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Conference paper

GENETIC DIVERSITY BETWEEN GENTILE DI PUGLIA, SOPRAVISSANA AND SARDA SHEEP BREEDS, USING MICROSATELLITE MARKERS

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Introduction

The Sarda sheep breed has played, in Italy, the same role as the Holstein Friesian, although on a smaller scale. In fact, being a high milk yielding indigenous breed of the Sardinian island, it spread out to the Italian peninsula half century ago, and was preferred by many sherpherds who abandoned their low producing sheep breeds: Gentile di Puglia and Sopravissana. These two multi-purpose indigenous sheep breeds represented up to 1963 the most important sheep resource in Southern and Central Italy respectively, numbering to about one million head of each of the two (ASSONAPA, 1972), and well fulfilled the market demand of sheep cheese; in 1992 there were about 300000 of each one (Sarti, 1992), while estimates for 2002 indicate that no more than 10 thousands are left. On the other side, the numbers of Sarda sheep, amounting to about 2.5 million head in 1963 (ASSONAPA, 1972), increased to over 4 million in 1992 (Sanna, 1992). This economic competition of the Sarda breed has lead to a dangerous loss of genetic diversity that nobody will be able to re-employ in the future. The average milk production of the Sarda, during one lactation, is high - 206 litres (AIA, 2001); no official recording exists for the other two indigenous breeds, although shepherds say that: 1. they produce 300-500 grams milk/day, 2. the length of the lactation is extremely variable (60 to 150 days), 3. the lamb has a very good meat conformation so that it can be sold to title butcher at a higher price than the Sarda lamb, 4. they are docile and have an excellent and "sparing" grazing system. They could then be proposed in the context of sustainable agriculture. When approaching the sustainability of a population, the knowledge of the extent of the genetic diversity should be the first step: in fact, it provides a basis for conservation

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decisions through investigating in the genes and gene combinations carried by each breed, that may be useful in the future for traits that are now difficult to define (e.g. the adaptation to poor environments and the quality of the products) (Hammond, 1998).

The present work aims to estimate the genetic diversity between these breeds, their inbreeding rate and the genetic markers that make them unique, in order to propose their sustainable use.

Materials and methods

Sheep for genotyping consisted of 25 Gentile di Puglia (GP), 20 Sopravissana (SV) and 15 Sarda (SA). Extracted DNA from frozen blood (GENOMIX extraction kit, Talent, Trieste) was PCR amplified at the following microsatellite loci: CSSM43, CSSM47, CSSM60, TGLA110, TGLA122, TGLA126, TGLA377, ETH3, ETH10, ETH225, NRAMP1, OARCP20 e SPS115. Primer sequences and amplification conditions were those referred in literature (Moore et al., 1994; Brezinsky et al., 1993; Solinas Toldo et al., 1993; Matthews and Crawford, 1998; Ede et al., 1995; National Centre for Biotechnology Information - GenBank). The selected microsatelliti are located on 7 different chromosomes (Moore et al., 1994; Brezinsky et al., 1993; Solinas Toldo et al., 1993; McGraw et al., 1997). Allele size was measured by Genescan software on the detected DNA fragments by the Perkin Elmer ABI Prism 310 DNA sequencer.

Allele frequencies and average heterozygosity, both expected and observed, for each breed were obtained with the FSTAT programme (Goudet, 1995). From allele frequencies, average genetic diversity at each locus was calculated according to Nei (1973). To estimate genetic distances between populations, Wright fixation indexes were used (Wright, 1943), obtained with FSTAT. These indexes rerpesent the probability that two alleles, chosen at random in one population, are identical by descent. The fixation index Fst between populations pairwise corresponds to the genetic distance between them. Slatkin (1993) suggests to use this parameter to express geographical isolation.

Results and discussion

In table 1, allele size and frequency are referred for each population and locus. Number of detected alleles ranges between 2 (CSSM60 and ETH10) and

14 (TGLA122), with average equal 7.5. Major differences between breeds in allele frequencies were found at TGLA377, TGLA122, OARCP20 and TGLA126, while at the remaining loci the alleles at the highest frequency are the same. Average gene diversity (Nei, 1973) over all loci and for the three breeds together was 0.58 (table 2). The microsatellite loci showing the highest gene diversity were TGLA126 (0.89); TGLA122 (0.83); TGLA377 (0.80). Of the three breeds, GP showed the highest gene diversity over all considered loci (0.59), followed by SA (0.53) and SV (0.51).

Table 1. - DETECTED ALLELE SIZE AND ALLELE FREQUENCIES (%) IN THE BREEDS GENTILE DI PUGLIA (GP); SOPRAVISSANA (SV) AND SARDA (SA)

Locus/	Freq. %			Locus/	Freq. %		
allele	GP	SV	SA	allele	GP	SV	SA
SCSM43				TGLA122			
232	0	0	3.3	129	0	0	46.7
234	4.0	0	0	131	54.0	27.5	3.3
244	10.0	15.0	6.7	133	6.0	0	0
246	6.0	0	10.0	135	4.0	2.5	0
248	0	7.5	6.7	137	2.0	12.5	0
252	6.0	0	10.0	141	2.0	0	0
254	26.0	65.0	33.0	143	0	0	3.3
256	10.0	7.5	6.7	147	0	0	10.0
258	4.0	0	20.0	149	16.0	15.0	3.3
260	26.0	2.5	3.3	151	4.0	30.0	3.3
262	6.0	2.5	0	153	4.0	7.5	30.0
264	2.0	0	0	155	0	5.0	0
ETH3				189	6.0	0	0
92	12.5	10.0	3.3	191	2.0	0	0
94	18.8	42.5	33.3	NRAMP1			
98	0	0	3.3	210	4.2	12.5	0
100	6.3	20.0	60.0	212	4.2	0	3.3
102	54.2	22.5	0	214	2.1	2.5	3.3
104	8.3	5.0	0	216	35.4	47.5	53.3
ETH225				218	8.3	22.5	26.7
130	2.0	0	0	220	39.6	0	3.3
136	2.0	5.0	0	222	4.2	15.0	10.0
138	30.0	45.0	20	224	2.1	0	0
140	46.0	47.5	36.7	CSSM60			
142	12.0	2.5	43.3	44	0	10.0	14.3
144	8.0	0	8	78	100.0	90.0	85.7

Locus/ allele	Freq. %			Locus/ allele	Freq. %		
	GP	SV	SA		GP	SV	SA
TGLA126				TGLA377			
112	14.0	0	0	86	2.0	17.5	6.7
114	12.0	7.5	12.5	92	12.0	5.0	36.7
116	20.0	10.0	12.5	94	10.0	5.0	16.7
118	0	22.5	0	96	2.0	0	0
120	2.0	0	13.3	98	14.0	0	10.0
122	0	0	3.3	100	4.0	0	6.7
124	12.0	7.5	10.0	102	36.0	32.5	30.0
126	6.0	7.5	6.7	104	16.0	40.0	0
128	2.0	0	0	106	4.0	0	0
132	8.0	0	10.0	OARCP20			
134	6.0	37.5	20.0	66	18.0	5.0	40.0
136	16.0	7.5	13.3	68	18.0	10.0	23.3
138	2.0	0	0	70	24.0	40.0	0
SPS115				74	26.0	45.0	30.0
230	0	0	3.3	78	2.0	0	0
232	22.0	0	10.0	80	6.0	0	0
238	0	2.6	6.7	84	4.0	0	0
240	0	0	3.3	86	2.0	0	6.7
242	16.0	0	3.3	ETH10			
244	26.0	42.1	13.3	209	100.0	91.7	100.0
246	36.0	55.3	60.0	211	0	8.3	0
CSSM47				TGLA110			
128	32.0	2.5	3.3	166	2.0	0	3.3
130	60.0	90.0	96.7	168	89.6	100.0	93.4
148	0	7.5	0	170	2.1	0	3.3
164	2.0	0	0	174	2.1	0	0
168	6.0	0	0	176	2.1	0	0
				178	2.1	0	0

The observed heterozygosity (table 3) was slightly lower than the expected one according to Hardy-Weinberg equilibrium, but not significantly different (P=0.55; 0.56 in the GP and SA; P=0.45 in the SV). This is an indication that the three breeds maintain a random-mating structure. In fact no intensive organised selection scheme for the improvement of the productivity has been systematically applied in the two endangered ones; while in the SA, the selection scheme that has been applied during the last two decades for the

improvement of milk yield is too recent to have made consistent genomic changes at breed level.

Table 2 - AVERAGE GENE DIVERSITY AT EACH LOCUS, IN THE BREEDS GENTILE DI PUGLIA (GP); SOPRAVISSANA (SV) AND SARDA (SA)

	Average gene diversity			
Locus	GP	SV	SA	Totale
CSSM43	0.85	0.56	0.84	0.79
CSSM47	0.54	0.19	0.07	0.35
CSSM60	0	0.18	0.25	0.12
TGLA122	0.68	0.81	0.69	0.83
TGLA 126	0.89	0.79	0.89	0.88
TGLA377	0.82	0.73	0.78	0.80
ETH3	0.66	0.74	0.55	0.75
ETH10	0	0.16	0	0.05
NRAMP1	0.75	0.72	0.70	0.73
OARCP20	0.82	0.64	0.71	0.77
SPS115	0.74	0.53	0.63	0.67
ETH225	0.69	0.59	0.67	0.68
TGLA110	0.20	0	0.13	0.11
Mean/locus	0.59	0.51	0.53	0.59

Table 3. - SAMPLE SIZE, NUMBER OF ALLELES PER LOCUS AND HETEROZYGOSITY AVERAGED OVER 13 MICROSATELLITE LOCI

Breed	No. animals	No. alleles/locus	Average heterozygosity	
			observed	expected
GP	25	5.3	0.127 ± 0.080	0.146 ± 0.082
SV	20	3.7	0.122 ± 0.064	0.141 ± 0.062
SA	15	4.4	0.121± 0.078	0.138 ± 0.076
Total	60	7.5		

Average inbreeding rate (table 4) was higher in the two endangered breeds (0.156 in GP; 0.159 in SV) than in the SA (0.138) which is obvious because this breed is 10 times larger in numbers than the other two. It is therefore the moment to start a conservation and improvement programme, while the inbreeding rate is still acceptable.

Genetic differentiation between GP and SV (table 4) is moderate (0.081); while the differentiation betwen the SA and the endangered ones is respec-

tively 0.111 and 0.107. According to Hartl (1989) these values indicate a medium-high differentiation rate; therefore, the disappearance of the two less productive breeds would entail a consistent loss of genetic diversity.

Table 4. - FIXATION INDEXES: TOTAL (ON DIAGONAL = INBREEDING WITHIN BREED) AND BETWEEN BREEDS (GENETIC DISTANCES).

Breed	GP	SV	SA
GP	0.156	0.081	0.111
SV		0.159	0.107
SA			0.138

Although many people are convinced that GP and SV are very similar, because their morphology and performances are almost the same, the genetic differentiation that was found in the present work shows that it is not true. This is confirmed by the fact that the two breeds have evolved separately in two different Italian areas, the Southern Apennine for the first, the Central Appennine and the Roman lowland for the second, with different socio-economic constraints and no exchange of breeding animals between them; this difference should be preserved through opportune programmes, and the polymorphic markers here presented could be the starting point to look for the candidate genes that make them different and that could lead to a sustainable use of such breeds.

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GENETSKA RAZNOLIKOST IZMEĐU PASMINA OVACA GENTILE DI PUGLIA, SOPRAVISSANA I SARDA PRIMJENOM MIKROSATELITSKIH MARKERA

Sažetak

Pasmina ovaca Sarda imala je istu ulogu u Italiji kao i Holstein frizijska, iako u manjem opsegu. U stvari, kao autohtona pasmina otoka Sardinije visokog prinosa mlijeka, proširila se na talijanski poluotok pred pola stoljeća pa su se mnogi ovčari odlučili za nju i napustili svoje pasmine ovaca niskog prinosa: Gentile di Puglia i Sopravissana. Ove dvije autohtone pasmine ovaca višestruke namjene predstavljale su do 1963. najvažnije resurse ovaca u južnoj, odnosno srednjoj Italiji a svaka je brojala pola milijuna grla, te su dobro zadovoljavale tržišnu potražnju za ovčjim sirom; 1992. godine bilo je oko 300000 svake od te dvije pasmine, dok procjene za 2002. godinu pokazuju da nije ostalo više od 10000 grla. S druge strane, broj ovaca Sarda, što je iznosio oko 2.5 milijuna grla 1963. g. porastao je na preko 4 milijuna 1992. g. Ovo ekonomsko takmičenje pasmine Sarda dovelo je do opasnog gubitka genetske raznolikosti koju nitko neće moći obnoviti u budućnosti. Prosječna proizvodnja mlijeka Sarde, u jednoj laktaciji, je visoka, 206 litara; ne postoje nikakvi službeni podaci za druge dvije autohtone pasmine, makar ovčari kažu da: 1. one proizvode 300 -500 grama mlijeka/dan, 2. duljina laktacije je vrlo različita (60-150 dana), 3. janje ima vrlo dobru strukturu mesa pa se može prodati mesaru za višu cijenu od janjetine Sarda, 4. vrlo su poslušne te imaju izvrstan i "štedljiv" sustav napasanja. Mogu se zato preporučiti za održivu poljoprivredu. Kada se govori o održivosti populacije saznanje o stupnju genetske raznolikosti trebao bi biti prvi korak: u u stvari ono pruža temelj pri odlučivanju za očuvanje istraživanjem gena i kombinacija gena koje nosi svaka pasmina, što može biti korisno u buduće za svojstva koja je sada teško odrediti (npr, prilagođavanje siromašnom okolišu i kakvoću proizvoda).

Cilj ovog rada je procijeniti genetsku raznolikost ovih pasmina, stopu inbreedinga i genetske markere koji ih čine jedinstvenima, kako bi se predložila održiva primjena.

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