Genetic diversity loss due to selection for scrapie resistance in the rare Spanish Xalda sheep breed

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Abstract

The effect of selection for scrapie resistance on genetic variability in the rare Xalda sheep breed was studied. Pedigree information comprised 1851 animals (1444 alive) at the moment of sampling. A total of 304 reproductive (or selected for reproduction) Xalda individuals were sampled and genotyped for 14 microsatellites. Genetic variability was assessed using: gene diversity (1−average kinship, GD), mean average relatedness (AR) and self-coancestry (ci) at the genealogical level; and expected heterozygosity (He), molecular mean kinship (Mk), molecular self-coancestry (si) and rarefacted average number of alleles per locus (A) at the molecular level. Two breeding strategies were evaluated: a) use of only young rams with genotype ARR/ARR and young ewes with low to moderate risk (risk groups R1 to R3); b) breeding without selection for PrP genotypes. The major cause of losses of genetic variability in the Xalda breed is the drift that occurs when a new group of reproductive individuals is selected. The loss of genetic variability is small in females compared to the males, where it is considerable. However, losses at the molecular level for young females with respect to adult females were above 5%. Young male individuals also retained most of the genetic variability assessed in adult rams. Selection against susceptibility to scrapie produced additional losses of more than 2% for He and A when rYF individuals are considered. As regards males, the situation becomes critical because of the scant number of available ARR/ARR young rams. The consequences for the management of the Xalda breed are discussed.

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1. Introduction

Scrapie is one of the diseases of the group of transmissible spongiform encephalopathies, which also includes Creutzfeldt–Jakob disease in humans and bovine spongiform encephalopathy (BSE) in cattle (Hunter, 1997; Prusiner, 1998).

In the light of scientific evidence relating mutation in the codons 136, 154 and 171 of the third exon of the PrP gene to the degree of susceptibility to scrapie in sheep (Hunter, 1997; Elsen et al., 1999; O'Doherty et al., 2002), the European Union has classified the alleles and the genotypes for scrapie in five categories from highly resistant (R1) to highly sensitive (R5) and has decided that breeding programs aimed at decreasing susceptibility to scrapie should be implemented in all the sheep breeds in its territory (European Commission, 2003; see also Gama et al., 2006, for a review). The goals of these programmes...
must include increasing the frequency of the ARR allele and the ARR/ARR homozygous genotype, which are considered highly resistant to scrapie, and the elimination of the VRQ allele, which has been shown to be highly sensitive to clinical scrapie (Hunter, 1997; Elsen et al., 1999; European Commission, 2003). These kinds of breeding programmes are criticised because they unavoidably reduce the available genetic variability in a breed, thus affecting selection and conservation programmes in sheep.

The protection of the genetic base of a population in risk has usually been measured by the rate of inbreeding (Windig et al., 2004). However, the computation of the individual coefficient of inbreeding is highly sensitive to the quality of the available pedigree information, thus making this parameter difficult to interpret (Goyache et al., 2003). Consequently, a number of recent studies (Caballero and Toro, 2000, 2002; Fernández et al., 2005) have used expected heterozygosity (Nei, 1987), usually called gene diversity (GD), as a criterion for quantifying genetic variability. In addition, recent studies (Caballero and Toro, 2002; Eding and Meuwissen, 2001) have formalised the way in which it is possible to obtain coancestry coefficients from molecular information by applying Malécot’s (1948) definition to the marker genes, though referring to identity by state instead of identity by descent. Owing to its straightforward relationship with genealogical coancestry, this parameter has been shown to be useful for conservation purposes (Toro et al., 2002, 2003; Royo et al., 2007). From a molecular perspective, another important measure of variability is allelic richness or average number of alleles per locus (4; Hurlbert, 1971; Fernández et al., 2005).

Several studies analysed how selection for scrapie resistance can affect performance traits (Brandsma and Visscher, 2004; De Vries et al., 2005; Vitezica et al., 2005). However, the assessments of the effect of such breeding programmes on the genetic variability in small-rare sheep breeds are scarce and have been only conducted at the genealogical level (Windig et al., 2004). Here, the effect of selection for scrapie resistance on genetic variability in the rare Xalda sheep breed is assessed, using theoretically equivalent parameters at the genealogical and molecular level. The study is done assuming the actual selective policy carried out by the Xalda breeders. The consequences on the management of the Xalda breed will be discussed.

2. Materials and methods

2.1. Flockbook information and risk groups

The Xalda (Álvarez Sevilla et al., 2004) is an endangered sheep breed mainly located in Asturias (Northern Spain) that can be included within the Spanish Celtic sheep breeds including Churra and Latxa breeds (Álvarez et al., 2004). During the 1990s, a conservation program commenced with the foundation of a flockbook, the recovery of reproductive individuals in isolated flocks and the implementation of pure breed mating policy (Álvarez Sevilla et al., 2004; Goyache et al., 2003). Apart from preservation, breeders select individuals for reproduction by their accordance to the breed standard (Álvarez Sevilla et al., 2004; Goyache et al., 2003) and a significant number of individuals (especially males) born each lambing season do not remain in the flocks.

The information registered in the Xalda flockbook since its foundation to June 1st 2005 was obtained from the breeders association (ACOXA). The flockbook included a total of 1851 animals (217 males) and more than 100 small sized flocks. The total number of flocks registered in the flockbook is significantly higher than the number of active flocks each year due to the short duration of some of them. Up to 1444 individuals (134 males) were alive at the moment of sampling and a total of 58 flocks were active. Flocks are of small size and usually have only one ram. Though the number of founders is large (325) the pedigree knowledge at population level is high (79.8% of the fathers and 55.1% of the mothers known). No Xalda individual can be traced more than nine generations back in its pedigree. From the living reproductive individuals over 39% aged between 2 and 3 years old and 43% aged between 4 and 6 years old. Rams have usually a large reproductive life and are commonly exchanged between flocks. In this respect, over 28% of the available rams is 7 or more years old.

PrP genotypes, analysed at the Central Veterinary Laboratory of the Spanish Ministry of Agriculture, corresponding to the individuals born in the last lambing season were also provided by ACOXA. Individual genotypes were classified in five risk groups (European Commission, 2003) from R1 (very low) to R5 (greatest risk). A more detailed description of the risk groups can be found in Table 1.

2.2. Definition of breeding groups and breeding strategies

In order to define breeding groups for genetic analyses, the living individuals registered in the flockbook were grouped in the following groups according to age and sex: a) adult males (AM); b) adult females (AF); c) young males (YM); and d) young females (YF). The AM and AF groups were formed by, respectively, 123
and 1128 individuals, totalling 1251 individuals. The YM and YF groups included, respectively, 11 and 182 individuals (totalling 193 individuals) that are those male and female individuals born in the last lambing season and selected for reproduction.

PrP genotype was considered in the groups of young individuals in order to account for additional losses of variation due to selection against susceptibility to scrapie. Breeding strategies with and without considering PrP genotype as the selective criterion were assessed and results were shown in a relative way with respect to the AM and AF groups.

When PrP genotypes are considered for selection the following criteria were taken into account: a) European rules aimed at increasing the frequency of the genotype ARR/ARR and elimination of the VRQ allele (European Commission, 2003); and b) the interest of the breeders association (ACOXA) in reducing the frequency of the ARQ allele, which has been shown to be associated with the highest risk of BSE in sheep (Baylis, 2002), thus reducing the risk to human health. Consequently, the additional losses of genetic variability due to selection for resistance to scrapie were assessed in a scenario in which only YM individuals with genotype ARR/ARR (risk group R1) and YF individuals with R1 to R3 risk group genotypes (which do not include either the VRQ allele or the ARQ/ARQ genotype) were expected to be used for reproduction. The assayed strategy coincides basically with that recently shown by Molina et al. (2006), consisting in genotyping rams and eliminating ARQ/ARQ and VRQ carriers, as the best strategy to improve the resistance and would cause minimal cost and loss of genetic variability.

Throughout the manuscript, the YF individuals with PrP genotype included in risk groups R1 to R3 and the R1 YM individuals will be considered ‘resistant’ and denoted as rYF and rYM, respectively. Results were compared with a breeding strategy in which no PrP genotype is used as the selective criterion, comparing the genetic variability retained by all the available YM and YF individuals or rYM and rYF individuals, always in reference to the AM and AF groups.

### Table 1

Classification of PrP genotype in risk groups, risk description, genotype frequencies and allelic frequencies for the PrP gene in the selected individuals for reproduction (see text) in the Xalda sheep breed of Asturias, by sex of the individuals (YM — males and YF — females)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Risk group</th>
<th>Risk description At individual level</th>
<th>Risk description At progeny level</th>
<th>Reference group</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARR/ARR</td>
<td>R1</td>
<td>Very low</td>
<td>Very low</td>
<td>YM 2&lt;sup&gt;a&lt;/sup&gt; (18.18)</td>
</tr>
<tr>
<td>ARR/AHQ</td>
<td>R2</td>
<td>Low</td>
<td>Low</td>
<td>YF 9&lt;sup&gt;b&lt;/sup&gt; (4.95)</td>
</tr>
<tr>
<td>AHQ/AHQ</td>
<td>R3</td>
<td>Low</td>
<td>Not low depending on the genotype of the other parent</td>
<td></td>
</tr>
<tr>
<td>ARR/AHQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHQ/ARH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARQ/ARQ</td>
<td>R4</td>
<td>Scrapie occasionally recorded</td>
<td>Higher risk than in R3 progeny</td>
<td>YM 1&lt;sup&gt;b&lt;/sup&gt; (0.55)</td>
</tr>
<tr>
<td>ARQ/VRQ</td>
<td></td>
<td></td>
<td></td>
<td>YF 60&lt;sup&gt;b&lt;/sup&gt; (32.97)</td>
</tr>
<tr>
<td>ARQ/ARH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHQ/VRQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARQ/VRQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARH/ARH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VRQ/VRQ</td>
<td>R5</td>
<td>Greatest risk</td>
<td>Greatest risk</td>
<td>YM 3 (1.65)</td>
</tr>
<tr>
<td>ARH/VRQ</td>
<td></td>
<td></td>
<td></td>
<td>YF 3 (1.55)</td>
</tr>
<tr>
<td>ARQ/VRQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHQ</td>
<td></td>
<td></td>
<td></td>
<td>YM 1 (0.27)</td>
</tr>
<tr>
<td>ARH</td>
<td></td>
<td></td>
<td></td>
<td>YF 8 (2.2)</td>
</tr>
<tr>
<td>ARQ</td>
<td></td>
<td></td>
<td></td>
<td>YM 18 (75)</td>
</tr>
<tr>
<td>ARR</td>
<td></td>
<td></td>
<td></td>
<td>YF 82 (22.53)</td>
</tr>
<tr>
<td>VRQ</td>
<td></td>
<td></td>
<td></td>
<td>YM 5 (1.37)</td>
</tr>
</tbody>
</table>

Frequencies are given in absolute values and as percentages (in brackets).

<sup>a</sup> These individuals form the rYM group.

<sup>b</sup> These individuals form the rYF group.

### 2.3. Genealogical analyses

Genealogical analyses on pedigree information were carried out using the program ENDOG (Gutiérrez and...
The variables given are genealogical gene diversity (GD), average relatedness (AR) and genealogical self-coancestry (c). The full coancestery (f) and GD are related in the following manner: \( AR = (1 + (AR/2))/2 \). Notice that these \( 2 \) individuals are a part of the YM individuals.

### Table 2

<table>
<thead>
<tr>
<th>Breeding group</th>
<th>Abbreviation</th>
<th>( N )</th>
<th>( n )</th>
<th>GD ( e )</th>
<th>AR</th>
<th>( H_e )</th>
<th>Mk</th>
<th>( s )</th>
<th>( A_{60} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult reproductive females</td>
<td>AF</td>
<td>1128</td>
<td>212</td>
<td>0.987</td>
<td>0.025</td>
<td>0.513</td>
<td>0.699</td>
<td>0.307</td>
<td>0.684</td>
</tr>
<tr>
<td>Female lambs selected for reproduction</td>
<td>YF</td>
<td>182</td>
<td>33</td>
<td>0.982</td>
<td>0.036</td>
<td>0.517</td>
<td>0.648</td>
<td>0.346</td>
<td>0.724</td>
</tr>
<tr>
<td>Female lambs selected for reproduction with desirable PrP genotype rYM</td>
<td>rYM</td>
<td>71</td>
<td>71</td>
<td>0.981</td>
<td>0.039</td>
<td>0.524</td>
<td>0.631</td>
<td>0.343</td>
<td>0.724</td>
</tr>
<tr>
<td>Adult reproductive males</td>
<td>AM</td>
<td>123</td>
<td>48</td>
<td>0.981</td>
<td>0.037</td>
<td>0.519</td>
<td>0.672</td>
<td>0.317</td>
<td>0.731</td>
</tr>
<tr>
<td>Male lambs selected for reproduction</td>
<td>YM</td>
<td>11</td>
<td>11</td>
<td>0.982</td>
<td>0.036</td>
<td>0.513</td>
<td>0.699</td>
<td>0.307</td>
<td>0.684</td>
</tr>
<tr>
<td>Male lambs selected for reproduction with desirable PrP genotype rYM</td>
<td>rYM</td>
<td>2⁶</td>
<td>2⁶</td>
<td>0.970</td>
<td>0.060</td>
<td>0.533</td>
<td>0.537</td>
<td>0.342</td>
<td>0.703</td>
</tr>
<tr>
<td>Total population</td>
<td></td>
<td>1444</td>
<td>304</td>
<td>0.987</td>
<td>0.026</td>
<td>0.513</td>
<td>0.685</td>
<td>0.316</td>
<td>0.695</td>
</tr>
</tbody>
</table>

The variables given are genealogical gene diversity (GD), average relatedness (AR) and genealogical self-coancestry (c), expected heterozygosity (\( H_e \)), molecular mean kinship (Mk), molecular self-coancestry (s), average number of alleles per locus (A) rarefacted to 6 copies.

\( a \) Computed as \( D = 1 - \bar{f} \), \( \bar{f} \) being the average coancestry.

\( b \) Notice that these \( 71 \) individuals are a part of the YF individuals.

\( c \) Notice that these \( 2 \) individuals are a part of the YM individuals.

### 2.4. Sampling and molecular analyses

Blood samples were randomly obtained from a total of 304 reproductive (or selected for reproduction) Xalda individuals, kept in the 58 active flocks included in the ACOXA flockbook at the period of sampling. Samples were assigned to the corresponding reference breeding group according to the aforementioned criteria. The number of young individuals sampled was 44 (11 YM and 33 YF), whilst the sampled adult individuals summed to 260 (48 AM and 212 AF). Throughout the paper, the sampled individuals will be considered to be representative of the corresponding YM, YF, AM and AF groups.

Total DNA was isolated from blood samples following standard procedures (Sambrook et al., 1989). A set of 14 microsatellites (BM8125, BM6526, CP34, BM757, INRA006, BM6506, BM1818, FCB128, CSSM31, CSSM66, ILSTS011, McMS3, RM006, ILSTS005) previously used in Álvarez et al. (2004, 2005c) was analyzed in all the sampled individuals. The PCR products were labelled using a fluorescent method (Cy5 labeled primer) and genotyping was performed on an ALFexpressII automated sequencer (Amersham Biosciences, Barcelona). The microsatellite set used here was tested for linkage disequilibrium in Álvarez et al. (2004).

Analyses on molecular information were carried out using the program MolKin (Gutiérrez et al., 2005a). The following parameters were computed at the reference population level: expected heterozygosity (\( H_e \); Nei, 1987) and average number of alleles per locus (A) corrected using Hurlbert’s rarefaction method (1971) as \( A(g) = \sum_{k=0}^{N} \left[ 1 - \left( 1 - \frac{A_{(g)} - 0.5}{N_k} \right) \right] / \frac{N_k}{N} \), where \( g \) is the specified sampled size, \( N \) the number of gene copies (alleles).
examined in a given locus \((N > g)\), and \(N_i\) the number of occurrences of the \(i^{th}\) allele among the \(N\) sampled gene copies to account for sample size. Notice that, in the case of no failure in genotyping, the maximum value of \(N\) in a given population is twice the sampling size and the maximum value of \(g\) is twice the number of individuals forming the population with the lowest sampling size.

The full molecular coancestry matrix between the genotyped individuals was computed. The molecular coancestry \((M)\) between two individuals \(i\) and \(j\) is the probability that two randomly sampled alleles from the same locus in two individuals are identical by state \((\text{Caballero and Toro, 2002})\). Molecular coancestry between two individuals \(i\) and \(j\) at a given locus can be computed using the following scoring rules \((\text{Caballero and Toro, 2002; Eding and Meuwissen, 2001})\):

\[
M_{ij,l} = \frac{1}{4} \left[ I_{11} + I_{12} + I_{21} + I_{22} \right],
\]

where \(I_{xy}\) is 1 when allele \(x\) on locus \(l\) in individual \(i\) and allele \(y\) on the same locus in individual \(j\) are identical, and zero otherwise. Note that this value can take only four values: 0, \(\frac{1}{4}\), \(\frac{1}{2}\) and 1.

The molecular coancestry between two individuals \(i\) and \(j\) \((M_{ij})\) can be obtained by simply averaging over \(L\) analyzed loci as \(M_{ij} = \frac{1}{L} \sum_{l=1}^{L} M_{ij,l}\). Moreover, the individual molecular self-coancestry \((s_i)\) was computed as \(s_i = \frac{1 + F_i}{2}\), where \(F_i\) is not individual inbreeding, but homozygosity. Both \(M\) and \(s\) were averaged within- and between-reference groups when necessary. The between-individuals average-distance \((D_k)\) matrix was computed as \(D_k = \frac{(s_i + s_j)}{2} - M_{ij}\) \((\text{Caballero and Toro, 2002})\), where \(s_i\) is the molecular self-coancestry for individual \(i\) and \(M_{ij}\) the molecular coancestry between individuals \(i\) and \(j\). When necessary, these parameters were averaged over all the within- or between-group pairs of individuals.

Notice that \(H_e\), \(M\) and \(s\) are related in a similar way than that reported for their theoretically equivalent genealogical parameters: \(M = \frac{2s - 1}{1 - H_e}\) and the \(s\) of the offspring of two individuals with molecular coancestry of \(M\) will be \(s = \frac{1 + M}{2}\).

### 3. Results

Genotypic and allelic frequencies on the PrP gene for young individuals are given in Table 1. The ARQ allele frequency was roughly 75%. The second most frequent allele was the favourable ARR (with a frequency of roughly 20%), whilst the undesirable VRQ allele was present at a frequency of 1.3%. From the PrP genotypes 55.4% (72.7% for the YM individuals) were ARQ/ARQ, which is classified at the undesirable risk level

\[
\text{Fig. 1. Ratios (in percentage with respect to adult individuals) of the genetic variability retained by the young Xalda individuals selected for reproduction with (black bars) and without (open bars) using the PrP genotype as selective criterion. Plot A.1 gives the ratios for expected heterozygosity (}H_e\text{), rarefacted (to six copies) average number of alleles per locus (}A_{ir}\text{) and genealogical gene diversity (}GD\text{) corresponding to female individuals (ratio }YF/AF\text{, black bars; and ratio }rYF/AF\text{, open bars), whilst plot B.1 gives the same information for male individuals (ratio }YM/AM\text{, black bars; and ratio }rYM/AM\text{, open bars). Plot A.2 gives the ratios for expected molecular self-coancestry (}s\text{) and genealogical self-coancestry (}c\text{) corresponding to female individuals (ratio }YF/AF\text{, black bars; and ratio }rYF/AF\text{, open bars), whilst plot B.2 gives the same information for male individuals (ratio }YM/AM\text{, black bars; and ratio }rYM/AM\text{, open bars).}
\]
Moreover, the beneficial ARR/ARR genotype is only present in 5.7% of individuals (18.2% of the males, comprising 2 young rams).

Genealogical and molecular parameters characterising the genetic variability in the reference groups and for the whole population are given in Table 2. The expected heterozygosity, molecular mean kinship, molecular self-coancestry and rarefied (to 6 copies) average number of alleles per locus for the whole data set were 0.685, 0.315, 0.695 and 3.2, respectively. At the genealogical level, GD was 0.987, mean AR was 0.026 and genealogical self-coancestry for the whole pedigree was 0.513. Notice that maker-based identity values are always higher than those computed from pedigree information due to the pedigree-based parameters assume that founder alleles are unique and in the present population they are only identical by descent (Malécot, 1948) whilst in the case of molecular-based information two alleles can be identical by state (Caballero and Toro, 2002).

The main results given in Table 2 were graphically represented in Fig. 1 as percentage of the genetic variability retained by the resistant groups with respect to the corresponding AF and AM groups. Plot A.1 in Fig. 1 shows that rYF individuals retained most of the genetic variability computed for YF individuals. In turn, the YF group retained 92.64%, 94.69% and 99.48% of the values observed respectively for $H_e$, $A_{(6)}$ and GD in the AF group. On the other hand, self-coancestry is slightly higher, respectively reaching ratios of 105.90% and 100.87% for $s$ and $c$ with respect to AF individuals (see plot A.2 in Fig. 1). Notice that self-coancestry is not diversity but an identity measure and then values above 100% characterise loss of diversity. The rYF individuals retained more than 97.5% of the variability computed for the YF group for $H_e$, $A_{(6)}$ and GD, the molecular self-coancestry levels were basically the same and the ratio rYF/YF for genealogical self-coancestry is only slightly higher (101.2%).

The ratios corresponding to males followed a similar pattern. The percentages of variability retained by YM individuals are comparable to those reported above for the YF group. YM individuals retained 97.07%, 99.07%, and 100.07% of the variability computed in the AM group for $H_e$, $A$ and GD, respectively (see plot B.1 in Fig. 1). Parameters $s$ and $c$ reached ratios of 105.90% and 100.87%, respectively, with respect to AF individuals (see plot B.2 in Fig. 1). With respect to the YM group, rYM individuals retained 79.89%, 94.12%, and 98.75% of the variability computed respectively for $H_e$, $A_{(6)}$ and GD. The corresponding ratios for $s$ and $c$ were respectively favourable at 96.1% and 102.1%.

<table>
<thead>
<tr>
<th></th>
<th>$D_{av}$</th>
<th>$D_k$</th>
</tr>
</thead>
<tbody>
<tr>
<td>YF–AF</td>
<td>0.499</td>
<td>0.373</td>
</tr>
<tr>
<td>rYF–AF</td>
<td>0.500</td>
<td>0.376</td>
</tr>
<tr>
<td>YM–AM</td>
<td>0.490</td>
<td>0.381</td>
</tr>
<tr>
<td>rYM–AM</td>
<td>0.476</td>
<td>0.552</td>
</tr>
</tbody>
</table>

Table 3
Differentiation between the YF and YM groups (considering – rY – the selective criterion of resistance to scrapie, or not) with respect to AF and AM individuals assessed through $D_{av}$ and $D_k$ for genealogical and molecular information, respectively (see text)

Differentiation assessed using $D_{av}$ and $D_k$, among YM and YF groups with the corresponding AF and AM groups is given in Table 3. The differentiation of the YF and rYF individuals with respect to AF individuals is virtually the same regardless of the source of information used (molecular or genealogical). However, with regard to the males, rYM individuals present a lower differentiation with respect to the AM group than the total available group of YM animals. $D_k$ is more than 31% lower for rYM than for YF (0.552 vs 0.381), whilst $D_{av}$ is 2.9% lower for the same pairs (0.476 vs 0.490). This scenario is consistent with the higher values of AR and Mk found in rYM individuals with respect to YM rams (see Table 2), thus highlighting the higher genetic representation of the rYM genotypes in the whole group. In fact, the rYM individuals have higher values of genealogical and molecular coancestry with the AM group than the whole YM individuals thus reducing the differentiation assessed using kinship-based genetic distances.

4. Discussion

The main goal of the present analysis is to assess the effect of selection for scrapie resistance on genetic variability in the rare Xalda sheep at the early stages of such a selective programme. To do so, we used a set of theoretically equivalent parameters at the genealogical and molecular level to obtain reliable conclusions from their analysis.

The predominant allele in the Xalda breed is ARQ, which has been shown to be also predominant in other Iberian coarse-wool sheep breeds (Álvarez et al., 2006; Gama et al., 2006; Álvarez et al., 2005a,b). The most frequent PrP genotype in the Xalda sheep is ARQ/ARQ, which is classified with an unacceptable risk to scrapie (R4). This scenario suggests the need to implement a breeding policy in order to increase genetic resistance to scrapie in the Xalda breed. However, frequencies of PrP genotypes with intermediate to low susceptibility to scrapie (from R1 to R3) comprise roughly a third of the
population and the proportion of ARR/ARQ heterozygotes represents a quarter of the individuals, thus allowing the implementation of breeding strategies leading to gradual rather than drastic selection of ARR genotypes. Recently, Alfonso et al. (2006), in the non-risk black-faced Latxa of Navarra dairy sheep breed, proposed a selection regime consisting in using only ARR/ARR reproductive males (or with a small proportion of ARR heterozygote rams), without selection for PrP genotype in females. However, this strategy cannot be explored in the Xalda sheep because this would not guarantee the elimination of the VRQ allele in the population. In this respect, since the presence of VRQ alleles in the population is limited, the elimination of VRQ-carrying animals would not result in extreme erosion of the genetic stock.

The present analysis was carried out assuming that the major cause of losses of genetic variability in the Xalda breed is the drift that occurs when a new group of reproductive individuals is selected. It can be argued that strict mating policies based on the minimisation of the average coancestry of the new reproductive individuals (Caballero and Toro, 2000) should be implemented in the breed in order to conserve its genetic variability. However, strict selective criteria based on individual coancestry coefficients are difficult to apply in the Xalda breed because selection depends on compliance with the breed standard, assignment of the individual to a founder line and the particular breeding situation of the flocks, among other factors. Consequently, most decisions aimed at avoiding losses of genetic diversity are taken at the flock level, computing average AR coefficients that may suggest the introduction of new, under-represented animals in a given flock (Goyache et al., 2003). Superimposed on this situation, the influence of the new requirements regarding selection against susceptibility to scrapie on the genetic variability of the Xalda breed must be assessed, first taking into account the drift due to selection.

In this respect, no losses of genetic variability at genealogical levels have been assessed for YF with respect to AF individuals, whilst losses at molecular levels are above 5%. YM individuals also retain most of the genetic variability assessed in AM individuals. Selection against susceptibility to scrapie produces additional losses of more than 2% for \( H_e \) and \( A \) when rYF individuals are considered. Regarding males, the situation becomes critical because of the scant number of available ARR/ARR young rams: \( H_e \) in rYM individuals is 20% lower than that of the whole group of young rams and \( A \) is roughly 6% lower, thus limiting the long-term evolutionary potential of the population. Moreover, the increase in self-coancestry with respect to the adult individual groups indicates that the diversity retained by young individuals is due more to between-individuals than to within-individuals diversity (Caballero and Toro, 2000, 2002). This general situation coincides with that observed for the parameter AR. This parameter and its molecular counterpart Mk provide information on the genetic representation of each individual in the population and, on average, can be used as an index to maintain the initial genetic stock, as well as to predict the long-term inbreeding (or homozygosity) of a population, thus suggesting modifications to management practice to conserve the genetic makeup of a population (Gutiérrez et al., 2003; Goyache et al., 2003; Gutiérrez et al., 2005a,b). In our study, both AR and Mk for rYM individuals largely exceed those for the YM group.

In order to compare the expected genetic variability of the offspring produced by crossing males and females of each reference group, expected heterozygosity (\( H_e \)), genealogical gene diversity (GD) and increases in inbreeding \( \Delta(1-GD) \) and homozygosity \( \Delta(1-H_e) \) were respectively computed from average allelic frequencies and genealogical coancestry values (Table 4). The increase in inbreeding and homozygosity for the rYF×rYM offspring is, respectively, four and two times higher than that obtained for the YF×YM offspring, thus pointing out a serious concern for the conservation program of the Xalda sheep breed. If selection aimed at increasing genetic resistance to scrapie needs to be applied, the maintenance of the genetic background from the founder population will need unbalanced contributions from the individuals selected for reproduction to the genetic background of the population, thus suggesting the implementation of a mating policy aimed at obtaining homozygous ARR/ARR rams from heterozygous ARR/ARQ individuals.

In general, sets of genealogical and molecular parameters computed here give similar information on

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Expected values of genealogical gene diversity (GD) and expected heterozygosity (( H_e )) computed for the progeny produced by crossing AF with AM individuals (AF×AM), YF with YM individuals (YF×YM) and rYF with rYM individuals (rYF×rYM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( H_e )</td>
<td>( \Delta(1-GD) )</td>
</tr>
<tr>
<td>AF×AM</td>
<td>0.982</td>
</tr>
<tr>
<td>YF×YM</td>
<td>0.971</td>
</tr>
<tr>
<td>rYF×rYM</td>
<td>0.940</td>
</tr>
</tbody>
</table>

Additionally the increase in inbreeding \( \Delta(1-GD) \) and homozygosity \( \Delta(1-H_e) \) with respect adult individuals are given for the YF×YM and rYF×rYM crosses.
the direction of the losses of genetic variability due to selective processes. However, relationship between genealogical parameters and their molecular counterparts is not straightforward and losses of variability assessed at the molecular level are higher. When sufficient pedigree information is available the use of molecular information in conservation programmes is not always recommended (Fernández et al., 2005). However, when genealogies are shallow, as usually happens at the beginning of a conservation program, the use of molecular parameters can be justified in order to accurately assess some scenarios of conservation.

5. Conclusions

Major losses of genetic variability are due to selection of new reproductive individuals. The implementation of strict selection strategies aimed at reducing susceptibility to scrapie in rare sheep populations affects conservation programs. As assessed here using genealogical and molecular parameters, the additional loss of variation due to selection for scrapie resistance is not dramatic for females. However, when the available number of rams resistant to scrapie is low, diversity levels of a rare sheep breed may become critical (Molina et al., 2006) affecting the long-term evolutionary potential of the population. A mating policy focused on obtaining resistant individuals from ARR heterozygote parents should be carefully implemented before the beginning of such a selective programme so as to avoid problems in current conservation programs in rare sheep breeds. The combined use of genealogical and molecular information in this scenario of conservation can be recommended. The implementation of a germplasm bank in order to manage the risks of novel ovine PrP polymorphisms and losses of genetic potential is also recommendable (Roughsedge et al., 2006).

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