Genetic variation of goat Y chromosome in the Sardinian population

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ABSTRACT – Sardinian goat population is commonly considered a crossbred of autochthonous animals with improved Mediterranean breeds, mainly the Maltese. It has been demonstrated by using autosomal microsatellites that the Sardinian goats can be divided into three subpopulations: Sardinian, crossbred with Maltese, and Maltese. The aim of this study was to evaluate sequence variation at Y chromosome in Sardinian bucks and to integrate autosomal microsatellites data. Blood from 190 bucks from 68 farms spread in the main Sardinian goat farming areas was sampled. Three ECONOGENE project primer pairs plus an additional one corresponding to a total of 7 SNPs were used. For all common SNPs, the most frequent allele corresponded to the ECONOGENE one. The additional analysed SNP showed allelic frequencies similar to the other markers. The comparison with haplotypes based on the 6 common SNPs showed that the Sardinian most frequent haplotype corresponded to the predominant one in Central Europe. Results of this study showed that the Sardinian goat population has 8 haplotypes resulting in a large diversity of paternal lineages. The next step will be linking autosomal information to Y chromosome data. In fact, up to date, it seems unfeasible to detect recent upgrading breeds by using Y chromosome variation only.

Key words: Goat, Y chromosome, SNP, Microsatellites.

Introduction – Studies on Y chromosome are of particular interest in livestock species because in common breeding strategies only a few males contribute genetically to the next generation (Lindgren *et al.*, 2004). The mammalian Y chromosome has two components, a pseudoautosomal region which frequently recombines with the X chromosome and a male-specific region (MSY). Markers on the MSY, which is paternally inherited in a haploid way, have been used for studying the origin of species, range expansion, admixture of populations, and migration in animals (Pidancier *et al.*, 2006). Molecular variation in the Y chromosome provides information about genetic diversity, since it reveals the pattern of distribution of paternal lineages. For instance, it may indicate stocks upgrading, which is often performed by using sires from breeds with the desired properties. Up to date, however, few phylogenetic surveys involving the Y chromosome have been reported in domestic species due to a lack of MSY variation. Indeed, very low rates of nucleotide diversity have been reported within the MSY of horse (Lindgren *et al.*, 2004), cattle (Hellborg and Ellegren, 2004), and sheep (Meadows *et al.*, 2006). In goat, latest studies based on mitochondrial DNA analyses revealed a complex pattern of caprine domestication (Luikart *et al.*, 2001; Naderi *et al.*, 2007). In the ECONOGENE project (http://econogene.eu/) the sequence variation at the Y chromosome was used to integrate information from mitochondrial and autosomal DNA to study the genetic diversity of several goat breeds.

Sardinian goat population is commonly considered a crossbred of autochthonous animals with improved Mediterranean goats, mainly the Maltese breed. The upgrading of the original population

has been likely made through imported Maltese breed males. Sechi *et al.* (2007) demonstrated by using autosomal microsatellites that the Sardinian goats can be divided into three subpopulations: Sardinian, crossbred with Maltese, and Maltese. The aim of this study was integrating autosomal microsatellites data by the observation of sequence variation at Y chromosome in Sardinian bucks.

Material and methods – Blood from 190 bucks from 68 farms spread in the main Sardinian goat farming areas was sampled. DNA was extracted by "Parco Genos" and "IGP" from fresh and frozen blood samples using the 5 PRIME GmbH ActivePure DNA purification system kit. Five primer pairs of the ECONOGENE project, which detected 8 SNPs in the ZFY, SRY, and DBY genes of the goat Y chromosome were used. One additional primer was designed using the free software Primer3 (http://frodo.wi.mit.edu/) to sequence a SRY gene portion where a further SNP was detected by Prashant et al. (2008). Amplified products were sequenced in both directions with ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction v.3.1 (Applied Bio-systems, Foster City CA, USA). Sequencing was carried out using an ABI PRISM® 3100-Avant Genetic Analyzer (Applera, Foster City, CA). Data were collected using the ABI PRISM Data collection software and analysed with DNA Sequencing Analysis software v.3.7 (Applied Biosystems, USA). The sequence data obtained from each primer pair were compared to genetic databases through a BLAST procedure, then aligned using Sequencher software v.4.8 (Gene Codes Corporation, USA) to identify SNPs, and then haplotype frequencies were calculated. Results were then compared to those found in the ECONOGENE project.

Results and conclusions – Two out of the 5 chosen primer pairs gave some analytical problems: SRY3 primer set did not amplify and the quality of ZFY1 sequence did not allow reaching the SNP location. Three ECONOGENE primer pairs plus the additional one, corresponding to 7 SNPs (2 in DBY gene and 5 in SRY gene), were used. Table 1 shows the number of analysed animals and the allelic frequencies for each SNP found. SRY2 and SRY5 primer pairs gave sequence products of variable length in both forward and reverse directions, thus preventing the determination of two involved SNPs (103 analysed animals for SRY_2_226 and 125 analysed animals for SRY_5_340) in a large portion of samples. SRY5_605 did not show polymorphism. The sole allele corresponded to the most frequent variant found in the ECONOGENE project. For all other SNPs, the most frequent allele corresponded to the ECONOGENE one, although with much higher frequencies. The SNP detected with the additional primer set (SRY_7_179) showed allelic frequencies similar to other markers. Haplotypes were reconstructed either considering all or only the most efficient SNPs (Table 2). ECONOGENE project identified three most frequent haplotypes defined by 8 SNPs. Haplotypes comparison based on the 6 SNPs shared with ECONOGENE project showed that the Sardinian most frequent haplotype corresponds to the predominant one in Central Europe. In addition, the comparison based on the four most efficient SNPs suggests that even the predominant Asian haplotype could be present in Sardinia. Indeed, this haplotype was previously detected in Sardinian goats analysed in the ECONOGENE project (unpublished data). All the remaining haplotypes showed very low frequencies. The analysis of polymorphism at SRY_7_179 added new information to the 6 SNPs haplotype although with a clear predominance of the T allele.

Table 1. Allelic frequencies for each SNP.

SNP	Allelic frequencies (%)		Analysed animals
DBY_1_238	16%C	84%A	183
DBY_1_424	15%T	85%C	173
SRY_2_226	17%T	83%G	103
SRY_5_340	4%A	96%T	125
SRY_5_605		100%T	188
SRY_5_732	19%A	81%G	188
SRY_7_179	23%A	77%T	189

Table 2. Analysed animals (N°) and Haplotypes[1] with 7 (A) and with 5 loci (B).

Haplotypes A	N°	Haplotypes B	N°
ACGATGT	3	ACTAA	6
ACGTTAA	1	ACTAT	4
ACGTTGA	1	ACTGA	5
ACGTTGT	55	ACTGT	127
ACTTTAA	1	ATTAA	3
ATTTTAA	3	CCTAA	1
CCTTTAA	1	CCTGT	1
CTTTTAA	4	CTTAA	16
Total	69	CTTGA	4
		CTTGT	2
		Total	169

[1]*Underlined base indicates SNP not in ECONOGENE panel*

As a whole, the results of this study showed that the Sardinian goat population has a large diversity of paternal lineages (Table 2). The next step will be to connect autosomal information with Y chromosome data. Indeed, up to date, it seems unfeasible to detect recent upgrading breeds by using Y chromosome variation only.

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