Impact of selection for scrapie resistance on genetic diversity of the Sambucana sheep breed (Piedmont, Italy)

Sartore S., Rasero R., Colussi S., Acutis, P.L., Peletto S., Soglia D., Maione S., Spalenza V., Sacchi P. (Dept. Animal Production, University of Torino, and Istituto Zooprofilattico del Piemonte, Liguria e Valle d'Aosta, Italy)

INTRODUCTION

In Italy, as in other European countries, since 2005 a program of selection for scrapie resistance in sheep, based on the PRNP polymorphism, has been implemented with the aim of increasing ARR 'resistant' allele and eliminating VRQ 'susceptible' allele. In a small breed, the ARR-carriers may be more related to each other than ranndomly chosen animals; as a consequence, for an equal number of reproducers the effective size may be smaller than expected in a pure genetic drift condition (1). In the Sambucana sheep breed (reared in Piemonte region, north-west Italy) the ARR allele frequency was higher than in other breeds, like Biellese for example, before the selection plan started (2). On the other hand, due to the reduced number of animals (3500), this breed is considered at risk of extinction (Piemonte Regional Rural Development Plan for 2007-2013 period). The aim of the present investigation was to evalute the impact of scrapie resistance selection on genetic variation of the Sambucana.

MATERIALS AND METHODS

Two subsets of animals were analysed: 80 born in 2004, before the selection for scrapie resistance began in 2005 ('before 2005' group) and 67 born in 2008 and 2009 ('after 2005' group). The period between the two groups represents about one generation. The rams were randomly chosen among the young candidate sires, which were subsequently selected for both genotype at PRNP locus and morphological traits related to meat production. The DNA extraction was performed as by (3). All the animals were genotyped for PRNP by "IZS-CEA" (4). The ISAG 2005 microsatellite panel (15 loci) (http://www.isag.org.uk/comptest.asp) was used as a sample of selectively scrapie-neutral markers (outside OAR 13 on which PRNP maps) and multiplex PCRs were developed according to standardised protocols (5). 13 randomly chosen rams were reanalysed in order to estimate the rate of technical and allele scoring errors.

RESULTS AND DISCUSSION

Seven individual samples and the SPS113 marker were discarded due to consitent failure of amplifying. Error rate per multilocus genotype and average error rate per allele were 0.55% and 0.41% respectively (6). Genetic variation found at the neutral marker loci, i.e. number of alleles (*A*), allele size range, and OAR location, are reported in Table 1.

Locus	*A*	Size range (bp)	OAR
CSRD247	14	207-243	14
D5S2	6	187-199	5
HSC	11	265-297	9
INRA23	10	197-219	1
INRA5	12	113-147	10
INRA63	13	167-207	14
MAF214	9	184-264	16
MAF65	7	111-137	15
MCM527	8	164-178	5
AE129	4	133-165	5
CP49	14	77-115	17
FCB11	10	122-148	2
FCB20	7	87-107	29
FCB304	11	146-190	19

Table1

No linkage disequilibrium was assessed for the markers located on the same chromosome. The average number of alleles were 8.637 and 9.091 before and after 2005, respectively. INRA5, MAF214 and AE129 showed a significant deficiency of heterozygosity both before and after 2005 (data not shown). Based on the estimate performed with the Micro-Checker software, they could be affected by presence of null alleles, so they were discarded.

Allelic richness (*Rs*), gene diversity (*H*), observed heterozigosity (H_{obs}), and F_{IS} at the neutral marker loci are reported in Table 2: average values (standard error) INRA5, MAF214, and OARAE129 excluded (n.s.=not significant, **P<0.01, ***P<0.001; Δ=difference; *Rs* based on a minimum sample size of 63 animals).

		Before 2005	After 2005	Δ
Neutral markers	*Rs*	8.481 (0.728)	9.008 (0.795)	+0.527 n.s.
	H	0.740 (0.025)	0.726 (0.024)	−0.014 n.s.
	H_{obs}	0.719 (0.020)	0.661 (0.019)	−0.058**
	F_{IS}	+0.028 n.s.	+0.090***	-

Table2

The selection does not affect neutral loci as far as allelic richness and gene diversity is concerned whereas observed heterozigosity significantly decreases after 2005. According to (3), some within-flock founder effect could explain this evolutive picture even thoug some rams were used in a rotational mating scheme from 2004 to 2009.

PRNP allele frequencies are reported in Table 3 (n.s.=not significant, *P<0.05, **P<0.01, ***P<0.001; Δ=difference

	Before 2005	After 2005	Δ
ARR	0.319	0.567	+0.248***
ARQ	0.544	0.366	−0.178**
AHQ	0.025	0.037	+0.012 n.s.
ARH	0.019	0.000	−0.019 n.s.
VRQ	0.093	0.030	−0.063*

Table 3

PRNP allelic richness , gene diversity, observed heterozigosity, and F_{IS} are reported in Table 4 (n.s.=not significant; Δ=difference)

	Before 2005	After 2005	Δ
Rs	4.996	4.000	−0.996
H	0.596	0.547	−0.049
H_{obs}	0.650	0.522	−0.128
F_{IS}	−0.090 n.s.	+0.044 n.s.	-

Table 4

PRNP observed genotype frequencies are reported in Table 5.

	Before 2005	After 2005	Δ
ARR/ ARR	0.063	0.343	+0.280
ARR/ ARQ	0.412	0.388	−0.024
ARR/ AHQ	0.013	0.045	+0.032
ARR/ ARH	0.025	0	−0.025
ARR/ VRQ	0.063	0.015	−0.048
ARQ/ ARQ	0.275	0.134	−0.141
ARQ/ AHQ	0.038	0.030	−0.008
ARQ/ ARH	0	0	0
ARQ/ VRQ	0.088	0.045	−0.043
AHQ/ AHQ	0	0	0
AHQ/ ARH	0	0	0
AHQ/ VRQ	0	0	0
ARH/ ARH	0	0	0
ARH/ VRQ	0.013	0	−0.013
VRQ/ VRQ	0.013	0	−0.013

Table 5

Effectivness of selection evident from the increase of ARR and decrease of ARQ and VRQ. ARH is not present after 2005. Heterozygosities decrease more markedly than at the neutral loci, even thogh no significant deficiencies are detected after 2005. ARR/ARQ is always the most widely encountred genotype.

Measures of genetic difference between the two groups of young rams are reported in Table 6 (n.s.=not significant, ***P<0.001).

	F_{ST}
Neutral marker *loci*	0.006 n.s.
PRNP	0.073***

Table 6

*F*ST index is significant for the PRNP gene but not for the neutral loci. These results show that genetic differentiation is high for the direct object of selection but not higher than expected by chance for the unlinked portion of genome.

Effects of removal of VRQ-carriers rams on allelic richness and gene diversity at neutral marker loci are reported in Table 7 (average values (standad error), n.s.=not significant, **P<0.01; N=number of animals; cr=carrier rams; Δ=difference; Rs based on a minimum sample size of 58 and 59 animals before and after 2005, respectively).

Group	*N VRQ-cr removed*	*Rs*			*H*		
		Whole group	After removal	Δ	Whole group	After removal	Δ
Before 2005 (N=73)	13	8.388 (0.714)	8.601 (0.747)	+0.213**	0.740 (0.025)	0.737 (0.024)	−0.003 n.s.
After 2005 (N=67)	4	8.909 (0.783)	9.002 (0.794)	+0.093**	0.726 (0.024)	0.727 (0.024)	+0.001 n.s.

Table 7

If all the VRQ-carrier rams are removed the allelic richness increases and the gene diversity is almost unchanged. The carriers of undesirable PRNP genotypes would not be essential to maintain the overall diversity in the Sambucana breed.

CONCLUSIONS

In one time generation a significant response to scrapie resistance selection has ben observed whereas no sign of strong diversity decrease are evident. A medium-long-term conservation strategy should provide for the sire rotational mating scheme to be increased.

REFERENCES

1. Palhière I, Brochard M, Moazami-Goudarzi K, Laloe D, Amigues Y, Bed'hom B, Neuts E, Leymarie C, Pantano T, Cribiu EP, Bibé B, Varrier E . *Gentetics Selection Evolution* 40 (2008), 663-680.
2. Acutis PL, Peletto S, Sbaiz L, Riina MV, Maniaci MG, Ru G, Moda G, Caramelli M. *Large Animal Review* 11-3 (2005), 39-43.
3. Acutis PL, Sbaiz L, Verburg F, Riina MV, Ru G, Moda G, Caramelli M, Bossers A. *Journal of General Virology* 85 (2004), 3156-3172.
4. Acutis P.L., (2005)
5. Soglia D, Rossi L, Cauvin E, Citterio C, Ferroglio E, Maione S, Meneguz PG, Spalenza V, Rasero R, Sacchi P. *European Journal of Wildlife Research* 56 (2010) DOI: 10.1007/s10344-010-0382-0.
6. Pompanon F, Bonin A, Bellemain E, Taberlet P. *Nature Reviews Genetics* 6 (2005), 847-859.
7. Alvarez I, Gutierrez JP, Royo LJ, Fernandez I, Gayache F. *Preventive Veterinary Medicine* 91 (2009), 172-178.