Genetic relationships between Chilota and Spanish native sheep breeds of Chile

R. de la Barra[1], M. E. Martínez[1*] and A. M. Carvajal[2]

[1]Instituto de Investigaciones Agropecuarias, Centro Experimental Butalcura. O´Higgins 415-A of. 14, Castro, Chiloé, Chile.
[2]Instituto de Investigaciones Agropecuarias, Centro Experimental Remehue. Panamericana Norte Km 8 SN, Osorno, Chile.

*Corresponding autor tel/ fax +56 65 630656. E-mail: eugemartinez.inia@gmail.com

Abstract

Chilota sheep is a typical animal population from Chiloé Archipelago in the south of Chile. First sheep were introduced in Chiloé in 1568 with Spanish conquerors. In 2010 Chilota sheep was recognized as the first original breed of Chile. Blood samples were obtained from 40 Chilota individuals from 40 flocks spread throughout Chiloé Archipelago. A maximum of one individual per flock were sampled. Up to 21 microsatellites, previously used to characterize both between- and within-breed genetic relationships in sheep were analyzed in all the individuals. Results show that Chilota sheep shows a population structure that doesn´t differ significantly from that presented in the Spanish racial groups previously analyzed. A deficit of heterozygotes is also observed possibly because of geographic isolation on the islands within the archipelago of Chiloé.

Keywords: sheep, microsatellite, genetic diversity, genetic differentiation.

Introduction

Sheep was introduced in Chiloé in 1568 with Spanish conquerors. In, 1788 there were near 90.000 head (Torrejón et al. 2004), and, at date, there is a total population of 150.000 head, from which only about 40.000 are estimated to be descendent from the first sheep (de la Barra 2008), even though only 600 of them are currently under genealogical control; this breed is considered as a candidate for conservation, since private alleles that are absent in other breeds in Chile have been identified (de la Barra et al. 2010). Chilota is a typical sheep breed inhabiting the Chiloé Archipelago in southern Chile which is used with meat and wool-purposes, although it has been also determined to have a high milk yield in early lactation (900) ml/d (de la Barra et al. 2011; Martínez et al 2011). In 2010 Chilota was recognized as the first original Chilean sheep breed.

Genetic relationships between American and Spanish sheep breed are of scientific interest in order to evaluate genetic diversity losses and to know the current genetic variability and its distribution among breeds, in addition to identify low-frequency alleles which are indicative of unique genetic variants (Aranguren-Méndez et al. 2001).

The aim of this work was to asses, by means of microsatellites, the genetic relationship among the Chilota and major native Spanish sheep breeds to contribute to the knowledge of the genesis and within-population genetic variability of Chilota breed.

Materials and methods

Samples -Blood samples were obtained from 40 Chilota individuals from 40 flocks spread throughout Chiloé Archipelago (one individual per flock). Sampled farms were distant enough one from another; therefore, the probability of animal exchange was low. Additionally, a total of blood samples from 160 individuals corresponding to four native Spanish sheep breeds were obtained; breeds and number of individuals sampled were 40 Castellana, 40 Churra, 40 Merina and 40 Manchega, the last used as the outgroup. In total 200 samples were analyzed. Genomic DNA was isolated from blood

samples following the standard procedure by Sambrook and Rusell (2001). Up to 21 microsatellites (BM8125, CSSM31, ILST005, D5S2, OarFCB0020, SPS113, HSC, INRA0063, ILST0011, INRA0026, MCM527, CSRD0247, OarCP0049, INRA005, OarFCB0304, MCM53, RM006, BM6526, OarAE129, INRA023 and MAF0065) were used to characterize both between and within-breed genetic relationships in sheep following the suggestions of Arranz et al (2001). DNA amplification was performed in automatic Genecamp PCR Systems 9600 and 9700 thermo cyclers (PE Applied Biosystems, Foster City, California, USA) following the protocol described by Arranz et al (2001), and then DNA fragments were separated by electrophoresis on a vertical acrylamide gel in denaturalizing conditions using automatic ABI PRISM 377 sequencers (PE Applied Biosystems). Genetic data was obtained by GEL Processor, GENESCAN Analysis and GENOTYPER computational programs.

Statistical analysis- Molecular information was analyzed using program MOLKIN 2.0 (Gutiérrez et al. 2005). The following parameters were estimated at the breed level: observed (Ho) and expected (He) heterozigosity of number of alleles per locus (A). Additionally, the within-and between-individuals molecular coancestry matrices were estimated. The molecular co-ancestry between two individuals was defined as the probability that two randomly sampled alleles from the same locus in two individuals are identical by state (Caballero and Toro, 2002) and can be estimated using the scoring rules proposed for Eding and Meuwissen (2001) and Caballero and Toro (2002). Between-individual kinship distance (Dk) matrix and within-and between-population molecular coancestry were estimated (Caballero and Toro, 2002).

Population structure and degree of admixture were assessed using STRUCTURE. This program was used to assess a possible cryptic genetic structure in the analyzed data set (Pritchard et al. 2000; Falush et al. 2003). For this analysis, only Hardy-Weinberg balanced markers were used. The program estimates, using the MARKOV chain Montecarlo method. The number of clusters was identified by means of the algorithm $\Delta K = L''(K)|/ s[L(K)]$ (Evanno et al. 2005). This ensures that the groups are, as representative as possible, samples from a single population, as the implemented algorithm uncovers hidden structure without using *a priori* knowledge about the number of clusters (population or breeds) present in data set. Eight different runs from K=1 to K=10 were carried out to identify most likely number of cluster present in data set. All runs used a burn-in period of 100.000 iterations and a period of data collection of 100.000 iterations.

Reynolds genetic distance was used as proximity descriptor since it characterizes the short-term evolution of populations (Álvarez et al. 2005).

Results

Parameters characterizing genetic variability of the analyzed sheep breeds are given in Table 1. Chilota breed showed the highest values for expected heterozigosity (0.77), an average number of 9.8 alleles per locus and the lowest values of within-breed molecular coancestry (0.23). All the sheep breeds analyzed showed high diversity (He > 0.7). Chilota breed showed the lowest values for observed heterozigosity (0.677) and the highest values of Fis (0.16).

Table 1 Number of individuals analyzed (N), expected heterozigosity (He), observed heterozigosity (Ho), within-population molecular coancestry (fii), heterozigosity deficiency within-population (Fis), average number of alleles per locus per analyzed breed and for the whole data set (A).

Breed	N	He	Ho	fii	Fis	A
Castellana	40	0.76	0.73	0.24	0.040	10.1
Churra	40	0.75	0.68	0.25	0.100	9.1
Manchega	40	0.77	0.69	0.23	0.12	9.0
Merina	40	0.76	0.69	0.24	0.11	8.9
Chilota	40	0.77	0.68	0.23	0.16	9.8
Total	200	0.79	0.69	0.25	0.09	10.0

The between-breeds Kinship distance (Dk) and molecular coancestry (fii) matrices are given in Table 2. Average molecular coancestry of Chilota with all the other breeds is 0.196, while average molecular coancestry between Spanish breeds is 0.201; this indicates a close proximity between them.

Table 2 Between-breeds Kinship distance (below diagonal) and between-breeds molecular co-ancestry (above diagonal).

Breed	Castellana	Churra	Manchega	Merina	Chilota
Castellana	-	0.218	0.199	0.207	0.206
Churra	0.430	-	0.180	0.209	0.194
Manchega	0.448	0.479	-	0.192	0.184
Merina	0.442	0.452	0.469	-	0.199
Chilota	0.450	0.472	0.483	0.471	-

Fig. 1 shows ΔK mean values for all STRUCTURE runs. The most likely K was four. This number is not coincident with the five sheep breeds analyzed. This indicates that some of the analyzed populations have a higher similarity and ascribe to a common cluster.
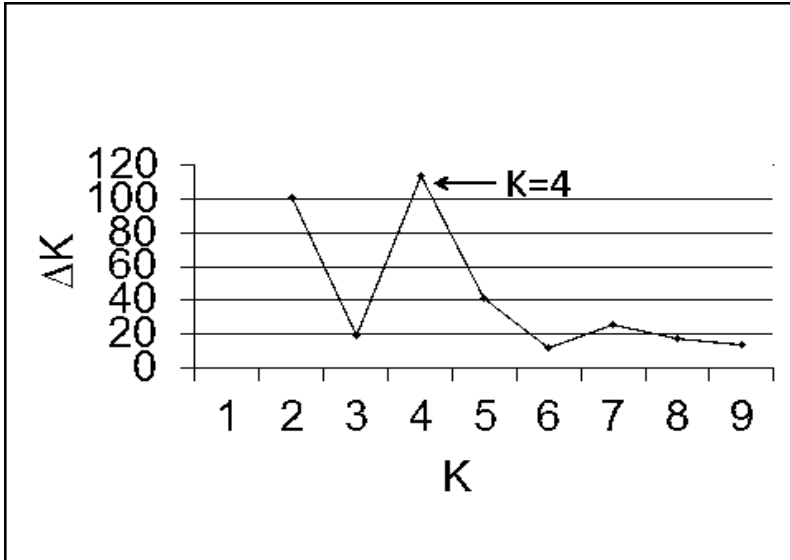

Fig1 Representation of the number of clusters identified by STRUCTURE software according to the methodology of Evano et al (2005). The analysis was based on 21 microsatellites utilized in the evaluation of the five sheep breeds.

Fig. 2 shows a graphic representation of how the five populations separate, assuming different K using STRUCTURE. In K=4 it can be seen the similitude between Churra and Castellana, while Manchega, Merina and Chilota breeds show evident differences.
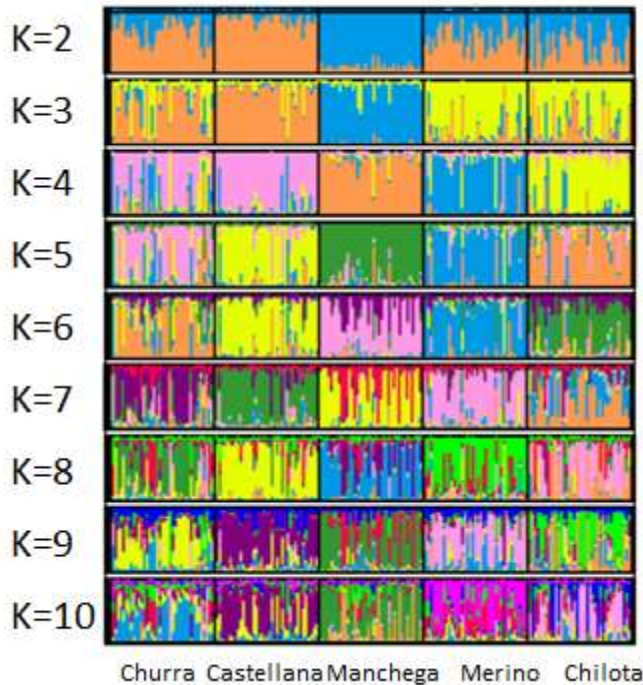

Fig. 2 Distribution of the groups according to the STRUCTURE software, based in 21 microsatellites used in the evaluation of five sheep breeds. The individuals were represented by vertical bars, each color being associated to a different group.

Figure 3 shows Reynolds' genetic distance. It is observed that Chilota breed is nearest to Churra and Castellana breeds, and more far to Manchega and Merina breeds.
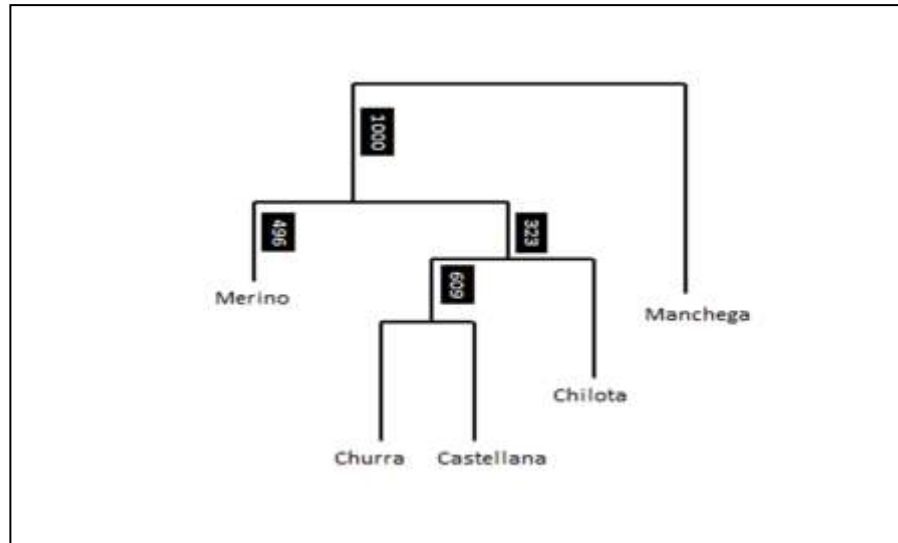

Fig. 3 Reynolds genetic distance among breeds: Castellana (CA), Churra (CHU), Manchega (MA), Merina (ME) and Chilota (CHI).

Discussion

In the 15th and 16th centuries, the structure of sheep breeds in the Iberian Peninsula was quite different from the present, only distinguishing between fine wool sheep, generically known as *Merinas*, and those sheep with coarse wool, called *Churras* (Sánchez, 1986). In absence of concrete data, we must assume that the first sheep heads brought to Central America were a representative sample of sheep existing in Spain at that time. In addition, it seems that *Merina* type did not develop well in Central America; consequently, first sheep introduced to South America were *Churra* type (Mason, 1981; Saucedo, 1984; Gratacos, 1998). Since those first *Churras* were brought to the Chiloé Archipelago to date, Chilota sheep have been physical and reproductively isolated, thus constituting a population that has evolved from that Spanish genetic pool which has originated the actual Spanish sheep breeds.

Genetic analyses showed that both expected and observed heterozigosity values (Table 1) are similar to those described for Iberian sheep breeds (Arranz et al. 2001; Legaz et al. 2008), for Spanish and Portugese Merina breeds (Diez-Tascón et al. 2000), and for Bordeleira, Serra da Estrela and Churra breeds (Oliveira et al., 2003, 2005).

Genetic variability found on Chilota breed is the same order of that found by other authors (Oliveira et al. 2003, 2005; Arranz et al. 2001) in different Spanish and Portuguese sheep breeds. Nevertheless, Chilota breed show, at the same time, the highest values for Fis (Table 1) which indicates the higher level of inbreeding. The observed deficit of heterozygotes has previously described on many Iberian and Creole sheep populations (Oliveira et al. 2003, 2005; Álvarez et al. 2004, 2005; Arranz et al. 2001; Martínez et al. 2005; Goyache et al 2006; Álvarez, 2007) since geographic isolation leads to mating between related individuals in a more frequent way than that expected by chance, generating poblational substructures, thus populations becoming endogamic.

Background of creole Chilota sheep population, which comes from a limited number of animals brought from Peru and maintained under a supposed strong reproductive isolation allow to deduct the existence of a high degree of inbreeding, which could explain this deficiency on heterozygotes. Islands in the Chiloé Archipelago are relatively isolated; sheep inhabiting these islands show a low heterozygosity as a consequence of the inbreeding. This situation is also observed in other small and isolated populations, as is the case of Xalda sheep breed in Spain (Álvarez et al. 2007), alpine sheep breeds in Switzerland (Dalvit et al. 2008) and in Italian sheep breeds (Bozzi et al. 2009).

The Kinship distance indicates that Chilota sheep breed is similar to the studied populations, in spite of the absolute lack of genetic flux between Chilota and the rest of the analyzed breeds. Chilota breed shows the highest proximity with Castellana breed and the lowest with Manchega breed.

The populations of Chilota, Manchega and Merina breeds (Fig. 2) are identified as genetically

82

homogeneous groups. In this sense, Chilota breed presents as a racial group different from Spanish breeds with which it is compared. On the other hand, Churra and Castellana breeds are identified as a part of the same cluster. This is a particularly striking aspect, since Chilota show the higher genetic proximity with these two breeds (Fig.3), in a context of a certain similarity with all the populations studied. The small difference between all the populations studied may be due to the large size of the populations or to the fact that they present genetic flows high enough to counteract the effects of genetic drift (Álvarez 2007).

Acknowledgements

References

1) Álvarez I, LJ Royo, I Fernández, JP Gutiérrez, E Gómez, F Goyache. 2004. Genetic relationships and admixture among sheep breeds from northern Spain assessed using microsatellites. J. Anim. Sci. 82, 2246-52.
2) Álvarez I, JP Gutiérrez, LJ Royo, I Fernández, E Gómez, JJ, Arranz, F Goyache. 2005. Testing the usefulness of the molecular coancestry information to assess genetic relationships in livestock using a set of Spanish sheep breeds. J. Anim. Sci. 83,737-44.
3) Álvarez I. 2007. Estructura genética de la raza Xalda. Tesis doctoral, Facultad de Ciencias Veterinarias, Universidad de León, España.
4) Aranguren-Méndez J, J Jordana, M Gómez. 2001. Genetic diversity in Spanish donkey breeds using microsatellite DNA markers. Genet. Sel. Evol. 33,433-22.
5) Arranz J, Y Bayón, F San Primitivo. 2001. Differentiation among Spanish sheep breeds using microsatellites. Genet. Sel. Evol. 33:529-42.
6) Bozzi R, P Degl'Innocenti, P Rivera, I Nardi, A Crovetti, C Sargentini, A Giorgetti. 2009. Genetic characterization and breed assignment in five Italian sheep breeds using microsatellite markers. Small Rumin. Res. 85, 50-57.
7) Caballero A, M Toro. 2002. Analysis of genetic diversity for the management of conserved subdivided populations. Conserv. Genet. 3, 289-99.
8) Dalvit C, E Sacca, M Cassandro, M Gervaso, E Pastore, E Piasentier. 2008. Genetic diversity and variability in Alpine sheep breeds. Small Rumin. Res. 80, 45-51.
9) De la Barra R. 2008. Efecto de la introducción de la ganadería en el archipiélago de Chiloé, Chile. Tesis doctoral, Universidad de León, España.
10) De la Barra R, A Carvajal, H Uribe, ME Martínez, C Gonzalo, JJ Arranz, F San Primitivo. 2011. El ovino criollo Chilote y su potencial productivo. Anim. Genet. Resource. 48, 1-7.
11) De la Barra R, H Uribe, E Latorre, JJ Arranz, F. San Primitivo. 2010. Genetic structure and diversity of four chilean sheep breeds. Chilean J. Agric. Res. 70(4), 646-51.
12) Diez-Tascón C, RP Littlejohn, PA Almeida, M Crawford. 2000. Genetic variation within the Merina sheep breed: Analysis of closely related populations using microsatellites. Anim. Genet. 31, 243-51.
13) Eding H, THE Meuwissen. 2001. Marker-based estimates of between and within population kinships for the conservation of genetic diversity. J. Anim. Breed. Genet. 118, 141-59.
14) Evanno G, S Regnaut, J Goudet. 2005. Detecting the number of clusters of individuals using the software Structure: a simulation study. Molecular Eco. 14, 2611-20.
15) Falush D, M Stephens, JK Pritchard. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164,1567-87.
16) Goyache F, J Carus, I Álvarez, J Gutiérrez, I Fernández, L Royo. 2006. Diversidad filogenética como método de utilidad en programas de conservación de recursos genéticos ganaderos. ITEA 120, 133-38.
17) Gratacos J. 1998. La ganadería en el descubrimiento de América. Resúmenes del I Congreso Iberoamericano de historia de la Veterinaria. Girona, España. pp 68.
18) Gutiérrez JP, LJ Royo, I Álvarez, F. Goyache. 2005. MolKin v2.0: a computer program for genetic analysis of populations using molecular coancestry information. J. Hered. 96, 718-21.
19) Legaz E, I Álvarez, L Royo, I Fernández, J Gutiérrez, F Goyache. 2008. Genetic relationships between Spanish Assaf (Assaf.E) and Spanish native dairy sheep breeds. Small Rumin. Res. 80, 39-44.
20) Martínez A, J Vega-Pla, M Bravo, J Caraballo, J Delgado. 2005. Caracterización genética de la raza Palmera con microsatélites. Arch. Zootec. 54, 263-67.
21) Martínez ME, C Calderón, R de la Barra, F de la Fuente, C Gonzalo. 2011. Udder morphological traits and milk yield of Chiloyta and Suffol sheep breeds. Chilean J. Agric. Res. 71(1), 90-95.

22) Mason I. 1981. Las Razas indígenas de Ovinos y Caprinos en América latina. Recursos genéticos animales en América latina: Ganado Criollo y especies de altura. FAO. Roma, Italia.

23) Oliveira C, E Barbosa, JC Lopes, JJ Arranz, Y Bayón, NV Brito, F San Primitivo. 2003. Genetic variability in portuguese sheep breeds Bordaleira de Entre-Douro e Minho and Serra da Estrela using microsatellites. Electronic J. Environ. Agri. Food Chem. 2, 484-86.

24) Oliveira C, B Gutiérrez-Gil, S Pedrosa, E Barbosa, R Dantas, J Lite, NV Brito, JJ Arranz, Y Bayón. 2005. Genetic characterization of Bordaleira de Entre Douro e Minho and Serra da Estrela sheep breeds: nuclear and mitochondrial DNA. Revista Portuguesa de Ciências Veterinárias 100, 175-80.

25) Pritchard JK, M Stephens, P Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945-59.

26) Sánchez A. 1986. Merinas precoces y razas afines en España. Asociación Española de Criadores de ovinos precoces. Madrid, España.

27) Sambrook J, D Russell. 2001. Molecular cloning: A laboratory manual. 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

28) Saucedo P. 1984. Historia de la ganadería en México. 1ª Ed. Universidad Nacional Autónoma de México. Dirección General de Publicaciones. México, D. F.

29) Torrejón F, M Cisternas, A Araneda. 2004. Efectos ambientales de la colonización española desde el río Maullín al archipiélago de Chiloé: Sur de Chile. Revista Chilena de Historia Natural. 77, 661-77.