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The genetic diversity in two local Italian sheep breeds: is selective breeding against scrapie susceptibility possible?

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ABSTRACT

The genetic variability and presence of population substructures in two conserved native Northern Italian sheep breeds (118 samples), Alpagota and Brogna, not involved into scrapie eradication plans imposed by the European commission (2003), were studied by investigating 17 microsatellite markers. Obtained data were used for computer simulation of successive generations of the two breeds, according to a pattern of within breed crossings and to the choice of breeding males based on the genotypic data for scrapie susceptibility. Assumptions were made for the mating scheme: the allelic frequencies of the sampled individuals are representative of the original populations, all rams and females have the same reproductive performances, males are selected by random drawing and used only for one round of mating, the number of individuals making up the sample population is fixed. Four new subsequent generations were simulated by using two different approaches: discarding males with unfavorable scrapie genotype (Risk Class V) and without selection of the males. The total number of alleles detected in Alpagota was 158 (mean 9.29±2.95), and in Brogna was 186 (mean 10.94±3.05). Differences in the mean number of alleles, expected and observed heterozygosity, and molecular coancestry were not detected for the selected and unselected populations of both breeds. Results show that, if assumptions are met, the selection against scrapie sensitivity is possible in low diffusion local breeds without compromising genetic diversity.
(Keywords: Alpagota, Brogna, sheep, scrapie, simulation)

INTRODUCTION

Scrapie is a fatal neurodegenerative disease belonging to the group of transmissible spongiform encelophathies, affecting sheep and goats. It has been shown that the susceptibility or resistance of sheep to scrapie is influenced mainly by mutation in the codons 136, 154 and 171 of the third exon of the prion protein (PrP) gene (*PRNP*) (*Hunter,* 1997). It is generally accepted that mutations coding for alanine at codon 136 (A_{136}) and for arginine at codon 171 (R_{171}) confer higher resistance, while those coding for valine (V_{136}) and glutamine (Q_{171}) render animals more susceptible to scrapie; polymorphisms in codon 154 are considered to have minor importance (*Hunter,* 1997; *Elsen et al.,* 1999). In the light of this scientific evidence, the European Union classified the genotypes at these three codons in five classes (Risk I: ARR/ARR, Risk II: ARR/AHQ AHQ/AHQ, Risk III: ARR/ARQ ARR/ARK ARR/ARH ARQ/ARQ ARQ/ARK ARQ/ARH ARQ/AHQ ARH/ARK AHQ/ARK AHQ/ARH, Risk IV:

ARR/VRQ ARK/VRQ AHQ/VRQ and Risk V: VRQ/VRQ ARQ/VRQ ARH/VRQ) from highly resistant (Risk I) to highly sensitive (Risk V) and established that breeding programmes aiming at decreasing scrapie susceptibility should be implemented in all European sheep breeds (*EC*, 2003). To achieve such goal breeding scheme must increase the frequency of the ARR/ARR genotype and eradicate completely the VRQ allele. However, application of these kind of schemes could be dangerous in many local breeds facing extinction as the available genetic variability will be reduced affecting conservation programmes as observed by several authors (*Alfonso et al.,* 2006; *Mann et al.,* 2007; *Van Kaam et al.,* 2008). In Italy today there is a statutory exception that permits the exclusion from the plan for the selection of rare breed sheep entered in the herd book; is optional the admission of many breeders to plan in these cases. The analysis of genetic variability and the estimation of its possible loss could be assessed using different criteria mainly based on pedigree information, anyway in sheep breeding and especially in small local populations, pedigree information are often unknown (*Goyache et al.,* 2003); in this case the use of molecular markers to evaluate molecular diversity could be a valuable alternative (*Alfonso et al.,* 2006; *Álvarez et al.,* 2007). The native breeds as Alpagota (ALP) and Brogna (BRO) analyzed, are historically tied to mountain communities of the Veneto Italian region and have always been used for the production of traditional dairy or meat products. The ALP is a small-sized sheep (Alpago mountains), and nowadays about 2.300 animals are enrolled in the herd book; it is listed in the FAO global databank for farm animal genetic resources where it is classified as endangered. It is a multipurpose breed (meat, milk, and wool), but now it is bred only for meat production due to its purported high quality. The BRO breed and is native to the Lessini mountains; at present the herd book accounts for about 1.450 animals. The BRO is a multipurpose breed (meat, milk, and wool), and its milk has been traditionally transformed to produce typical cheeses. The animals are medium to small in size and are very prolific, with a twinning rate of 58% as reported by *Pastore and Fabbris* (1999). The local sheep breeds as Alpagota (ALP) and Brogna (BRO), are reared in north-east Italy and nowadays they can be considered meat type. Their importance is due to their possibility to exploit marginal areas and to the production of typical products of economic interest in niche markets (es. "Alpago Lamb", Slow Food Presidia in the 2008). At present they are considered as endangered and are part of a conservation programme but no data on their susceptibility to scrapie are available.

Aim of this study was to simulate a mating scheme to evaluate the loss of heterozygosity in local sheep breeds under conservation scheme for reducing scrapie susceptibility.

MATERIALS AND METHODS

In this study we have analyzed 118 animals, 23 males and 42 females for BRO and 27 males and 26 females for ALP. From each individual, 250 µl of blood was transferred on paper, FTA cards (FTA Nucleic Acid Collection, Storage and Purification, Whatman) for more rapid DNA extraction. Two disks obtained punched the FTA card were treated by running 3 washes of 5 minutes each in 200 µl of FTA Purification Reagent and then a wash of 5 minutes 200 µl TE Buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). The stage of purification was made by treating punches with 70% ethanol washes in cold running two centrifugal to 4000 RPM for 5 minutes. Once dry, the disks were used directly in the amplification mix. The extracted DNA was amplified by PCR at 17 microsatellite loci (*Table 1*). Two punches were added 15 µl total reaction mixture

containing 1X PCR buffer (16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl pH 8.8, 0.01% Tween 20), 0.35 µl of forward and reverse primer, 0.2 mM dNTPs, 0.3 mM of $MgCl_2$ and 0.01 U/µl of *Taq* DNA polymerase, in a final volume of 15 µl. Details on the primer annealing temperatures can be found in *Dalvit et al.* (2008). Allele size was determined with a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA). A panel of 17 microsatellite markers was chosen according to *ISAG/FAO Standing Committee* (2004) recommendations and to previous studies (*Baumung et al.*, 2006) to investigate highly polymorphic markers spread throughout the genome.

Table 1

Microsatellite markers
with corresponding fragment size and chromosomal location

Locus	Fragment size	Ch.me
CSRD247	213–259	14
ILSTS87	138–178	6
OarCP34	100–128	3
OarFCB304	150–198	19
McM527	171–189	5
OarAE54	122–156	25
OarFCB20	92–122	2
URB058	161–209	13
OarAE129	137–157	5
OarAE119	147–185	19
INRA063	162–210	14
HSC	263–299	20
INRA023	196–224	1
MAF214	185–261	16
MAF65	121–143	15
OarCP49	69–119	17
TGLA53	140–168	12

Moreover, as a complete pedigree was absent, the investigated breeds were genotyped at 17 microsatellite loci to assess their genetic variability and to evaluate through simulations generations the possible variability loss if selection against sensitive genotypes will be carried out. The approach of simulation obtained by generating successive populations through Hybridlab software version 1.0 proposed by *Einar et al.* (2006), is used in this work to obtain information on temporal genetic populations of sheep which has available only a representative sample of the initial population but without adequate reference data. The following assumptions were made: a) the allelic frequencies of the sampled individuals are representative of the original populations, b) all rams and females have the same reproductive performances, c) males are selected by random drawing and used only for one round of mating, d) the number of individuals making up the sample population is fixed. For each real subject information about sex, scrapie genotype and the genotype at the 17 microsatellite loci and was used for selection and for the simulated mating process.

Population of 1000 samples was generated from the original individuals (real) (521 F and 479 M for ALP; 503 F and 479 M for BRO); these were considered all females was chosen as a number equal to 1/5 the number of females to males. By these males was extracted from a number equal to 1/20 (actual sex ratio in the populations investigated) the number of females from representative subgroups of allele frequencies (investigated for scrapie) real males. In this way it could create a generation simulated (pop_T0) Representative actual frequency of males, consisting of adult females (Fα), adult males (Mα) and male comeback year for domestic (Mβ). In the *Figure 1* shows the pattern of intersections adopted. Composition in the column are shown the respective compositions of the populations placed under review, taking stock of the study at the end of each of the 4 hypothesized reproductive events after the selections and the lambs before the next breeding. 2 cases were postulated, the absence of selection (NO selection) and selection for scrapie eradication (YES selection). In the first case the males are sampled randomly from the entire gene pool of the offspring, while in the latter case, the sample of subjects from which to extract the animals for the comeback is reduced because it eliminates the class V. There is selection in females. The number of offspring equals the number of breeding females on average prolificacy of the breed (if 145% for ALP and BRO)

Figure 1

Mating scheme used

Time line (year)	Event	Population	Composition
0	Mα + Mβ + Fα		
0	Mα **x** Fα	=> **pop_T0**	Mα + Mβ + Fα
1	Mγ + Fβ		
1	Mβ **x** Fα	=> **pop_T1**	Mβ + Fα + M**γs** + F**βs**
2	Mδ + Fγ		
2	M**γs x** (F**αs** + F**βs**)	=> **pop_T2**	M**γs** + (F**αs** + F**βs**)**s** + M**δs** + F**γs**
3	M**εs** + F**δs**		
3	M**δs x** ((F**αs** + F**βs**)**s** + F**γs**)	=> **pop_T3**	M**δs** + ((F**αs** + F**βs**)**s** + F**γs**)**s** + F**δs** + M**εs**

M = males, F = females, **+** = and (no reproduction), **x** = mating (yes reproduction), α-β-γ-δ-ε = progressive number generation, ***s*** = selection [F**αs** = 90% Fα; F**βs**-F**γs**-F**δs** = 10% Fβ-Fγ-Fδ (respectively); (F**αs**+F**βs**)**s** = 90% (F**αs**+F**βs**); (F**αs** + F**βs**)**s** + F**γs**)**s** = 90% (F**αs** + F**βs**)**s** + F**γs**); M**γs**-M**δs**-M**εs** = 10% (the number of Fα) of Mγ-Mδ-Mε (respectively)]

Number of alleles per locus, allelic frequencies, and observed and expected heterozygosity were calculated using Molkin version 3.0 and Genetix version 4.05.2

(*Belkhir et al.,* 1996-2004). A test for population differentiation was performed, as implemented in GENEPOP version 4.0. Molecular coancestry coefficients within and between breeds were measured according to *Caballero and Toro* (2002) using Molkin version 3.0 (*Gutiérrez et al.,* 2005). The differences for frequency scrapie genotype between observed and expected heterozygosity are tested by $\chi 2$ test.

RESULTS AND DISCUSSION

The total number of alleles detected in Alpagota (NO selection and YES selection) was 158 (mean 9.29, SD±2.95), and in Brogna (NO selection and YES selection) was 186 (mean 10.94, SD±3.05). The largest number of alleles was found at loci URB058 (16) for Alpagota (NO selection and YES selection) and OarCP49 (17) for Brogna (NO selection and YES selection); the smallest at locus McM527 (4) for Alpagota (NO selection and YES selection) and OarAE129 for Brogna (NO selection and YES selection). There is no difference between the number of alleles of the gene pool consists of 4 populations selected and the 4 selected for both Alpagota for Brogna. This indicates that if the assumptions were met would be possible to exclude the Risk Class V by selection without loss of genetic diversity. The genetic variability of each population was studied in terms of number of observed alleles and molecular coancestry, as shown in *Table 2.* The results of the analysis on simulated populations were consistent with those made by real people on *Dalvit et al.* (2009). The observed and expected heterozygosity were constant. Also the values of molecular coancestry constants are within-population. Considering then the percentages of allele frequencies (*Table 3*) is known as both slightly decreased the frequency of the VRQ allele in generations of populations subjected to selection for elimination of Risk Class V in both ALP and in BRO, while observing the opposite phenomenon in populations not subject to selection. Overall the differences for frequency scrapie genotype between observed and expected heterozygosity in all 4 populations was not significant ($\chi 2$ test).

Table 2

Number of analyzed sample (sample size), expected (H. exp.) and observed (H. obs.) heterozygosity, within-breed molecular coancestry (Kinsub) for each population analyzed

Population		**ALP_T0**	**ALP_T1**	**ALP_T2**	**ALP_T3**	**BRO_T0**	**BRO_T1**	**BRO_T2**	**BRO_T3**
Sample size		573	625	625	625	547	597	597	597
NO selection	H. obs.	0.7555	0.7548	0.7541	0.7520	0.7894	0.7892	0.7846	0.7820
	SD	±0.1595	±0.1564	±0.1577	±0.1549	±0.105	±0.1005	±0.0964	±0.0975
	H. exp.	0.7385	0.7387	0.7392	0.7388	0.7640	0.7643	0.7656	0.7668
	SD	±0.1524	±0.1509	±0.1516	±0.1495	±0.088	±0.0883	±0.0873	±0.0868
	Kinsub	0.2615	0.2613	0.2608	0.2612	0.2360	0.2357	0.2344	0.2332
YES selection	H. obs.	0.7572	0.7523	0.7494	0.7443	0.7876	0.7851	0.7797	0.7783
	SD	±0.1586	±0.1587	±0.1594	±0.1576	±0.107	±0.1081	±0.105	±0.1041
	H. exp.	0.7385	0.7380	0.7384	0.7372	0.7639	0.7630	0.7627	0.7624
	SD	±0.1515	±0.1531	±0.1534	±0.1529	±0.0881	±0.0887	±0.0885	±0.0901
	Kinsub	0.2615	0.2620	0.2616	0.2628	0.2361	0.2370	0.2373	0.2376

Table 3

Percentage of total alleles

	Pop	Sample	% of total alleles					
			ARR	**ARQ**	**AHQ**	**ARH**	**ARK**	**VRQ**
NO selection	ALP_T0	1146	15.36	63.00	9.16	0.79	0.87	10.82
	ALP_T1	1250	14.88	64.16	8.88	0.64	0.88	10.56
	ALP_T2	1250	15.84	63.20	8.16	0.48	0.96	11.36
	ALP_T3	1250	15.92	63.36	7.92	0.40	1.04	11.36
	BRO_T0	1094	23.03	48.54	0.91	2.83	1.55	23.13
	BRO_T1	1194	23.28	48.07	0.84	2.93	1.68	23.20
	BRO_T2	1194	23.53	47.82	0.75	2.60	1.68	23.62
	BRO_T3	1194	22.95	48.66	0.75	2.18	1.68	23.79
YES selection	ALP_T0	1146	16.06	62.83	8.64	0.79	1.05	10.65
	ALP_T1	1250	16.56	62.80	8.72	0.72	0.96	10.24
	ALP_T2	1250	16.56	62.16	9.20	0.72	1.12	10.24
	ALP_T3	1250	16.40	61.92	9.60	0.96	1.28	9.84
	BRO_T0	1094	23.86	49.09	0.91	2.83	1.65	21.66
	BRO_T1	1194	23.37	49.75	0.84	3.35	1.76	20.94
	BRO_T2	1194	23.87	49.25	0.92	3.27	2.26	20.44
	BRO_T3	1194	24.54	49.83	0.84	3.10	1.93	19.77

CONCLUSION

In the Veneto local sheep breeds the selection plan for reducing scrapie susceptibility (*EC*, 2003) is not applied, according to Italian law which allows exemption of farm management. Moreover, the reduced number of the population is the problem that a possible selection as that prohibiting the use of sheep belonging to Risk Class V should gradually increasing the loss of heterozygosity in local sheep breeds. In this study it was possible to verify that the results of analysis on simulated populations were consistent with those made in previous study. The assumptions used in this study were upheld in reality, the plan coupled with selection and elimination of males belonging to the Risk Class V may be used, because:

- there is no difference between the number of alleles of the gene pool compost from 4 populations selected by the 4 selected for both Alpagota for Brogna,
- the molecular coancestry values are constant within-population,
- the simulation shows a decrease in the frequency of the VRQ allele in generations of populations subjected to selection, unlike those not subject to selection.

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REFERENCES

Alfonso, L., Parada, A., Legarra, A., Ugarte, E., Arana, A. (2006). The effects of selective breeding against scrapie susceptibility on the genetic variability of the Latxa Black-Faced sheep breed. Genet. Sel. Evol., 38. 495-511.

Álvarez, I., Royo, L.J., Gutiérrez, J.P., Fernández, I., Arranz, J.J., Goyache, F. (2007). Genetic diversity loss due to selection for scrapie resistance in the rare Spanish Xalda sheep breed. Livestock Science. 111. 204-212.

Baumung, R., Cubric-Curik, V., Schwend, K., Achmann, R., Sölkner, J. (2006). Genetic characterisation and breed assignment in Austrian sheep breeds using microsatellite markers information. J. Anim. Breed. Genet., 123. 265-271.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F. (1996-2004). GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions. CNRS UMR 5000, Université de Montpellier II, Montpellier, France. http://www.genetix.univ-montp2.fr/genetix/genetix.htm

Caballero, A.,Toro, M.A. (2002). Analysis of genetic diversity for the management of conserved subdivided populations. Conserv. Genet., 3. 289-299.

Dalvit, C., De Marchi, M., Zanetti, E., Cassandro, M. (2009). Genetic variation and population structure of Italian native sheep breeds undergoing in situ conservation. J. Anim. Sci., 87. 3837-3844.

Dalvit, C., Saccà, E., Cassandro, M., Gervaso, M., Pastore, E., Piasentier, E. (2008). Genetic diversity and variability in Alpine sheep breeds. Small Rumin. Res., 80. 45-51.

EC (2003). Commission decision (2003/100/EC) of 13 February 2003 laying downminimum requirements for the establishment of breeding programmes for resistance to transmissible spongiform encephalopathies in sheep. Official Journal of the European Union, 14.2.2003, L41/41.

Elsen, J.M., Amigues, Y., Schelcher, F., Ducrocq, V., Andreoletti, O., Eychenne, F., Tien Khang, J.V., Poivey, J.P., Lantier, F., Laplanche, J.P. (1999). Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov. Archives of Virology. 144. 431-445.

Gutiérrez, J.P., Royo, L.J., Álvarez, I., Goyache, F. (2005). MolKin v2.0: A computer program for genetic analysis of populations using molecular coancestry information. J. Hered., 96. 718-72.

Hunter, N. (1997). Molecular biology and genetics of scrapie in sheep. In: Piper, L., Ruvinsky, A. (Eds.): The Genetics of Sheep. CAB International, Wallingford, 225-240.

ISAG/FAO Standing Committee. (2004). Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans. Measurement of Domestic Animal Diversity (MoDAD): Recommended Microsatellite Markers. http://dad.fao.org/cgi-bin/getblob.cgi?sid=ca53b91a6f7c80be8e7066f4a5066dc1,50006220

Man, W.Y.N., Lewisb, R.M., Boultonc, K., Villanueva B. (2007). Predicting the consequences of selecting on PrP genotypes on PrP frequencies, performance and inbreeding in commercial meat sheep populations. Genet. Sel. Evol., 39. 711-729.

Nielsen, E.E.G., Bach, L.A., Kotlicki, P. (2006). Hybridlab (version 1.0): a program for generating simulated hybrids from population samples. Molecular Ecology Notes. 6. 4. 971-973.

Pastore, E., Fabbris, L. (1999). L'allevamento ovi-caprino nel Veneto, analisi e prospettive future di un settore ricco di storia. Veneto Agricoltura ed., Legnaro (PD), Italy.

Van Kaam, J.B.C.H.M., Finocchiaro, R., Vitale, M., Pinelli, F., Scimonelli, M., Vitale, F., Portolano, B., Oltenacu, P.A., Caracappa, S. (2008). Prion protein gene frequencies in three Sicilian dairy sheep populations. Ital. J. Anim. Sci., 7. 87-94.

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