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Effect of selection for scrapie resistance on genetic diversity in a rare and locally adapted sheep breed: The case of Sambucana

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ABSTRACT

In Italy, since 2005 a breeding plan to increase scrapie resistance has been adopted. The impact of this selection on genetic diversity was assessed on Sambucana, an autochtonous sheep breed reared in southern Piedmont, by analysing the evolution of allele frequencies at different levels: PRNP (prion protein) gene, microsatellite loci on OAR13 (where PRNR maps), and microsatellite loci on other chromosomes, not subjected to selection for scrapie resistance. A total of 147 young rams, 80 born in 2004 and 67 in 2008–2009 were analysed. Evidence of diversity loss was observed for PRNP gene as a consequence of the directional selection. Diversity was affected in the immediate vicinity of PRNP but the effect on more distant loci on the same chromosome was trivial. With regard to neutral markers, lack of heterozygosis with no changeover of allele frequencies was observed suggesting an increase of inbreeding. Mating policies would be sufficient to solve these problems. A selection scheme based on genotyping rams and eliminating carriers of both susceptible and high susceptible alleles is the best way to improve natural resistance to scrapie with low costs and minimal problems in the current conservation programmes targeting rare breeds.

1. Introduction

In sheep, susceptibility to scrapie is influenced by mutations in the PRNP (prion protein) gene, located on OAR13 (sheep chromosome 13). Haplotypes-alleles VRQ and ARQ are associated with susceptibility, whereas ARR has been linked to resistance (Belt et al., 1995; Bossers et al., 1996; Hunter et al., 1996). Accordingly, the European Union has implemented programmes for genetic control of scrapie susceptibility in sheep (European Commission, 2003).

The impact of selection for scrapie resistance has been investigated frequently. Most studies predict effects and costs of selection in term of diversity using both simulated and real datasets (Alfonso et al., 2006; Álvarez et al., 2007, 2009; Drögemüller et al., 2004; Man et al., 2009; Molina et al., 2006; Roden et al., 2006; Windig et al., 2004; Wiśniewska et al., 2010), whereas only few investigation analyse the achieved effects after the selection programmes have been applied (Palhière et al., 2006, 2008). Various concerns have been raised regarding possible unintended consequences of widespread selection on PRNP, including risk of genetic diversity loss (Dawson et al., 2008; Parada et al., 2007).

Sambucana is a Piedmontese sheep breed devoted to meat production. Since 1985, several initiatives have been carried out to safeguard this breed and to enhance the value of its derived productions. An important step was the creation of the 'Agnello Sambucano Garantito' brand, which certifies the origin of the lambs and guarantees the meat quality. In 2001, the Sambucano lamb was added to the "Presidia" list of Slow Food. In 1993, the breed was classified as 'at limited diffusion' (FAO-UNEP, 1993). In 2005, 168 rams and 3995 ewes were registered (Associazione Nazionale della Pastorizia, http://www.assonapa.it).

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Before the selection programme started, the Sambucana breed showed an ARR frequency slightly above the threshold to comply with European regulations, in spite of this it could benefit of some derogations as a rare and locally adapted breed. Nevertheless, the selection programme for scrapie resistance has been applied since 2005 (Regione Piemonte, 2005). This decision meets customer expectations of consuming a high-quality meat that is also safe to eat.

Currently, great attention is paid to avoid excessive inbreeding or genetic drift. Genealogical information can be used, however the quality of pedigree information is often inadequate in a population kept in an extensive breeding system. For Sanbucana breed, pedigrees are not available at all and, in such cases, the evolution of genetic diversity can be assessed only using a molecular approach.

The aim of the present investigation was to use Sambucana as a model to evaluate the consequences of selection for scrapie resistance on molecular diversity in a rare and locally adapted breed. Three processes may play a key role on genetic diversity: direct selection on PRNP gene, frequency changes of loci on OAR13 due to linkage disequilibrium, and genetic drift as a consequence of the use of a limited part of the population for breeding. Therefore, the evolution of allele frequencies was analysed at these different levels: PRNP gene, microsatellite loci on OAR13, and microsatellites mapped on chromosomes others than OAR13.

2. Materials and methods

2.1. Sample collection

A total of 147 Sambucana young rams (candidate sires), 80 born in 2004 (denoted as cohort 1) and 67 in 2008–2009 (denoted as cohort 2), were randomly chosen from different flocks among all animals genotyped at the PRNP locus by the IZSTO-CEA (Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italian Reference Centre for Animal Transmissible Spongiform Encephalopathies). The between-sampled cohorts period length (4 years) represents about one generation. The analyses were performed on rams only because, before 2005, males were genotyped exclusively.

Each cohort was divided into two risk groups according to the selection criteria adopted in the Italian breeding plan against VRQ and in favour of ARR (Decreto Ministeriale, 17 dicembre 2004). 'Low risk' rams are ARR/ARR and ARR/non-ARR, except ARR/VRQ, all other animals being considered as 'high risk'.

2.2. Molecular techniques

DNA was extracted from blood samples using the NucleoSpin QuickPure extraction kit (Macherey-Nagel, Dueren, Germany). Fourteen microsatellites were chosen on OAR13 (Table 1). PRNPS11, -15, and -24 map within PRNP whereas PRNPS04 and -05 map at about 40 kb upstream of the 5′ end of the gene (GenBank U67922.1). The other nine OAR13 markers were chosen outside the gene at various distances (Geldermann et al., 2003; Isler et al., 2006; Lühken et al., 2006; Palhière et al., 2008; Preuss et al., 2005). Other 12 microsatellites (http://www.isag.us/; http://www.econogene.eu/) were selected to avoid synteny with PRNP (neutral microsatellites in Table 1). PCR assays were performed as previously described (Soglia et al., 2010).

An error assay was performed by replicating the genotyping on a randomly chosen 10% of individual samples. The average error rate per allele was computed (Pompanon et al., 2005). Individual markers were tested for deviations from Hardy–Weinberg (HW) proportions with F_{IS} statistics (FSTAT 2.9.3.2, Goudet, 1995). The presence of

Table 1
Location and values of F_{IS} for the OAR13 and neutral microsatellites.

OAR13 microsatellites				Neutral microsatellites			
	Dist.[a]	Cohort 1	Cohort 2		OAR[b]	Cohort 1	Cohort 2
BMC1222	−35.1	+0.080 n.s.	+0.089 n.s.	CSRD247	14	+0.031 n.s.	+0.1972;**
MCM152	−18.5	−0.081 n.s.	−0.028 n.s.	D5S2	5	+0.058 n.s.	+0.2432;**
ILSTS59	−17.0	−0.105 n.s.	+0.025 n.s.	HSC	9	+0.068 n.s.	+0.1422;**
HUJ616	−8.2	+0.065 n.s.	−0.1151;*	INRA23	1	+0.084 n.s.	+0.102 n.s.
URB58	−1.4	−0.089 n.s.	−0.009 n.s.	INRA5	10	+0.4293;***	+0.2123;***
BMS1669	−0.6	−0.059 n.s.	+0.096 n.s.	INRA63	14	+0.072 n.s.	−0.006 n.s.
PRNPS04	0	−0.085 n.s.	−0.077 n.s.	MAF65	15	+0.010 n.s.	+0.011 n.s.
PRNPS05	0	−0.2483;***	−0.2863;***	MCM527	5	+0.047 n.s.	+0.064 n.s.
PRNPS11	0	−0.045 n.s.	+0.2901;*	OarCP49	17	−0.070 n.s.	+0.017 n.s.
PRNPS15	0	+0.166 n.s.	+0.018 n.s.	OarFCB11	2	+0.005 n.s.	+0.130 1;*
PRNPS24	0	+0.166 n.s.	−0.128 n.s.	OarFCB20	2	+0.062 n.s.	+0.121 n.s.
CTSBJ12	+21.0	−0.025 n.s.	+0.002 n.s.	OarFCB304	19	−0.088 n.s.	−0.051 n.s.
MMP9	+27.9	+0.074 n.s.	+0.2593;***				
BMS2319	+32.5	+0.1101;*	+0.3993;***				

n.s. not significant.

[a] Distance of any locus from PRNP (deviation in Mb, Ovine version 2.0 Genome Assembly map provided by the International Sheep Genomic Consortium, http://www.livestockgenomics.csiro.au/cgi-bin/gbrowse/oarv2.0/).

[b] Chromosome location.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.