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Genetic characterization of Colombian indigenous sheep¤

Caracterización genética de ovinos criollos colombianos

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Abstract

Background: indigenous breeds are important for poor farmers because of their natural selection against harsh environments and adaptation to regional conditions. However, inbreeding of indigenous sheep populations has increased in Colombia due to indiscriminate cross-breeding with foreign animals and lack of reproductive controls, with subsequent loss in productivity, which poses a great risk for the conservation of valuable genes. **Objective:** to determine the genetic diversity in Colombian indigenous sheep by using a panel of 10 microsatellite molecular markers. **Methods:** blood samples from 362 individuals from 43 farms in 11 Colombian provinces were genotyped and analyzed for a panel of 10 microsatellite markers. **Results:** a total of 134 alleles were found (13.4 alleles/locus on average) with a range of observed and expected heterozygosity of 0.428 to 0.831 and 0.615 to 0.855, respectively, and 0.742 polymorphic information content (PIC). The average Wright F-statistics (F_{IS}) of the breeds was 0.107, suggesting moderate levels of inbreeding. Colombian sheep showed a low level of genetic differentiation among breeds (F_{ST} = 0.054) and STRUCTURE analysis showed complex patterns of admixture in the breeds. **Conclusion:** overall, Colombian sheep have high genetic variability, which is very important for future conservation programs and genetic improvement.

Keywords: *conservation, DNA, genetic diversity, ovine cattle, population structure.*

Resumen

Antecedentes: las razas animales autóctonas son importantes para los agricultores de escasos recursos a causa de su selección natural contra el duro ambiente y su adaptación a condiciones regionales. Sin embargo

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en Colombia, debido al cruce indiscriminado con razas foráneas y a la falta de control de la reproducción, ha aumentado la consanguinidad en las poblaciones de ovinos criollos y por lo tanto la pérdida en la productividad, lo que supone un gran riesgo para la conservación de genes valiosos. **Objetivo:** determinar la diversidad genética en razas criollas de ovinos colombianos utilizando análisis por microsatélites. **Métodos:** se visitaron 43 granjas localizadas en 11 departamentos del país, en las cuales se tomaron muestras de sangre a 362 individuos. Las muestras fueron genotipadas y analizadas para un panel de 10 marcadores microsatélites. **Resultados:** un total de 134 alelos fueron encontrados (13,4 alelos/locus en promedio), con un rango de heterocigocidad observada y esperada de 0,428 a 0,831 y 0,615 a 0,855, respectivamente, y un contenido de información polimórfica (PIC) promedio de 0,742. El Wright F-statistics (F_{IS}) promedio de las razas evaluadas fue 0,107, lo cual sugiere que las razas tienen niveles moderados de consanguinidad. Las ovejas colombianas presentaron un bajo grado de diferenciación genética entre las distintas razas (F_{ST} = 0,054) y el análisis de STRUCTURE mostró complejos patrones de mezcla en las razas estudiadas. **Conclusión:** en términos generales, las ovejas colombianas presentan una alta variabilidad genética lo cual es muy importante para futuros programas de conservación y mejoramiento genético.

Palabras clave: *ADN, conservación, diversidad genética, estructura poblacional, ganado ovino.*

Resumo

Antecedentes: as raças crioulas, devido a sua seleção natural em ambientes hostis e adaptação às condições regionais, são importantes para os agricultores de poucos recursos econômicos. Porém, na Colômbia, devido ao cruzamento indiscriminado com raças estrangeiras e a falta de controle na reprodução, tem aumentado a consanguinidade nas populações de ovinos crioulos e por tanto tem gerado perda na produtividade, o que faz supor um grande risco para a conservação de genes valiosos. **Objetivo:** determinar a diversidade genética em raças crioulas colombianas utilizando um painel de 10 marcadores moleculares microssatélites. **Métodos:** visitaram-se 43 granjas localizadas em 11 departamentos do país, nos quais foram amostrados 362 indivíduos, que foram genotipados e analisados para um painel de 10 marcadores microssatélites. **Resultados:** um total de 134 alelos foram encontrados (média de 13,4 alelos/locus), com um rango de heterocigocidade observada e esperada de 0,428 a 0,831 e 0,615 a 0,855, respectivamente, e um conteúdo de informação polimórfica (PIC) médio de 0,742. O Wright F-statistics (F_{IS}) médio das raças avaliadas foi de 0,107, o qual sugere que as raças apresentam níveis moderados de consanguinidade. As ovelhas colombianas apresentaram um baixo grau de diferenciação genética entre as diferentes raças (F_{ST} = 0,054) e o análise de STRUCTURE mostrou complexos patrões de mistura nas raças estudadas. **Conclusão:** em termos gerais, as ovelhas colombianas apresentaram uma alta variabilidade genética, o qual é muito importante para futuros programas de conservação e melhoramento genético.

Palavras chave: *conservação, diversidade genética, DNA, estrutura populacional, gado ovino.*

Introduction

Sheep play an important role in food production systems and are frequently recognized as a multi-purpose species capable of improving the rural economy. Rusticity, high fertility, and adaptability to harsh conditions are noticeable traits of these animals (Radhikag *et al*., 2015).

Sheep farming represents a profitable production system in Colombia due to the favorable conditions of the market, in which the demand for products from these animals has increased over time. This situation makes sheep one of the most promising livestock species in the rural sector of the country (Barrios, 2005).

According to the national inventory of Instituto Colombiano Agropecuario (ICA), for 2016 Colombia had a population of 1,423,466 sheep across the country. However, Guajira, Boyacá, Córdoba, and Cesar provinces concentrate 74% of the total sheep population.

The origins of Colombian sheep date back to the indigenous wool sheep resulting from crosses between European breeds and indigenous hair sheep breeds

which, in turn, descended from African breeds. These animals were introduced to the country 500 years ago during the conquest and have experienced a process of adaptation to Colombian tropics (Delgado *et al*., 2009).

Other foreign breeds such as Katahdin, Santa Inés, Dorset, Dorper, and Hampshire are also present in Colombia. These breeds have been extensively used because of their higher productive performance compared with local indigenous breeds, however, the former do not have the adaptation level of the latter (Egito *et al.,* 2002). On the other hand, the use of only a few genetic superior males for intensive mating might cause a reduction of the effective population size, higher levels of inbreeding and finally, decreased genetic diversity within breeds (Pastrana *et al*., 1996).

The increasing interest in the conservation of genetic resources has encouraged studies to characterize the genetic diversity of several livestock species and breeds (Sodhi *et al*., 2006). Microsatellite molecular markers have been extensively used with this purpose by several authors (Kugonza *et al*., 2011; Dixit *et al*., 2012; Souza *et al*., 2012; Awobajo *et al*., 2015; Jiang *et al*., 2015) taking advantage of their high polymorphism, codominant inheritance, abundant presence with wide dispersion in the genome, and high repeatability (Dodgson *et al*., 1997).

The objective of this study was to estimate the genetic diversity of three Colombian sheep breeds using a panel of 10 microsatellite markers and to determine the genetic structure between these populations.

Materials and methods

Ethical considerations

The protocol used to obtain blood samples was approved by the ethics committee for animal experimentation of the Universidad de Antioquia (Act 71, June, 2012).

Animals and samples

Blood samples were collected from 362 sheep of the following Colombian breeds: ovino de pelo colombiano (OPC), criollo de lana (CRL), and Mora Colombiana (MORA). The animals were located in 43 farms in 11 Colombian provinces (Antioquia, Boyacá, Caldas, Cesar, Córdoba, La Guajira, Magdalena, Nariño, Santander, Sucre, and Valle del Cauca). The samples were taken following recommendations by the MoDAD (FAO, 1996).

DNA extraction and genotyping

Genomic DNA was extracted from white blood cells using MOBIO commercial kit (Ultra Clean DNA Blood Isolation Kit, Calalog # 12000-100, Los Ángeles, California, USA). The quantity and quality of the extracted DNA was measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA).

The panel used for genotyping the animals contained 10 microsatellites (Table 1) recommended by the Food and Agriculture Organization (FAO, 2011), and the International Society of Animal Genetics (ISAG) for diversity studies in sheep. The markers were chosen according with their polymorphism level detected in previous studies (Bozzi *et al*., 2009; Zhong *et al*., 2010; Álvarez *et al*., 2012; Ben Sassi *et al*., 2014) and their location in different chromosomes.

Microsatellites were amplified in two multiplex PCR assay: Multiplex 1 (SRCRPS9, MCM140, OARCB226, MAF214, ILSTS5, SRCRSP1) and Multiplex 2 (MAF33, ILSTS28, SRCRSP5, ILSTS11). Each PCR tube with a final volume of 10 µL contained ~120 ng genomic DNA, 0.2 mM of each dNTP, 1.85 U Taq Hot Start polymerase (ABM), 0.2 µM fluorolabeled forward primer and unlabeled reverse primer, 1.14 µL GC Enhancer (Applied Biosystems, Foster City, California), 1.5 mM MgCl2 and miliQ water up to adjust the final volume.

Amplification was carried out in a PTC 100 thermocycler (MJ Research Inc., Waltham, Massachusetts, USA) and PCR cycling conditions were as follows: initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 75 sec, annealing at 55°C for 45 sec, and extension at 72°C for 60 sec, and a final extension at 72°C for 10 min.

The fluorescent labelled PCR products were mixed with Hi Di formamide and Liz 500 TM internal size

standard, denatured at 95°C for 5 min and genotyped on a capillary electrophoresis ABI PRISM® 310 DNA analyser (Applied Biosystems). The analysis for allele identification was performed using GENEMAPPER 4.1 software (Applied Biosystems).

Statistical analysis

The allele frequencies for all loci, number of alleles (NA) and the effective number of alleles (ENA) observed per locus, observed (OH) and expected (EH) heterozygosities were estimated for each marker with GenAIEx 6.5 software (Peakall y Smouse, 2012). The polymorphic information content (PIC) was calculated with the Excel Microsatellite Toolkit V 3.1.1 (Park, 2001).

The tests to obtain the deviations from the Hardy-Weinberg (HW) were estimated for each breed using Markov Chain Monte Carlo simulations with Genepop 4.2 software (Rousset, 2008). The population genetic structure was analysed by obtaining the Wright F-statistics (F_{IS}, F_{ST}, and F_{IT}) following the methods described by Weir y Cockerham (1984) and using Fstat 2.9.3.2 software (Goudet, 2002).

The genetic differentiation was examined in two steps. First, the genetic distances between breeds were determined using the method of Nei (1987) with GenAIEx 6.5 and Genepop 4.2. Software. These values were used to create a consensus tree through the un-weighted pair group method with arithmetic mean (UPGMA) included in MEGA 6 software (Tamura *et al*., 2013).

Subsequently, the population genetic structure was calculated with a Bayesian iterative algorithm using STRUCTURE software (Pritchard *et al*., 2000), which randomly assigns individuals to a pre-determined number of genetic groups K, according with the genotype of the multiple loci evaluated, measuring the admixture levels within individuals and

Table 1. Description of the microsatellite markers used in PCR.

Microsatellite	Chromosome	Primer sequence (5'-3')	Range	Fluorochrome
SRCRSP 9	3	F: TCCAGATTTTGTACCAGACC R: GTCATGTCATACCTTTGAGC	107-133	6-FAM
MCM 140	6	F: GTTCGTACTTCTGGGTACTGGTCTC R: GTCCATGGATTTGCAGAGTCAG	169- 193	6-FAM
ILSTS 11	9	F: GCTTGCTACATGGAAAGTGC R: CTAAAATGCAGAGCCCTACC	262-292	6-FAM
SRCRSP 5	18	F: GGACTCTACCAACTGAGCTACAAG R:GTTTCTTTGAAATGAAGCTAAAGCAATGC	128-156	6-FAM
OarCB226	2	F: CTATATGTTGCCTTTCCCTTCCTGC R: GTGAGTCCCATAGAGCATAAGCTC	115-159	VIC
MAF 33	9	F: GATCTTTGTTTCAATCTATTCCAATTTC R: GATCATCTGAGTGTGAGTATATACAG	117-143	VIC
MAF 214	16	F: GGGTGATCTTAGGGAGGTTTTGGAGG R: AATGCAGGAGATCTGAGGCAGGGACG	178-224	VIC
ILSTS 28	3	F: TCCAGATTTTGTACCAGACC R: GTCATGTCATACCTTTGAGC	117-167	NED
ILSTS 5	7	F: GGAAGCAATGAAATCTATAGCC R: TGTTCTGTGAGTTTGTAAGC	176-214	NED
SRCRSP 1	13	F: TGCAAGAAGTTTTTCCAGAGC R: ACCCTGGTTTCACAAAAGG	116-144	NED

determining the K value for the parental populations. The analysis of the present study included a correlated allele frequencies model, assuming a level of non-independence (Pritchard *et al*., 2000; Zuccaro *et al*., 2008; Ciani *et al*., 2013).

A representative K value for the sample set was obtained by running three independent simulations for three pre-selected K values ($1 \leq K \leq 3$). All runs were performed with 200,000 burn-in period and 1,000,000 MCMC iterations after burn-in. Finally, the most appropriate K value in the populations was calculated with the ΔK algorithm used by Evanno *et al*. (2005).

Results

Population genetic variability

In total, 134 alleles were identified in the 362 genotyped individuals. The NA ranged from 9 to 21 for loci SRCRSP1 and ILSTS28, respectively, with an average NA of 13.4. Parameters evaluating population genetic variability by locus are presented in Table 2.

The EH ranged between 0.615 (ILSTS 5) and 0.855 (OarCB2256), with a population mean of 0.63, indicating high levels of genetic variability in the breeds.

The OH ranged between 0.428 (SRCRSP 5) and 0.831 (ILSTS 28), with an average of 0.672 for all the loci.

The average PIC for the 10 markers was 0.742 with no values lower than 0.5, suggesting that all loci were highly polymorphic. The average F_{IS} value was different from zero (0.102; $p<0.05$). Several microsatellites such as SRCRSP 5, ILSTS 11, MAF 214, and OarCB2256 showed F_{IS} values higher than 0.1, indicating an excess of homozygote individuals for these markers in the populations.

The F_{IT}, which measures individual heterozygosis respect to that of the total population was 0.151 ($p<0.05$), indicating a 15.1% general deficit of heterozygote individuals in these populations. The F_{ST}, which measures the genetic variability explained by differences between breeds was 0.054. Therefore, most of the total genetic variability comes from differences among individuals (94.6%), while only 5.4% is due to differences between breeds. This suggests a very low level of genetic differentiation between the populations studied.

Genetic variability within breeds

The MNA for all breeds was 9.6. The OPC sheep had the highest MNA (12.40), while MORA showed

Table 2. Summary statistics in Colombian indigenous sheep population analysed by 10 microsatellite loci.

LOCUS	NA	ENA	OH	EH	PIC	F_{IS}	F_{ST}	F_{IT}
OarCB 2256	20	6.530	0.757	0.855	0.844	0.110	0.019	0.126
MAF 33	13	4.702	0.782	0.852	0.835	0.060	0.071	0.127
ILSTS 28	21	4.130	0.831	0.819	0.797	-0.049	0.092	0.047
ILSTS 11	11	5.208	0.627	0.804	0.776	0.216	0.016	0.228
MCM 140	11	5.749	0.713	0.798	0.772	0.097	0.032	0.127
SRCRSP 1	9	3.161	0.666	0.738	0.697	0.064	0.102	0.159
SRCRSP 5	10	3.406	0.428	0.726	0.687	0.405	0.025	0.420
SRCRSP 9	12	3.251	0.768	0.688	0.650	-0.159	0.103	-0.039
MAF 214	15	3.623	0.591	0.735	0.647	0.182	0.051	0.224
ILSTS 5	12	2.438	0.561	0.615	0.661	0.005	0.027	0.032
Mean	13.4	4.220	0.672	0.763	0.742	0.102*	0.054*	0.151*

*$p<0.05$. NA: number of alleles. ENA: effective number of alleles. OH: observed and EH: expected heterozygosities. PIC: polymorphic information content. F-statistics (F_{IS}, F_{ST}, F_{IT}).

the lowest MNA (7.90). Different parameters to evaluate genetic variability for each breed are presented in Table 3.

Table 3. Summary statistics for three Colombian indigenous sheep breeds analysed by 10 microsatellite loci.

Breed	N	MNA	MENA	OH	EH	F_{IS}
OPC	292	12.40	4.44	0.678	0.752	0.100
CRL	36	8.50	4.50	0.689	0.762	0.110
MORA	34	7.90	3.72	0.606	0.671	0.111

N: number of animal sampled. MNA: mean number of alleles. MENA: mean effective number of alleles. OH: observed and EH: expected heterozygosities. F_{IS}: F-statistic. OPC: ovino de pelo colombiano. CRL: criollo de lana.

The lowest values of OH and EH were observed in MORA breed (0.606 and 0.671, respectively), while the highest OH and EH were obtained for the CRL breed (0.689 and 0.762, respectively). There were no large differences between EH and OH, with the CRL and MORA breeds showing the highest and lowest differences, respectively. In general, all breeds showed high genetic diversity.

Inbreeding parameters (F_{IS}) for each breed had a mean of 0.107. All breeds showed F_{IS} levels higher than zero, indicating a general deficit of heterozigote individuals.

From a total of 30 tests to evaluate HW equilibrium in each breed (Table 4), 15 showed significant deviations ($p<0.05$), which is explained by the heterozygosity deficit.

Figure 1 shows the phylogenetic tree constructed from the matrix of Nei standard genetic distances. The CRL and MORA breeds are grouped in the same node, separated from the OPC individuals.

Population structure was analyzed using STRUCTURE program with the expected number of clusters (K) ranging from 1 to 3. According with the method proposed by Evanno *et al.* (2005), it was assumed that K = 3 is the most likely number of ancestral populations that contribute to the genetic diversity observed in Colombian sheep breeds. There was no clear differentiation between populations under any of the K values tested (Figure 2), which indicates high admixture levels.

Table 4. Hardy Weinberg equilibrium test by breed.

Loci breed	OarCB 2256	MAF 33	ILSTS 28	ILSTS 11	MCM 140	SRCRSP 1	SRCRSP 5	SRCRSP 9	MAF 214	ILSTS 5
OPC	0.000*	0.002*	0.000*	0.000*	0.000*	0.084	0.000*	0.000*	0.000*	0.949
CRL	0.000*	0.550	0.865	0.028*	0.992	0.591	0.024*	0.985	0.081	0.993
MORA	0.681	0.059	0.987	0.039*	0.000*	0.774	0.005*	0.000*	0.382	0.720

* Significant deviation from Hardy Weinberg equilibrium ($p<0.05$). OPC: ovino de pelo colombiano. CRL: criollo de lana.

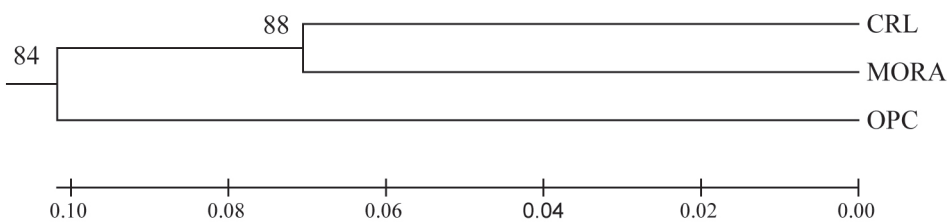

Figure 1. Phylogenetic tree (UPGMA) representing Nei's standard genetic distances between CRL (criollo de lana), MORA and OPC (ovino de pelo colombiano) breeds.

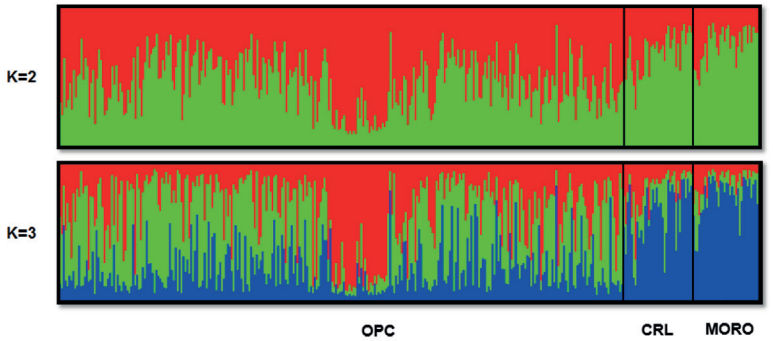

Figure 2. Population structure estimated with STRUCTURE in CRL (criollo de lana), MORA and OPC (ovino de pelo colombiano) breeds for values of K = 2 and K = 3.

Discussion

We studied the genetic diversity of three Colombian indigenous sheep breeds by using microsatellite molecular markers. According with reference values reported by ISAG/FAO (2011) for the minimum number of alleles using this panel of microsatellites, all loci were highly polymorphic. The average NA found in this study (13.4) is higher than that obtained by Nanekarani *et al*. (2010) using 15 microsatellites in a population of 360 sheep of four breeds in Iran. The EH, best estimator of the genetic diversity present in a population (Kim *et al*., 2002), had a value of 0.63, indicating that the indigenous sheep population in Colombia has a high genetic variability. Except for SRCRSP 9 and ILSTS 28 loci, the OH was always lower than the EH. Similar results were found by Bozzi *et al*., (2009), Arora *et al.,* (2010), Zhong *et al*. (2010), and Gornas *et al*., (2011) in sheep populations of Sudan, India, China, and Italy, respectively.

Polymorphic information content (PIC), a parameter indicative of the degree of informativeness of a marker, ranged from 0.647 (MAF214) to 0.844 (OarCB2256). In the present study all markers were highly informative (PIC>0.5; Arora *et al.,* 2006). These observations suggested the additional utility of these markers for population assignment (Arora *et al.,* 2010) in indigenous sheep and gene mapping (Kayang *et al*., 2002).

On the other hand, it is important to note the low level of differentiation observed among breeds (F_{ST} = 0.054). Similar results were found by Bozzi *et al*. (2009), Zhong *et al*. (2010), *Álvarez et al*. (2012), and Ben Sassi *et al*. (2014), who reported F_{ST} ranging between 0.09 and 0.3. Genetic similarity between Colombian sheep breeds could be explained by a high flow of genes between farms that borrow or lease rams and also because of the poor control in breeding programs within sheep farms.

All three breeds showed high MNA. The OPC breed had the highest MNA (12.40), which in turn is higher than the value reported in Mexican indigenous sheep (7.8 alleles/locus; Quiroz *et al*. (2007), Paraguayan indigenous sheep (7.25 alleles/locus; Ochipinti *et al*. (2012), American indigenous sheep (7.71 alleles/locus; Blackburn *et al*. (2011), Kenyan indigenous sheep (6.16 alleles/locus; Muigai *et al.* (2009), and Indian indigenous sheep (5.48 alleles/locus; Arora *et al.* (2010). It is important to note that OPC individuals represented 80.66% of the total population sampled, however, this percentage is similar to the proportion of this breed in the sheep national inventory (Martinez *et al*., 2009).

The significant deviations found in the HW tests and the high values of F_{IS} found in each breed indicate a general deficit of heterozygote individuals. This could have been caused by nonrandom mating (Arora *et al.,* 2010), selection (Álvarez *et al*., 2012) and the Wahlund effect which means the presence of substructures in the sheep populations (Nei, 1987).

Nevertheless, it is highly probable that the main origin of this heterozygosity deficit is the high levels of endogamy in the flocks, which is caused by mating individuals with closely related ancestors, which in Colombia is related with poor animal identification,

lack of mating control (Martínez *et al*., 2009), and the lack of clear breeding objectives. Moreover, most sheep farms in Colombia only have one or two rams, which must be mated with all the females of the flock, thus increasing endogamy and reducing genetic diversity (Tolone *et al*., 2012).

The standard genetic distances allowed grouping CRL and MORA breeds in a same node. It is essential to highlight that MORA breed was developed 50 years ago by ICA by crossing CRL (50%), Hampshire (23%), Romney Marsh (19%), and Corriedale individuals (8%) to obtain animals for meat production but also recessive homozygotes for black coloured wool (Pastrana *et al*., 1996). On the other hand, these are wool sheep breeds with a common geographical origin, since they are descendent of Churra and Manchega Spanish sheep breeds. In addition to this, CRL and MORA breeds are closely bred in the high tropics of Colombia, and the crossing between individuals of these breeds is frequent. Therefore, the results of the distance matrix and the phylogenetic tree can be explained by the common geographical location, the origin of these breeds and the breeding practices to obtain genetically improved individuals through crossbreeding strategies.

The Bayesian analysis using STRUCTURE program assumed a value of K = 3 as the most likely number of ancestral populations to explain the genetic diversity and structure of the Colombian sheep populations. As Figure 2 showed, none of the clusters had a clear differentiation, which suggests the presence of high admixture levels in these sheep breeds. This could be considered as a phenomenon of introgressive hybridization in which there is a gene flow from one population into the gene pool of another, but in this case, this introgression would occur between different proportions of the genetic material of three ancestral breeds, as showed by the most likely K value. The group of wool sheep imported from Europe 500 years ago (Churra and Manchega breeds, mainly) would make up the first ancestral population, from which the current Colombian sheep are descendants (Pastrana and Calderon, 1996). African sheep brought to America after the colonization would make up the second ancestral population, and from which breeds like OPC, Black Belly, and Pelibuey would be descendants. European breeds introduced to the region long after the conquest times with the aim of genetically improving wool sheep could make up the third ancestral group.

In conclusion, all three Colombian sheep breeds showed high genetic variability, evidenced by high polymorphism and number of alleles per locus for the molecular markers used for genotyping. However, a heterozygosity deficit was present to some extent in all breeds, something mainly attributed to endogamy within sheep flocks. In order to maintain the existing genetic diversity, breeding strategies (i.e. seed stock exchange oriented) aiming to maintain effective population size while minimizing inbreeding and genetic drift, should be implemented, especially for MORA and CRL. Besides, a conservation program is recommended for MORA and CRL breeds, including *in vitro* (e.g. germplasm bank) and *in situ* (e.g. maintenance of flocks) approaches.

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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