RESEARCH

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# GENETIC STRUCTURE AND DIVERSITY OF FOUR CHILEAN SHEEP BREEDS

**Rodrigo de la Barra[1*], Héctor Uribe[2], Etel Latorre[3], Fermín San Primitivo[4], and Juan Arranz[4]**

## ABSTRACT

Chilean sheep breeds have a significant founder effect since they descend from very few parents, in some cases population inbreeding has increased and in others there have been significant differentiation processes as compared to their original population. The objective of this study was to estimate the current status of genetic diversity in sheep herds, which comprise the main sheep breeds in Chile, using molecular methods. Animals from four breeding herds were sampled and analyzed. The breeds studied were Corriedale, Suffolk Down, Romney Marsh and Chilota, these are the predominant numerical pure breeds in Chile. From each breed population 40 animals were sampled. Genetic characterization was done using nine microsatellite molecular markers (SSR) recommended by FAO-ISAG. Results showed that in the Chilean sheep herds there are low genetic complementarities among breeds and a high risk of losing genetic diversity due to inbreeding in Chilota and Romney Marsh breeds.

**Key words**: Microsatellite, diversity, sheep, Chile.

## INTRODUCTION

Chile has about 3.9 million head of sheep. The numerically largest breeds are Corriedale, Suffolk Down, Romney Marsh and Chilota. Corriedale was introduced in the XIX century; Suffolk Down and Romney Marsh arrived in the last century, and Chilota was introduced in Chile by the end of the XVI century (Avendaño *et al*., 2005). From 2001 to date, Chile projects itself as a net meat and wool sheep exporter, and this is why there have been huge efforts in order to increase breeding numbers and to improve production efficiency.

The four breeds have a significant founder effect since they descend from a reduced number of founder parents which were introduced into the country by Spanish conquerors. Population inbreeding rate has been intensified, and in some cases, jointly with other reasons, has led to significant differentiation as compared to their original founder population.

Breeding structure of the Chilean sheep sector follows a hierarchical - pyramidal structure; management decisions involving a very few sheep herds can have a high impact in genetic diversity in most of the active breeding herds, because they purchase breeding stock from the few elite herds (De la Barra and Uribe, 2009).

Due to the lack of accurate evaluation and monitoring systems, it is difficult to measure the current status of genetic diversity and inbreeding in the breeding populations. This lack of information could be a barrier for future sustainable sheep industry development in Chile, since further improvements rely on significant and sustained yield increments which depend on additive genetic merit of selected breeding stock. Losses of genetic diversity endanger the genetic selection potential of maintaining and improving yield traits. To minimize potential risk of genetic diversity losses it is necessary to know the current genetic variability and its distribution among breeds, and to identify low frequency alleles which are indicative of unique genetic variants (Aranguren-Méndez *et al*., 2001).

The objective of this study was to estimate, using molecular methods, the current status of genetic diversity in sheep herds which make up the main Chilean breeds.

[1]Instituto de Investigaciones Agropecuarias INIA, Centro Experimental Butalcura, Dalcahue, Chiloé, Chile. [*]Corresponding author (delabarrarodrigo@gmail.com).
[2]Consejo Nacional de Mejoramiento Genético, Valdivia, Chile.
[3]Instituto de Investigaciones Agropecuarias INIA, Angamos 1056, Casilla 277, Punta Arenas, Chile.
[4]Universidad de León, Facultad de Veterinaria, Campus de Vegazana, 24071, León, España.
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## MATERIAL AND METHODS

Thirty-seven Corriedale, 38 Suffolk Down, 36 Romney Marsh and 40 Chilota sheep from four genealogically controlled breeding stock herds were randomly sampled. Animals were destined to breeder production. Nine microsatellite molecular markers (SSR) were chosen based on their polymorphism and simplicity to be used in multiplex and used for genetic characterization: BM8125, CSSM31, ILST005, ILST011, INRA026, MCM527, BM6526, MCM53 and RM006. The protocol followed was as described by Arranz *et al*. (2001). DNA was obtained from blood samples vacuum stored in 10 mL assay tubes containing EDTA-K3 anticoagulant after jugular punction. Samples were stored at -20 ºC (Satsangi *et al*., 1994). DNA extraction was done by blood red and white cell lysis followed by DNA recovery. Amount of DNA was based on 260 nm sample wave length absorbance (Sambrook y Russell, 2001). Isolated DNA was suspended on T10E1 buffer (Tris 10 mM, EDTA 1 mM) to keep it as stock solution at 4 ºC from which the samples were diluted with T10E0.2 to reach a final concentration of 10 ng $\mu L^{-1}$ which was used for the analyses. After amplification of DNA by means of Polymerase Chain Reaction (PCR), fragments were separated by electrophoresis on a vertical acrylamide gel under denaturalizing conditions.

Automatic ABI PRISM 377 and ABI 3100 sequencers, automatic Genecamp PCR Systems 9600 and 9700 thermo cyclers (PE Applied Biosystems, Foster City, California, USA) were used. Genetic data was done by GEL Processor, GENESCAN Analysis and GENOTYPER computational programs incorporated on the ABISPRISM 377 sequencer. Allelic frequency and relationship distance were estimated by MOLKIN 3.0 software (Gutiérrez *et al*., 2005). Population genetic structure was assessed based on Bayesian clustering using the STRUCTURE software (Pritchard *et al*., 2000; Falush *et al*., 2003). This software identifies clusters of similar genetic structure assigning animals within breeds included in the analysis.

## RESULTS AND DISCUSSION

The smallest polymorphic information contents (PICs) were 0.545 and 0.614 for INRA026 and BM8125 (Table 1), respectively. Most of the markers had PIC above 0.80 which is considered high by several researchers (Arranz *et al*., 2001; Peter *et al*., 2007; Dadi *et al*., 2008; Dalvit *et al*., 2008).

Structural analysis of the metapopulation of these four Chilean breeds allows evaluation of internal consistency of the breeds included. Based on maximum likelihood inference, the presence of four clusters corresponds to the four breeds involved in the analysis (Figure 1).

**Table 1. Polymorphic information content (PIC), alleles number and size range for locus in used markers.**

| Marker | PIC | Alleles number | Size range (bp) |
|---|---|---|---|
| BM8125 | 0.614 | 11 | 107-123 |
| CSSM31 | 0.870 | 25 | 126-165 |
| ILST005 | 0.804 | 17 | 187-207 |
| ILST011 | 0.882 | 23 | 264-288 |
| INRA26 | 0.545 | 9 | 85-99 |
| MCM527 | 0.863 | 17 | 161-182 |
| BM6526 | 0.901 | 21 | 153-176 |
| MCM53 | 0.842 | 22 | 69-98 |
| RM006 | 0.861 | 20 | 113-138 |

bp: base pair.

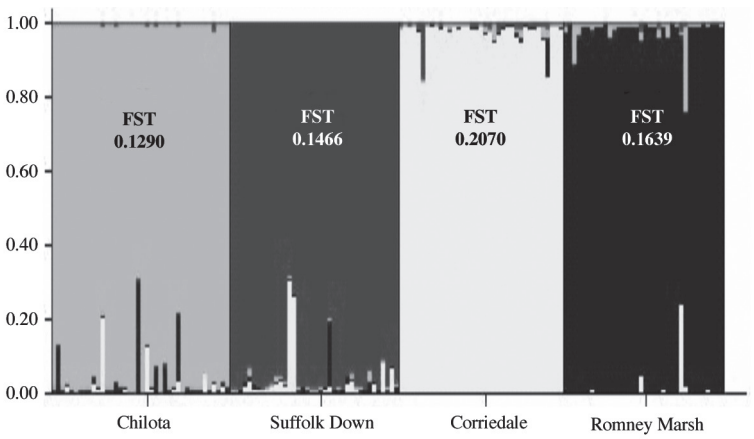



**Figure 1. Genetic structure of the main Chilean sheep breeds, based on maximum likelihood inference (log P(X/K)) according to Pritchard *et al.* (2000).**

Genetic diversity Fisher's estimator (FST) attributable to subpopulation allotment for each population shows a high degree of differentiation among breeds (Figure 1). Global FST for the metapopulation was 0.1129, which confirms a high degree of subdivision within the metapopulation. 11.3% of the allelic frequencies variance can be attributed to differences among subpopulations.

Table 2 shows the degree of fit of each population to clusters inferred by maximum likelihood. There is a high consistency for each breed, mainly for Romney Marsh and Corriedale.

Table 3 shows expected heterozygosity as a genetic variability indicator. This value shows that Chilota is the most genetically diverse breed, while Romney Marsh is the least. In contrast with this, observed heterozygosity is less than expected for Chilota breed (0.6956 *vs*. 0.7553), which indicates that this population shows a high inbreeding level which is corroborated by an Inbreeding coefficient (Fis) value of 0.1982. This situation could be due to the presence of an isolated subpopulation within the breed with high genetic variability across subpopulations and within populations. Inbreeding is the more likely explanation.

Regarding to Romney Marsh, greater inbreeding is compatible with expressed low genetic diversity. However, Corriedale and Suffolk Down show more heterozygosity than expected, which could be due to new genetic material brought into the population through artificial insemination and directed mating. Data show genetic diversity losses as a consequence of inbreeding in Chilota and Romney Marsh breeds.

In this study, average heterozygosity was higher than reported for the main European breeds (Rendo *et al*., 2004; Tapio *et al*., 2005; Peter *et al*., 2007), which reveals higher global genetic diversity for Chilean sheep breeds and similar to Alpine sheep breeds (Dalvit, 2008).

Individual contribution of each breed to national sheep genetic diversity can be evaluated by maximizing observed heterozygosity (Gain Diversity Breed, GDB) and the mean number of alleles in each *locus* adjusted by sample size and their divergence from the other populations.

According to maximization of observed heterozygosity for Chilean sheep breeds (Table 4), total retained genetic diversity does not contribute enough to the diversity of each separated breed. This shows a loss of genetic diversity in each breed, which is not compensated by the global diversity of the metapopulation; in other words, there is low genetic complementarities among the breeds studied. According to the divergence level of each population compared to the other populations (Table 4), Chilota and Romney Marsh have a genetic diversity which is divergent from the other breeds and cannot be preserved by the metapopulation global diversity. If this

**Table 2. Proportion of animals to inferred clusters according to Pritchard *et al.* (2000) for the main Chilean sheep populations.**

| | Inferred clusters (K = 4) | | | | |
|---|---|---|---|---|---|
| **Population** | **1** | **2** | **3** | **4** | **N** |
| Chilota | 0.005 | 0.952 | 0.026 | 0.016 | 40 |
| Suffolk Down | 0.954 | 0.006 | 0.011 | 0.029 | 38 |
| Corriedale | 0.013 | 0.007 | 0.007 | 0.974 | 37 |
| Romney Marsh | 0.006 | 0.018 | 0.964 | 0.012 | 36 |

**Table 3. Observed and expected heterozygosity and Wright's Fis value of the main Chilean sheep breeds.**

| Breed | Observed heterozigosity | Expected heterozigosity | Fis |
|---|---|---|---|
| Chilota | 0.605 | 0.755 | 0.198 |
| Suffolk Down | 0.809 | 0.716 | -0.130 |
| Corriedale | 0.774 | 0.714 | -0.085 |
| Romney Marsh | 0.595 | 0.695 | 0.143 |
| Total | 0.6961 | 0.8129 | 0.040 |

**Table 4. Retained breed diversity or breed contribution to population diversity and contribution to population allelic richness by Hulbert's estimator.**

| Breed | GDB (Caballero y Toro, 2002) | Divergence (Petit *et al*., 1998) |
|---|---|---|
| Chilota | -2.076 | 0.995 |
| Suffolk Down | -1.289 | -1.128 |
| Corriedale | -1.196 | -1.140 |
| Romney Marsh | -0.479 | 1.159 |

GDB: gain diversity breed.

is the case, both populations would be candidates for conservation if the aim is to preserve global diversity of the Chilean sheep population.

It is observed that 42% of the 165 analyzed alleles in the Chilean breeds correspond to unique alleles for the Chilean breeds (Figure 2a), showing that they are found exclusively in one of the breeds and absent in all the others. This value shows the amount of genetic diversity across populations, which can only be supplied by the population having the unique alleles toward the metapopulation global diversity. This finding reveals a low genetic similarity in the origin of the sheep breeds studied here. This is also supported by Figure 2b, where only 9.7% are common alleles for the four breeds. Therefore, genetic diversity preservation of Chilean breeds must be managed simultaneously to optimize total diversity of the metapopulation (Caballero y Toro, 2002).

Figure 2c shows absolute value of private alleles for each population. Chilota sheep is the breed contributing with the larger amount of private alleles to the metapopulation, followed by Romney Marsh and Suffolk Down. In contrast to the other breeds, Corriedale contributes with very few private alleles to the metapopulation, therefore its contribution to global diversity is low. Consequently, for these breeds, molecular co-ancestry could be an adequate method to select breeding candidates which would retain genetic diversity (Álvarez *et al*., 2005; Gutiérrez *et al*., 2005).

Fifteen out of twenty-five unique alleles of Chilota breed have allelic frequency below to 5%, eight of them have frequencies between 5% and 10%, and only two alleles have frequencies above 10% (Figure 3). Consequently, most of the unique alleles of this breed have low frequencies and thus are under an extinction threat. For Suffolk Down (Figure 3), 11 out of 21 private alleles have frequencies below 5%, one has frequencies between 5% and 10% and six of them have allelic frequencies above 10%. This means that there are some alleles not exposed to extinction. However, for most of them the risk of allelic variants decline exists, and they cannot be supplied by another population.

Corriedale breed (Figure 3) has only six private alleles, five of them having allelic frequencies below 5%, and one has frequencies between 5% and 10%. This is the highest number of alleles found in low frequency and with high risk of declining for unique allelic variants which cannot be supplied by other populations.

Romney Marsh breed (Figure 3) has 21 private alleles, 15 of them having allelic frequencies below 5%, three of them show frequencies from 5% to 10%, and three of them have frequencies above 10%. As in the other breeds most of the private alleles are found in low frequencies and this implies a risk of loss for private allelic variants which cannot be supplied by the other breeds.

In the four breeds studied, most of the private alleles were found at frequencies below 5%, which is similar to that reported for most of the European sheep breeds (Peter *et al*., 2007).

To preserve genetic diversity in sheep breeds it is necessary to minimize inbreeding in each generation; consequently, genealogical records must be maintained to optimize mating designs. This is most relevant for animals carrying unique alleles and would help in retaining genetic

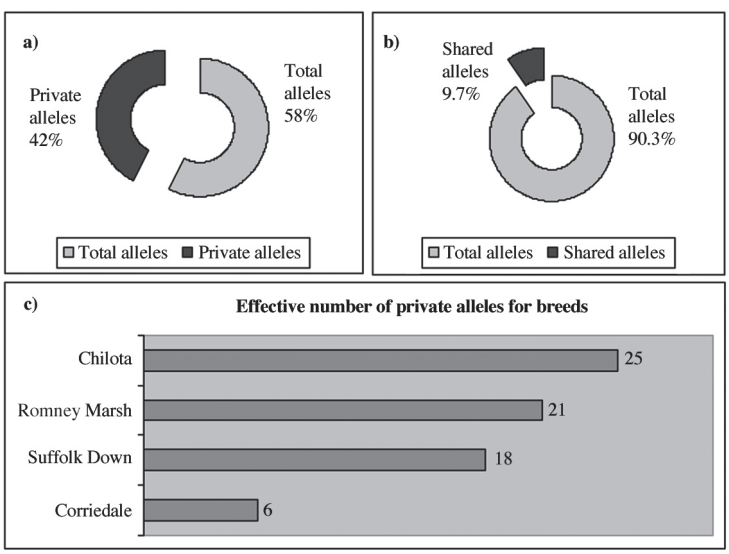
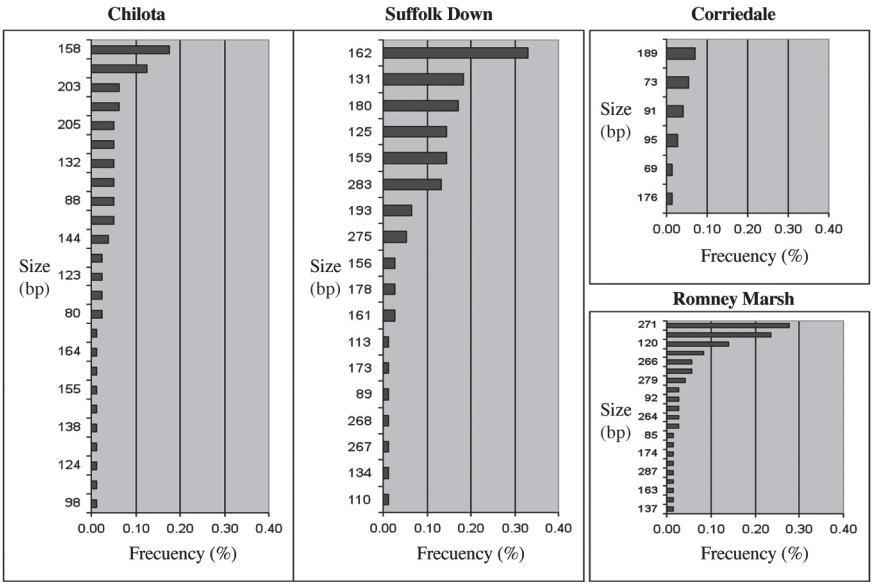



**Figure 2. a) Private and total alleles, b) shared and total alleles, and c) effective number of private alleles, for each breed.**




bp: base pair.

**Figure 3. Private allelic frequencies in Chilota, Suffolk Down, Corriedale, and Romney Marsh breeds.**

variability in future generations (Caballero y Toro, 2002; Bustamante *et al*., 2006).

## CONCLUSIONS

It is concluded that in the Chilean sheep herds there is a lack of genetic complementarities among the four breeds included in this study, and low genetic similitude in the origin of the breeds. Metapopulation is not allowed to compensate, across breeds, allelic variants losses. Each sheep breed studied reflects a loss of genetic diversity; furthermore in Chilota and Romney Marsh breeds there exists a potential risk of further loss due to inbreeding. Therefore, both breeds are candidates to conservation if the aim is to preserve Chilean global genetic diversity. Romney Marsh breed can be complemented by introducing new breeding material of the same breed from outside the country, while Chilota is a unique breed found only in Chiloé Archipelago.

## RESUMEN

**Estructura genética y diversidad de cuatro razas ovinas chilenas.** Las razas ovinas presentes en Chile presentan un importante efecto fundador, al descender de un reducido número de progenitores. En algunos casos se sospecha que ha aumentado el grado de consanguinidad poblacional, mientras que en otros se observan procesos significativos de diferenciación respecto a sus poblaciones de origen. El objetivo del presente estudio fue estimar el estado actual de la diversidad genética de los planteles que gestionan las principales razas utilizadas en la ganadería ovina chilena. Para ello se utilizaron las razas Corriedale, Sufolk Down, Romney Marsh y Chilota, dado que son las predominantes en Chile como poblaciones puras. Se analizaron 40 animales de cada raza, pertenecientes a cuatro planteles de reproductores. Para la caracterización genética de los animales se utilizaron nueve marcadores moleculares de tipo microsatélite (SSR) recomendados por FAO-ISAG. Los resultados indican una baja complementariedad genética entre las razas ovinas predominantes en Chile, advirtiéndose además una potencial pérdida de diversidad genética en todas las razas así como un elevado riesgo de incremento en esta pérdida por efecto de la endogamia en las razas Chilota y Romney Marsh.

**Palabras clave:** microsatélite, diversidad, ovejas, Chile.

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