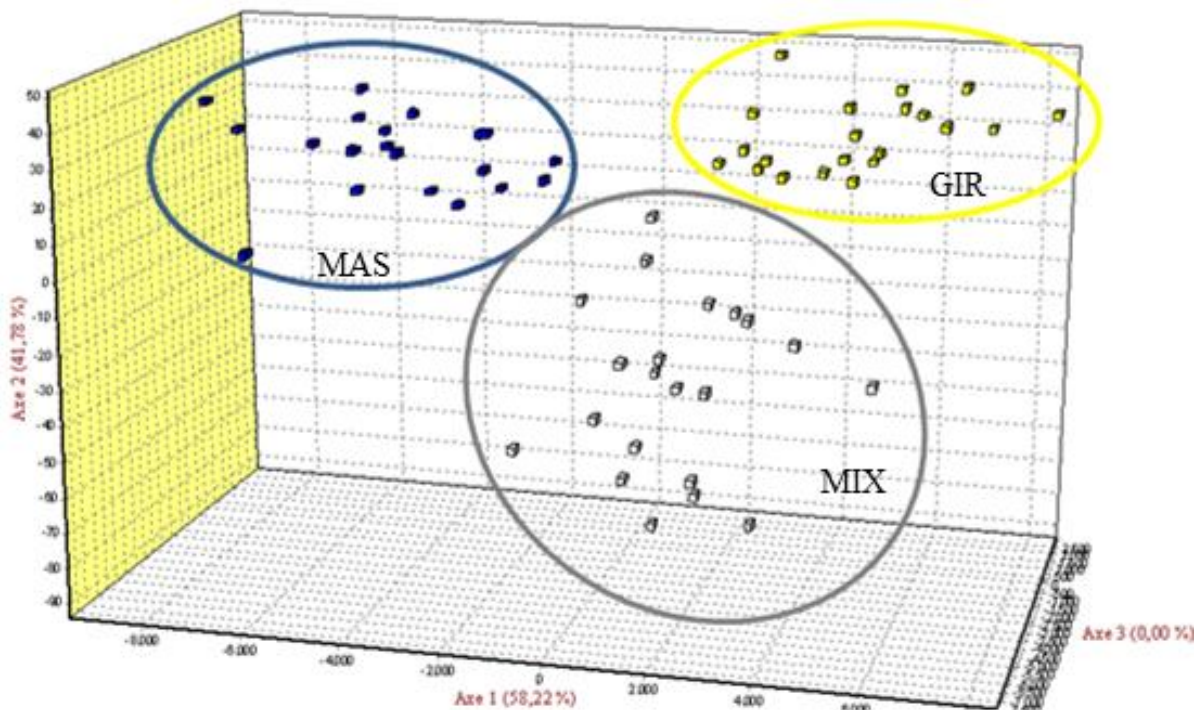
presence of private alleles is an important element of genetic differentiation among populations, in order to distinguish the MAS goat group from other populations.

To evaluate inter-population relationships, Nei and Reynolds genetic distances were estimated using the allelic frequency data. The lowest values were observed between GIR and MIX groups (0.018), whereas the highest ones between GIR and MAS groups (0.055) (Table 4). The Reynolds genetic distances were also used to reconstruct the unrooted neighbor-joining dendrogram (Figure 1). The MAS group formed a defined cluster and was differentiated from the other groups.

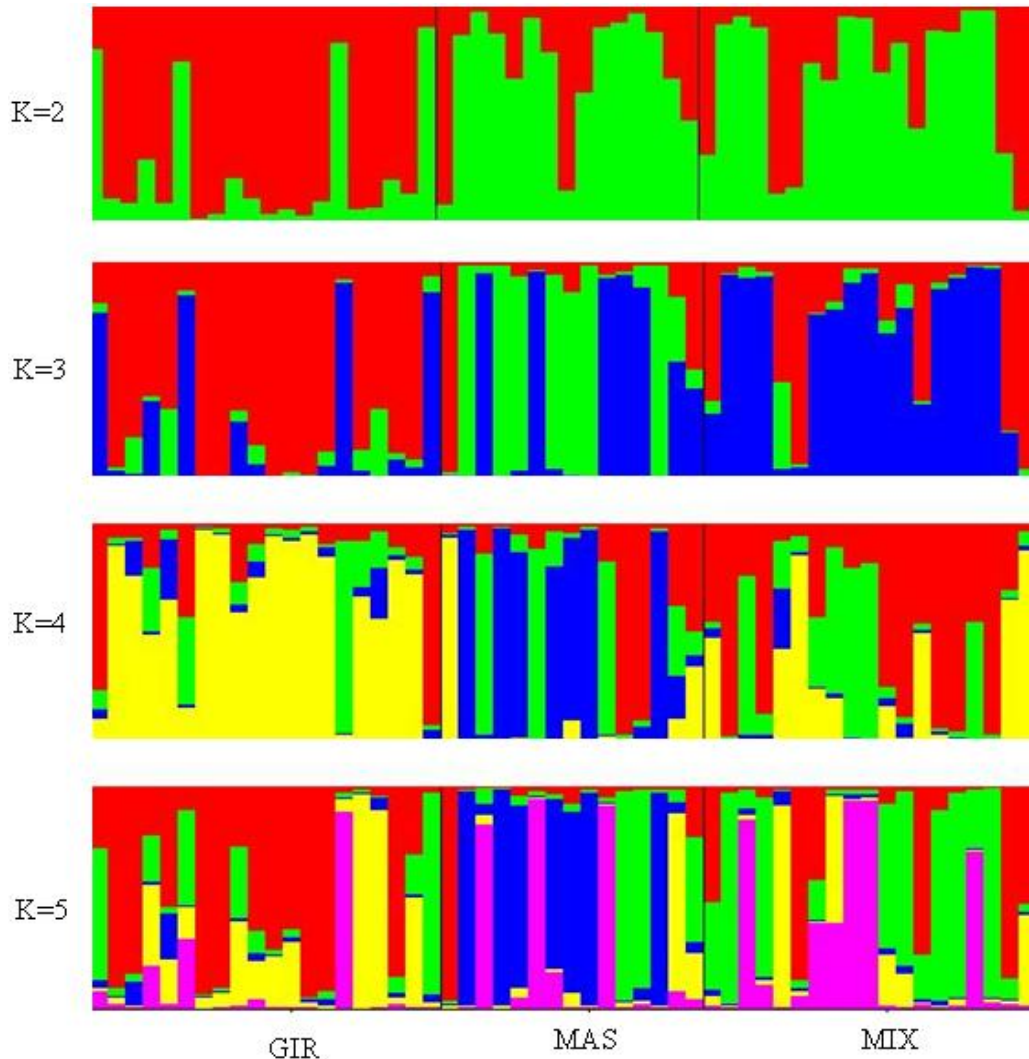
Figures 2 and 3 showed the genetic relationship assessed using FCA analysis among populations and among individuals, respectively. The first two components explained the 100% of the total variation, 58.43% of which explained by Axe 1 that clearly distinguished the MAS population from the other populations; 41.57% explained by Axe 2 that separated the other two populations (Figure 2). The MAS population was clearly separated from the GIR and MIX populations. These results were confirmed by individual analysis where MAS individuals are separated from the others and formed a clearly



**Figure 2.** Spatial representation of the populations as defined by the Factorial Correspondence Analysis. GIR=Girgentana, MAS=Mascaruna, MIX=Mixed population.



**Figure 3.** Spatial representation of the individual multilocus genotypes as defined by the Factorial Correspondence Analysis. GIR, girgentana; MAS, mascaruna; MIX, mixed population.



**Figure 4.** Estimated population structure of the goat for  $K$  ranging from 2 to 5. GIR, Girgentana; MAS, Mascaruna; MIX, Mixed population.

distinct group (Figure 3).

The analysis of genetic distances indicated that the MAS population was the most distanced group, a result that was confirmed by the unrooted neighbor-joining dendrogram, the FCA, and the presence of several private alleles. The results obtained on the genetic differentiation underline the hypothesis that MAS goat could be a population with a defined genetic structure although the absence of a herd book or breed registration societies, the possibility of influx from other genomes, and in some case the non-availability of pure bucks for breeding.

Based on definition of FAO (2007), a breed is “either a subspecific group of domestic livestock with definable and identifiable external characteristics that enable it to be separated by visual appraisal from other similarly defined groups within the same species or a group for which geographical and/or cultural separation from phenotypically similar groups has led to acceptance of its

separate identity”. In this context, molecular biology techniques to characterize populations are very useful tools in order to obtain correct conclusions depending on the species (Quiroz et al., 2008).

Assignment test was performed using the STRUCTURE software with the number of expected population ( $K$ ) ranging from 2 to 6. The most likely number of  $K$  was estimated by comparing the log-likelihood of each  $K$ -value. The  $\ln \Pr(G|K)$  increased from  $K=2$  to  $K=5$  and then decreased for  $K=6$ . For  $K=5$ , the  $\ln \Pr(G|K)$  was maximized and also mean variance of the  $\ln \Pr(G|K)$  estimates was the lowest one. For  $K=2$  the GIR separates from other two groups. With  $K$  increasing from 3 to 5, the GIR breed maintained a separate cluster, whereas the MIX group showed higher level of admixture and a more complex structure because its genome is shared among several populations (Figure 4). The MAS group showed a cluster, less distinct than the GIR breed,



**Table 5.** Proportion of membership of each breed in each of the 5 clusters inferred. GIR, Girgentana; MAS, Mascaruna; MIX, Mixed population.

Breed	1	2	3	4	5
GIR (n=20)	0.506	0.154	0.033	0.220	0.087
MAS (n=20)	0.098	0.131	0.476	0.085	0.210
MIX (n=20)	0.240	0.365	0.018	0.147	0.230

but more clearly defined than the MIX group. Table 5 shows the assignment proportion of each population to the five clusters inferred, choosing the iteration with the minimum variance. The GIR breed was the most differentiated group with 50.6% of the individuals assigned to cluster one and most MAS individuals (47.6%) belong to a specific cluster (three). Pariset et al. (2006) obtain similar results of proportion of assigned individuals considering Italian goat breeds with a defined genetic structure.

Furthermore, 22% of the GIR individuals were assigned to cluster four and 21% of the MAS individuals to cluster five. The MIX group showed the highest level of genetic admixture with different proportion of individuals belonging to cluster one, two and five (24, 36.5, and 23%, respectively). This could be due to the crossbreeding and introduction of genetic material from other populations. However, the MAS group, despite the possible influxes from other populations, presents a certain degree of distinction. There are studies of population structure in goats (Pariset et al., 2006) and sheep (Arora et al., 2011) that showed the lowest proportions of assignment and a clear evidence of admixture in herd book registered animals. The analysis with STRUCTURE software and the genetic clustering was consistent with the unrooted neighbor-joining dendrogram and the FCA results, confirming the separation of the MAS group.

## Conclusion

This study presented the first results on characterization of genetic variability of the Mascaruna goats through molecular markers analyses. The high frequencies of strong alleles at casein genes in Mascaruna goat population indicated that selection strategies for these alleles could be an important breeding objective. The preservation of small populations and of breeds in danger of extinction could be achieved by establishing economic reasons for their survival and, therefore, through their utilization.

For the microsatellite markers, our results suggest that admixture was low for the Girgentana breed, and this was confirmed by the highest value of inbreeding and the lowest genetic diversity. Despite the influxes from others populations, the genetic homogeneity for Mascaruna population was comparable with the level observed for standardized breeds, confirming the hypothesis that the Mascaruna is not a mixed population, but can be

considered as a population with particular genetic background. To confirm the results obtained, further analyses will be conducted on a wider sample, involving other breeds, and using mitochondrial DNA in addition to other markers for better clarifying the origins and the genetic structure of Mascaruna goat.

## REFERENCES

- Agha SH, Pilla F, Galal S, Shaat I, D'Andrea M, Reale S, Abdelsalam AZA, Li MH (2008). Genetic diversity in Egyptian and Italian goat breeds measured with microsatellite polymorphism. *J. Anim. Breed. Genet.* 125:194-200.
- Arora R, Bhatia S, Mishra BP, Joshi BK (2011). Population structure in Indian sheep ascertained using microsatellite information. *Anim. Genet.* 42: 242-250.
- Arranz JJ, Bayon Y, Primitivo FS (2001). Genetic variation at microsatellite loci in Spanish sheep. *Small Rumin. Res.* 39: 3-10.
- ASSONAPA (2012). <http://www.assonapa.it/Consistenze/>
- Bruno-de-Sousa C, Martinez AM, Ginja C, Santos-Silva F, Carolino MI, Delgado JV, Gama LT (2011). Genetic diversity and population structure in Portuguese goat breeds. *Liv. Sci.* 135:131-139.
- Baumung R, Cubric-Curik V, Schwend K, Achmann R, Solkner J (2006). Genetic characterization and breed assignment in Austrian sheep breeds using microsatellite markers information. *J. Anim. Breed. Genet.* 123: 265-271.
- Belkhir K, Borsa P, Goudet J, Chikhi L, Bonhomme F (1996). GENETIX 4.05, logiciel sous Windows™ pour la génétique des populations. Laboratoire Génome Populations, Interactions. CNRS UMR 5000. Université de Montpellier II: Montpellier, France.
- Canón J, García D, García-Atance MA, Obexer-Ruff G, Lenstra JA, Ajmone-Marsan P, Dunner S, ECONOGENE Consortium (2006). Geographical partitioning of goat diversity in Europe and the Middle East. *Anim. Genet.* 37: 327-334.
- Chessa S, Budelli E, Chiatti F, Cito AM, Bolla P, Caroli A (2005). Short Communication: Predominance of  $\beta$ -Casein (*CSN2*) C Allele in Goat Breeds Reared in Italy. *J. Dairy Sci.* 88: 1878-1881.
- Chessa S, Rignanese D, Kuppe J, Pagnacco G, Erhardt G, Caroli A (2008a). The  $\beta$ -Casein (*CSN2*) Silent Allele C1 Is Highly Spread in Goat Breeds. *J. Dairy Sci.* 91: 4433-4436.
- Chessa S, Rignanese D, Chiatti F, Radeghieri A, Gigliotti C, Caroli A (2008b). Simultaneous identification of *CSN1S2* A, B, C, and E alleles in goats by polymerase chain reaction-single strand conformation polymorphism. *J. Dairy Sci.* 91:1214-1217.
- Chiatti F, Chessa S, Bolla P, Cigalino G, Caroli A, Pagnacco G (2007). Effect of the k-casein polymorphism on milk composition in the Orobica goat. *J. Dairy Sci.* 90: 1962-1966.
- Cosenza G, Pappalardo M, Pastore N, Rando A, Di Gregorio P, Masina P, Ramunno L (2001). An AS-PCR method for identification of carriers of the goat *CSN1S1* 01 allele. In: 'Proceedings of the XIV ASPA Congress'. pp 64-66. (Scientific Association of Animal Production, Firenze, Italy).
- Dettoni ML, Vacca GM, Carcangiu V, Pazzola M, Mura MC, Rocchigiani AM (2009). A reliable method for characterization of the goat *CSN1S1* E allele. *Liv. Sci.* 125: 105-108.
- Felsenstein J (2009). Phylogeny Inference Package PHYLIP. Version 3.69. Department of Genome Sciences and Department of Biology,

- University of Washington: Seattle, WA, USA.
- FAO (2007). The state of the world's animal genetic resources for food and agriculture, edited by B. Rischkowsky & D. Pilling. Rome (<http://www.fao.org/docrep/010/a1250e/a1250e00.htm>).
- Gigli I, Maizon DO, Riggio V, Sardina, MT, Portolano B (2008). Casein haplotype variability in Sicilian dairy goat breeds. *J. Dairy Sci.* 91: 3687-3692.
- Glowatzki-Mullis ML, Muntwyler J, Baumle E, Gaillard C (2008). Genetic diversity measures of Swiss goat breeds as decision-making support for conservation policy. *Small Rumin. Res.* 74: 202-211.
- Goudet J (1995). FSTAT (version 1.2), a computer program to calculate F-statistics. *J. Hered.* 8: 485-486.
- Hoda A, Biçoku Y, Cara M (2010). Polymorphism of microsatellite loci in MHC complex for two populations of Shkodrane sheep breed in Albania. *Biotechnol. Biotech. Eq.* 24: 360-364.
- Iamartino D, Bruzzone A, Lanza A, Blasi M, Pilla F (2005). Genetic diversity of Southern Italian goat populations assessed by microsatellite markers. *Small Rumin. Res.* 57: 249-255.
- Lagonigro RE, Pietrolà E, D'Andrea M, Veltri C, Pilla F (2001). Molecular genetic characterization of the goat  $\alpha$ S2 casein allele. *Genet. Sel. Evol.* 25: 403-408.
- Marletta D, Bordonaro S, Guastella AM, Criscione A, D'Urso G (2005). Genetic polymorphism of the calcium sensitive caseins in Sicilian Girgentana and Argentata dell'Etna goat breeds. *Small Rumin. Res.* 57: 133-139.
- Marletta D, Criscione A, Bordonaro S, Guastella AM, D'Urso G (2007). Casein polymorphism in goat's milk. *Lait* 87: 491-504.
- Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998). Statistical confidence for likelihood-based paternity influence in natural populations. *Mol. Ecol.* 7: 639-655.
- Martin P, Szymanowska M, Zwierzchowski L, Leroux C (2002). The impact of genetic polymorphisms on protein composition of ruminant milks. *Reprod. Nutr. Dev.* 42: 433-445.
- Miller SA, Dykes DD, Polesky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 16: 1215.
- Negrini R, D'Andrea M, Crepaldi P, Colli L, Nicoloso L, Guastella AM, Sechi T, Bordonaro S, Ajmone-Marsan P, ECONOGENE Consortium (2012). Effect of microsatellite outliers on the genetic structure of eight Italian goat breeds. *Small Rumin. Res.* 103: 99-107.
- Nei M (1987). *Molecular Evolutionary Genetics*. Columbia University Press: New York, USA.
- Ng-Kwai-Hang KF, Grosclaude F (2003). Genetic polymorphism of the milk proteins. In: *Advance Dairy Chemistry Volume 1.3 edition*. (Ed Fox PF). pp 737-814. New York, USA.
- Page RDM (1996). TREEVIEW, An application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.* 12: 357-358.
- Pariset L, Cappuccio I, Ajmone Marsan P, Dunner S, Luikart G, England PR, Obexer-Ruff G, Peter C, Marletta D, Pilla F, Valentini A, ECONOGENE Consortium (2006). Assessment of population structure by single nucleotide polymorphisms (SNPs) in goat breeds. *J. Chromatogr. B.* 833: 117-120.
- Pariset L, Cuteri A, Ligda C, Ajmone-Marsan P, Valentini A, ECONOGENE Consortium (2009). Geographical patterning of sixteen goat breeds from Italy, Albania and Greece assessed by Single Nucleotide Polymorphisms. *BMC Ecology.* 9: 20.
- Prinzenberg EM, Gutsher K, Chessa S, Caroli A, Erhardt G (2005). Caprine kappa-casein (CSN3) polymorphism: new developments in molecular knowledge. *J. Dairy Sci.* 88: 1490-1498.
- Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure using multilocus genotype data. *Genetics.* 155: 945-959.
- Quiroz J, Martinez AM, Zaragoza L, Perezgrovas R, Vega-Pla JL, Delgado JV (2008). Genetic characterization of the autochthonous sheep populations from Chiapas, Mexico. *Liv. Sci.* 116: 156-161.
- Ramamoorthi J, Thilagam K, Sivaselvam SN, Karthickeyan SMK (2009). Genetic characterization of Barbari goats using microsatellite markers. *J. Vet. Sci.* 10: 73-76.
- Ramunno L, Cosenza G, Pappalardo M, Pastore N, Gallo D, Di Gregorio P, Masina P (2000a). Identification of the goat CSN1S1 F allele by means of PCR-RFLP method. *Anim. Genet.* 31: 342-343.
- Ramunno L, Pappalardo M, Cosenza G, Pastore N, Gallo D, Grimaldi G, Rubino R, Calandrelli M, Rando A (2000b). Genetic characterization at casin loci  $\alpha$ S<sub>1</sub> and  $\alpha$ S<sub>2</sub> in a goat population reared in Sorrento Peninsula. In: 'Proceedings of the XIV S.I.P.A.O.C. Congress'. (Ed. Società Italiana di Patologie Ovi-Caprine) pp 321-324. (Vietri sul mare, SA, Italy).
- Ramunno L, Longobardi E, Pappalardo M, Di Gregorio P, Cosenza G, Mariani P, Pastore N, Masina P (2001a). An allele associated with a non-detectable amount of  $\alpha$ S2 casein in goat milk. *Anim. Genet.* 32: 19-26.
- Ramunno L, Cosenza G, Pappalardo M, Longobardi E, Gallo D, Pastore N, Di Gregorio P, Rando A (2001b). Characterization of two new alleles at the goat CSN1S2 locus. *Anim. Genet.* 32: 264-268.
- Rousset F (2008). Genepop'007: a complete re-implementation of the Genepop software for Windows and Linux. *Mol. Ecol. Resour.* 8: 103-106.
- Reynolds J, Weir BS, Cockerham C (1983). Estimation of the coancestry coefficient basis for a short term genetic distance. *Genetics.* 105: 767-779.
- Sacchi P, Chessa S, Budelli E, Bolla P, Cerotti G, Soglia D, Rasero R, Cauvin E, Caroli A (2005). Casein haplotype structure in five Italian goat breeds. *J. Dairy Sci.* 88: 1561-1568.
- Siwek M, Finocchiaro R, Curik I, Portolano B (2010). Hierarchical structure of the Sicilian goats revealed by Bayesian analyses of microsatellite information. *Anim. Genet.* 42: 93-95.
- Vacca GM, Ouled Ahmed Ben Ali H, Pazzola M, Sanna M, Dettori ML, Carcangiu V (2009). An investigation on allele frequency at the CSN1S2 locus and its relationship with milk parameters in the Sarda goat. *J. Anim. Feed Sci.* 18: 628-637.
- Weir BS, Cockerham CC (1984). Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358-1370.