PUBLICACIONES
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Short communication

Possible association of bovine chromosome 5 markers with growth and fat traits in Hereford cattle raised under extensive conditions

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1. Introduction

Different cattle management systems were recently tested searching for QTL (Hagen et al., 2005; Morris et al., 2009), and differences between post-weaning environments were found in the significance of some QTL. However, most research on DNA markers is done under conditions of intensive production; therefore, there is a need to establish the association of these markers in pasture based production systems.

Bovine chromosome 5 (BTA5) has been widely studied because several QTLs have been detected there (Allan et al., 2009; Casas et al., 2000; Li et al., 2004a; Stone et al., 2005). In particular, three chromosomal regions (0 to 30 cm, 55 to 70 cm, and 70 to 80 cm) were identified as having significant associations with the growth traits, while the region between 62 and 82 cm seems to be important for fat traits (Casas et al., 2000; Li et al., 2004a). Within them, two genes were reported as candidates for growth and fat traits: Myogenic factor 5 (Myf5), between 0 and 30 cm, and Insulin-like Growth Factor 1 (IGF1), between 55 and 80 cm.

The objective of the present work was to test for a possible association between BTA5 markers in previously reported QTL for growth and fat, in a pasture based system. In order to do this, four microsatellites, mapped to two BTA5 chromosomal regions that include Myf5 and IGF1, were genotyped. Genotypic data was tested against Estimated Breeding Values (EBVs) for traits related with growth: 400 Days Weight (W400), 600 Days Weight (W600), Rib Eye Area (REA); and traits related with fat: Rib Fat (Rib), Rump Fat (Rump) and Intra Muscular Fat (IMF).
2. Materials and methods

2.1. Samples

Samples were taken from a commercial Hereford type herd that includes approximately 400 dams, and produces 160 bulls every year to be sold. The herd had been selected using BreedPlan® Genetic evaluation for almost 15 years, with the objectives of low birth weight and a moderate 600 days weight (personal communication). The animals are bred in an extensive pasture system, with ad libitum access to cultivated grass. Pastures available include Lolium spp., Bromus spp., Festuc a spp., Trifolium spp. and natural species, with higher productivity on autumn and spring. DNA was extracted from samples collected from 56 calves and 16 dams (blood), and from 18 sires (stored semen).

2.2. Genotyping

Four BTA5 microsatellites were genotyped (PCR protocols as supplementary): BPI (14,382,552 bp), close to the Myf5 gene, and ETH10 (60,836,475 bp), IGF1 (71,198,741 bp) and RM029 (80,417,234 bp) near the IGF1 gene (www.ncbi.nlm.nih.gov/projects/mapview). Fragments were resolved in a MegaBACE1000 sequencer and the data analyzed with Fragment Profiler Software (GE healthcare, USA).

2.3. Pedigree, trait data and genotype inferring

Pedigree data included 1754 animals, born between 1980 and 2007. The sampling included animals belonging to a single family, from the 1st to the 8th generation of one of the founder sires. HHH ENF 972. The herd was evaluated for 400 (W400) and 600 (W600) Days Weight, Rib Eye Area (REA), Rib Fat, Rump Fat and Intra Muscular Fat. 2008 database BreedPlan Estimated Breeding Values (EBVs) were provided by the herd owner. Genopro 2.0 (Thallman et al., 2001) was used to infer pedigrees following the rule that a genotype was accepted if pGmax>0.95.

2.4. Genetic and statistical analysis

Allele frequencies, expected heterozygosity (H_e) and Hardy-Weinberg equilibrium (HWE) were calculated using Genepop 4 (Rousset, 2008), considering only the genotyped animals. Association between EBVs and genotypes (including inferred genotypes) was performed using SAS 9.0 GLM with the following model: Y_{ij} = G_i + e_{ij}, where Y_{ij} is i analyzed trait of j animal, G_i = genotype effect (considering one or two genes) and e_{ij} = random error.

3. Results and discussion

Allele frequencies and H_e for the genotyped animals are presented as Supplementary Table S1. After the Genoprob run, 91 new inferred genotypes were accepted (21% more genotypes) belonging to 73 not sampled animals (82% more animals). Those animals were also included in the association tests. Table 1 resumes all tests between traits and markers.

Previous studies (Li et al., 2004a) have detected three BTA5 regions associated with growth traits (0 to 30 cm, 55 to 70 and 70 to 80 cm). Myf5 gene and BPI microsatellite are located between 0 and 30 cm region; IGF1 gene (and microsatellite), ETH10 and RM029 microsatellites are mapped between 60 and 82 cm. Our study detected significant association of all markers with different growth traits. In particular, BPI was associated (p≤0.05) with REA and explains 1.5 points in REA EBV and 18.5% of the whole variability (see Supplementary Tables S2 and S3). BPI is close to the Myf5 gene, whose product is an intramuscualr factor that enhances myocyte development and muscularity, whereas REA is an ultrasound measurement of the amount of muscle in the carcass. These results are related to those that associate an SNP of Myf5 with Pre-weaning Average Daily Gain and Average Daily Gain on Feed in Bos taurus (Li et al., 2004b). Furthermore, we also found an association (p≤0.1) of BPI with W400 and W600 EBVs. In BTA5 region between 60 and 82 cm, IGF1 (p≤0.05) and RM029 (p≤0.1) microsatellites were associated with W600. When considering two genotypes, IGF1-ETH10 shows a strong association with W400 and W600 EBVs. Moreover, HWE deviation for these three markers (Table 1), suggests that this region could be influenced by selection. Multiple QTL in 50 to 82 cm BTA5 region were detected for yearling weight (Li et al., 2004a), and in IGF1 for growth traits (Machado et al., 2003). In addition, IGF1/SnaB1 polymorphism was associated with weight gain after weaning (Ge et al., 2001).

For fat traits, prior data suggest that the region between 62 and 82 cm would be important. A QTL was found near 60 cm for fat depth (Casas et al., 2006), and other for dressing percentage between 30 and 80 cm (Stone et al., 2005). Li et al., 2004a found a haplotype between 65.4 and 70.0 cm associated with backfat. In this study, ETH10 was associated with Rib and Rump fat, explaining 1.18 and 1.67 points of the

<table>
<thead>
<tr>
<th>Marker</th>
<th>F_e (W&amp;C)</th>
<th>P-val</th>
<th>W400</th>
<th>W600</th>
<th>REA</th>
<th>R1 B FAT</th>
<th>RUMP FAT</th>
<th>IMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP1</td>
<td>0.057</td>
<td>0.474</td>
<td>0.065</td>
<td>0.082</td>
<td>0.031</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<tr>
<td>ETH10</td>
<td>0.065</td>
<td>0.051</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.031</td>
<td>0.032</td>
</tr>
<tr>
<td>IGF1</td>
<td>0.273</td>
<td>0.015</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>RM029</td>
<td>0.794</td>
<td>0.000</td>
<td>0.035</td>
<td>0.024</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>ETH10-IGF1</td>
<td>-</td>
<td>-</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>IGF1-RM029</td>
<td>-</td>
<td>-</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>
EVBs variation, respectively; in both traits allele 221 appeared to be beneficial (see Supplementary Table S2).

In conclusion, QTL for growth and fat traits previously reported in two regions of BTA5 are also expressed in a commercial pasture based system, where animals are not always fed to express their maximum genetic potential.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.livsci.2010.06.001.

References

Association of bovine chromosome 5 markers with birth and weaning weight in Hereford cattle raised under extensive conditions


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ABSTRACT

Genetic markers have been used to assess the association of economically important traits with cattle under intensive feeding conditions; however, there is still the need to ascertain the usefulness of these markers under extensive production systems. Bovine chromosome 5 has been widely studied because several QTLs have been detected. Microsatellite BP1 neighboring the Myogenic factor 5 gene (Myf5), and microsatellites ETH10, IGF1 and RM029 near Insulin-like Growth Factor 1 gene (IGF1), were selected to establish their association with BLUPs (Best Linear Unbiased Predictor) for direct Birth Weight (dBW), direct Weaning Weight (dWW) and maternal Weaning Weight (mWW). Two herds were used for this objective, one commercial and the other experimental. Associations (P ≤ 0.05) between dBW and all BTAS loci (BP1, ETH10, IGF1, and RM029) were detected. Additional associations were observed between mWW and BP1. dBW was significantly associated (P ≤ 0.05) with ETH10 genotypes and with the interaction IGF1*Herd. In particular region near BP1 could be contributing to the rare positive correlation between dBW and mWW previously found in the INTA Balcarce Station experimental herd. We confirmed marker associations with growth traits in two BTAS regions close to previously reported QTL obtained in intensive feeding conditions; these regions affect dBW, dWW and mWW in a pasture-based system.

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1. Introduction

A limited number of markers are available to be used by producers, and they explain a relatively small proportion of the genetic variation for a limited number of traits (Dekkers, 2004). Recently, Morris et al. (2009) have searched QTL expressed in different cattle management systems. Most marker associations have been evaluated under intensive productive conditions. Therefore, there is a need to establish

the association of these markers in pasture-based production systems.

Bovine chromosome 5 (BTAS) has been widely studied because several QTLs have been detected for many traits, i.e. growth, fat, male and female reproduction (Casas et al., 2000; Li et al., 2002, 2004; Casas et al., 2003; Stone et al., 2005; Allan et al., 2009). In particular three chromosomal regions (0 to 30 cM, 55 to 70 cM, and 70 to 80 cM) were identified as having significant associations with the growth traits. Within those regions at least two genes were reported as candidates. Myogenic factor 5 (Myf5), which maps within the 0 to 30 cM region, is capable to convert non-muscle cells into muscle, thus, is a potential candidate for growth and meat quality related traits (Daubas et al., 2000; Maek et al., 2006).
Insulin-like Growth Factor 1 (IGF1) gene, between 55 and 80 cM, has a physiological role in growth and development of mammals (Werner et al., 1994). Its polymorphisms have been associated with growth traits (Moody et al., 1996; Andrade et al., 2008).

Because productive systems in developing countries are extensive pasture-based and funds are not always available for sampling and genotyping, it is required to use the available data as much as possible. Quantitative data have been used for genetic improvement since the 50s, and a considerable amount of information is stored. Breeders have kept frozen semen since then, so these reservoirs are a source of DNA. Much more information can be gleaned by calculating genotypic probabilities for individuals with missing marker data, and tracking markers over an extended pedigree in commercial or long-term experimental populations (Thallman et al., 2001a).

Genotypic data from four BTAS microsatellites were tested against BLUPs (Best Linear Unbiased Predictor) for direct Birth Weight (dBW), direct Weaning Weight (dWWW) and maternal Weaning Weight (mWWW). This association test was performed to prove if the QTLs previously detected, in the chromosomal regions that include Myf5 and IGF1, are expressed in Hereford populations raised under extensive pasture-based conditions.

2. Materials and methods

2.1. Samples and herd description

Two Hereford herds, one experimental (EH) and the other commercial (CH), were sampled, blood was collected from calves and dams and semen from bulls. Additionally, blood samples were collected from 17 unrelated Hereford bulls, born between 2005 and 2007 from 6 different studs, and were only used as reference population (RP) for allele diversity and frequencies. These bulls were INRA Balcarce Experimental Station Performance Test participants (www.inta.gov.ar/balcarce/index.htm).

EH was previously described by Melucci et al. (2009). Briefly, the herd was established in 1960 and selected between 1986 and 2006 to increase weaning weight without increasing birth weight. It was a closed stable herd of 100 cows and 4 bulls. Two older bulls (3 years-old) were replaced every year with the 2-year-old males selected. Samples taken from this herd include 66 calves, born between 2005 and 2007, 83 dams, and 9 bulls. These animals belonged to generations between 2.19 and 8.16; and had an average inbreeding coefficient of 0.047.

The CH was a typical stud that included approximately 400 dams, and produced 160 bulls every year to be sold. The herd was selected using BreedPlan® Genetic evaluation (EVBS) for almost 15 years with an objective of a low birth weight and a moderate 600 day weight (personal communication). All sampled animals presented genetic connection with a founder sire born in 1980, except for 6 bulls that were used to introduce new genetics to the herd. Animals belonging to first to eighth generations from founder sire were included in the sampling. This included 56 calves (born in 2006 and 2007), 17 dams, and 18 bulls. Dams were selected from those that had several progenies in the herd, and the sires samples included semen stored from bulls used since 1986. This kind of sampling was performed to maximize the genotype inferring.

2.2. DNA extraction

Semen was digested using an extraction buffer containing 0.4 mg/ml proteinase K and 25 mM DTT, and DNA was purified by chloroform organic extraction technique. DNA was extracted from blood samples using DNAzol® reagent (Invitrogen™, Carlsbad, CA, USA) following manufacturer’s instructions.

2.3. Genotyping

Four microsatellites markers surrounding the selected genes were genotyped: BPI (14382552 bp), close to Myf5 gene, ETH10 (60836475 bp), IGF1 (71198741 bp) and RM029 (80417234 bp) near IGF1 gene (www.ncbi.nlm.nih.gov/projects/mapview). Additionally, BM1824 microsatellite (BTAS1, 122 cM) was included to evaluate a random, multiallelic polymorphism, in a different chromosome and not associated with a known structural gene. Microsatellite PCR multiplex was performed in 12.5 μl final volume, including 1.5 pmol, 2.5 pmol, 3.0 pmol, 6.0 pmol and 10 pmol of ETH10, BM1824, RM029, BPI and IGF1 fluorescent labeled primers, respectively (www.ncbi.nlm.nih.gov/projects/mapview); buffer 1× (Invitrogen™), MgCl2 2.5 mM, 100 mM of each dNTP, 0.04 U/μl Taq Platinum (Invitrogen™), and 2 ng/μl DNA. PCR program was: 2 min 94 °C, 10 cycles of 30 s 94 °C, 45 s 60 °C, 30 s 72 °C, followed by 30 cycles of 30 s 94 °C, 45 s 58 °C, 30 s 72 °C, and final extension of 7 min 72 °C. Fragments were resolved in a MegaBACE 1000 sequencer (GE healthcare, USA) and data analyzed with Fragment Profiler Software (GE healthcare).

2.4. Genetic variability

Genepop software (Rousset, 2008) was used to calculate allele number, gene frequencies, and unbiased expected heterozygosity (He) for each locus and over all loci (Hs). Hardy–Weinberg equilibrium (HWE), estimated by Fis statistic, and linkage disequilibrium, were carried out using the Markov chain method included in the same package. To evaluate a genetic variation in time intervals, the experimental herd was divided into 3 contemporary groups: animals born between 1996 and 1999, 2000 and 2003, 2004 and 2007; this four year distribution was made to avoid animals sired by a particular bull and his son, in the same contemporary group. Genepop 4 exact test was performed to assess group and/or population differentiation based on its genetic data. Phases of linkage disequilibrium were reconstructed with Phase v2.1.1 (Li and Stephens, 2003) using default options; phases were accepted when the probability was higher than 0.95.

2.5. Pedigree, trait data and genotype inferring

Pedigree data included 2474 animals from EH, born between 1960 and 2008; and 1754 animals from CH, born between 1980 and 2007. Both herds were evaluated for direct Birth Weight (dBW), direct Weaning Weight (dWWW), and
maternal Weaning Weight (mWW), in separate analysis. The EH Best Linear Unbiased Predictor (BLUP) analysis was described by Melucci et al. (2009); while CH BLUPs were provided as EBVs by the herd owner and correspond to 2008 BreedM@ analysis. Usually molecular association studies use phenotype value, even though some research has been done using Breeding Values (Pereira et al., 2005; Schumman et al., 2008). In this sense, although using phenotypic data, Miquel et al. (2009) envisage the advantages of using Breeding Values in association studies, because they would consider the genetic correlations within different traits.

Genoprob 2.0 (Thallman et al., 2001a,b) was used to infer genotypes, in four microsatellites from chromosome 5, using genotypic and pedigree data. The genotyped individuals in each population, 157 for EH and 91 for CH, were used as reference to generate genotypes in the 2474 animals and 1754 animals included in the respective pedigrees. Default meiosis probabilities and errors were set, complete penetrance and no null alleles were considered, and finally 10 iterations were allowed. A genotype was accepted if pmax (posterior probability that the unordered genotype is correct) was higher than 0.95. Animals with BLUPs and genotypes (detected or inferred) were included in the association analysis.

2.6. Association analysis

For the association studies, the traits of interest were analyzed using the General Linear Model (GLM) procedure of the SAS program (Statistical Analysis System, 1999). Single-locus association analysis between markers and BLUPs was performed. Two models were defined depending on the number of alleles found for each marker. In markers with more than two alleles (considering only CH and EH), the interaction between herd (H) and the genetic marker (GM) couldn’t be estimated. That was the case of BM1824 (BTA1), BP1 and ETH10 that were analyzed using a model with the fixed effect of herd (H) and genetic marker (GM) nested in H. For RM029 and IGF1, biallelic markers, the model included fixed effect of H; GM and H×GM interaction. This same analysis was performed for BP1, excluding animals with 310 allele that were only detected in CH, and allowed the marker to fit in a biallelic analysis. Additive effects were estimated by the difference between two homozygous genotypes, and dominance effects were estimated by subtracting the average of solutions for homozygous genotypes from that for heterozygous genotype. In addition, both models were used to analyze pairwise genotypes from close genes in chromosome (ETH10, IGF1 and RM029), as well as reconstructed phases for IGF1-RM029. For statistically significant main effects (P<0.05), least squares means were reported and Bonferroni’s means separation test at P<0.05 was used to determine differences between genotypes.

3. Results and discussion

3.1. Allele frequencies and heterozygosity

A total of 1180 genotypes of 5 microsatellites were typed from 266 sampled animals. Allele frequencies are presented in Fig. 1 and Table S1. The number alleles were 7 for BP1, 6 for ETH10, 3 for RM029, 2 for IGF1, and 5 for BM1824. Unbiased expected heterozygosity values for each locus and the average heterozygosity over all loci are given in Table S2, h_E ranged from 0.222 to 0.709; and H_E was 0.579, 0.475 and 0.497 for the reference, commercial, and experimental herd, respectively. This genetic variability let us use most of the genotypes in the association tests (see below).

Differences in gene frequencies during generations can be consequence of genetic drift, migration or selection. Neutral markers close to chromosome regions containing causative genes could be affected by selection (as example see Pereira et al., 2005). To evaluate frequencies variation during the time, the Experimental Herd was divided into three groups. Fig. 1 clearly illustrates differences among herd gene frequencies and evidences a tendency toward increase or decrease of some of the allelic frequencies across time. Within this herd, the groups 1996–1999 and 2004–2007 were statistically different (P≤0.05) for all BTA5 markers except for IGF1 (P≤0.08); 2000–2003 and 2004–2007 groups were different (P≤0.08) only for RM029: finally 1996–1999 and 2000–2003 show no significant differences (Table S3). The observed differences could be consequence of genetic drift, especially considering that is a closed herd with a reduced effective size number. Even though, BM1824 (control marker located on BTA1) did not show significant differences between groups. This support the hypothesis that BTA5 marker frequencies could have been affected by selection and that there could be causative genes in the surroundings of these markers (see association discussion).

3.1.1. Hardy–Weinberg equilibrium and linkage disequilibrium
A total of 10 HWE tests were performed (see Table S2); for the commercial herd, HWE for ETH10, IGF1 and RM029 were rejected (P≤0.05), and for the experimental herd only RM029 was rejected (P≤0.05). Reasons for HWE deviations could be methodological (null alleles and sampling bias), or population effects (population structure, consanguinity, and selection). Here the RM029 disequilibrium for the three populations could suggest null allele presence for that marker but, as commercial herd showed disequilibrium for the three loci contained in a 22,000 kbp region that include RM029 (between 60,000 kbp and 82,000 kbp), selection could be the responsible for this.

A total of 60 disequilibrium tests (10 for each population or group) were performed. Two of them showed an important disequilibrium: ETH10/IGF1 (P=0.0043) in the commercial herd, and BP1/RM029 (P=0.0031) in the experimental herd due to group 2004–2007 that presents a P=0.0010 for this pair of markers. Only 3 out of 60 tests (5%) were in disequilibrium, probably the amount of samples is not enough to detect linkage because distance between markers is higher than 5 cM.

3.1.2. Genotype inferring

To exploit phenotypic stored data from 4288 animals included in both pedigrees, single locus analysis of Genoprob 4.0 was used to infer genotypes, 375 genotypes were inferred (97 BP1, 29 ETH10, 165 RM029, and 84 IGF1) and 262 not sampled animals could be included in the association tests. Even though the used version of Genoprob package didn’t
consider phases in the chromosome, the strategy was successful, as it was reported by Allan et al. (2009); we could obtain 15% more genetic information and 99% more animals were included in the association test. This kind of strategy is important to increase the number of animals included in an association test, especially when BUP are from older animals for which DNA was not available for genotyping.

3.2. Association

Previous data (Li et al., 2004) have detected three chromosomal regions significantly associated with growth traits in BTA5, between 0 and 30 cM, were Myf5 gene and BP1 microsatellite are located; and between 55 to 70 and 70 to 80 cM were IGF1 gene and microsatellite, and ETH10 and RM029 microsatellites are mapped. Table 1 summarizes all
### Table 1
Average BLUPS and its Standard Error (SE) in kilograms for direct Birth Weight (DBW), direct Weaning Weight (dWW) and maternal Weaning Weight (mWW), for each BTA5 marker are presented. Only significant results are presented. BP1, IGFI and RM029 results correspond to the analysis that include Genetic Marker, Herd, and CM'H interaction effect. When CM'H was significant, results are presented separated for each herd. For ETH10 the model includes H effect and CM effect nested in H, only genotypes founded in both herds are shown (see Table S3 for the complete set). Additive and dominance effects for each biallelic analysis are also presented.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Herd (N)</th>
<th>Trait</th>
<th>Genotype (N)</th>
<th>Average BLUP of genotype ± SE</th>
<th>Additive effect a</th>
<th>Dominance b</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP1</td>
<td>Both herds (311)</td>
<td>dWW*</td>
<td>312/312 (78)</td>
<td>7.68 ± 0.40</td>
<td>2.37 ± 1.08***</td>
<td>0.08 ± 0.63 NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mWW**</td>
<td>312/322 (150)</td>
<td>8.95 ± 0.33</td>
<td>10.05 ± 1.01</td>
<td>Not estimable</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>322/322 (83)</td>
<td>7.96 ± 1.27</td>
<td>Not estimable</td>
<td></td>
</tr>
<tr>
<td>IGFI</td>
<td>Commercial Herd (93)</td>
<td>dBW</td>
<td>229/229 (38)</td>
<td>1.70 ± 0.28</td>
<td>-2.33 ± 0.20</td>
<td>0.84 ± 0.12 NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dWW*</td>
<td>229/231 (12)</td>
<td>14.76 ± 5.06</td>
<td>-1.43 ± 0.18</td>
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</tr>
<tr>
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<td></td>
<td></td>
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<td>13.05 ± 5.88</td>
<td>-1.29 ± 0.27</td>
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<tr>
<td></td>
<td>Experimental Herd (201)</td>
<td>dBW</td>
<td>231/231 (39)</td>
<td>14.76 ± 5.06</td>
<td>2.81 ± 2.21</td>
<td>Not estimable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dWW*</td>
<td>231/231 (39)</td>
<td>13.05 ± 5.88</td>
<td>1.59 ± 2.72</td>
<td>Not estimable</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>231/231 (39)</td>
<td>20.67 ± 2.52</td>
<td>Not estimable</td>
<td></td>
</tr>
<tr>
<td>RM029</td>
<td>Commercial Herd (126)</td>
<td>dBW</td>
<td>82/82 (2)</td>
<td>16.78 ± 1.23</td>
<td>82/82 (17)</td>
<td>82/84 (21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dWW*</td>
<td>82/82 (17)</td>
<td>3.14 ± 0.80</td>
<td>3.14 ± 0.80</td>
<td>Not estimable</td>
</tr>
</tbody>
</table>

**NS**, not significant.

*Genotypes are expressed allele1/allele2, in brackets are the total numbers of observations of each marker analysis, and for each genotype included in the analysis.

* a Estimated by the difference between the two homoygous genotypes.

* b Estimated by subtraction of the average solutions for homozygous genotypes from that for heterozygous genotype.

* Significance < 0.05,

** Significant < 0.01,

Tests between traits and markers: the least square means of BLUPS for each genotype with its standard deviation, the additive and dominance effect are presented. The association tests revealed a significant association (P < 0.05) between dWW and all BTA5 loci (BP1, ETH10, IGFI, RM029), and between mWW and BP1. Besides, dBW was significant associated (P < 0.05) with ETH10 genotypes and with the interaction IGFI’Herd.

In particular, for BP1 microsatellite the first tests performed, that include the entire data set and consider the genotype nested in herd, showed significant association with dWW and mWW. The second analysis, performed excluding genotypes 310/− present only in commercial herd, showed that dWW model was significant for herd (P < 0.01) and for genotype (P < 0.05), furthermore, a significant additive effect (P < 0.05) was detected. Calves with genotype 312/322 had a mean value 16.53% larger (P < 0.05) than 312/312, while the additive effect was 2.37 ± 1.08 kg. For mWW, the model was significant for genotype (P < 0.001), showing a significant additive effect (P < 0.001) too; genotypes 312/322 and 322/322 had a significant mean value 73.96% (P < 0.05) and 176.39% (P < 0.01) higher than 312/312, respectively, and the additive effect was 5.06 ± 1.38 kg. BP1 marker is close to My5 gene, and the association with dWW and mWW is in concert with the results obtained by Li et al. (2004), that associate an SNP of My5 with Pre-weaning Average Daily Gain (PADG) in commercial lines of Bos taurus. Previous work in this experimental herd (Melucci et al., 2009) described a low value of R²dWW (0.05), and a positive correlation between dWW and mWW (R² = 0.37), while Meyer (1997) found more frequent negative estimates of R²dWW/mWW in field data than in those data sets that originated from experimental herds. BP1 322 allele was significantly associated with higher WW (direct and maternal) in both herds, even though experimental herd had undergone intensive selection for increased WW and commercial herd had not. Furthermore, this allele was the most frequent in experimental herd and showed a significant increase (P < 5 e − 5) during generations (Fig. 1). Tanking all this into account, we conclude that a BTA5 locus close to BP1 is affecting WW, and could be contributing to the rare positive correlation between dWW and mWW found in the experimental herd. Moreover, a Marker Assisted Selection using this marker (or other in the region) could help to select animals that “break the curves”. My5, which product is an intramuscular factor that enhances myocytes development and muscularity, could be candidate gene to explain the observed association with WW. Alternatively, other genes in the surroundings of BP1 marker could be causative gene for that trait differences: i.e. Myogenic factor 6, Inhibitor of growth family 4 and Cell division cycle associated protein 3 (www.ncbi.nlm.nih.gov/projects/mapview).
Within 55 and 80 cM BTA5 region, ETH10 showed significant association (P < 0.01) with dBW and dWW. As the microsatellite is multiallelic, the analysis is not powerful and few significant differences (P < 0.05) were found only in the experimental herd (Table 1 and Table S4), but for both traits the presence of alleles 216 or 222 produces lower average values and 214 and 218 greater ones. RM029 showed significant association (P < 0.05) for dWW, but in this case interaction RM029 × herd was significant too; as a result the 82/82 genotype dWW average value was 35% higher than 84/84 genotype within commercial herd but not in the experimental one. IGF1 microsatellite interaction with herd was significant for dBW (P < 0.05) and dWW (P < 0.01), while genotype association was significant only for dWW (P < 0.01). Even though similar results were found for both herds, in the case of dBW the experimental herd 229/229 genotype was 0.9 kg lower than 229/231 (P < 0.05) and 1.04 kg lower than 231/231 (P < 0.01), but no significant differences (P > 0.05) were found for the commercial herd. In case of dWW significant differences were only found in the commercial herd, 229/229 genotypes were 5.91 kg lower than 231/231 (P < 0.01). When considering two marker genotypes together and the phases (only for IGF1/RM029), no significant effects (P > 0.05) were detected, this was due to data structure and poor estimability. Other possible candidate genes in this region that could explain the association are: actin-related protein, growth arrest-specific 2 like 3 protein, myosin binding protein C, myosin IA, myosin light chain 6B, integrin, myoglobin and glycosyltransferase (www.ncbi.nlm.nih.gov/projects/mapview).

Previous studies have associated IGF1 microsatellite with BW EDP (Expected Progeny Differences) and 180 day gain from birth to weaning in Hereford cattle (Moody et al., 1996), and they found a 15% effect of 229 allele. Andrade et al. (2008) found that BW and 240 day weight were associated with IGF1 genotypes in Canchim cattle (Charolais-Nelore × 5/8). For both traits, IGF1 microsatellite genotypes that included the 231 and 225 alleles were associated with low and high body weights respectively (also found by Pereira et al., 2005); 225 allele is of Ceubane breed origin so it is not surprising that this allele is related with high body weight. In our study, genotypes 229/229 and 231/231 were associated with low and high dBW and dWW, respectively. Andrade et al. (2008) didn’t find the homozygote 231 genotype and reported that differences between 229/229 and 229/231 genotypes were not large, furthermore, for dWW 229/229 genotype was lower than 229/231; considering this, their result and ours could be equivalent. Earlier, Machado et al. (2003) had found a QTL for growth traits in the neighborhood of IGF1 studying Canchim cattle too, even though, they hypothesized that the IGF1 gene is not directly responsible for variations in growth traits. In addition, Li et al. (2004) detected a dominance effect (P < 0.01) of IGF1 on BW in B. taurus cattle, Ge et al. (2001) found IGF1/SnaBl polymorphisms associated with weight gain during the first 20 days after weaning in Angus cattle but Curi et al. (2005) suggested that the alleles of the IGF1 microsatellite and those of the IGF1/SnaBl polymorphism do not show strong linkage disequilibrium, despite their close location.

Despite this kind of analysis could lead to an over-estimation of the effect of some individual alleles, the results across both herds and previous literature are in agreement. Special consideration should be done in marker ETH10 (multiallelic), as the number of animals in some genotype classes is quite low (see Table 1 and Table S4); therefore associations and size of effect should be considered as tenuous. BM1824 (BTA7, 122 cM) was included as control. There are not much QTLs for growth traits in that chromosome, only Casas et al. (2003) detected a QTL for BW between 100 and 135 cm. In this work we didn’t find any association between BM1824 and the traits tested.

In conclusion, we confirm marker association with growth traits in two BTA5 regions close to previously reported QTLs for growth obtained in intensive feeding conditions. Furthermore, we showed that these regions are affecting dBW, dWW and mWW also in a pasture-based system, where animals are not always able to express their maximum genetic potential. These findings were also tested in a commercial herd, which are the potential candidates for a Marker Assisted Selection, so these findings can be used to support such schemes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.livsci.2010.06.160.

References

within commercial lines of *Bos taurus* and their associations with growth traits. J. Anim. Sci. 80, 1187–1194.


History and selection imprinting on genetic relationships among bovine breeds analyzed through 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 (DGAT1), thymoglobin (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 triacylglycerol 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 (Grissart 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 divides the ‘2′ (C) and ‘3′ (T) alleles. Barendse et al. (1999, 2004) found that the ‘2′ 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

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-acyltransferase 1 (DGAT1), thymoglobin (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).

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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 ‘2′ 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
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 homeostasis (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 G.H.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), 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_bee1f.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 flavor 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 enzyme'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 \(\omega_3\)-cas, \(\kappa\)-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 Brahman 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 (Argentina Crecle, Patagonian Creole, Saavedroche Creole, Chaqueque Boliviano, Chucho, Valle Grande 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>
<thead>
<tr>
<th>Breed</th>
<th>Acronym</th>
<th>N</th>
<th>Breed origin</th>
<th>Sample origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereford</td>
<td>HE</td>
<td>21</td>
<td>England</td>
<td>Argentine</td>
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<tr>
<td>Aberdeen Angus</td>
<td>AA</td>
<td>59</td>
<td>Scotland</td>
<td>Argentine-Urugua</td>
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<td>Galloway</td>
<td>G</td>
<td>10</td>
<td>Scotland</td>
<td>Argentine</td>
</tr>
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<td>Netherlands</td>
<td>Argentine</td>
</tr>
<tr>
<td>Jersey</td>
<td>J</td>
<td>10</td>
<td>Island of Jersey</td>
<td>Argentine-Urugua</td>
</tr>
<tr>
<td>Charolais</td>
<td>CH</td>
<td>14</td>
<td>France</td>
<td>Argentina</td>
</tr>
<tr>
<td>Retinta</td>
<td>T</td>
<td>26</td>
<td>Spain</td>
<td>Spain</td>
</tr>
<tr>
<td>Argentine Creole</td>
<td>AM</td>
<td>20</td>
<td>Argentina</td>
<td>Argentine</td>
</tr>
<tr>
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<td>20</td>
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<td>Argentine</td>
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<tr>
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<td>Chucho</td>
<td>PA</td>
<td>7</td>
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<tr>
<td>VaccumCreole</td>
<td>Y</td>
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<td>Argentina</td>
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<tr>
<td>Brahman</td>
<td>BZ</td>
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<td>EEU</td>
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<tr>
<td>Nelore</td>
<td>NE</td>
<td>33</td>
<td>Brasil</td>
<td>Argentine-Bolivia</td>
</tr>
<tr>
<td>Wagyu</td>
<td>W</td>
<td>22</td>
<td>Japan</td>
<td>Uruguay</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>SNP</th>
<th>Method</th>
<th>Analyzed mutation</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGAT1</td>
<td>PCR-SSCP</td>
<td>K232A (eighth exon)</td>
<td>Ripoll et al. (2006)</td>
</tr>
<tr>
<td>TG</td>
<td>PCR-RFLP</td>
<td>C -&gt; T (5 leader sequence)</td>
<td>Barendse et al. (2001)</td>
</tr>
<tr>
<td>LEP</td>
<td>PCR-RFLP</td>
<td>A52V (third exon)</td>
<td>Liefers et al. (2002)</td>
</tr>
<tr>
<td>GH</td>
<td>PCR-RFLP</td>
<td>L217 V (fifth exon)</td>
<td>Yao et al. (1996)</td>
</tr>
<tr>
<td>SCD</td>
<td>PCR-RFLP</td>
<td>Val -&gt; Ala (878 ORF position)</td>
<td>Taniguchi et al. (2004)</td>
</tr>
</tbody>
</table>

* This transition defines the ‘2’ (C) and ‘3’ (T) alleles.
2.4. Statistical analysis

2.4.1. Measures of genetic variability

GENEPOL 1.2 software (Gou and Thompson, 1982; Raymond and Rouset, 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 Fst statistics (Weir and Cockerman, 1984), using the exact test included in GENEPOL 1.2 software.

2.4.2. Genic differentiation and population’s subdivision

Genetic subdivision and genic differentiation among breeds were studied with Wright’s Fst statistic, using the variance-based method of Weir and Cockerman (1984) and with the exact G test (Goudet et al., 1996). These parameters were calculated using the GENEPOL 1.2 software package (Raymond and Rouset, 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 software (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_s) was calculated from allele frequencies. Dendrograms 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, 1995).

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 < 0.05) (Table 4). These deviations comprise two loci in Hereford, Valde Grande Creole and Wagyu cattle, and one locus in Jersey, Patagonian Creole, and Table 3: Estimated gene frequencies for five analyzed SNPs in Hereford (HE), Holstein (HD), Jersey (J), Charolais (CH), Aberdeen Angus (AA), Retinta (T), Galloway (G), Patagonian Creole (CA), Savadorelo Creole (SAA), Chaqueño Bolivia (ES), Valde Grande Creole (V), Argentine Creole (AM), Chusco (PH), Varamelo Creole (Y), Wagyu (W), Brahman (BR), and Nelore (NE) cattle breeds.

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_o 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 Fst index and the exact G test for population differentiation were used to analyze the degree of genetic differentiation among the cattle breeds studied. The Fst parameter showed significant differences across the cattle populations (Fst = 0.1593), ranging from 0.070 to 0.2053 for each locus (Fst, LEP = 0.070; Fst, TG = 0.1374; Fst, SCD = 0.1682; Fst, DGAT1 = 0.1818; Fst, 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 < 0.0001).

AMOVA analysis allowed to partition genetic variability between different groups of breeds based on 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 populations was for LEP locus (92.93%).

Moreover, AMOVA was calculated grouping the breeds in four groups according to their European, Asian, Creole or Zebu origin. Considerable levels of variation among groups were observed (90–95%), while variation 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).
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.

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 other 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 phylogenetic 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, Chaqueno Boliviano Creole; NE, Nellore; BZ, Brahman; AM, Argentine Creole; SAA, Saavedra 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, Chaqueno Boliviano Creole; NE, Nellore; BZ, Brahman; AM, Argentine Creole; SAA, Saavedra 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 (Liñón et al., 2002, 2006). Liñón 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, Liñón 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 (Liñón 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 Zebuine breeds had a low frequency of the A allele, in agreement with previous 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, Valле...
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 rooted 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, lack the tree of 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; Martin-Burriel et al., 1999; Cañón 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 Argentinian Creole cattle were located in an intermediate position between the two Taurine groups. One of these Taurine clades included Gallovay, Retinta and some Creole breeds (Chaqueño Boliviano, Vacuno Criollo 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 (Saavedrea 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 reason 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 far 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 Cebuene 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 population (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 Cebuene breeds may be consequence of the introgression process of alleles of Cebuene 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 FST 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 FST 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 those 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 DGAT2 casein where variant B were predominant 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 marbleing 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 phylogenetic reconstruction of the studied breeds obtained with STRs, probably due to the effect of selection for marbleing 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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