

Original

# Genetic diversity of Colombian Creole sheep

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## ABSTRACT

**Objective.** To characterize genetically the Colombian Creole sheep and their relations with breeds of European origin. **Materials and methods.** 261 blood samples from the following populations were collected: Wool Creole (WC), Colombian Mora (CM), Hair Creole (HC), Mestizos (Mes), Hampshire (Hamp), Corriedale (Corr), Katahdin (Kath), Pelibuey (Pel), in 40 farms of eight departments (Córdoba, Magdalena, Cesar, Atlántico, Valle del Cauca, Nariño, Boyacá and Tolima) and 30 samples of Spanish Merino (SM), Merino Precoz (MP), Merinofleischschaf (MF), Segureño (Seg) and Uda (UD) of Nigeria. A total of 15 microsatellite markers were included in this study. **Results.** In Creole sheep, the average number of alleles found was  $6.20 \pm 1.48$  (CL),  $7.27 \pm 1.39$  (CP) and  $3.60 \pm 1.55$  (MC); genetic diversity was also high in them (heterozygosities greater than 75%), negative values in the  $F_{IS}$  revealed a high degree of introgression. In addition, the  $F_{ST}$  revealed genetic structure in both the Creole groups ( $F_{ST}=0.02^{**}$ ) and in the sampled departments ( $F_{ST}=0.039^{**}$ ). According to the genetic distance, the Colombian Creole sheep show differences with the foreign sheep. **Conclusions.** The results obtained recommend protecting the Creole ovine culture since it is threatened by constant crossbreeding with foreign breeds, which would lead to loss of the genetic identity and the adaptation characteristics of the Creole animals.

**Keywords:** DNA; conservation; genetics (*Source: CAB*).

## RESUMEN

**Objetivo.** Caracterizar genéticamente los ovinos criollos colombianos y sus relaciones con razas de origen europeo. **Materiales y métodos.** 261 muestras de sangre de las siguientes poblaciones, fueron colectadas: Criollos de Lana (CL), Mora Colombiana (MC), Criollo de Pelo (CP), mestizos (Mes), Hampshire (Hamp), Corriedale (Corr), Katahdin (Kath), Pelibuey (Pel), En 40 fincas de ocho departamentos (Córdoba, Magdalena, Cesar, Atlántico, Valle del Cauca, Nariño, Boyacá y

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Tolima) y 30 muestras de Merino Español (ME), Merino Precoz (MP), Merinofleischschaf (MF), Segureño (Seg) y Uda (UD) de Nigeria. Un total de 15 marcadores microsatélites fueron incluidos en este estudio. **Resultados.** En ovinos criollos, el número promedio de alelos encontrado fue  $6.20 \pm 1.48$  (CL),  $7.27 \pm 1.39$  (CP) y  $3.60 \pm 1.55$  (MC); hallándose también alta diversidad genética en ellos (heterocigosidades superiores al 75%), valores negativos en el  $F_{IS}$  revelaron alto grado de introgresión; además el  $F_{ST}$  reveló estructura genética tanto en los grupos criollos ( $F_{ST}=0.02^{**}$ ), como en los departamentos muestreados ( $F_{ST}=0.039^{**}$ ). Según la distancia genética, los ovinos criollos colombianos presentan diferencias con los ovinos foráneos. **Conclusiones.** Los resultados obtenidos recomiendan proteger la ovinocultura criolla puesto que se encuentra amenazada por los constantes cruzamientos con razas foráneas, lo que conllevaría a pérdida de la identidad genética y de los rasgos de adaptación propios de los animales criollos.

**Palabras clave:** ADN; conservación; genética (*Fuente: CAB*).

## INTRODUCTION

The sheep, like other domestic animals, are not native to the American continent, they arrived from the Iberian Peninsula, first as food for the navigators and conquerors, then as breeding stock for the first settlers and religious and the racial base of the wool cattle was constituted in America. Due to the reduced number of heads and environmental conditions, the animals were raised without a precise selection scheme, which produced a miscegenation that lasted for centuries, giving rise to the so-called Creole sheep breeds that persist today in the form of small nuclei, usually in marginal areas. It is demonstrated that the diffusion of the unwool races in the American continent was developed from the Caribbean bases. The major positive characteristics of Creole are its rusticity, its adaptation to marginal environments, difficult climates and its longevity.

In Colombia, animals that entered the Caribbean coast, probably along the Guajira, gave rise to the so-called Colombian Creole Ovine (CCO). Its wide adaptation makes it easily found from the arid zones of the Guajira to the humid moors of the Andean zone. The Colombian Creole sheep population is made up of: Wool Creole sheep (WS) of economic importance for Boyacá, Cundinamarca, Nariño and Santander, the Mora Colombian synthetic sheep breed (MC) in the departments of Boyacá, Cundinamarca and Santander and the Creole sheep of Hair or African (CH), which is found mainly in the Atlantic Coast, Eastern plains, Tolima, Valle del Cauca and Huila. In some areas of the country three varieties of CH are recognized: Colombian Creole sheep Sudan (CS) whose coat goes from yellow to white, Ethiopian (CE) of cherry red color and Abyssinian (CA) of black color; this denomination by varieties is taken into account mainly by

the producers of the department of Córdoba and Valle del Cauca. The Instituto colombiano Agropecuario (ICA) reports that in Colombia there are 1.423.274 animals. Production in Colombia is of low use of inputs and is generally related to traditional and artisan production systems both in the case of wool sheep (WS) and hair sheep (HS) (1).

In the present work the genetic diversity of the Colombian Creole sheep was evaluated, the differences between the varieties of Creole hair sheep were evaluated and the genetic relationships of the Colombian Creole with sheep of European and African origin were studied, by means of the use of microsatellites molecular markers.

## MATERIALS AND METHODS

**Sampling.** Blood samples were taken from 169 HC sheep and 30 WS sheep, in 40 farms (Table 1). According to the criteria of the producers, unrelated animals were sampled. In vacutainer tubes with EDTA, 10 ml of blood were taken from each animal by means of puncture in the jugular vein. The HC contributed the largest sample size, because this is the first work in the country, which is done to know its genetic diversity. Cruised creole sheep (Mix) were also sampled, with Pelibuey of Mexican origin ( $n=16$ ), with Hampshire ( $n=2$ ), with Katahdin ( $n=5$ ) and with Romney Marsh ( $n=2$ ). Foreign sheep breeds in meat production (FM) were used as reference samples: Katahdin and Mexican Pelibuey were used and in wool production (WP), Corriedale and Hampshire. Samples of Spanish Merino ( $n=5$ ), Merino Premature of French origin ( $n=5$ ), Merino Fleischschaf of German origin ( $n=5$ ), Segureño ( $n=5$ ) of Spanish origin and Uda ( $n=10$ ) of Nigerian origin were included.

In the department of Córdoba, the HC were classified by Sudan (CS), Ethiopian (CE) and Abyssinian (CA) varieties.

**Table 1.** Number of samples per genetic group, abbreviation (Abbrev.), Sample size (N) and location of the sheep used.

Genetic Group	Abbrev.	N	Location
Wool creole	WS	30	Boyacá, Nariño
Colombian Mora	CM	4	Boyacá
Total wool creole sheep	WS	34	
Creole Sudán	CS	30	Atlántico, Cesar, Córdoba, Magdalena.
Ethiopian Creole	CE	37	Atlántico, Cesar, Córdoba, Magdalena.
Abyssinian Creole	CA	13	Atlántico, Cesar, Córdoba, Magdalena.
Unclassified Hair creole in variety	UHC	89	Valle del Cauca, Tolima
Total creole hair sheep	HC	169	
CL x Foreign	MesL	4	Boyacá
CP x Foreign	MesC	21	Córdoba, Valle del Cauca
Pelibuey of México	Pel	8	Valle del Cauca
Hampshire	Hamp	5	Boyacá
Katahdin	Kath	5	Córdoba, Valle del Cauca
Corriedale	Corr	4	Boyacá
Merino Spanish	MS	6	Spain, Colombia
Merino fleischschaf	MF	5	Germany
Merino Premature	MP	5	France
Segureño	Seg	5	Spanish
UDA	UD	10	Nigeria
<b>Total</b>		<b>281</b>	

**Microsatellite markers.** Now the 15 microsatellites used and their position in the genome: *OarCP34(3)*, *McM527(5)*, *D5S2(5)*, *RM006(5)*, *OarAE129(5)*, *ETH225(9)*, *INRA35(12)*, *TGLA53(12)*, *INRA63(14)*, *TGLA126(16)*, *BM8125(17)*, *OarFCB48(17)*, *OarFCB304(19)*, *OarCP20(21)*, *BM6526(26)*, of a panel of 32 used in the Biovis project, chosen among those proposed by FAO (Food and Agricultural Organization) and ISAG (International Society for Animal Genetics) (2).

**Extraction, PCR and Amplification.** The molecular characterization was carried out in the laboratories of Animal Genetics and Molecular Biology of Universidad Nacional de Colombia,

Palmira. The Salting Out-protocol (1988) was used to extract DNA. For the PCR, 2.0 X of Taq Tampon were used; 4 mM MgCl<sub>2</sub>; 0.4 mM of dNTPs; 0.2 mg/ml BSA; 0.2 uM of primers; 0.75 U of Taq and 40 ng of DNA for a final volume of 25 ul. The thermal PCR profile was: initial denaturation 5 min at 95°C, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 58°C, 90 seconds at 72°C and a final extension at 72°C 15 minutes. For the visualization of the amplified products, 6% polyacrylamide gels (29:1 acrylamide - bisacrylamide) were used in Fisher sequencing cameras of 35 x 45 cm and 10 bp marker.

**Statistic analysis.** The number of alleles (NA), the average number of alleles per locus (UHC), the values of observed, expected heterozygosity and  $F_{IS}$ , the Hardy-Weimberg equilibrium deviation test (EHW), were estimated using ARLEQUIN software 3.11; the content of polymorphic information (PIC), with the program Microsatellite Toolkit software for Excel; allelic richness, through the FSTAT program, see. 2.9.3. Molecular variance analysis (AMOVA) was performed with the ARLEQUIN program see 3.11.

To estimate the distances and phylogenetic relationships between groups, the minimum Nei distance was used, by means of TFPGA® and the  $F_{ST}$  by pairs of populations with ARLEQUIN see 3.11. Two dendograms were constructed, the first with all the genetic groups and the second with the eight departments sampled using only the data of the creole races, by means of the algorithm UPGMA (Unweighted Pair Group Method with Arithmetic mean), by means of TFPGA®.

The structure of the populations was explored with the cluster analysis method, based on models implemented in the program STRUCTURE v.2.3.1, which calculates a probability value for a number of K populations (or cluster) predetermined, and assigns the part of the genome of each individual that derives from each cluster. The population structure was tested from K = 1 to K = 16 with 15 microsatellite markers to analyze the relationships between Colombian Creole sheep and foreign breeds. When the Nigerian race UDA was included, it was tested with K = 1 up to K = 17 with seven microsatellite markers.

The total number of interactions was 500000 after 100000 *burning-in* with three replicates for each K. It was assumed that the allelic frequencies of

the current populations were correlated and that they could have originated from more than one ancestral population. The most probable value of K was determined according to the method of Evanno implemented in the program Structure Harvester v0.6.94.

## RESULTS

For the 15 markers, the number of alleles ranged from five to nine, being the markers ETH225, INRA63, RM006 and OarFCB48 the ones with the highest number of alleles. The PIC values were very informative, since they oscillated between 0.62 (OarFCB304) and 0.84 (INRA63, BM6526); the average for all the markers was 0.76. In general, for each marker the expected heterozygosity (He) was lower than the observed heterozygosity (Ho). The Ho in all loci was high except for OarCP34 (0.40) and OarFCB304 (0.44). The genetic diversity found per marker was high (0.78±0.01). The OarFCB304 was the only marker that was not found in HW equilibrium. The fixation index (F<sub>IS</sub>) did not show significant values for most markers; only the OarCP34 and OarFCB304 systems showed a reduction in the number of heterozygotes (p<0.001) (Table 2).

Table 3 shows the descriptive statistics among the Colombian Creole wool and hair sheep. We found 93 alleles in WS and 109 alleles in HC, the latter presented a higher average in the number of alleles (7.27±1.39) with respect to

CL (6.20±1.48), which may be affected by the sample size. For both genetic groups, the He was less than the Ho. The genetic groups HC and WS presented similar values of both Ho (0.90±0.18 and 0.88±0.21) and He (0.79±0.06 and 0.78±0.07) respectively. High genetic diversity was found, higher than 75%.

**Table 2.** Number of alleles (NA), allelic richness (AR), polymorphic information content (PIC), observed heterozygosity (OH), expected heterozygosity (EH) and fixation index (F<sub>IS</sub>) for 15 markers.

Marker	NA	AR	PIC	OH	EH	F <sub>IS</sub>
BM8125	6	5.95	0.74	0.98	0.76	-0.30
INRA35	8	7.58	0.81	0.96	0.83	-0.15
ETH225	9	7.85	0.79	0.96	0.81	-0.18
INRA63	9	8.65	0.84	0.93	0.80	-0.16
TGLA126	7	6.34	0.75	0.97	0.80	-0.21
TGLA53	7	6.74	0.78	0.94	0.80	-0.18
BM6526	8	7.95	0.84	0.97	0.86	-0.13
RM006	9	8.39	0.83	0.94	0.79	-0.18
OarCP34	7	4.99	0.67	0.40	0.70	0.43**
OarFCB304	5	4.71	0.62	0.44	0.63	0.30**
OarCP20	6	5.46	0.76	0.92	0.78	-0.17
OarAE129	5	4.92	0.72	0.96	0.77	-0.25
McM527	7	6.25	0.78	0.99	0.80	-0.23
D5S2	7	6.08	0.64	0.94	0.71	-0.34
OarFCB48	9	7.73	0.77	0.97	0.78	-0.24
<b>Average</b>	6.73	6.64	0.76	0.88	0.78	-0.14
<b>Deviation</b>	1.39			0.05	0.01	0.05

\*\*p<0.001

**Table 3.** Descriptive statistics for Creole wool and hair sheep, hair and half-breed varieties: sample size (N), number of alleles (NA), number of total alleles per marker (NTA), average number of alleles (ANA), effective number of alleles (N<sub>e</sub>), observed heterozygosity (H<sub>o</sub>), expected heterozygosity (H<sub>e</sub>) y (F<sub>IS</sub>).

	N	NA	NTA	NPA	N <sub>e</sub>	H <sub>o</sub>	H <sub>e</sub>	F <sub>IS</sub>
WS	30	8	93	6.20±1.47	4.58	0.88±0.21	0.78±0.07	-0.13 ns
MC	4	7	54	3.60±1.55	3.34	0.97±0.09	0.75±0.15	-0.45 ns
HC	169	9	109	7.27±1.39	4.96	0.90±0.18	0.79±0.06	-0.14ns
Total	203		109	5.69±1.47	4.29	0.92±0.16	0.77±0.09	-0.24 ns
CS	30	9	95	6.33±1.45	4.46	0.91±0.18	0.77±0.09	-0.18 ns
CE	37	8	94	6.27±1.28	4.60	0.89±0.23	0.77±0.10	-0.15 ns
CA	13	8	89	5.93±1.16	4.48	0.87±0.24	0.79±0.09	-0.10 ns
CPN	89	9	105	7.00±1.61	4.86	0.91±0.15	0.79±0.06	-0.15 ns
Total	169		105	6.38±1.38	4.60	0.90±0.20	0.78±0.09	-0.15 ns
MesL	4	8	68	4.53±1.64	3.44	0.93±0.15	0.76±0.11	-0.29 ns
MesC	21	8	89	5.93±1.34	4.73	0.92±0.15	0.80±0.05	-0.15 ns
<b>Total</b>	<b>228</b>	<b>9</b>	<b>105</b>	<b>5.23±1.49</b>	<b>4.08</b>	<b>0.93±0.15</b>	<b>0.78±0.08</b>	<b>-0.22 ns</b>

ns: not significant. F<sub>ST</sub>= 0.02\*\*; F<sub>IS</sub>= -0.16; F<sub>IT</sub>= -0.14

In another analysis where hair sheep were separated by variety, a maximum of 105 alleles were found in these. The UHC group obtained an average of alleles of  $7.00 \pm 1.61$ , being the highest of all the groups of Creole sheep; in addition, the populations CS (6.33), CE (6.27) and WS (6.2), presented the highest average number of alleles with regarding the groups MC, CA, MesL and MesC (5.93).

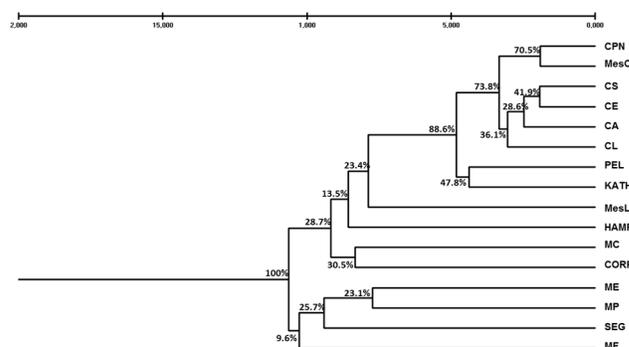
A private allele was found in the CA population (124bp) in the OarCP34 marker and in the CPN population (167bp) in the McM527 marker, with a frequency of 5.9% and 1.7% respectively.

The  $H_o$  presented higher values than the  $H_e$  in all the sheep populations (Table 3). The genetic diversity ( $H_e$ ) in all the Creole populations was high, higher than 75%.  $H_e$  values ranged between  $0.75 \pm 0.15$  and  $0.80 \pm 0.05$ . The varieties CS ( $0.77 \pm 0.09$ ), CE ( $0.77 \pm 0.010$ ) and CA ( $0.79 \pm 0.09$ ) presented high values of genetic diversity, as did the UHC group ( $0.79 \pm 0.06$ ).

The values of  $F_{IS}$  (Table 3), were not significant in any of the groups, while the value of  $F_{ST}$  found was 0.02 being highly significant, indicating that there is little genetic differentiation between the groups.

The  $F_{ST}$  between population pairs (Table 4), were low but significant in almost all combinations, except between MC with CL and MesL; CA with CE and UHC with MesC. The WS group differed from the Creole hair populations (CS, CE, CA, CPN) since all the  $F_{ST}$  showed highly significant values ( $p < 0.001$ ). The  $F_{ST} = 0.000$  confirmed that there are no genetic differences between CA and CE varieties.

Figure 1 shows the dendrogram performed with the minimum distance of Nei, which includes all the Creole, half-breed and foreign genetic groups sampled. It can be seen that the Colombian Creole sheep are genetically separated from the Hampshire, Corridale and Mora Colombian breeds. The races ME, MP, MF and Seg, were in a different group from the Creole races. The Colombian creole races and the Pelibuey and Katahdin races were grouped, this may be due to the fact that they have the same origin in common. When observing the Creole hair sheep, the UHC and the MesC were located in the same group, while in another group the CE, CA and CS hair varieties were located. Suggesting genetic differences between different hair biotypes.



**Figure 1.** Dendrogram performed with the minimum distance of Nei, using the UPGMA classification method, using data from 15 microsatellite markers in Colombian Creole sheep and foreign breeds.

To know the genetic structure by departments of the genetic groups WS and HC with their varieties, the  $F_{ST}$  was found by pairs of populations, the

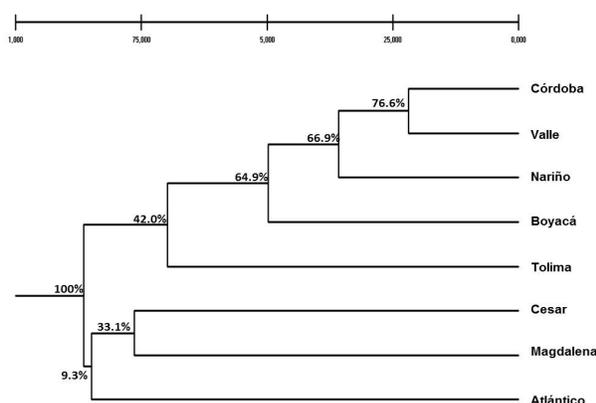
**Table 4.** Estimates of genetic differentiation for Creole wool populations (WC), Colombian Mora (CM), Sudan hair Creole (CS), Ethiopian hair Creole (CE), Abyssinian hair Creole (CA), unclassified hair creole (UHC), creole half-breed wool (MesL) and creole half-breed meat (MesC).

	WC	MC	CS	CE	CA	CPN	MesL	MesC
WC	*****							
MC	0.007ns	*****						
CS	0.012*	0.060*	*****					
CE	0.020*	0.031*	0.010*	*****				
CA	0.024*	0.041*	0.010*	0.000ns	*****			
UHCN	0.023*	0.035*	0.023*	0.023*	0.022*	*****		
MesL	0.011*	-0.083ns	0.047*	0.025*	0.035*	0.041*	*****	
MesC	0.025*	0.040*	0.019*	0.011*	0.014*	0.001ns	0.041*	*****

\*\*( $p < 0.001$ ); \*( $p < 0.05$ ); ns: not significant

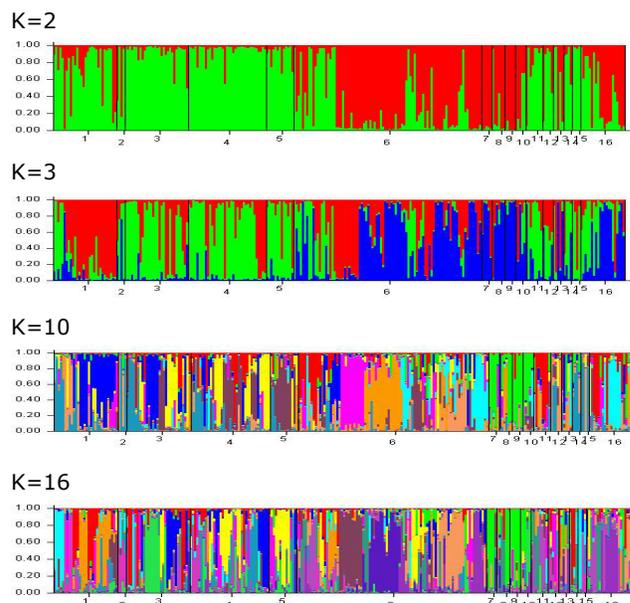
result obtained was: 0.039 ( $p < 0.0001$ ), which indicates little genetic differentiation among the animals sampled by department (Table 5). Population structure was found in all the combinations made, the largest genetic structure was found between the departments of Tolima and Magdalena (0.103), (moderate genetic differentiation).

In general, in almost all the combinations in Table 5, the genetic structure values were small, which suggests that there is little differentiation by departments despite the difference in climate and ovine phenotype (WS and HC). The same results were observed in the dendrogram (Figure 2), where both wool and hair breeds were grouped in the same group.



**Figure 2.** Dendrogram made with the minimum distance of Nei, through the UPGMA classification method using 15 microsatellite markers in eight Colombian departments.

An analysis of the structure of the population was carried out using the Bayesian algorithm. In figure 3, when the existence of two ancestral populations is assumed ( $K=2$ ), a common ancestor can be seen for all the Creole sheep WS, CS, CE, CA also for the Pel, Hamp, Corr and for the MesL, all the previous ones differed from the other groups.



**Figure 3.** Graphic representation of the results of the analysis of the genetic structure of six Colombian Creole sheep populations compared to foreign and mixed breeds ( $K=2$  to  $K=16$ ), using 15 microsatellite markers. 1: CL; 2: MC; 3: CS; 4: CE; 5: CA; 6: CPN; 7: MF; 8: ME; 9: MP; 10: Seg; 11: Pel; 12: Hamp; 13: Kath; 14: Corr; 15: MesL; 16: MesC.

**Table 5.** Genetic differentiation by department.

	Boyacá	Nariño	Córdoba	Valle	Tolima	Atlántico	Cesar	Magdalena
<b>Boyacá</b>	*****							
<b>Nariño</b>	0.035*	*****						
<b>Córdoba</b>	0.027*	0.019*	*****					
<b>Valle</b>	0.025*	0.037*	0.021*	*****				
<b>Tolima</b>	0.050*	0.058*	0.038*	0.030*	*****			
<b>Atlántico</b>	0.080*	0.072*	0.056*	0.040*	0.079*	*****		
<b>Cesar</b>	0.071*	0.072*	0.063*	0.049*	0.078*	0.074*	*****	
<b>Magdalena</b>	0.082*	0.055*	0.061*	0.060*	0.103*	0.048*	0.056*	*****

Between departments  $F_{ST} = 0.039^{**}$  ( $**p < 0.001$ ).

When it is assumed that there are three ancestral populations ( $K=3$ ) a common ancestor can be found for all creole sheep, which agrees with the mixed origin of these populations; the WC, CS, CE, CA and Pel (green color) are differentiated, sharing a greater resemblance in their structure to the UHC and the MesC with the foreign races MF, ME, MP, Seg (blue color), it can be seen that mainly the CPN and the MesC have a mixture of both foreign and Creole races, as do the MesL, Hamp, Kath and Corr, when  $K=10$  and  $K=16$  is observed that in general all the populations, both Creole and foreign, sampled in the country present a large mixed composition.

## DISCUSSION

The average number of alleles (ANA) found in sheep WS and HC was 6.20 and 7.27 respectively, this could be influenced by the sample size of the groups. The values found in the average number of alleles in CL are also close to those found in Mexican Creole breeds: Chiapas (ANA = 6.10), Chamula (ANA = 6.90) and Brown (ANA = 7.80), (3) already reported in the Ibero-American ovine biodiversity project - Biovis (ANA = 6.90). Likewise, the average number of alleles found in this study is similar to the Creoles of Paraguay (4), Mexico (5) and the United States (6).

The  $H_e$  in both groups was similar and revealed high levels of variability for both WS (0.78) and HC (0.79).

The high values of  $H_e$  found in these two Colombian genetic groups coincide with those found with microsatellites in Creole sheep from America: in Mexico with the sheep breeds from Chiapas (3), in the eastern region of Paraguay with sheep from the wetlands (4), in the Chilean sheep breed Chilota (7), in Creole and improved sheep of the United States (6).

The high levels of variability found, could be explained by their diverse origin since it is probable that the sheep had followed the same route of entry to the country as the cattle. According to different authors, cattle entered through three routes: from the Caribbean coast, from Ecuador and also from Nicaragua, via the Panama-Buenaventura route.

According to Delgado et al (8), during the fifteenth century several sheep introductions were made, therefore Ibero-American populations were founded on a very diverse animal base, imported

from Spain and Portugal; subsequently, the effects of natural and artificial selection, genetic drift and continuous migrations of genotypes from the Iberian Peninsula itself, as well as from other European countries, from Africa and Asia, constituted a high genetic richness. On the other hand, throughout the history of creole sheep, low selection pressure has been exerted.

The value of  $F_{IS}$  that estimates the excess or deficit of heterozygotes was not significant, it showed negative values in both WS (-0.13) and HC (-0.14), suggesting the presence of exogamy in Creole sheep production in the country. The high genetic diversity and absence of intrapopulation endogamy may be due mainly to the constant use of indiscriminate crosses between Creole sheep and foreign breeds which was observed in the field.

It is important to note that wool creole (WC) and hair (HC) creoles showed very little genetic differentiation ( $F_{ST} = 0.014$  ( $p < 0.0001$ )) probably due to the introduction of Canarian sheep in America. The Canary Islands played a very important role in the distribution of animal genetic resources after the discovery of America, becoming a crossroads for Spanish navigators and other European countries with their overseas colonies and there are multiple historical evidences that confirm the participation of the Canarian sheep in the first colonization of America. According to Delgado et al (8), the Canarian sheep could have its origin in the hybridization between pre-Hispanic sheep and wool sheep introduced in the Canary Islands by Spaniards from the southern ports of the Iberian Peninsula or the African coast.

The genetic closeness between HC and WC was also observed in the analysis by departments where it was found that Boyacá and Nariño that correspond to high tropics were grouped with Valle del Cauca, Tolima and Córdoba (low tropic) regardless of the geographic location and the phenotype of sheep (wool or hair), while the departments of the North Coast of Colombia (Atlántico, Cesar and Magdalena), formed another group of hair sheep only.

The average number of alleles found in Creole hair sheep CS (ANA= 6.33), CE (ANA= 6.27) and CA (ANA= 5.93) was similar, despite the difference in sample size used in each variety: 30, 37 and 13 respectively; were lower than those reported in the Cuban hair sheep (ANA= 7.2), (9), similar for the Brazilian race Morada

Nova (ANA = 5.09), (10). The three varieties of hair sheep showed high values of heterozygosity, greater than 75%, indicating high genetic diversity. No significant values were found for the  $F_{IS}$  fixation index in the CS, CE and CA groups.

The phenotypic differentiation of the Creole hair varieties is a difficulty, since the specimens CS, CE, CA are very crossed and only pure specimens can be found in a few flocks. However when you observe their color, type of head, ears and other physiognomic features one variety can be distinguished from another.

The above is corroborated with the results found in this work, where the population structure was highly significant for these three varieties ( $F_{ST} = 0.02$ ), indicating that there is a genetic differentiation according to the scale of values proposed by Wright (11). The comparison CS with CE and CA differs significantly, but CE and CA are similar. Therefore, it can be concluded that there are only two biotypes of Creole sheep of hair, CE and CS in the country while the variety CA is possibly the same CE. CS and CE varieties have been reported by different authors (1).

It is important to clarify that the names of the varieties of Colombian, Ethiopian, Sudan and Abyssinian hair sheep have no relationship with the countries of origin; no justifiable reason was found in the literature, since these countries belong to East Africa. With the exception of Venezuela, no other country that uses this denomination was found either. In 1940 a nucleus of hair sheep arrived, coming from Abyssinia to the department of Tolima; it is likely that these names originated from this event.

The UHCUs are a reflection of the country's sheep production, since in this work they represent the largest sample size of different departments (Magdalena, Atlántico, Cesar, Valle del Cauca and Tolima). The genetic distances group the unclassified hair Creoles (UHC) with the half-breed (Month), which is confirmed in the Bayesian analysis and in the high values of diversity ( $0.79 \pm 0.06$ ). This finding generates an alert, because it is a vulnerable population because of the constant hybridization to which it is exposed, which may lead in the short future to absorption by foreign breeds, taking into account the low generational interval.

The CS, CE and CA hair Creole varieties formed a different group that moves away from the UHC and MesC, suggesting that the former remain

relatively free of introgression, with respect to the hair Creoles of the rest of the country.

Creole sheep represent an important resource for the small farmer. Its high value as a genetic resource is reflected in the constant increase of the population that has developed without any encouragement or support from associations or governmental organizations.

It was found, as observed in the Bayesian analysis and in the dendrogram, that the group of all the Colombian Creole sheep both wool and hair (CL, CS, CE, CA, UHC) and also the group of foreign breeds sampled in Colombia (Corr, Hamp, Pel, Kath) show: 1. Genetic similarity between them which suggests introgression in the Creole genetic groups, appearing in a greater proportion in the UHCs as explained above. 2. The Creole sheep have differences with the foreign breeds ME, MF, MP, Seg and UD, which suggests that the Creoles have genetically separated from their ancestors.

When observing the results in the Colombian Creole sheep of hair, it is important to recognize the difference found between the hair varieties (CS, CE and CA) and the UHCs, the former have a clear tendency to be a homogeneous group and different from the second. CS, CE and CA varieties have a lower degree of introgression with other breeds when compared with the CPN group, this last group is remarkably close to the group of half-breed creole sheep (Mes) and the introgression of other breeds is clearly observed in the bayesian analysis both in the  $F_{ST}$  and in the minimum distance of Nei. The UHCs are sheep that belong to the departments of Atlántico, Cesar, Magdalena, Tolima and Valle del Cauca and are a reflection of the country's sheep population, which mostly show crosses, only the animals sampled in Córdoba showed little introgression.

In general, the information that exists in the tropical regions of the world about the genetic sheep ovine resource and its importance is limited; taking into account that the OCC have had an adaptive process to the tropical environment of the country (12, 13, 14); it is necessary to promote their recognition and production: the ovine associations, the academy and the state should encourage in the producer the knowledge and conservation of these genetic groups, since their adaptation process over 500 years, has allowed them to survive the tropical climate turning them into a source of valuable

wealth and genetic diversity that must be further explored.

The set of the 15 microsatellite markers used provided information that was important for the analysis of genetic diversity and population structure of the Colombian Creole sheep and its relationship with some foreign breeds. It provides a starting point for future actions aimed at preserving the race as genealogical control, allocation of individuals to populations, product traceability, etc. It is one of the first works in Genetic Diversity of Colombian Creole hair sheep Ovine. High genetic diversity was found due to constant hybridisations of the Creole breeds with other breeds, therefore the Creole biotypes are vulnerable to absorption by foreign breeds, this is mainly due to the fact that introduced breeds, Creole breeds and alternate crosses in order to strengthen hybrid vigor for production (15).

High genetic closeness was found between the Creole sheep of wool and hair, probably due to a common origin in the Canary Islands and the crossing between both biotypes in recent times. In the group of non-classified hair creole

(UHC), a high degree of introgression was found, which leads to the loss of genetic identity. Hair sheep from the department of Córdoba CS, CE and CA differed from UHC group because they have genetic identity which makes them distant from the UHC. In the Creole hair sheep of the department of Córdoba, the CS differed from the CE and CA, while these last two were very similar genetically. The hair sheep of the department of Córdoba have their own genetic identity, being relatively purer than the rest of the country. Genetic differences were found between Creole sheep and breed ME, MP, MF, Seg and Uda.

### Conflict of interests

The authors affirm that there are no potential conflicts of interest regarding the research, authorship, or publication of this article.

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