Genetic diversity of Senepol cattle in Colombia using ten multiplexed microsatellites

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Abstract

An automated multiplex PCR assay of 10 polymorphic microsatellite loci was optimized here in Senepol bovine cattle. Allele frequencies were estimated for Colombian herds. The power of this set of markers for parentage and kinship analyses was also tested. Population genetic parameters, combined probability of identity (CPI) and combined probabilities of exclusion (CPE) were estimated.

The average number of alleles per locus was 14, the effective number of alleles was 5, the observed and expected heterozygosities were 0.635 and 0.776, respectively, the Shannon Index was 2.70 and the polymorphic information content was 0.751. These values evidenced elevated levels of polymorphism and an underestimation of genetic diversity through the use of manual genotyping methods. Inbreeding coefficient (*F*is=0.206) in Colombian Senepol herds was moderate. Altogether, data from the used set of microsatellite loci yielded a CPI between two random samples of 1.07E-12. The CPE in parentage testing were >0.998 for the first parent and >0.999 for both the second parent and parent pairs. This set of polymorphic markers can be genotyped in a relatively easy, effective and reliable way for relatedness and forensic analyses. Finally, we recommend our approach for population genetic analyses of other Senepol populations and future genetic monitoring of inbreeding in Senepol herds.

Key words: *Bos taurus, genetic relatedness, genetic variation, molecular marker, parentage*

Diversidad genética de ganado Senepol en Colombia empleando diez microsatélites combinados

Resumen

Se optimizó en ganado bovino Senepol una prueba automatizada de PCR multiplex de 10 loci microsatélite polimórficos. Adicionalmente, se estimaron las frecuencias alélicas en hatos colombianos y se evaluó el poder de este grupo de marcadores en pruebas de paternidad y parentesco. Se estimaron parámetros genético-poblacionales, probabilidad de identidad combinada (CPI) y probabilidades de exclusión combinadas (CPE).

El número promedio de alelos por locus fue 14, el número efectivo de alelos fue 5, las heterocigocidades observada y esperada fueron 0.635 y 0.776, respectivamente, el índice de Shannon fue 2. 70 y el contenido de información polimórfica fue 0.751. Estos valores evidenciaron altos niveles de polimorfismo y una subestimación de la diversidad genética cuando se usan métodos manuales de genotipificación. El coeficiente de endogamia en hatos colombianos de Senepol fue moderado (*F*is=0.206). En general, los datos del grupo utilizado de microsatélites arrojaron un CPI entre dos muestras aleatorias de 1.07E-12. El CPE in las pruebas de parentesco fue >0.998 para el primer parental y >0.999 tanto para el segundo parental como para el par de parentales. Este grupo de marcadores microsatélite es relativamente fácil de genotipificar en una forma efectiva y confiable para análisis forenses y de parentesco. Finalmente, recomendamos nuestra aproximación para análisis genéticos-poblacionales de otras poblaciones de Senepol y futuros monitoreos genéticos de endogamia en hatos de Senepol.

Palabras clave: *Bos taurus, marcador molecular, parentesco genético, paternidad, variación genética*

Introduction

Senepol is a *Bos taurus* cattle breed endemic to the New World (Hupp 1978), that emerged in 1810's in St. Croix, Virgin Islands, after crossing N'Dama and Red Poll. It was introduced in Colombia from the United States, Virgin Islands, Venezuela and Brazil in the early 2000's and it is mainly distributed in the North West in the departments of Antioquia, Córdoba and Cundinamarca. Senepol was

subsequently introduced in other departments and the current populations size is estimated at around 2700 individuals (Asosenepol 2015). Two previous population genetic studies of Colombian Senepol have made use of ten polymorphic microsatellite loci recommended by the International Society for Animal Genetics (ISAG) (Montoya et al 2010; Sepúlveda et al 2012). These studies revealed remarkable levels of genetic variability and evidenced the utility of such markers for forensic and relatedness analyses. Such analyses have been based on single amplification of each marker followed by manual genotyping and allele scoring through analysis of polyacrilamede gel electrophoresis (PAGE) profiles. However, this approach is expensive as well as time and effort consuming, but also allele scoring is prone to errors, thus limiting practicity, replicability and reliability.

Therefore, the aim of this study was to optimize a routine methodology of multiplex amplification and automated genotyping of the same ten polymorphic microsatellite loci in a sample of the Senepol Colombian population and assess the potential of these markers for parentage and individual identification analyses.

Materials and methods

Sampling and genotyping

We selected 302 Senepol individuals, representing over 10% of the current population. Genomic DNA of 302 Senepol individuals from 25 farms in seven Colombian departments (Cundinamarca, Antioquia, Boyacá, Casanare, Córdoba, Meta and Tolima) had been previously extracted from blood samples kindly provided by Asociación Colombiana de Criadores de Ganado Senepol y sus Cruces (ASOSENEPOL) (Sepúlveda et al 2012).

A set of ten polymorphic microsatellites previously used in parentage tests and recommended by The International Society of Animal Genetics (ISAG) was selected for this study. PCR reactions were carried out in a total volume of 15µl containing 1X PCR buffer, 0.2 mM dNTPs, between 1.0 and 3.0 mM $MgCl_2$, 0.2µM of each primer, 0.5U de *Taq* polymerase (Thermo Fisher Scientific, Waltham, MA, USA) and 30 to 50µg of template DNA (Table 1). Initial denaturation at 94°C for 5 min was followed by 35 cycles of denaturation at 94°C for 30 s, primer annealment for 30 s (between 50 and 65°C) and extension at 72°C for 1 min with a final extension step at 72°C for 10 min.

Primer sets with similar thermal profiles and $MgCl_2$ requirements were pooled in the same tube and products were coamplified. To do this, we first adjusted the amplicon size of four loci by redesigning one or both primers from the reference *Bos taurus* genome (Genbank Accession number GCA_000003055.3) with the software Primer3 v4.0 (Untergrasser et al 2012). The 5' end of all forward primers was labeled with one of the commercial dyes FAM™, VIC®, PET® or NED™ (Applied Biosystems, Foster City, CA, USA), making sure that coamplified markers had different labels (Table 1).

Amplicons were run in an ABI genetic Analyzer 3130 (Applied Biosystems) using LIZ500® as molecular weight standard (size range between 35-500 bp). Genotype editing and allele calling was performed making use of the software Geneious v6.1.6 (Biomatters Ltd., Auckland, New Zealand).

Data analysis

Deviation from the Hardy-Weinberg equilibrium (HWE) was tested across all loci with a modified version of the Markov chain random walk algorithm (Guo and Thompson, 1992). HWE, observed and expected heterozygosities (*Ho* and *He*, respectively) and Inbreeding coefficient (*F*is) were estimated in the package Arlequin v3.11 (Excoffier and Lischer 2010).

Table 1. Primer sequences, chromosome location, allele size and multiplexed amplification conditions of ten microsatellite loci in Senepol cattle.

Locus	Primer sequence 5'-3' (Fluorochrome) **	Chm ***	Size range (bp)	[$MgCl_2$] mM	Temp. (°C)	Multiplex
BM2113	F:GCTGCCTTCTACCAAATACCC (FAM) R:CTTCCTGAGAGAAGCAACACC	2	118-148			
SPS115	F:AAAGTGACACAACAGCTTCTCCAG (PET) R:AACGAGTGTCCTAGTTTGGCTGTG	15	240-260			
TGLA126	F1:CCCAGATTTCTTGACTCATCG * (VIC) R1:AGTCCCAGCCTCTGATCTCT *	20	240-318	2.0	65.0	M1
ETH225	F1:GGGGATTATCTGTGGCATAA * (FAM) R:ACATGACAGCCAGCTGCTACT	9	184-212			
ETH10	F:GTTCAGGACTGGCCCTGCTAACA (NED) R:CCTCCAGCCCACTTTCTCTTCTC	5	200-218			
TGLA122	F:CCCTCCTCCAGGTAAATCAGC (VIC) R:AATCACATGGCAAATAAGTACATAC	21	132-178	2.0	65.0	M2
INRA32	F:AAACTGTATTCTCTAATAGCTAC (NED) R:GCAAGACATATCTCCATTCCTTT	11	158-196	2.5	62.5	M3

INRA64	F1:GCATCAACACAGGGTTGTTTA * (PET) R:CTGAAAGCAGAATGAGGTGC	23	218-228			
BM1824	F:GAGCAAGGTGTTTTTCCAATC (PET) R:CATTCTCCAACTGCTTCCTTG	1	136-192	2.5	59.5	M4
INRA37	F1:GGCGAAGGTTTGGATACAT * (FAM) R:AAAATTCCATGGAGAGAGAAAC	10	254-288			

** Modified from original primer sequence. ** Fluorochrome colors: Blue (FAM), Green (VIC), red (PET) and yellow (NED). *** Chromosome.*

Polymorphism level and genetic diversity were assessed through the estimation of allele frequencies, total number of alleles per locus (*N*a), effective number of alleles (*N*e) and log2-base Shannon Index (SI) in the software GenAlEx v6.5 (Peakall and Smouse 2006). We also calculated Polymorphic Information Content (PIC), Probability of Identity (PI) and Probability of Exclusion (PE) with the software Cervus v3.0.6 (Kalinowsky et al 2007). PE is defined as the probability of excluding a candidate parent or parent pair from parentage of a given offspring in three possible scenarios: testing one parent when the other parental genotype is unavailable (PE1 or first parent); testing one parent when the genotype of the other parent is available (PE2 or second parent) and; when a parent pair is tested (PE3 or parent pair) (Jamieson and Taylor, 1997; Marshall et al 1998).

Results

The ten microsatellite loci were successfully amplified and scored in four multiplex reactions consisting on one set of four (M1) and three sets of two (M2, M3 and M4) pooled markers (Table 1). The average *N*a was 14.3, which ranged between six (*INRA64*) and 28 (*TGLA126*) alleles per locus. *N*e ranged from 1.9 to 12.1 with an average of 5.6. Average values for SI and PIC were 2.70 and 0.752, respectively (Tables 2 and 3). All ten tested loci exhibited significant deviation from the Hard-Weinberg expectations ($p<0.001$) as determined by observed heterozygosity deficit (*H*o vs *H*e) and inbreeding levels that reached 21% (*F*is = 0.206) and. The average PI per locus was 0.087 with a range between 0.013 and 0.302. The Combined PI (CPI) of the ten-locus set was 1.07E-12. As far as the probability of exclusion, PE1 ranged between 0.126 and 0.716, PE2 between 0.279 and 0.834 and, PE3 between 0.447 and 0.955. The combined analysis of all ten microsatellite loci yielded a CPE1 >0.998, whereas both CPE2 and CPE3 were >0.999.

Table 2. Genetic diversity indices and power for parentage testing of ten microsatellite loci in Colombian Senepol cattle.

	TGLA 122	***TGLA 126***	***INRA 32***	***ETH 10***	***BM 2113***	***ETH 225***	***INRA 37***	***BM 1824***	***INRA 64***	***SPS 115***	**Average**
N	295	298	297	297	299	298	299	297	298	298	
N a	16	28	18	8	14	12	16	15	6	10	14.3
N e	4.69	12.1	7.51	5.46	5.15	3.78	7.03	3.52	1.93	4.43	5.56
Ho	0.732	0.762	0.670	0.801	0.696	0.517	0.696	0.609	0.205	0.661	0.635
He	0.788	0.919	0.868	0.818	0.808	0.737	0.859	0.717	0.484	0.776	0.777
SI	2.83	3.93	3.27	2.55	2.77	2.48	3.06	2.24	1.42	2.44	2.70
PIC	0.762	0.912	0.853	0.790	0.784	0.710	0.842	0.673	0.448	0.741	0.752
PI	0.070	0.013	0.031	0.060	0.060	0.095	0.036	0.123	0.302	0.084	0.087
CPI	Combined Probability of Identity								1.07E-12		
PE1	0.432	0.716	0.580	0.455	0.457	0.359	0.502	0.313	0.126	0.309	0.425
CPE1	Combined Probability of Exclusion (first parent)								>0.998		
PE2	0.609	0.834	0.735	0.631	0.633	0.545	0.714	0.488	0.279	0.568	0.604
CPE2	Combined Probability of Exclusion (second parent)								>0.999		
PE3	0.803	0.955	0.896	0.808	0.820	0.750	0.878	0.679	0.447	0.754	0.779
CPE3	Combined Probability of Exclusion (parent pair)								>0.999		

N: Sample size; Na: number of alleles; Ne: effective number of alleles; Ho: observed heterozygosity; He: expected heterozygosity; SI: Shannon Index; PIC: polymorphic information content; PI: probability of identity; PE1: probability of exclusion (first parent); PE2: probability of exclusion (second parent); PE3: probability of exclusion (parent pair).

Table 3. Allele frequencies and allele sizes (in parenthesis) of ten microsatellite loci in Colombian Senepol cattle.

Allele	***TGLA 122***	***TGLA 126***	***INRA 32***	***ETH 10***	***BM 2113***	***ETH 225***	***INRA 37***	***BM 1824***	***INRA 64***	***SPS 115***
N	295	298	297	297	299	298	299	297	298	298
1	0.008	0.003	0.020	0.003	0.007	0.003	0.003	0.007	0.008	0.003
	(132)	(240)	(158)	(200)	(118)	(184)	(254)	(136)	(218)	(240)
2	0.017	0.003	0.010	0.224	0.012	0.008	0.002	0.002	0.035	0.008
	(134)	(242)	(160)	(206)	(120)	(186)	(256)	(140)	(220)	(244)
3	0.015	0.032	0.005	0.202	0.035	0.029	0.007	0.002	0.176	0.304
	(136)	(244)	(162)	(208)	(122)	(188)	(258)	(142)	(222)	(246)
4	0.017	0.020	0.005	0.039	0.192	0.015	0.012	0.012	0.693	0.138
	(138)	(246)	(164)	(210)	(124)	(190)	(260)	(144)	(224)	(248)
5	0.053	0.035	0.007	0.013	0.050	0.154	0.025	0.003	0.057	0.300
	(140)	(248)	(170)	(212)	(126)	(192)	(262)	(152)	(226)	(250)
6	0.253	0.042	0.066	0.150	0.129	0.060	0.007	0.002	0.030	0.144
	(142)	(250)	(172)	(214)	(128)	(194)	(264)	(158)	(228)	(252)
7	0.025	0.097	0.133	0.177	0.012	0.458	0.055	0.005		0.029
	(144)	(252)	(174)	(216)	(130)	(196)	(266)	(172)		(254)
8	0.005	0.035	0.098	0.192	0.104	0.092	0.196	0.007		0.022
	(146)	(254)	(176)	(218)	(132)	(198)	(268)	(178)		(256)
9	0.015	0.029	0.19		0.100	0.015	0.092	0.133		0.045
	(148)	(256)	(178)		(134)	(206)	(270)	(180)		(258)
10	0.361	0.022	0.221		0.339	0.128	0.169	0.288		0.007
	(150)	(258)	(180)		(136)	(208)	(272)	(182)		(260)
11	0.078	0.027	0.099		0.007	0.034	0.134	0.421		
	(152)	(260)	(182)		(138)	(210)	(274)	(184)		
12	0.068	0.002	0.069		0.01	0.003	0.002	0.057		
	(154)	(262)	(184)		(140)	(212)	(278)	(186)		
13	0.007	0.003	0.019		0.002		0.003	0.005		
	(156)	(264)	(186)		(142)		(282)	(188)		
14	0.017	0.002	0.005		0.002		0.005	0.056		
	(158)	(266)	(188)		(148)		(284)	(190)		

15	0.059 (160)	0.003 (268)	0.040 (190)	0.100 (286)	0.002 (192)
16	0.002 (178)	0.002 (276)	0.003 (192)	0.189 (288)	
17		0.002 (280)	0.003 (194)		
18		0.002 (284)	0.007 (196)		
19		0.002 (286)			
20		0.002 (290)			
21		0.002 (304)			
22		0.077 (306)			
23		0.084 (308)			
24		0.136 (310)			
25		0.087 (312)			
26		0.143 (314)			
27		0.067 (316)			
28		0.035 (318)			

Discussion

The microsatellite data showed a large number of alleles and high degree of polymorphism that nonetheless contrast with moderate levels of inbreeding and heterozygosity deficit that lead to departure from the Hardy-Weinberg equilibrium. In particular, this is expected to occur as a consequence of finite size of individual herds, strong trait selection and limited genetic exchange among herds. In case this trend remains unchanged over time, inbreeding levels could substantially increase and facilitate loss of genetic diversity.

Even though only a few previous studies have assessed levels of genetic variation in Senepol, these seem nonetheless to be relatively high in Colombian herds as compared with other populations. Brenneman et al (2007) estimated an average *N*a of 6.6 from 26 microsatellite loci in a herd in Florida (USA) that was initially established from Caribbean founders. Also, Silva Filho et al (2013) assessed the genetic diversity of a herd in Minas Gerais (Brazil) making use of ten polymorphic microsatellites that yielded an average *N*a of 5.3 and *N*e of 3.1. Unlike the present study in Colombia, which showed a *N*a of 14.3 and *N*e of 5.6, both analyses were focused on single herds and evidenced genetic diversity

levels below the values in Colombian herds. Whether the smaller diversity diversity observed in previous studies is determined by limited sample sizes or historical factors of those populations remains to be determined in future studies.

Overall, combined multilocus genotypes provide high power for both individual identification and parentage testing in the Colombian Senepol population (Radko 2010; Souza et al 2012; Stevanović et al 2009). Unlike our two previous genetic surveys of the Colombian Senepol population, which were based on PAGE genotyping (Montoya et al 2010; Sepúlveda et al 2012), the present study implemented an automated approach of multiplex genotyping and allele scoring. Interestingly, the number of alleles per locus increased nearly 100% in the present study, which unveils an underestimation of genetic diversity using manual methods. The mismatch between these two genotyping assays is reasonable given the limited resolution of PAGE-based methods. This limitation is especially evident in the detection of allelic variants in microsatellites with dinucleotide repeat units, which are a common type of repeat in many species and the case of all ten loci implemented in the present study (Schug et al 1998; Li et al 2002).

In summary, the protocol presented here is cost and time effective since it provides a routine alternative to amplify ten microsatellite loci in four multiplex PCR reactions. This marker set revealed substantial levels of polymorphism and evidence of inbreeding in Colombian herds of Senepol cattle. These markers yielded low probability of identity, which is suitable for forensic analysis as well as individual recognition, but they also showed high probability of exclusion under three different scenarios in paternity tests (first parent, second parent and parent pair), which will be useful for future population genetic analysis of Senepol in Colombia and elsewhere.

Acknowledgements

To the University of Antioquia (CODI-Sostenibilidad UdeA 2016 and CODI-Menor 2009) and the Colombian Association of Senepol Cattle Breeders (ASOSENEPOL) for financial support and supply of biological samples.

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Received 7 June 2016; Accepted 7 July 2016; Published 1 August 2016