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History and selection imprinting on genetic relationships among bovine breeds analyzed trough five genes related with marbling

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ABSTRACT

Many candidate genes have been suggested as responsible for marbling in beef cattle, for instance diacylglycerol O-acyltransferase 1, thyroglobulin, growth hormone, leptin and stearoyl CoA desaturase. The objective of the present work was to evaluate the polymorphisms of five SNPs of these candidate genes in 389 animals of 18 *Bos Taurus* and *Bos indicus* breeds. The obtained results were compared with ones previously obtained with STRs and loci related to milk production in these populations. Moreover we analyzed whether the phylogenies reconstructed using SNPs associated with marbling resulted in the known tree topology. The tree constructed with UPGMA, using genetic distance D_A, exhibit a topology partially consistent with the historical origin of breeds. The result observed in the Correspondence Analysis coincided with the topology of the UPGMA tree. This work allowed us to evaluate the five SNPs genetic diversity and to demonstrate that the grouping of the breeds may be the result of its history, selection process, or both at once.

1. Introduction

Marbling is an important trait for meat quality because confers juiciness, flavor and tenderness to beef hence it contributes directly to the price of beef on international markets. Many candidate genes have been suggested as responsible for marbling in beef cattle, such as diacylglycerol O-acyltransferase1 (DGAT1), thyroglobulin (TG), growth hormone (GH), leptin (LEP) and stearoyl-coenzyme A desaturase (SCD) (Barendse et al., 1999, 2001, 2004, 2006; Buchanan et al., 2002; Thaller et al., 2003; Nkrumah et al., 2004a,b; Taniguchi et al., 2004; Sorensen et al., 2006; Oka et al., 2002; Tatsuda et al., 2008).

The DGAT1 is a microsomal enzyme that catalyzes the final step of triglyceride synthesis. The DGAT1 gene has been mapped to bovine chromosome 14. A lysine/alanine (K232A) substitution on the protein encoded by the bovine DGAT1 gene has been shown to be associated with milk fat content in different breeds such as Holstein–Friesian, Fleckvieh and Jersey (Grisart et al., 2002; Spelman et al., 2002; Winter et al., 2002), and with fat deposition in beef cattle. Thaller et al. (2003) showed that the lysine allele of DGAT1 has also a positive effect on intramuscular fat content in the Charolais and Holstein breeds. Moreover, Sorensen et al. (2006) reported that the DGAT1 activity in *longissimus dorsi* muscle in individuals with K/K genotype was about five fold greater than for either the K/A or A/A genotypes in Holstein and Charolais bulls. In contrast, Moore et al. (2003) detected no association of the SNP in the DGAT1 gene (K232A mutation) with fat thickness in a commercial line of *Bos taurus*. In addition, Casas et al. (2005) reported no significant associations of DGAT1 alleles with carcass composition and meat quality traits in *Bos indicus*.

The T3 and T4 thyroid hormones have an important role in the metabolic regulation, and among other functions, they affect the lipid metabolism. TG is the precursor of T3 and T4 in the thyroid gland and its gene has been mapped to bovine chromosome 14. By this reason, the TG gene has been considered as a candidate gene to explain differences in marbling. Barendse et al. (2001) reported the C to T transition in the thyroglobulin 5′ leader sequence to be highly associated with intramuscular fat deposition in long-fed cattle. This transition defines the '2' (C) and '3' (T) alleles. Barendse et al. (1999, 2004) found that the TG '3' allele was more frequent in animals with higher marbling scores. However, this marker appears to be useful in Wagyu cattle specifically. In other beef cattle breeds this marker has not proved to be a good predictor of marbling (Rincker et al., 2006; Barendse et al., 2004; Casas et al., 2005, 2007).

As was mentioned above, DGAT1 and TG genes have been mapped to the centromeric region of chromosome 14. The presence of a quantitative trait locus (QTL) in the centromeric end of chromosome 14 associated with production traits in cattle has

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been supported by many studies (Coppieters et al., 1998; Heyen et al., 1999; Riquet et al., 1999; Looft et al., 2001; Boichard et al., 2003).

GH is a polypeptide hormone secreted by the anterior pituitary gland and it plays a major role in tissue growth, fat metabolism and homeorhesis (Shingu et al., 2004; Beauchemin et al., 2006; Thomas et al., 2007). The GH gene is the regulator of the animal growth and metabolism and it has been mapped to bovine chromosome 19. Different polymorphisms have been identified in the bovine GH gene (Lucy et al., 1991; Zhang et al., 1993; Kirkpatrick et al., 1993). Most of these polymorphisms have been associated with differences in carcass composition, marbling and milk production (Lee et al., 1996; Yao et al., 1996; Lechniak et al., 2002; Di Stasio et al., 2005; Curi et al., 2005; Barendse et al., 2006; Thomas et al., 2007). In the present report the analyzed polymorphism was GH6.1, also known as AluI RFLP (Yao et al., 1996). It is caused by a C to G nucleotide change in the fifth exon of the gene, which gives rise to two alleles that are responsible for alternative forms of bovine GH with a Leucine or Valine amino acid residue at position 127.

LEP is a protein hormone product of the obese gene synthesized and secreted predominantly by white adipocytes (Zhang et al., 1994; Ji et al., 1998). The role of LEP as a lipostatic signal that regulates whole-body energy metabolism makes it one of the best physiological markers of body weight, food intake, energy expenditure (Houseknecht et al., 1998; Woods et al., 1998), reproduction (Cunningham et al., 1999; Garcia et al., 2002), and certain immune system functions (Lord et al., 1998). LEP gene has been mapped to bovine chromosome 4 (Stone et al., 1996). Polymorphisms in the coding regions of the leptin gene in cattle have been associated with serum leptin concentration (Liefers et al., 2003), feed intake (Liefers et al., 2002; Oprzadek et al., 2003), milk yield (Liefers et al., 2002; Buchanan et al., 2003), body fatness (Buchanan et al., 2002; Nkrumah et al., 2004a,b) and with marbling scores (http://ca.igenity.com/igenity_beef1.html). We analyzed the polymorphism situated in exon 3 of the leptin gene (Liefers et al., 2002) which causes an amino acid change from Alanine to Valine amino acid residue at position 59.

SCD is the enzyme responsible for the conversion of saturated fatty acids to Δ9-monounsaturated fatty acids. Inhibition of desaturase activity leads to an accumulation of stearic acid in bovine adipose tissue, which can cause a substantial increase in fat hardness (Smith et al., 1998; Yang et al., 1999). The fatty acid composition of bovine fat has an impact on the visual manifestation of marbling during processing, the softness of the fat, and the flavour of the meat on the consumers plate (Melton et al., 1982; Smith et al., 1998). Due to its important role in fatty acid oxidation, SCD is a candidate for genetic variation in fatty acid composition. Taniguchi et al. (2004) reported in Japanese Black cattle an aminoacid substitution on the SCD gene that may change the enzymes's catalytic activity. This SNP, observed in the ORF (position 878) of SCD gene, causes an amino acid replacement from Valine (V) to Alanine (A).

On the other hand, several studies have reported geographical clines in polymorphism on genes related with production traits, such as α_{S1}-cas, κ-cas, GH, serum albumin, several microsatellites and Y-chromosome polymorphisms. These gradients have been shown to be related to different causes, such as domestication centre, population origin, migration route, gene introgression and/or adaptive effects of a particular allele (Baker and Manwell, 1980; Medjugorac et al., 1994; MacHugh et al., 1994, 1997; Lirón et al., 2002; Beja-Pereira et al., 2002, 2003).

The objective of the present work was to evaluate the polymorphisms of five SNPs of candidate genes related with marbling: DGAT1, TG, LEP, GH and SCD in 389 animals of 18 *B. Taurus* (European, Asian and Creole) and *B. indicus* breeds, in order to evaluate the genetic diversity within and among studied populations and the phylogenetic relationship of the analyzed breeds. The results obtained from the analysis of the SNPs were compared with ones previously obtained with five loci related to milk production (Lirón et al., 2002) and nine microsatellites (Lirón et al., 2006) in the same populations. Moreover we wanted to check whether the phylogenies reconstructed using SNPs associated with marbling resulted in the known tree topology.

2. Materials and methods

2.1. Sample collection

Blood samples were collected from 389 animals belonging to 18 *B. Taurus*, *B. indicus* (Brahman and Nelore) and synthetic Brangus breeds (Table 1). The *B. Taurus* breeds were grouped according to their geographical origin in European (Hereford, Aberdeen Angus, Galloway, Holstein, Jersey, Charolais and Retinta), Asian (Wagyu) and American Creole breeds (Argentine Creole, Patagonian Creole, Saavedreño Creole, Chaqueño Boliviano, Chusco, Valle Grande Creole, Yacumeño Creole).

2.2. DNA extraction

Total DNA was extracted from blood samples using the DNAzol® reagent (Invitrogen, Carlsbad, CA, USA) following manufacturer instructions.

2.3. SNPs genotyping

The five SNPs of candidate genes related with marbling were analyzed by PCR-RFLP or PCR-SSCP as detailed in Table 2.

Table 1
Summary of cattle breeds sampled.

Breed	Acronyms	*N*	Breed origin	Sample origin
Hereford	HE	21	England	Argentine
Aberdeen Angus	AA	59	Scotland	Argentine–Uruguay
Galloway	G	10	Scotland	Argentine
Holstein	HO	20	Netherlands	Argentine
Jersey	J	10	Island of Jersey	Argentine
Charolais	CH	14	France	Uruguay
Retinta	T	26	Spain	Spain
Argentine Creole	AM	20	Argentine	Argentine
Patagonian Creole	CA	20	Argentine	Argentine
Saavedreño Creole	SAA	20	Bolivia	Bolivia
Chaqueño Boliviano	ES	20	Bolivia	Bolivia
Chusco	PA	7	Bolivia	Bolivia
Valle Grande Creole	V	20	Bolivia	Bolivia
Yacumeño Creole	Y	35	Bolivia	Bolivia
Brangus	BR	12	EE.UU	Argentine
Brahman	BZ	20	EE.UU	Bolivia
Nelore	NE	33	Brasil	Argentine–Bolivia
Wagyu	W	22	Japan	Uruguay

Table 2
Genotyped method, analyzed mutation and reference for each studied SNP.

SNP	Method	Analyzed mutation	Author
DGAT1	PCR-SSCP	K232A (eighth exon)	Ripoli et al. (2006)
TG	PCR-RFLP	C → T (5́ leader sequence)*	Barendse et al. (2001)
LEP	PCR-RFLP	A59 V (third exon)	Liefers et al. (2002)
GH	PCR-RFLP	L217 V (fifth exon)	Yao et al. (1996)
SCD	PCR-RFLP	Val → Ala (878 ORF position)	Taniguchi et al. (2004)

* This transition defines the '2' (C) and '3' (T) alleles.

2.4. Statistical analysis

2.4.1. Measures of genetic variability

GENEPOP 1.2 software (Gou and Thompsom, 1992; Raymond and Rousset, 1995) was used for calculation of allele frequencies, for each locus in each studied population. The unbiased expected heterozygosity (h_e) for each locus and the average heterozygosity over all loci (H_e) were calculated according to Nei (1978), using the ARLEQUIN 2.0 software package (Schneider et al., 2000). Hardy–Weinberg equilibrium (HWE) for each locus within populations was estimated by F_{IS} statistics (Weir and Cockerham, 1984), using the exact test included in GENEPOP 1.2 software.

2.4.2. Genic differentiation and population's subdivision

Genetic subdivision and genetic differentiation among breeds were studied with Wright's F_{ST} statistic, using the variance-based method of Weir and Cockerham (1984) and with the exact *G* test (Goudet et al., 1996). These parameters were calculated using the GENEPOP 1.2 software package (Raymond and Rousset, 1995). A hierarchical analysis of the variance was carried out, after defining groups of breeds based on their historical origin, using the AMOVA software implemented in the ARLEQUIN 2.0 package (Schneider et al., 2000). Structure 2.0 software (Pritchard et al., 2000) was used for inferring population structure from genotype data. To analyze the population structure with this software were considered admixture and no admixture ancestry models, correlated or independent gene frequencies models, and was used a burning period of 100,000 followed by 1,000,000 Markov chain Monte Carlo (MCMC) repeats. In the case of no admixture model, the number of genetic clusters (K) ranged from 1 to 17.

Nei's standard genetic distance (D_A) was calculated from allele frequencies. Dendograms were constructed from the distance matrix using the unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973) and the Neighbor-Joining (NJ) (Saitou and Nei, 1987) algorithms. Distance and tree were computed using Populations 1.2.28 software (Langella, 1999). The tree was visualized using TreeView (Page, 1996). To condense the genetic variation revealed for the five SNPs, principal components analysis (PCA) was performed from all allele frequencies according to Cavalli-Sforza et al. (1994) using PAST software (Palaeontological Statistics; Hammer et al., 2001).

3. Results

3.1. Allele frequencies

Allele frequencies for all breeds are presented in Table 3. As a rule for the loci studied, the same alleles were predominant in most of the cattle breeds. At the SCD and LEP loci variant A was the most abundant in all studied populations. In addition, alleles DGAT1 A, TG 2 and GH L were the most common variants in most of the cattle breeds analyzed. The exceptions were DGAT1 A variant in Argentine Creole, Nelore and Brahman breeds, TG 2 allele in Galloway and Wagyu breeds, and GH L allele in Wagyu.

3.2. Hardy–Weinberg equilibrium

From a total of 90 locus-population possible combinations, only 71 HWE tests could be calculated. Sixteen locus-population combinations were excluded because one variant was fixed or almost fixed (frequency higher than 0.975), while three locus-population combinations had not data. A total of 10 locus-population combinations were statistically significant ($P \leqslant 0.05$) (Table 4). These deviations comprise two loci in Hereford, Valle Grande Creole and Wagyu cattle, and one locus in Jersey, Patagonian Creole, Chaqueño Boliviano and Charolais breeds. Non-significant deviations from HWE were observed for the other ten analyzed breeds.

Table 3
Estimated gene frequencies for five analyzed SNPs in Hereford (HE), Holstein (HO), Jersey (J), Charolais (CH), Aberdeen Angus (AA), Retinta (T), Galloway (G), Patagonian Creole (CA), Saavedreño Creole (SAA), Chaqueño Boliviano (ES), Valle Grande Creole (V), Argentine Creole (AM), Chusco (PA), Yacumeño Creole (Y), Wagyu (W), Brangus (BR), Brahman (BZ) and Nelore (NE) cattle breeds.

BREED	DGAT1 A	TG 2	LEP A	GH L	SCD A
HE	0.95	0.98	0.63	0.83	1
HO	0.81	0.78	0.85	0.85	0.95
J	0.65	0.50	0.86	0.78	1
CH	0.89	0.92	0.50	0.75	1
AA	0.90	0.74	0.89	0.57	1
T	0.71	0.71	0.80	0.64	0.71
G	0.85	0.40	0.81	1	0.60
CA	0.79	0.50	0.81	0.91	0.98
SAA	0.75	0.72	0.86	0.74	1
ES	0.55	0.77	0.50	0.87	0.76
V	0.50	0.79	0.83	0.53	1
AM	0.47	0.75	0.83	0.97	0.89
PA	0.64	0.50	0.50	1	0.80
Y	0.60	0.91	0.60	0.72	0.61
W	0.50	0.32	0.58	0.19	0.95
BR	0.62	0.70	0.80	0.82	0.96
BZ	0.20	0.89	0.69	1	0.98
NE	0.31	0.90	0.54	0.90	1

3.3. Heterozygosity

The values of observed (h_o) and unbiased expected (h_e) heterozygosities for each locus of the 18 breeds, calculated from gene frequencies, are given in Table 4. The h_e ranged from 0.047 for TG in Hereford, to 0.571 for TG in the Chusco breed. The average heterozygosity (H_e) was also estimated for each population, varying from 0.182 in Hereford breed to 0.484 in Chusco Creole cattle.

3.4. Genetic distances

The F_{ST} index and the exact *G* test for population differentiation were used to analyze the degree of genetic differentiation among the cattle breeds studied. The F_{ST} parameter showed significant differences across the cattle populations (F_{ST} = 0.1593), ranging from 0.070 to 0.2053 for each locus (F_{ST} LEP = 0.070; F_{ST} TG = 0.1374; F_{ST} SCD = 0.1682; F_{ST} DGAT1 = 0.1818; F_{ST} GH = 0.2053). The exact G test for population differentiation indicated that gene distributions are significantly different among populations (exact *p* value for all loci $p \leqslant 0.0001$).

AMOVA analysis allowed to partitionate genetic variability between different groups of breeds based in their historical origin. At first, this analysis was performed for each locus considering only one group. These results evidence that difference among populations account for 7–21% of genetic diversity, while difference within populations account for 78–92% of genetic variance (Table 5). The highest percentage of variation among populations was for GH gene (21.11%), whereas the biggest percentage within population was for LEP locus (92.93%).

Moreover, AMOVA was calculated grouping the breeds in four groups according to their European, Asiatic, Creole or Zebu origin. Considerable levels of variation among groups were observed (90–95%), while variance among populations within each group only explained between 5% and 10% of the genetic variance. These analyses evidenced that the greatest percentage of variation among groups was for DGAT1 and TG loci (15.47% and 13.42% respectively).

Table 4
Observed (h_o) and expected (h_e) heterozygosities, and significant Fis index for five SNPs in the eighteen analyzed populations.

Breed	DGAT1			LEP			TG			GH			SCD	
	h_o	h_e	Fis (P value)	h_o	h_e	Fis (P value)	h_o	h_e	Fis (P value)	h_o	h_e	Fis (P value)	h_o	h_e
HE	0	0.102	1 (0.026)	0.737	0.478	−0.565 (0.039)	0.048	0.047		0.333	0.285		0	0
AA	0.1	0.185		0.220	0.198		0.317	0.386		0.387	0.494		0	0
HO	0.263	0.309		0.176	0.258		0.444	0.357		0.3	0.261		0.1	0.097
J	0.300	0.479		0	0.264		1	0.526	−1 (0.006)	0.444	0.366		0	0
BZ	0.294	0.337		0.500	0.436		0.214	0.198		0	0		0.048	0.048
AM	0.278	0.513		0.167	0.290		0.500	0.387		0.05	0.050		0.208	0.191
CA	0.176	0.337		0.125	0.325		0.818	0.524		0	0.169	1 (0.002)	0.050	0.050
SAA	0.500	0.384		0.143	0.254		0.437	0.417		0.260	0.394		0	0
ES	0.789	0.508	−0.058 (0.022)	0.538	0.520		0.467	0.370		0.263	0.235		0.474	0.371
PA	0.714	0.494		1	0.513		1	0.571		0	0		0	0.356
V	0.900	0.513	−0.791 (0.001)	0.333	0.290		0.412	0.337		0.118	0.513	0.776 (0.002)	0	0
NE	0.461	0.443		0.267	0.514		0.200	0.189		0.115	0.177		0	0
BR	0.583	0.489		0.200	0.337		0.600	0.442		0.364	0.312		0.083	0.083
CH	0.214	0.198		0.429	0.538		0	0.159	1 (0.043)	0.214	0.389		0	0
W	0.800	0.513	−0.583 (0.023)	0.167	0.500	0.673 (0.010)	0.529	0.451		0.381	0.316		0.091	0.089
T	0.571	0.423		0.400	0.355		0.579	0.422		0.454	0.474		0.579	0.422
Y	–	–		–	–		0.187	0.175		0.5	0.413		0.643	0.495
G	–	–		0.231	0.323		0.200	0.505		0	0		0.609	0.433

Table 5
Percentage of variation obtained by AMOVA test.

Source of variation in% of variation	DGAT1	LEP	TG	GH
Among populations	17.84	7.61	13.26	21.11
Within populations	82.16	92.93	86.74	78.89
Among groups	15.47	0.76	6.02	13.42

3.5. Genetic distances and relationships between populations

Allele frequencies were used to generate the D_A genetic distances for each pair of 18 cattle populations. Distance matrix was used in order to build phylogenetic trees using UPGMA and the NJ algorithms. Depending on the clustering algorithm used, different topologies were obtained. Only the tree constructed with UPGMA, using genetic distance D_A, exhibit a topology partially consistent with the historical origin of breeds since the tree illustrates the main divergences observed between the Asiatic Taurine, European Taurine and Zebu clades. The UPGMA tree constructed from a matrix of D_A distances is shown in Fig. 1. Due to the spatial distortion caused by Wagyu a second UPGMA tree was generated without considering alleles frequencies of this breed. However, in both cases, the outcome of multivariate analysis was similar.

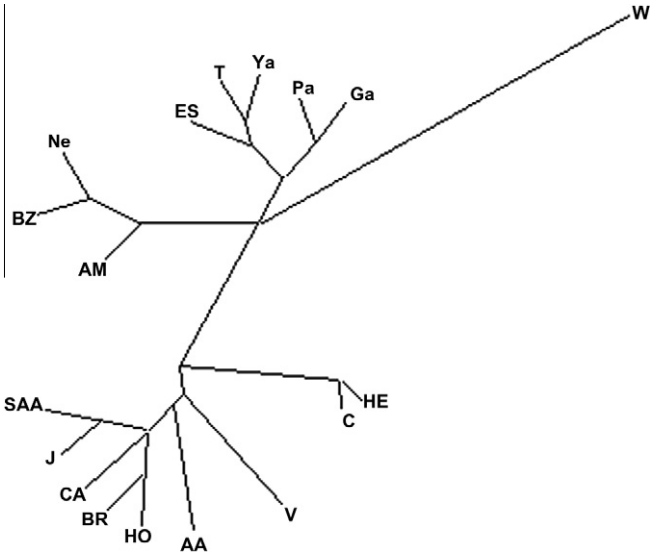

Fig. 1. UPGMA tree constructed from a matrix of DA distances (W, Wagyu; Ga, Galloway; PA, Chusco Creole; Ya, Yacumeño Creole; T, Retinta; ES, Chaqueño Boliviano Creole; Ne, Nelore; BZ, Brahman; AM, Argentine Creole; SAA, Saavedreño Creole; J, Jersey; CA, Patagonian Creole; BR, Brangus; HO, Holstein; AA, Aberdeen Angus; V, Valle Grande Creole; C, Charolais and HE, Hereford cattle breeds).

3.6. Principal components analysis (PCA)

The PCA was performed from allele frequencies. Fig. 2 illustrates the first and the second PCs for the five SNPs frequency distributions in 16 cattle breeds, since the Galloway and Yacumeño Creole breeds were excluded from the PCA analysis because it distorting the spatial representation. The first two components were selected, accounting cumulatively for 67.45% of the variability in the data. The first PC accounts for 37.29% of the total variance and clearly distinguishes the Wagyu breed and the others groups. The second PC summarizes 29.16% of the variation, and shows a differentiation pattern with the Zebu group in one side and the Taurine breeds in the other. In both cases the differences were explained by the gene frequencies of GH, TG and DGAT1 loci. The third PC, which accounts for 15% of the variance, was not represented in the figure because did not provide any relevant information about relationships between populations.

A second PCA was performed without the Wagyu gene frequencies since this breed position in the first PCA was far away from other races (Fig. 3). In this case the first two components accounting cumulatively for 67.46% of the variability in the data. The first PC accounts for 44.73% of the total variance and shows a differentiation pattern with the Zebu group in one side and the Taurine breeds in the other. This difference was explained by gene frequencies of DGAT1 locus. The second PC summarizes 22.73% of the variation, and distinguishes Charolais and Hereford of the other breeds. The third PC, which accounts for 15% of the variance, did not provide any relevant information.

The two PCA results obtained largely coincided with the topology of the phylogentic tree constructed with UPGMA using the classical genetic distance D_A.

4. Discussion and conclusions

In this work we evaluated the polymorphisms of five SNPs related with marbling in *B. taurus* and *B. indicus* breeds, in order to calculate the genetic diversity within and among studied popula-

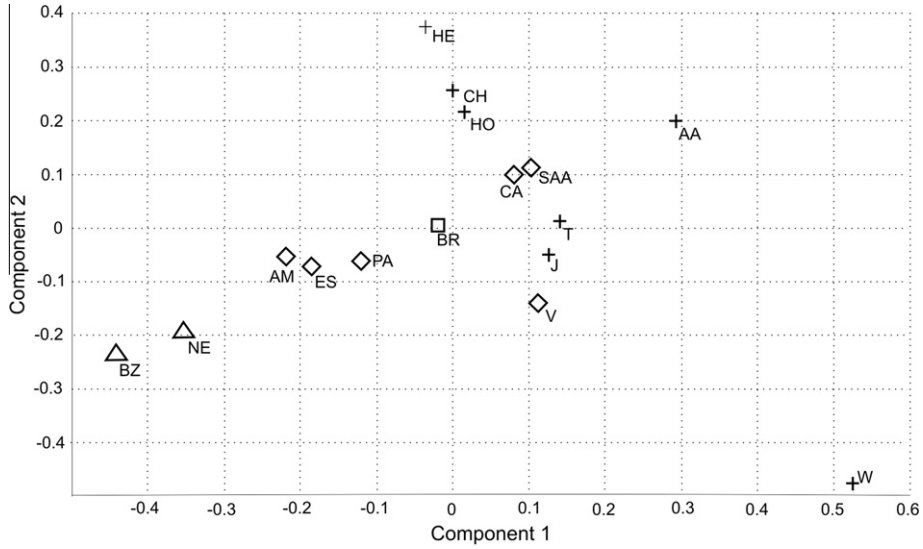

Fig. 2. Principal components analysis (PCA) of allele frequencies from five genotyped SNPs in sixteen analyzed populations (W, Wagyu; PA, Chusco Creole; T, Retinta; ES, Chaqueño Boliviano Creole; NE, Nelore; BZ, Brahman; AM, Argentine Creole; SAA, Saavedreño Creole; J, Jersey; CA, Patagonian Creole; B, Brangus; HO, Holstein; AA, Aberdeen Angus; V, Valle Grande Creole; CH, Charolais and HE, Hereford cattle breeds).

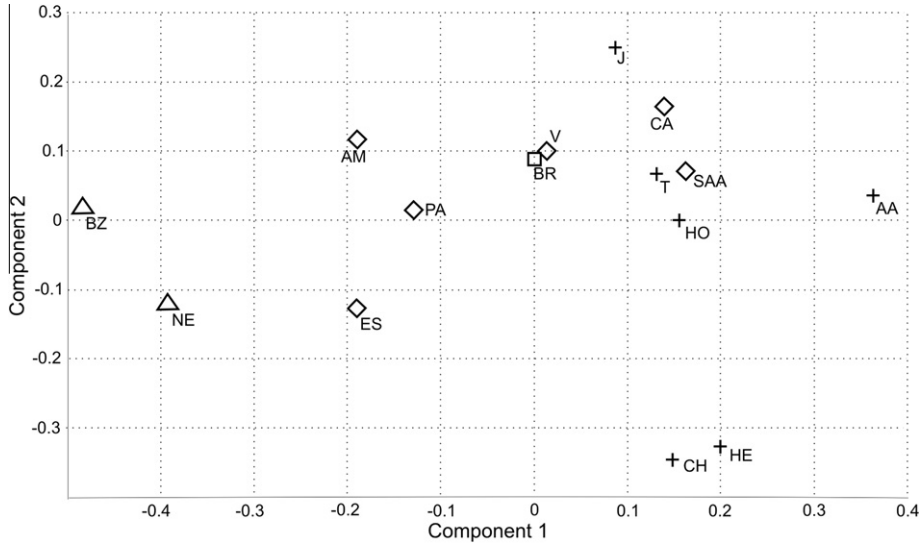

Fig. 3. Principal components analysis (PCA) of allele frequencies from five genotyped SNPs in fifteen analyzed populations (PA, Chusco Creole; T, Retinta; ES, Chaqueño Boliviano Creole; NE, Nelore; BZ, Brahman; AM, Argentine Creole; SAA, Saavedreño Creole; J, Jersey; CA, Patagonian Creole; B, Brangus; HO, Holstein; AA, Aberdeen Angus; V, Valle Grande Creole; CH, Charolais and HE, Hereford cattle breeds).

tions, and the phylogenetic relationship of the analyzed breeds. We also compared the results obtained from the analysis of these SNPs with ones previously obtained with loci related to milk production and STRs in these populations (Lirón et al., 2002, 2006). Lirón et al. (2002), studied the genetic diversity and population structure in Argentine and Bolivian Creole cattle by analysis of five loci related to milk production. Moreover, Lirón et al. (2006) assessed the genetic diversity and relationships of European taurine, Zebuine and American Creole cattle breeds through nine microsatellites.

Our second objective was to assess whether the SNPs reflected the phylogenetic relationship like the microsatellite markers (Lirón et al., 2006), since the SNPs analyzed were subjected to artificial selection processes, and the phylogenetic relationships may be distorted by the effect of selection.

The analysis of observed gene frequencies evidenced that GH and TG loci in Wagyu breed presented values away from those observed in the other breeds. This aspect could be explained by the fact that this breed is selected for marbling and present the highest values of marbling (Zembayashi et al., 1995). The allele frequencies analysis of DGAT1 locus evidenced a geographical cline that was given by high frequencies for the A allele in European breeds (0.95–0.81) followed by Creole and Retinta (0.79–0.47), Wagyu (0.50) and lastly Zebu breeds (0.31–0.20). The similar frequencies in Creole and Retinta may be consequence of the origin of Creole breeds from Iberian cattle. Allele frequencies of Zebu breeds were very different with respect to Taurine breeds due to Cebuine breeds had a low frequency of the A allele, in agreement with previously reported results (Winter et al., 2002; Ripoli et al., 2006). The allele frequencies analysis of TG, LEP, GH and SCD loci did not evidenced a so marked geographical cline.

The HWE tests evidenced that 10 locus-population combinations were statistically significant (two loci in Hereford, Valle

Grande Creole and Wagyu cattle and one locus in Jersey, Patagonian Creole, Chaqueño Boliviano and Charolais breeds), including five combinations with excess of heterozygotes and five combinations with excess of homozygotes. These deviations may be consequence of some possible causes like selection, small number of samples, population stratification and inbreeding.

When we compared the UPGMA unrooted tree with that obtained with microsatellites by Lirón et al. (2006), some differences were observed. While the STRs UPGMA tree showed that all Creole cattle breeds are clustered together, Brahman and Nelore (*B. indicus* breeds) were a separate group and Holstein was clustered with Angus and Hereford (*B. taurus* breeds), the marbling SNPs markers UPGMA tree did not evidence well defined groups. The lack of consistent topology of the phylogenetic trees is commonly explained by several factors. First, the construction of trees using admixture population, such as Creole cattle, contradicts the principles of phylogeny reconstruction (Felsenstein, 1982). The second issue that should be considered is the number of markers analyzed. Based on theoretical studies, Takesaki and Nei (1996) have shown that one of the important factors for analyzing the correct phylogenetic position of populations in a genetic study is the number of loci used. The number of markers genotyped here are probably insufficient for complete resolution. Nevertheless, various taurine phylogenetic analyses performed with a higher number of microsatellites also evidenced a weak topology in Taurine breeds (Moazami-Goudarzi et al., 1997; Martín-Burriel et al., 1999; Cañon et al., 2001). Another way of understanding the lack of structure is assuming that populations have differentiated according to a radiative scheme of divergence (Moazami-Goudarzi and Laloe, 2002). According to this model, it is expected that genetic distances between breeds would be equivalent, and any casual differences among them might be due to random genetic drift. Furthermore, this scheme anticipates discrepancies among topology exhibited by each marker. Increasing the number of loci does not necessarily enhance the reliability of the phylogeny. In contrast to the large divergence between the Taurine and Zebu cattle, the European breeds and American Creole, which originated around four centuries ago, could be considered to be closely related, and the main factor describing their genetic variability is random drift and selection.

However, the UPGMA tree topology was partially consistent with the historical origin of breeds since the tree illustrates the main divergences observed between the Asiatic Taurine, European Taurine and Zebu clades. The Wagyu breed was located at one end of the tree, and the Zebu group (Nelore and Brahman) with Argentine Creole cattle were located in an intermediate position between the two Taurine groups. One of these Taurine clades included Galloway, Retinta and some Creole breeds (Chaqueño Boliviano, Yacumeño Creole and Chusco). This last fact probably can be explained due to Retinta breed was one of the breeds introduced in America by the Spanish conquerors during the first 50 years of colonization. In the other Taurine clade we found Jersey, Brangus, Holstein, Angus with some Creole breeds (Saavedreño Creole, Patagonian Creole, Valle Grande Creole). Hereford and Charolais were grouped together but close to the latter clade. Within Zebu breeds, Brahman and Nelore were more similar to each other than with Brangus. This fact is consistent with the origin of Brahman (obtained crossing Nelore, Guzerat and Gir) and Brangus. This latter breed is 3/8 Brahman and 5/8 Angus and that is why gene frequencies in Brangus are more similar to Taurine breeds than to Zebu breeds.

The grouping of the breeds may be the result of its history, selection process, or both at once, the latter case may be the situation that explains the UPGMA tree topology obtained. Probably the fact that Wagyu was at one end may be the result of selection processes for higher marbling score. Furthermore, the clustering of Hereford with Charolais would be a consequence of their lower level of marbling.

The result observed in terms of the Principal Components Analysis also matched with the topology of the phylogenetic tree and was in concordance with the marbling score of breeds and with the historical origin of the breeds, dividing Wagyu from the others breeds, and Zebu from Taurine breeds. The first principal component (PC) of the PCA performed with all gene frequencies, clearly distinguishes the Wagyu breed and the others groups, and this was explained by the gene frequencies of TG and GH loci, whose values in Wagyu breed were found away from those values observed in the remaining breeds. This aspect could be explained by the fact that this breed is selected for marbling and present the highest values of marbling (Zembayashi et al., 1995). The second PC shows a differentiation pattern with the Taurine breeds in one side and the Zebu group in the other. The DGAT1 frequencies explain this principal component as Cebuine breeds had a low frequency of the A allele, in agreement with previously reported results (Winter et al., 2002; Ripoli et al., 2006). Interestingly, European breeds had the A allele at a very high frequency. It is worth noting that the A allele is considered part of the ancestral haplotype of DGAT1 in European populations (Winter et al., 2002). Native Creole breeds tended to have higher frequencies of the A allele, but always below those of European breeds. The fact that native breeds have allele frequencies intermediate to those of European and Cebuine breeds may be consequence of the introgression process of alleles of Cebuine origin in Creole herds (Lirón et al., 2006; Ripoli et al., 2006).

The first principal component (PC) of the PCA performed without Wagyu gene frequencies, distinguishes the Zebu group in one side and the Taurine breeds in the other and this is explained by the gene frequencies of DGAT1 locus. The second PC distinguishes the Charolais and Hereford from the other breeds. This differentiation was explained by TG gene frequencies and would be a consequence of their lower level of marbling. This result was consistent with that observed in the UPGMA tree.

When the AMOVA analysis was performed for each locus considering only one group the difference among populations account for 7–21% of genetic diversity, while difference within populations account for 78–92% of genetic variance. This result was coincident with the F_{ST} estimation. This last index indicated that around 16% of the total genetic variation corresponded to differences between breeds while the other 84% corresponded to differences among individuals. Otherwise, the degree of population structure, measured with F_{ST} index, was higher than the value previously reported for microsatellites (9%) (Lirón et al., 2006) and for loci related to milk production (11%) (Lirón et al., 2002) in some of these populations. In all three cases, the analyzed markers showed significant differences among the populations studied. The STRs showed genetic differentiation among breeds and between the two major types of cattle (Zebu and Taurine groups) (Lirón et al., 2006). These results were a consequence of the presence of some Zebu diagnostic alleles. In the case of loci related to milk production (Lirón et al., 2002), all the variants of the loci analyzed were found in nearly all cattle breeds studied, hence the significant differences observed across Creole cattle populations were consequence of the allelic distribution, rather than diagnostic alleles.

The SNPs associated with marbling distinguished the Wagyu breed from the other groups, and differentiated the Taurine and the Zebu breeds based on the allele frequencies patterns because they are biallelic markers. In the case of DGAT1 locus, similar gradient in gene frequencies were previously reported for other genes such as α_{S1} casein where variant B were predominat in Taurine while variant C were more abundant in Zebu breeds (Baker and Manwell, 1980; Giovambattista, 1996; Postiglioni et al., 1998; Arranz Santos, 1994; Arranz Santos et al., 1996; Ripoli, 2001). The differences observed for TG and GH allele frequencies would be

consequence of marbling selection process and may be this fact explains the UPGMA tree topology obtained.

In conclusion, the main findings of this study were: (i) the partial confirmation of the phylogeny reconstruction of the studied breeds obtained with STRs, probably due to the effect of selection for marbling reflected in PCA and UPGMA tree analyses; (ii) the interaction of history, selection process and random genetic drift in the determination of grouping of the breeds.

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