= ANIMAL GENETICS =

Genetic Characterization of Colombian Brahman Cattle Using Microsatellites Markers¹

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Abstract—Genetic structure and diversity of 3789 animals of the Brahman breed from 23 Colombian regions were assessed. Considering the Brahman Zebu cattle as a single population, the multilocus test based on the HW equilibrium, shows significant differences (P < 0.001). Genetic characterization made on the cattle population allowed to examine the genetic variability, calculating a $H_0 = 0.6621$. Brahman population in Colombia was a small subdivision within populations ($F_{it} = 0.045$), a geographic subdivision almost non-existent or low differentiation ($F_{st} = 0.003$) and the F_{is} calculated (0.042) indicates no detriment to the variability in the population, despite the narrow mating takes place or there is a force that causes the variability is sustained without inbreeding actually affect the cattle population. The outcomes of multivariate analyses, Bayesian inferences and interindividual genetic distances suggested that there is no genetic sub-structure in the population, because of the high rate of animal migration among regions.

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INTRODUCTION

Indicus breed have different geographic origins and suffered various mixtures to over the centuries, confronted with processes of environmental adaptation, selection, migration and reproductive [1, 2]. According to existing data in the information system of domestic animal diversity (DAD-IS), 25 cattle breeds are reported in Colombia including the Brahman cattle. The origin of Brahman is the result of crossbreeding of taurine (presumably Shorthorn) and zebu (indubrasil, Gyr and Guzerá), but most of their phenotypic and genetic characteristics [3, 4] correspond to the latter. The Brahman cattle arrived in the Cauca Valley region of Colombia in the 1900s where it was crossed with Creole breeds to obtain racially mixed animals (Pinzon, 1984). These cattle are known for having the highest genetic quality in the word. Currently about 90% of meat production in Colombia comes from Zebu, which highlights the Brahman cattle and theirs crosses, as the ideal breed for meat production in tropical countries. In 1946 was created the Asociación Colombiana de Criadores de Ganado-Cebú (Asocebú), an entity with a systematic and clear target for improvement and purification of different zebu breeds, which has an evident development of beef and milk cattle throughout Colombia, particularly in tropical areas.

MATERIALS AND METHODS

Animals. Samples obtained were pure Brahman cattle registered in the Asociación Colombiana de Criadores de Ganado Cebú (Asocebú). These samples were sent to the Laboratorio de Biotecnología y Genética (BIOTECGEN S.A.) to perform genotyping test. A total of 3789 unrelated cattle of both sexes (3594 males and 195 females) were analyzed. The breeds studied came from 23 different Colombian regions.

STR genotyping. DNA extraction was made from hair follicle according to the modified protocol of Troy et al. [10]. We analyzed a total of 11 microsatellites loci (TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA122, TGLA126, INRA23, BM1824, ETH3 and

Currently, most reports in population genetics at the species has focused on taurine breeds [5-10] and to a lesser degree zebu breeds [1, 11-15]. Genetic characterization in tropical zebu breeds are underage [16-19], reporting a single population analysis to Colombian Brahman breed [4]. The goal of this work was perform an allelic characterization and estimation of population genetic parameters of Brahman breed raised in Colombia by using microsatellites markers, nine of which are approved by the international Society of Animal Genetic (ISAG) and determine the structure population of Brahman breed in Colombia.

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ETH225). Nine of these markers are recommended by ISAG for paternity testing in cattle and have been widely used in other studies of genetics characterization.

The StockMarks[®] for Cattle Bovine Genotyping Kit of Applied Biosystems[®] (Foster city, CA, United States) and Bovine GenotypesTM-Panel 3.1 of FINNZYMES Diagnostics were used to make multiplex PCR. PCR reactions were carried out according to the commercial kit instruction. The amplified products were evaluated in the automated genetic analyzer ABI PRISM 310 and HITACHI 3500 (Applied Biosystems[®]). The allocation of alleles was carried out by FlexiBinV2 software [20] and Applied Biosystems GeneMapper v. 3.2. and v. 4.1 software (Applied Biosystems[®]). The nomenclatures of the alleles were assigned according to size in base pairs (bp) of each allele, using as reference samples of the ISAG.

Measures of genetic variability. Allele frequencies for all loci as well as the number of population-specific alleles found (private alleles, PAs) was determined with Fstat v. 2.9.3. The software Genetix v. 4.03 was used to estimate observed (H_0) and unbiased withinbreed expected (H_e) heterozygosities, and mean number of alleles (MNA) by population. An exact test for deviations from Hardy-Weinberg equilibrium (HWE) was performed using the GENEPOP package, version 3.0 [21]. Both global tests across populations and loci and tests per locus per population were done using the method of Guo and Thompson [22], and the P values were obtained using a Markov chain of 10 000 dememorization steps, 500 batches, and 5000 iterations. Genotypic linkage disequilibrium (LD) was also calculated with this software and the same Markov chain settings. Significance levels were adjusted using the sequential Bonferroni method to take into account multiple tests on the same data set.

Differentiation, structure and population's subdivision. Fstat v. 2.9.3 was used to estimate the F statistics per locus by Weir and Cockerham [23] and P values were obtained based on 1000 randomizations. These values were compared with results obtained with GENEPOP v. 3.0 [21]. CERVUS v. 2.0 [24] was used to estimates the probability of exclusion and the combined probability of exclusion according to Jamieson and Taylor [25] and the polymorphic information content (PIC) was determined as described by previous workers [25]. Standard genetic distance (DS) [26], chord distance (DC) and stepwise-weighted genetic distance (DSW) [27] were calculated from allele frequencies. Dendograms were constructed from the distance matrix using the unweighted pair group method with arithmetic mean (UPGMA) and the Neighbor-Joining (NJ) algorithms. Bootstrap values were calculated from 1000 replications of resampling loci. Distance and trees were computed using the POPULA-TIONS v. 1.2.28 software. The trees were visualized using TREEVIEW [28]. Genetic structure and the degree of admixture of 23 different Colombian regions of Brahman cattle were investigated using the Bayesian clustering procedure of STRUCTURE v. 2.0 software [29], with *burn-in* length and MCMC iterations of 50000 and 100000, respectively. The parameter alpha (degree of admixture) was inferred from the data using the default settings and an admixture model with correlated allele frequencies [30]. The most probable number of populations (K) given the observed genotypic data was estimated according to the monophyletic groups in the phylogenetic tree generated from genetic distance computed by POPULATIONS v. 1.2.28 software.

RESULTS

A total of 153 alleles were detected among 11 loci evaluated, there were 17 private alleles present in Antioquia (7), Córdoba (4), Cesar (2), Tolima (2), Santander (1) and Guaviare-Meta (1). The allelic frequencies and the private alleles are summarized in the Table 1. The average number of alleles for these loci was 13.9 which is considered high. The gene diversity was calculated using the observed heterozygosis (H_{o}) compared with the expected heterozygosis (H_e) in the 11 loci tested (see Table 5), in the 23 regions analyzed and the average number of alleles per locus or Na. In general, gene diversity was low in most of the markers with an H_0 and H_e mean of 0.6621 and 0.6929 respectively, but without significant differences ($P \le 1$; $\alpha =$ 0.05) between these two values. The ETH225 is the only marker with a lowest H_0 of 0.3142. Concerning to the 23 regions analyzed, its shows that the H_0 was low in all regions with a slight increase in Nariño, Putumayo and Sucre ($H_0 \sim 0.7$) but without significant differences (P > 0.909; $\alpha = 0.05$) between H_0 and H_e parameters.

HW equilibrium analysis in each region shows significant differences between regions and the total population. Specifically we can see that in 3 different regions (Caquetá, Arauca, Cauca and Casanare) only show highly significant HW disequilibrium ($P \ll 0.01$; $\alpha = 0.05$) for a single locus of the 11 analyzed (ETH10, TGLA227 and ETH10 respectively). In these regions, in the other 10 loci evaluated, multilocus test is not significant (P > 0.05; $\alpha = 0.05$). Considering the Brahman cattle population as a single population in Colombia, the multilocus test shows that have highly significant differences (P < 0.001; $\alpha = 0.05$). After analyzing linkage disequilibrium, there were detected seven pairs of loci in linkage disequilibrium (Table 2); however, these couples do not have a physical linkage, because they are in different chromosomes.

The polymorphic information content (PIC) calculated showed that zebu Brahman population evaluated in Colombia have high information content in five polymorphic loci (TGLA122, INRA23, TGLA126, BM2113 and TGLA53), but the average is less than 0.6531 PIC. This value is reduced by other systems that have small PIC (Table 3). In all cases, the

$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Allele#	Size	Freq.	Private	Allele#	Size	Freq.	Private							
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	TGLA53	•			10	123	0.0001	Córdoba							
2 156 0.0004 12 127 0.0001 Antioquia 3 158 0.0089 13 129 0.0001 N. de Santander 4 160 0.4006 1 121 129 0.0001 N. de Santander 5 162 0.0870 1 121 0.0020 N. de Santander 6 164 0.0399 2 125 0.0032 7 166 0.0400 3 127 0.0368 8 168 0.2815 4 129 0.1542 9 170 0.0199 5 131 0.0004 10 172 0.0033 6 133 0.0510 11 173 0.0003 Sucre 10 141 0.4251 15 180 0.0047 12 143 0.0747 16 182 0.0004 13 160 0.0003 17 183 0.0004 13 160 0.0046 20 186 0.0004	1	154	0.0302		11	125	0.0182								
3 158 0.0089 13 129 0.0001 N. de Santander 4 160 0.4006 BM2113 I 121 0.0020 IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	2	156	0.0004		12	127	0.0001	Antioquia							
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$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	4	160	0.4006		BM2113	BM2113									
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	5	162	0.0870		1	121	0.0020								
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	6	164	0.0399		2	125	0.0032								
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	7	166	0.0400		3	127	0.0368								
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	8	168	0.2815		4	129	0.1542								
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	9	170	0.0199		5	131	0.0004								
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	10	172	0.0039		6	133	0.0510								
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	11	173	0.0003		7	135	0.1266								
13 176 0.0004 9 139 0.1158 14 178 0.0003 Sucre 10 141 0.4251 15 180 0.0035 11 143 0.0747 16 182 0.0047 12 145 0.0003 17 183 0.0004 13 160 0.0003 Antioquia 18 184 0.0222 13 160 0.0046 2 246 0.2677 20 186 0.0069 2 246 0.2677 3 248 0.3946 22 190 0.0061 4 250 0.1640 4 252 0.0061 24 194 0.0003 6 254 0.0434 4 255 0.0661 24 194 0.0028 7 256 0.0853 26 0.8653 26 10 262 0.0005 4 264 0.0025 26 28 290 0.0008 9 260 0.0225 25 266 0.0853 4 264 </td <td>12</td> <td>174</td> <td>0.0046</td> <td></td> <td>8</td> <td>137</td> <td>0.0098</td> <td></td>	12	174	0.0046		8	137	0.0098								
14 178 0.0003 Sucre 10 141 0.4251 15 180 0.0035 11 143 0.0747 16 182 0.0047 12 145 0.0003 Antioquia 17 183 0.0004 13 160 0.0003 Antioquia 18 184 0.0222 13 160 0.0046 2 246 0.2677 21 188 0.0303 13 248 0.3946 2 246 0.2677 21 188 0.0303 3 248 0.3946 2 246 0.2677 23 192 0.0010 5 252 0.0061 4 250 0.1640 23 192 0.0010 5 252 0.0061 6 254 0.0434 25 196 0.0028 7 256 0.0853 26 10 262 0.0005 28 290 0.0004 10 262 0.0005 11 264 0.0002 V. del Cauca <td>13</td> <td>176</td> <td>0.0004</td> <td></td> <td>9</td> <td>139</td> <td>0.1158</td> <td></td>	13	176	0.0004		9	139	0.1158								
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BM1824 11 264 0.0002 V. del Cauca	28	290	0.0004		10	262	0.0005								
	BM1824	1		•	11	264	0.0002	V. del Cauca							
1 1/2 0.0001 Antioquia IGLA22/	1	172	0.0001	Antioquia	TGLA227	I									
2 178 0.0346 1 77 0.6433	2	178	0.0346		1	77	0.6433								
3 180 0.4619 2 79 0.0394	3	180	0.4619		2	79	0.0394								
4 182 0.4205 3 81 0.1961	4	182	0.4205		3	81	0.1961								
5 184 0.0008 4 83 0.1116	5	184	0.0008		4	83	0.1116								
6 188 0.0462 5 85 0.0018	6	188	0.0462		5	85	0.0018								
7 190 0.0001 Cundinamarca 6 87 0.0012	7	190	0.0001	Cundinamarca	6	87	0.0012								
8 192 0.0357 7 89 0.0005	8	192	0.0357		7	89	0.0005								
ETH3 8 91 0.0004	ETH3				8	91	0.0004								
1 101 0.0008 9 93 0.0025	1	101	0.0008		9	93	0.0025								
2 103 0.1013 10 95 0.0018	2	103	0.1013		10	95	0.0018								
3 109 0.0020 ETH10	3	109	0.0020		ETH10										
4 111 0.0001 Antioquia 1 209 0.2760	4	111	0.0001	Antioquia	1	209	0 2760								
5 113 0.0040 2 211 0.1815	5	113	0.0040	1	2	20)	0.1815								
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7 117 0.4273 4 215 0.0001 Valle del Cauca	7	117	0 4273		4	215	0.0001	Valle del Cauca							
8 119 0.0152 5 217 0.0172	8	119	0.0152		5	217	0.0172	and all cuudu							
9 121 0.0007 6 219 0.0456	9	121	0.0007		6	219	0.0456								

Table 1. Allelic frequencies and private alleles by locus of the Zebu Brahman in Colombia

Allele#	Size	Freq.	Private	Allele#	Size	Freq.	Private
7	221	0.0011		9	210	0.0764	
8	223	0.0003	Cundinamarca	10	212	0.0004	
9	237	0.0003	Antioquia	11	214	0.3317	
TGLA122				12	216	0.1063	
1	137	0.1642		13	218	0.0016	
2	141	0.0020		14	222	0.0022	
3	143	0.0763		ETH225			1
4	145	0.1138		1	140	0.0204	
5	147	0.0160		2	142	0.0071	
6	149	0.1038		3	144	0.0097	
7	151	0.1646		4	146	0.0009	
8	153	0.1486		5	148	0.1633	
9	155	0.0023		6	150	0.0326	
10	157	0.0311		7	152	0.0008	
11	159	0.0017		8	154	0.0214	
12	161	0.0609		9	156	0.0045	
13	163	0.0124		10	158	0.0012	
14	165	0.0003		11	160	0.7320	
15	167	0.0655		12	162	0.0060	
16	169	0.0340		13	164	0.0001	Córdoba
17	171	0.0011		TGLA126			1
18	173	0.0001	Antioquia	1	109	0.0176	
19	175	0.0009		2	113	0.0003	
20	194	0.0004	Cundinamarca	3	115	0.1680	
INRA23				4	117	0.1288	
1	122	0.0001	Antioquia	5	119	0.1241	
2	194	0.0334	-	6	121	0.0827	
3	196	0.1691		7	123	0.2533	
4	198	0.1024		8	125	0.2189	
5	202	0.0491		9	127	0.0039	
6	204	0.0445		10	129	0.0005	
7	206	0.0102		11	131	0.0016	
8	208	0.0726		12	137	0.0003	

 Table 1. (Contd.)

Table 2. Pairs of loci in linkage disequilibrium in the population of Brahman Zebu cattle in Colombia evaluated the *P*-value (α : 0.05)

Locus 1	Locus 2	<i>P</i> -value
BM1824	ETH3	0.02778
ETH225	ETH3	0
ETH3	TGLA126	0.04165
BM1824	INRA23	0.00491
BM2113	INRA23	0.0011
ETH225	INRA23	0.0162
ETH3	INRA23	0.00888

exclusion probability for pairs of parental and identity was higher in TGLA122 followed by INRA23 and TGLA126 (0.975, 0.951 and 0.943 respectively) and the probability of exclusion accumulated was 0.9999. Similar behavior was observed for the probability of exclusion identity (Table 4).

The analysis of population structure of Brahman cattle in Colombia (as one) showed an undetected preferential inbreeding ($F_{is} = 0.042$). Only for ETH225 locus, there was a visible variation coefficient of 0.277 F_{is} . On the other hand, Brahman cattle population in Colombia there sowed a small subdivision within populations ($F_{it} = 0.045$) and a geographic sub-

Markers	N. alelles ¹	N. ind. ²	PIC ³	NE-1P ⁴	NE-2P ⁵	NE-PP ⁶	NE-I ⁷	NE-SI ⁸	H-W ⁹
BM1824	8	3784	0.526	0.805	0.672	0.515	0.235	0.506	NS
BM2113	13	3777	0.731	0.618	0.434	0.235	0.085	0.393	***
ETH10	9	3494	0.604	0.761	0.598	0.424	0.172	0.463	NS
ETH225	13	3778	0.404	0.898	0.754	0.596	0.350	0.620	NS
ETH3	13	3761	0.546	0.796	0.656	0.497	0.219	0.494	NS
SPS115	11	3292	0.696	0.664	0.487	0.298	0.109	0.409	***
TGLA122	20	3717	0.873	0.382	0.235	0.085	0.025	0.314	***
TGLA126	12	3741	0.796	0.532	0.358	0.180	0.057	0.354	**
TGLA227	12	3783	0.489	0.848	0.693	0.524	0.262	0.549	***
TGLA53	28	3610	0.714	0.632	0.454	0.255	0.096	0.401	NS
INRA23	14	3787	0.805	0.507	0.335	0.151	0.049	0.351	***

 Table 3. Estimates of Brahman Zebu cattle in 11 microsatellite PIC values, Probability and Non-Exclusion Probability of identity

¹ Number of alleles, ² Number of individuals, ³ Polymorphic Information Content, ⁴ Non-Exclusion Probability (first parental), ⁵ Non-Exclusion Probability (second parent), ⁶ Non-Exclusion Probability (parental pair), ⁷ Non-Exclusion Probability (identity), ⁸ Non-Exclusion Probability (paired identity), ⁹ Test of Hardy-Weinberg Equilibrium, NS = not significant, *P < 0.05, ***P < 0.001.

Table 4. Writght *F* statistic of the 11 loci of Brahman Zebu cattle population evaluated. The coefficients F_{it} , F_{st} and F_{is} estimated by Weir and Cockerham [23], the coefficient is the ratio relat estimated according to Queller & Goodnight [35]; relate relationship is corrected according to Pamilo [36, 37]; sig_a, sig_b and sig_w are the components of variance between samples, between individuals within samples and within the total population respectively

Markers	F _{it}	F _{st}	F _{is}	Relat	Relatc	Sig_a	Sig_b	Sig_w	Nm
BM1824	0.029	0.004	0.025	0.009	-0.050	0.003	0.015	0.587	4.8339
BM2113	0.020	0.003	0.017	0.005	-0.035	0.002	0.013	0.741	7.7327
ETH10	0.012	0.002	0.010	0.003	-0.021	0.001	0.007	0.652	7.8875
ETH225	0.279	0.003	0.277	0.005	-0.767	0.001	0.120	0.313	6.9351
ETH3	0.018	0.003	0.016	0.005	-0.032	0.002	0.010	0.609	5.2470
SPS115	0.032	0.001	0.031	0.003	-0.064	0.001	0.023	0.712	4.8055
TGLA122	0.023	0.002	0.021	0.003	-0.043	0.002	0.018	0.862	5.8965
TGLA126	0.004	0.002	0.002	0.004	-0.004	0.002	0.002	0.816	5.0845
TGLA227	0.076	0.008	0.069	0.015	-0.147	0.004	0.036	0.493	5.6682
TGLA53	0.099	0.001	0.099	0.002	-0.219	0.001	0.073	0.668	5.8749
INRA23	0.009	0.002	0.007	0.003	-0.015	0.001	0.006	0.815	6.4126
TOTAL	0.045	0.003	0.042	0.005	-0.015	0.019	0.323	7.268	5.8738

division almost non-existent or low differentiation $(F_{\rm st} = 0.003)$. This means that the population of these cattle in Colombia is not structured and that the present flow between the populations analyzed is moderately high (Nm = 5.8738). These results are summarized in Table 5. Considering the 23 subpopulations (K = 23), the Bayesian analysis confirmed that all subpopulations tend to form a single population structure, which is visible in the clustering analysis by using genetic distances of these subpopulations. This result is consistent with the analysis of population structure, using the $F_{\rm st}$ coefficient found for this same population.

Using the matrix of genetic distances from subpopulation in general, a dendrogram was generated to display the population hierarchy. Although it is not presented a defined population structure using Bayesian analysis and the coefficient of F_{st} in Brahman cattle population in Colombia there is a small structure of 4 well-defined populations (Fig. 1). This phylogeny showed two monophyletic groups that shared a common ancestor. Furthermore, this phylogeny showed a close genetic link between geographically close populations, while others do not. This could be explained because of the constant marketing of this breed between distant regions and between them. Addition-



Fig. 1. Dendrogram based on genetic distance [34] and constructed with the UPGMA method with the distance of 11 microsatellite allele frequencies of the 23 regions of Brahman cattle in Colombia.

ally, this analysis showed the subpopulation of Norte de Santander as the population that possesses the genetic ancestral type. Based on the results obtained in the previous phylogenetic tree (Fig. 1), we performed a new analysis of population structure using the pro-

Table 5. Values of genetic variability of 11 loci evaluated for Brahman cattle. Expected heterozygosis (H_e), observed heterozygosis (H_o) and the number of individuals (N) analyzed by locus in Brahman cattle population. P > 0.05; α : 0.05

Markers	$H_{\rm e}$	H _o	P (0.95)	Ν
BM1824	0.6052	0.5880	1.000	3784
BM2113	0.7563	0.7413	1.000	3777
ETH10	0.6602	0.6523	1.000	3494
ETH225	0.4354	0.3142	0.897	3778
ETH3	0.6217	0.6107	1.000	3761
SPS115	0.7359	0.7123	1.000	3292
TGLA122	0.8838	0.8639	1.000	3717
TGLA126	0.8205	0.8174	1.000	3741
TGLA227	0.5338	0.4941	0.952	3783
TGLA53	0.7464	0.6726	0.908	3610
INRA23	0.8230	0.8159	1.000	3787
TOTAL	0.6929	0.6621	1.000	

gram STRUCTURE, but now considering only four subpopulations of Brahman cattle in Colombia (K = 4). This analysis is summarized (Fig. 2).

DISCUSSION

The 11 markers tested ranged between 8 and 28 alleles, the average number per locus was 13.9, which is within the parameters recommended by the Food and Agricultural Organization of the United Nations (FAO), that suggest at least 5 different alleles per locus to estimate genetic distances. The higher values are compared with data from other studies [4]. The distribution of allele frequencies shows that the population is represented by a few alleles at some loci, like allele 160 in the ETH225 system with a frequency of 73.2%, allele 77 in the microsatellite TGLA227 with a frequency of 64.37%, among others alleles in other systems with percentages close to 60%. Similarly, the frequencies of the most frequent alleles for each system are similar to the presented by other authors [4], although they used a more reduce populations. On the other hand, 19 of 23 regions had a H_0 lower than H_e indicating that the number of heterozygotes is low compared with the number of alleles detected. Such conduct could be explained by preferential crossbreeding between individuals with similar alleles in the

population sampled, resulting in the absence of the less common alleles. At the same time, this event leads to an increased formation of homozygotes and the corresponding decrease in the diversity indicators. Comparing the alleles of each of the 11 markers with those reported in American Zebu breeds including the Brahman, the number of alleles, their frequencies and the inbreeding coefficient (F_{is}), are different. By comparing the observed and expected heterozygosity in observed with that reported in other analyzes, it appears that behave similarly (Table 6).

Comparing the observed heterozygosity between our study with other studies described [4, 19], it was found that this value in our study is slightly higher than the other two studies. This small difference may be associated with the impact that generates a larger sample size. There are alleles of 3 loci (ETH225: 153, 157 and 159, TGLA227, 79 and TGLA122, 136), characteristic of Zebu Brahman by being in a high frequency in this breed and absent in the breeds of taurine origin [31]. However, our study were conducted with individuals identified like pure and the frequency for these alleles was less than 15%. The absence of these alleles characteristic of Zebu, may be due to mixing with other races ancient. As the maintenance of pure Zebu has few years in Colombia, may have been altered the allele frequencies characteristic of this breed. Another option that cannot be ignored is that the allelic assignment made between studies would not be similar, so this would explain the differences detected.

Genetic characterization made in Brahman cattle population from different regions of Colombia allowed to examine the genetic variability, calculating an $H_e = 0.6929$ with 11 markers remain high when is compared with other Zebu breeds, taurine [9, 32] and native breeds of cattle [33]. Most of the allelic diversity evaluated would be explained because when entering the Brahman cattle in the country could not have had a strict control on their mating, crossing with other breeds. However, the Brahman cattle are a breed that comes from the recent mixing of Nelore, Gyr and Guzerat. Therefore must be regarded as *Bos indicus* has a contribution from the *Bos taurus* breed according to studies of mtDNA [3].

When all loci were evaluated for the whole sample, there was a high deviation from HWE ($P \le 0.0001$; $\alpha = 0.005$ with Bonferroni correction), so statistical test rejected the null hypothesis of random union of gametes in the population and for the 11 systems in general. This fact can be explained by the deficit of heterozygotes considering both populations as evaluated microsatellite systems. Linkage disequilibrium was detected for seven pairs of loci, although this linkage is not physical because the loci are on different chromosomes. The most probable explanation for these imbalances is that the alleles of a locus have frequencies showing a statistical association, without requiring a physical connection or selection close to a locus. Therefore, when making the joins between the



Cluster 1



Fig. 2. Analysis of triangle points in the 4 subpopulations (K = 4) of Brahman cattle in Colombia. The points of the same color represent an individual of the same subpopulation.

alleles of higher frequency could be estimated as dependent, and therefore may be considered a rating on the association of alleles at these loci for the population.

The inbreeding coefficient is strongly influenced by the pedigree of the obtained data, since this measure is calculated by the genetic information provided in accordance with this the F_{is} calculated indicates no detriment to the variability in the population, despite narrow mating takes place or if there is a force that causes it to hold the variability without inbreeding actually affect the cattle population. The inbreeding level expected is high under the addressed reproduction system of these animals. Nevertheless, the inbreeding coefficient (F_{is}) shows low levels. These results may be explained considering the migration rate resulting from the commercialization of individuals between the estates, the reproductive processes of artificial insemination, in vitro fertilization and embryo transfer, the samples brought from other countries, among other components, would lead to homogenize the frequencies between different regions. To assess inbreeding more accurately, particularly in the sense of identity by descendent, it would be necessary to estimate F (individual inbreeding coefficient) from pedigree information.

Parentage tests are based on the genetic characterization of each system in a reference population to calculate the probability of exclusion of the population. We observed a probability value stored under exclusion at loci BM1824, ETH225 and TGLA227, which do not favor the exclusion, while TGLA122 and INRA23 TGLA126 markers are more effective. This efficiency

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Markers	ANT	ATL	BOL	ВОҮ	CAL	CAQ	ARA	CAU	CESAR	COR	CUN	HUIL	MAG	GUA	NOR	NAR	QUI	RIS	SAN	SUC	TOL	VALL	VIC	Tot	Prom
BM1824	7	5	5	5	5	5	4	5	5	7	5	5	5	5	4	3	4	3	5	5	6	5	2	110	4.783
BM2113	12	8	7	9	9	7	7	9	10	11	10	7	8	10	7	5	4	8	12	7	10	9	5	191	8.304
ETH10	7	5	5	6	6	4	3	4	6	6	6	3	3	5	4	4	3	5	5	4	6	4	3	107	4.652
ETH225	12	2	6	5	9	3	7	4	11	11	10	4	6	8	5	1	5	4	10	4	9	6	2	144	6.261
ETH3	11	4	4	5	6	5	5	5	9	8	8	3	3	7	6	2	3	3	7	4	6	5	5	124	5.391
SPS115	10	6	8	8	7	6	7	7	9	10	9	4	6	9	6	4	6	3	9	6	11	7	5	163	7.087
TGLA122	18	12	12	14	13	12	10	11	16	18	13	9	11	14	11	5	8	9	15	11	16	12	8	278	12.087
TGLA126	12	7	6	8	10	6	7	7	8	10	8	6	9	8	6	5	6	5	10	6	8	8	6	172	7.478
TGLA227	10	4	6	7	6	4	5	5	8	8	7	4	4	7	4	4	4	3	11	4	6	5	3	129	5.609
TGLA53	23	9	9	16	16	12	9	12	19	24	18	8	8	16	11	6	5	7	19	11	17	12	8	295	12.826
INRA23	13	10	9	11	10	9	11	9	13	11	12	9	8	12	9	7	7	8	13	9	10	10	7	227	9.870
TOTAL	135	72	77	94	97	73	75	78	114	124	106	62	71	101	73	46	55	58	116	71	105	83	54		

Table 6. Number of alleles sampled locus both in the 11 and the 23 regions of the Colombian Brahman cattle population

depends on the marker polymorphism and genetic variability of the breed. Therefore, ETH225 affects the exclusion probability because most of the population has the same genotype and polymorphic information content is low as well as PE. The calculation of the probability of exclusion would help establish the reliability of these systems as tools in the resolutions of paternity test as well as other indicators such as the paternity index and probability of paternity (W). The last, calculates the probability that the pair examined share alleles because there is a consanguineous relationship rather than by chance. In Colombia has not been established a value of accumulated exclusion probability (AEP) for farm animals. This genre study of 11 systems showed an AEP = 0.9999, that is translates as the exclusion of 999 cattle at random from 1000 individuals tested. This is similar to the *P*-value >0.99 that is generally used in other species such as horses, which has similar conditions of breeding and reproductive management. This similarity in the values of AEP between two different species occurs due to the use of polymorphic systems for prescribing a case of filiation as exclusion.

The results of this study confirm that the Brahman breed in Colombia is a strong artificial selection process, in search of an optimal genotype and phenotype compared to different environmental conditions and trade of the country. This selection process is evidenced by the increasing trend of consanguinity by nonrandom mating of animals that has been going on for many years. Furthermore, evidence in this study suggests the existence of a single genetic Brahman population in Colombia. This would allow reliable paternity tests in all the territory because there is not a substructure in the population.

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