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Genetic characterization of the Hartón del Valle, Angus, Brangus, Holstein, and Senepol cattle breeds in Colombia, using ten microsatellite markers¤

Caracterización genética de las razas Hartón del Valle, Angus, Brangus, Holstein y Senepol en Colombia, usando 10 marcadores microsatélites

Caracterização genética das raças Hartón del Valle, Angus, Brangus, Holandês e Senepol na Colômbia, usando 10 marcadores microsatélites

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Summary

The objective of this paper is to establish a genetic characterization of the Senepol (S, n=49), Holstein (H, n= 60), Hartón del Valle (HV, n=60), Angus (A, n=61) and Brangus (Br, n=60) cattle breeds in Colombia, by using the following microsatellite markers: SPS115, INRA64, ETH225, ETH10, BM1824, INRA37, TGLA122, TGLA126, INRA32, and BM2113. A total of 142 alleles were obtained for ten analyzed loci, considering the five cattle breeds as a whole. The number of alleles per locus ranged from 9 (INRA64 and 1824) to 22 (TGLA122). The expected heterozygosity was between 0.79 (INRA32) and 0.90 (INRA37) in all the cattle breeds, respectively; and medium heterozygosity was 0.84. The average number of alleles per breed varied from 9.2 in the Senepol breed to 10.3 in the Holstein breed. The expected heterozygosity range varied from 0.75 in the Hartón del Valle breed and 0.82 in the Holstein breed, with an average of 0.79. Hardy Wienberg disequilibrium was observed (p>0.05) when the populations were analyzed with all the markers. All the populations presented a heterozygote deficit, which could be the result of a strong endogamy tendency within all the herds. The markers used in this study allowed a genetic characterization of the analyzed populations. The microsatellites panel in

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the Hartón del Valle breed should be increased in order to increase the reliability value. Microsatellite panels could solve parenthood cases for the remainder breeds.

Key words: *cattle, genetic variability, molecular marker.*

Resumen

El objetivo de este trabajo fue caracterizar genéticamente las razas bovinas Senepol (S, n=49), Holstein (H, n= 60), Hartón del Valle (HV, n=60), Angus (A, n=61) y Brangus (Br, n=60) en Colombia, con los marcadores microsatélites SPS115, INRA64, ETH225, ETH10, BM1824, INRA37, TGLA122, TGLA126, INRA32 y BM2113. En total, 142 alelos fueron encontrados en los diez loci analizados, considerando las cinco razas como un todo. El número de alelos por locus estuvo entre 9 (INRA64 y BM1824) y 22 (TGLA122). La Heterocigosidad esperada a través de todas las razas varió entre 0.79 (INRA32) y 0,90 (INRA37) y heterocigosidad media esperada de 0.84. El número promedio de alelos por raza varió de 9.2 en la raza S a 10.3 en la raza H. El rango de la Heterocigosidad esperada entre las razas varió entre 0.75 en la raza HV y 0.82 en la raza H, con una media de 0.79. Al analizar las poblaciones con el total de marcadores, todas se encontraron en desequilibrio de Hardy Weinberg (p>0.05). Todas las poblaciones presentaron un déficit de heterocigotos, para todas las poblaciones, lo que podría ser el resultado de la fuerte tendencia a la endogamia dentro de los diferentes hatos. Los resultados indicaron que los marcadores utilizados en este estudio permitieron caracterizar genéticamente las poblaciones analizadas. En el caso de la Raza HV, se debe aumentar el panel de microsatélites para aumentar el valor de confiabilidad. Para las demás razas el panel de microsatélites permitiría resolver casos de filiación.

Palabras clave: *ganado, marcadores moleculares, variabilidad genética.*

Resumo

O objetivo do presente trabalho foi caracterizar geneticamente as raças Senepol (S, n=49), Holandês (H, n= 60), Hartón del Valle (HV, n=60), Angus (A, n=61) e Brangus (Br, n=60) na Colômbia, com os marcadores microsatélites SPS115, INRA64, ETH225, ETH10, BM1824, INRA37, TGLA122, TGLA126, INRA32 e BM2113. Em total, 142 alelos foram encontrados nos 10 satélites analisados nas cinco raças. O número de alelos esteve entre 9 (INRA64 e BM1824) e 22 (TGLA122). A heterocigosidade esperada a través de todas as raças variou entre 0.79 (INRA32) e 0.90 (INRA37) e heterocigosidade esperada de 0.84. O número médio de alelos por raça variou de 9.2 na raça S a 10.3 na raça H. O rango de heterocigosidade esperada entre raças variou entre 0.75 na raça HV e 0.82 na raça H, com una media de 0.79. Ao analisar as populações com o total de marcadores encontraram-se o desequilíbrio Hardy Weinberg (p>0.05). Todas as populações apresentaram um déficit de heterocigotos, o que poderia ser o resultado da forte tendência de endogamia nos diferentes rebanhos analisados. Os resultados indicaram que os marcadores utilizados em este estúdio permitiram caracterizar geneticamente as populações analisadas. No caso da raça HV deve-se aumentar o número de microsatélites para aumentar o valor de confiabilidade. Para as demais raças os microsatélites analisados permitiriam resolver casos de paternidade.

Palavras chave: *gado, marcador molecular, varibilidade genética.*

Introduction

Livestock breeds have been the product of centuries of natural and artificial selection. Breeds have been selected to fit a wide range of environmental conditions and human needs. The selection of a few highly productive breeds has caused the decrease of many other non-productive breeds (Maudet *et al.*, 2002).

In Colombia there is a large variety of native and foreign breeds; most of them have adapted to the diversity of geographical conditions of these country. Therefore, it is thought that these breeds present an enormous genetic diversity due to the fact that they come from different regions could explain their genetic variability.

Microsatellite markers (STR) have made possible the study in numerous breeds around de

world, something like that. Currently, there are more than 1200 STR known in cattle (Kappes *et al*., 1997) some of them approved by the International Society of Animal Genetics (ISAG) and the Food and Agriculture Organization (FAO, 2007). The usefulness of microsatellite markers have been documented in bovine breeds for the estimation of genetic diversity (Ciampolini *et al*., 1995; Peelman *et al*., 1998; Zamorano *et al*., 1998b; Steigleder *et al*., 2004; Barrera *et al*., 2006a; Martínez *et al*., 2006) and the relationships among livestock breeds (Moreno *et al*., 2001; Hansen *et al*., 2002; Maudet *et al*., 2002; Radko *et al*., 2005; Barrera *et al*., 2006b), as well as genetic characterization (Zamorano *et al*., 1998a; Lara *et al*., 2005; Martínez *et al*., 2005; Calvo *et al*., 2009; Martínez *et al*., 2009), individual profiles, and paternity tests (Giovambattista *et al*., 2001; Mommens *et al*., 1998; Sanz *et al*., 2002) and genealogical-registries control (Jamieson, 1994).

In general, the distribution of genetic polymorphisms tends to be quite homogeneous among the different populations involved. Nevertheless, in the variability extend, specific characteristics for each population group are usually noted. (Barrera *et al*., 2006a; Barrera *et al*., 2006b, Calvo *et al*., 2009, Hansen *et al*., 2002; Martínez *et al*., 2009; Maudet *et al*., 2002; Mommens *et al*., 1998; Moreno *et al*., 2001; Radko *et al*., 2005; Steigleder *et al*., 2004; Zamorano *et al*., 1998a; Zamorano *et al*., 1998b). This could be achieved through the genetical characterization of the animals in each region.

This paper was conducted to study genetic diversity among the *Hartón del Valle*, *Angus*, *Brangus*, *Holstein*, and *Senepol* cattle breeds, all of them found in Colombia and well adapted to the diversity of its weather and geographical conditions, using ten microsatellite markers located in different chromosomes.

Materials and methods

Population Sample

Sample was constituted as follows: *Senepol* (S, n=49) from Hacienda Río Piedras, municipality of Jericó, Antioquia; *Holstein* (H, n= 60) from the municipality of San Pedro de los Milagros, Antioquia; Hartón del Valle (HV, n=60) from Hacienda Sanjonhondo, located in Tuluá municipality, Valle del Cauca; *Angus* (A, n=61) and *Brangus* (Br, n=60), both from Centro de Investigación en Biotecnología y Reproducción (Cibre) of the Universidad San Martín in Montería. Blood samples were taken from all specimens and DNA isolation carried out using salting out protocol (Miller *et al.*, 1988), procedures took place at Animal Genetics Laboratory, Universidad de Antioquia.

Genotyping

Ten microsatellite markers were used for this study. These were taken from the ISAG's recommended list for studies on genetic variability (Table 1) and were used applying multiplex systems (duplex or triplex) according to each marker's amplification conditions.

Table 1. Chromosomal location, range of alleles of each one of the markers used and microsatellites alleles (specie - specific) of the Hartón del Valle (HV), Angus (A), Brangus (Br), Holstein (HO) and Senepol (S) cattle breeds.

Microsatellite marker	Chromosome	Range	Private alleles (Breed)
BM1824	1	242-264	160 (A)
ETH225	9	171-187	163 (BR), 135 (HV), 157 (HV)
ETH10	5	133-163	208 (BR)
BM2113	2	208-228	147 (BR), 121 (S), 123 (S)
SPS115	15	160-188	242 (A), 264 (A)
INRA037	11	114-156	118 (BR), 156 (BR), 114 (HO)
INRA032	11	135-179	167 (HV), 149 (S)
INRA064	23	112-134	187(BR)
TGLA122	21	149-189	175 (BR), 173 (HV), 167 (S), 179 (S)
TGLA126	20	121-149	112 (HV)

Amplification conditions included approximately 50 ng of bovine genomic DNA, 10X reaction buffer (10mM Tris-HCL pH 9.0; 50mM KCl; 0.1% Tritón ® X-100), 1.5 mM of $MgCl_2$; dNTPs; 0.125 µM of forward and reverse primers and 1 U of *Taq* DNA Polymerase in a total volume of 15 µL. The reaction was carried out in a T-Personal 48 thermocycler (Biometra GMBH, D-37079 Goettingen, Germany).

INRA32 and BM2113 markers were amplified in duplex reaction at 58 ºC; the rest in duplex or triplex reaction at 56 ºC, under the following conditions: 94 °C for 2 min. for denaturation; next, 35 cycles at 94 °C for 45 s, 45 s for alignment, 72 °C for 45 s and final extension at 72 °C for 15 min. Amplification products were visualized in a 6% denaturing polyacrylamide gels and stained with 1% silver nitrate (Budowle *et al.*, 1991). Genotypes designation was made by comparison of a molecular ladder.

Statistical Analysis

GDA, Genetic Data Analysis software was used for the genetic analysis (Lewis y Zaykin, 2001). This program allowed the calculation of the average number of alleles, the observed and expected heterozygosities and the *Fis* per breed and per locus. Arlequín software (Schneider *et al.*, 2000) was used for the analysis of allele frequencies (data available at request), observed and expected heterozygosities and *Fis analysis.*

Polymorphic Information Content (PIC) for each marker was estimated in each breed according to Botstein and White's (1980) description; the exclusion probability (EP) was calculated for both individual markers and for the group of markers per breed, according to Jamieson (1994) suggestion. These procedures were undertaken to determine whether these microsatellite markers are informative to study breed characterization and paternity tests or not.

Results

A total of 142 alleles were found in the ten analyzed loci, considering the five breed as a whole. The number of alleles per loci was between 9 (INRA64 and BM1824) and 22 (TGLA122), with an average of 14.2 (Table 2). Expected heterozygosities throughout all the breeds ranked between 0.79 (INRA32) and 0.90 (INRA37), and the average expected heterozygosity was 0.84.

Tabla 2. Observed (Ho) and expected (He) heterozygosities average per locus. Average of the number of alleles (NA) and inbreeding coeficient (Fis) per locus in Colombian cattle breeds. Average of observed and expected heterozygosities per locus.

		Heterozygosity average		
Locus	**NA**	**He**	**Ho**	**Fis**
SPS115	12	0.84	0.57	0.32
INRA64	9	0.8	0.6	0.25
ETH225	15	0.88	0.73	0.17
ETH10	11	0.83	0.65	0.21
BM1824	9	0.85	0.66	0.22
INRA37	20	0.9	0.58	0.35
TGLA122	22	0.81	0.65	0.2
TGLA126	12	0.85	0.49	0.42
INRA32	17	0.79	0.61	0.22
BM2113	15	0.89	0.79	0.12
Average	14,2	0.84	0.63	0.25

The average number of alleles per breed ranked from 9.2 in *Senepol* breed to 10.3 in *Holstein* breed (Table 3). The expected heterozygosity range among the breeds ranked between 0.75 in *Hartón del Valle* breed and 0.82 in *Holstein* breed, with an average of 0.79.

Table 3. Observed (Ho) and expected heterozygosity average (He). Average of the number of alleles per breed (NA) and inbreeding coefficient (Fis) per breed.

		Heterozygosity average		
Breed	**NA**	**Ho**	**He**	**Fis**
Hartón del Valle	9.7	0.63	0.75	0.17
Angus	9.3	0.60	0.77	0.23
Brangus	10.3	0.67	0.80	0.15
Holstein	10.3	0.64	0.82	0.23
Senepol	9.2	0.62	0.80	0.23
Average	9.7	0.63	0.79	0.20

Values for the number of alleles, expected heterozygosity (He), observed heterozygosity (Ho), *Fis*, polymorphic information content (PIC) and exclusion probability (EP) calculated for each marker in each breed are shown in table 4. *Senepol* breed presented the lowest alleles number, 6 for INRA64 locus. The highest were in *Brangus* for INRA37 and in *Holstein* for TGLA122, both with 16. *Brangus* breed showed the highest observed heterozygosity for the BM2113 (0.845) microsatellite and *Angus* breed showed the lowest one in the INRA32 locus (0.410). A slight heterozygote excess was found in the *Brangus* breed in the INRA032 locus (-0.023) and in the *Hartón del Valle* breed in the BM1824 locus (-0.123). PIC varied from 0.537 for INRA32 in *Senepol* breed and 0.899 for INRA37 in the same breed. The lowest combined EP was 0.9997 for *Hartón del Valle* breed, the remaining breeds presented values higher than 0.9999.

Table 4. Number of alleles, Expected (He) and observed heterozygosities (Ho), Fis polymorphic information content (PIC), exclusion probability (EP) and combine exclusion probabilidade (PEC) for the analyzed microsatellites per breed.

			Heterozygosity average					
Breed	**Locus**	**NA**	**He**	**Ho**	**Fis**	**PIC**	**EP**	**PEC**
HARTÓN	SPS115	8	0.725	0.550	0.243	0.719	0.517	
	INRA64	8	0.803	0.600	0.255	0.797	0.599	
	ETH225	14	0.861	0.733	0.150	0.854	0.718	
	ETH10	8	0.632	0.617	0.025	0.627	0.423	
	BM1824	8	0.610	0.683	-0.123	0.604	0.346	
	INRA37	7	0.800	0.567	0.294	0.794	0.598	
	TGLA122	14	0.724	0.600	0.173	0.718	0.544	
	TGLA126	8	0.750	0.450	0.399	0.741	0.538	
	INRA32	12	0.812	0.712	0.124	0.805	0.621	
	BM2113	10	0.830	0.750	0.102	0.816	0.649	0.9997
ANGUS	SPS115	11	0.850	0.655	0.231	0.843	0.690	
	INRA64	7	0.756	0.576	0.240	0.749	0.52	
	ETH225	10	0.820	0.667	0.188	0.813	0.626	
	ETH10	7	0.753	0.672	0.110	0.747	0.535	
	BM1824	9	0.863	0.640	0.261	0.854	0.709	
	INRA37	10	0.802	0.460	0.430	0.795	0.605	
	TGLA122	9	0.657	0.656	0.002	0.651	0.439	
	TGLA126	10	0.853	0.475	0.446	0.844	0.693	
	INRA32	9	0.542	0.410	0.245	0.537	0.335	
	BM2113	11	0.857	0.803	0.063	0.849	0.699	0.9999

Table 4. *Cont...*

Breed	Locus	NA	Heterozygosity average		Fis	PIC	EP	PEC
			He	Ho				
BRANGUS	SPS115	8	0.835	0.527	0.371	0.824	0.663	
	INRA64	7	0.786	0.571	0.274	0.778	0.569	
	ETH225	11	0.899	0.796	0.114	0.891	0.779	
	ETH10	8	0.841	0.760	0.097	0.833	0.674	
	BM1824	7	0.830	0.815	0.019	0.821	0.646	
	INRA37	16	0.754	0.520	0.310	0.746	0.575	
	TGLA122	13	0.657	0.610	0.069	0.646	0.446	
	TGLA126	8	0.768	0.570	0.261	0.760	0.554	
	INRA32	11	0.716	0.732	-0.023	0.709	0.507	
	BM2113	12	0.885	0.845	0.046	0.877	0.754	0.9999
HOLSTEIN	SPS115	10	0.837	0.559	0.334	0.83	0.664	
	INRA64	8	0.802	0.678	0.156	0.795	0.604	
	ETH225	9	0.822	0.746	0.090	0.815	0.642	
	ETH10	9	0.750	0.570	0.244	0.743	0.547	
	BM1824	8	0.817	0.556	0.320	0.809	0.632	
	INRA37	13	0.846	0.750	0.114	0.838	0.698	
	TGLA122	16	0.856	0.768	0.103	0.848	0.711	
	TGLA126	8	0.795	0.444	0.443	0.786	0.591	
	INRA32	10	0.830	0.560	0.325	0.818	0.647	
	BM2113	12	0.877	0.735	0.164	0.868	0.735	0.9999
SENEPOL	SPS115	9	0.818	0.519	0.370	0.802	0.618	
	INRA64	6	0.621	0.478	0.234	0.607	0.391	
	ETH225	8	0.821	0.667	0.190	0.807	0.623	
	ETH10	10	0.791	0.630	0.208	0.781	0.618	
	BM1824	8	0.730	0.591	0.191	0.720	0.527	
	INRA37	15	0.913	0.660	0.283	0.899	0.799	
	TGLA122	10	0.846	0.618	0.273	0.834	0.678	
	TGLA126	7	0.740	0.555	0.254	0.719	0.518	
	INRA32	10	0.852	0.650	0.243	0.839	0.682	
	BM2113	9	0.862	0.830	0.040	0.849	0.704	0.9999

The results for EHW per breed and per locus showed that most of the loci showed disequilibrium, except for two of them (ETH10 and INRA32) in *Hartón del Valle*, two loci (ETH10 and TGLA122) in *Angus*, two loci (ETH225 y BM2113) in *Holstein*, two loci (INRA64 and BM2113) in *Senepol* and five loci (ETH10, BM1824, TGLA122, INRA32 and BM2113) in *Brangus* ($p>0.05$).

When analyzing the ten microsatellites for every breed's specific alleles, a total of three alleles were found in the TGLA122, ETH225 and INRA32 loci for *Hartón del Valle*; 4 alleles in the TGLA126, BM1824 and SPS115 loci for *Angus*; six alleles in the INRA37, BM2113, TGLA122, ETH225, INRA64 and ETH10 loci for *Brangus*; three alleles in BM2113, TGLA122 and INRA32 loci in *Holstein* and one allele in INRA37 in *Senepol* (Table 1). In most of the loci, the allelic frequency varied among breeds. The highest frequency was 0.20 for the 132 allele from the TGLA126 marker in *Angus* breed.

Discussion

In all the included breeds, all the analyzed microsatellites were polymorphic, showing between 9 (INRA64 y BM1824) and 22 alleles (TGLA122) (Table 2). In a study by Maudet *et al.* (2002) in native French cattle-breeds, the TGLA122 microsatellite turned out to be the most polymorphic too, with 19 alleles. Heterozygosity per locus was high and ranked between 0.79 and 0.90 (Table 2). Thus, it shows the variation degree within and among the different Colombian breeds. The difference between the average number of alleles per locus and the average number of alleles per breed might have resulted from the disparity of the analyzed samples (49 *S;* 60 *HV*, *B* and *H*, and 61*A*).These results show the great genetic diversity in cattle breeds in Colombia.

Genetic diversity in populations of domestic animals (cattle, buffaloes, goats, guinea pigs, etc.) has great importance from the point of view of conservation of endangered breeds. It is important to preserve genetic diversity in order for the breeds to withstand environmental changes or threats from emergent diseases, to guarantee the future supply of meat for the human population (FAO, 2007).

For most of the systems, *Holstein* breed showed the highest average number of alleles (Table 3), and the highest heterozygote deficit, probably due to the fact that this breed count for a huge number of specimens, but high endogamy is observed because few males are use for breeding with their daughter and granddaughters.

Senepol is a breed recently introduced to our country with a low number of specimens and even less breeding males, therefore a few numbers of alleles per locus are expected in this breed, concordant with our findings that showed a low average number of both alleles and private alleles (species - specific), in addition to the highest heterozygote deficit (Table 3). Similar results were found in Hartón del Valle breed, which is a Colombian native breed, exclusive to the Valle del Cauca region and represented by a few specimens as well, beside of this fact, this breed has been subject of conservation programs tending to preserve it because its resistance to diseases and high productivity.

Brangus crossbreed, which has been obtained from crosses between *Angus* and *Brahman breeds*, exhibited high heterozygosity values, due to the fact that has its origin from two different breeds, increasing the chance that different alleles for each locus enhance the genetic variability in *Brangus* breed, this might explain why *Brangus* breed showed the lowest heterozygotes deficit (Table 3).

An interesting outcome was the overall deficit of heterozygotes. For all the populations, the observed heterozygosity (Ho) was lesser than expected, when analyzed per population, as well as per locus (Tables 2 and 3). This might be related with an endogamy tendency in the different herds. Artificial insemination (Maudet *et al.*, 2002) and embryo transfer techniques also influence genetically these results.

Most of the loci used in this study have been analyzed in previous studies with different breeds (Barrera *et al.*, 2006a; Calvo *et al.*, 2009; Hansen *et al.*, 2002; Martínez *et al.*, 2006; Maudet *et al.*, 2002; Mommens *et al.*, 1998; Radko *et al.*, 2005). A deficit of heterozygotes only in the insemination bull population was reported by Maudet *et al.* (2002). In previous studies a deficit of heterozygotes was found in the Colombian native breeds *Romosinuano* and *Hartón del Valle*. (Barrera *et al.*, 2006a), in the Colombian native breeds *Romosinuano* and *BON* (Calvo *et al.*, 2009), A deficit of heterozygotes in Italian cattle breeds analyzed with another kind of microsatellite markers was reported by Ciampolini *et al.* (1995). All of these deficits have been attributed to the selection criteria for bulls chosen as sires in crossbreeding programs.

Table 4 shows the PIC values indicating the discriminatory value of each locus; values higher than 0.5 can be considered as highly discriminatory, what means that all of them allow us to value genetic diversity within and among each population. INRA37 marker for *Senepol* breed presented the highest PIC value and INRA32 for *Angus* breed presented the lowest one with 0.537.

The results of combined EP indicate that microsatellites panel must be increased for *Hartón del Valle* breed to a reliability minimum value of 0.9999, since its total value was 0.9997. The EP for the remaining breeds is 0.9999 and thus, this microsatellite panel could be useful to solve filiation cases (Martinez *et al.*, 2005). Hence that the appropriate microsatellite panel for these cattle breeds parentage testing could not be adequate for other cattle breeds or vice versa; the research group considered necessary to establish a set of highly informative markers according to each breed to be evaluated.

Although some private breed alleles were found, its frequency was relatively low, except for allele 132 in *Angus* breed. Therefore, the possibility of using alleles for characterizing a breed would be, in principle, improbable; however, Hanotte *et al.* (2000) quoted by Hansen *et al.* (2002), has shown that it is possible to characterize a breed with relative accuracy in studies involving several cattle breeds. Steigleder *et al.* (2004) report the 161 allele for the ETH225 marker in the Brazilian native breed as a private allele. Further studies based on analysis involving more microsatellites or increasing the number of specimen are required in order to improve the analysis.

In conclusion, the results obtained with these microsatellites show considerable genetic diversity among the analyzed breads present in Colombia. Furthermore, microsatellites are a powerful tool for genealogy controlling. They also allow to control or organize mating systems in order to preserve their high genetic variability, and reinforce their environmental adaptation to the tropics as well as to solve filiation's cases. The combined power of exclusion for those markers exceeding 0.9999, for most of the analyzed breeds, highly suggests that this panel of markers could be theoretically considered as greatly useful for parentage verification of the analyzed cattle breeds.

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