GENETIC VARIABILITY OF THE ZEBU CATTLE BREED (*Bos indicus*)
IN THE DEPARTMENT OF HUILA, COLOMBIA USING MICROSATELLITE MOLECULAR MARKERS

Variabilidad genética de Zebú (*Bos indicus*) en el departamento del Huila, Colombia, usando marcadores moleculares por microsatélites

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ABSTRACT

The polymorphism of 11 microsatellites from zebu cattle (*Bos indicus*) was studied using a commercial multiplex system to estimate genetic variability. Allele frequencies polymorphism information content and heterozygosis were calculated. Allele frequencies revealed that in the analyzed sample the markers were not equally polymorphic. The average allele was 14.2 with the highest values for the TGLA122 microsatellites. The mean heterozygocity was 0.7056 and the polymorphism information content was 0.668. This multiplex analysis could be used for pedigree information and for adequate genetic improvements in breeding programs and paternity test.

Key words: alleles, microsatellite, polymorphism, zebu.

RESUMEN

Para estimar la variabilidad genética, el polimosfismo de 11 microsatélites de vacunos zebú (*Bos indicus*) fue estudiado mediante el sistema comercial multiplex system. Se calcularon frecuencias alélicas, contenido de información polimórfica y heterocigosidad. Las frecuencias alélicas de la muestra analizada revelan que los marcadores no fueron equitativamente polimórficos. El alelo promedio fue 14,2 con el mayor valor para los microsatélites TGLA122. El promedio de heterocigosidad fue 0,7056 y el contenido de información polimórfica de 0,668. Este tipo de análisis puede ser usado para información de pedigri y mejoramiento genético en programas de cria y pruebas de paternidad.

Palabras clave: alelos, microsatélite, polimorfismo, zebú.
INTRODUCTION

The *Bos indicus* Zebu herd with the Brahman (Red and White) and Gyr breeds is one of the largest commercial beef herds in the world and is well-adapted to tropical regions. The Zebu cattle breed is the most important beef herd in Colombia, where the total number of purebred and crossbred Zebu cattle totals 95% of the bovine population. Accurate pedigree information is essential to maintain the quality of breed improvement programs and molecular markers have become an important genetic tool in genetics studies, allowing the analysis of genetic variability within and between herds. Microsatellites have been widely used as genetic markers in bovine population studies and pedigree verification (Visscher *et al*., 2002; Hansen *et al*., 2002; Ibeagha-Awemu and Erhardt, 2005), mainly because of their large polymorphism information content, widespread distribution in the eukaryotic genome (Tautz and Renz, 1984) and the robust methodology available. Microsatellites have been effective in evaluating differences within cattle breeds and in determining population substructures (MacHugh *et al*., 1998; Ciampolini *et al*., 1995). More than 1400 microsatellites have been mapped in the cattle genome (Luikart *et al*., 1999) and some of them have been employed in population genetics studies and kinship verification. The aim of the study described in this paper was to characterize Zebu cattle through the analysis of the genetic variability of eleven microsatellite markers and to evaluate if these markers provide information on the genetic variability and parentage test of this herd. This study presents preliminary information to determine the possible use of microsatellite molecular markers in evaluating parentage test in a zebu cattle population (*Bos indicus*) in the Huila region.

MATERIALS AND METHODS

**SAMPLE COLLECTION AND DNA EXTRACTION**

Eighty unrelated adult Zebu cattle (47 Brahman and 33 Gyr breeds) were sampled. They were registered in their breeding associations and randomly selected from private and research herds belonging to 7 farms located in different regions of department Huila (Colombia). Blood samples were collected in EDTA with tubes and total genomic DNA was isolated by the Salting out method (Aljanabi and Martinez, 1997) and stored at -20 °C.

**MICROSATELLITE AMPLIFICATION**

As recommended by the International Society of Animal Genetics (ISAG), eleven microsatellites (Table 1) were selected for the analyses using the Stockmarks for Cattle Bovine Genotyping Kit (*Applied Biosystems*, Division Perkin-Elmer, Foster City, CA). Multiplex amplification was carried out in a final volume of 15 μL containing 50 ng template DNA, 0.5 units AmpliTaq Gold™ polymerase (PE *Applied Biosystems*, Foster City, CA), 3.0 μL Stockmarks Buffer, 400 μM each dNTP and 5.5 μL primer mix (Table 1). A Programmable Thermal Controller PTC-100 (*MJ Research, INC*) was used in an initial denaturation phase of 15 min at 95 °C, followed by 31 cycles of 45 s at 94 °C, 45 s at 61 °C and 1 min at 72 °C. A final extension was programed at 72 °C for 1 h and then at 25 °C for 2 h. After amplification, 90 μL water were added to each tube and 0.4 μL of this solution was mixed with 2 μL loading mix (DI formamide: dye: GS 500 Rox - 6:1:1)
and analyzed using an ABI PRISM 3100 DNA Sequencer. The fluorescence data was collected by GeneScan™ Analysis 3.0 and analyzed using Genotyper™ 3.0 software. Parentage testing was carried out by assessing compatibility between alleles present in a calf and those found in the assumed parents. As suggested by Luikart et al., 1999 and Weller et al., 2004, an assigned parent was excluded if its genotype was incompatible in two or more loci with that of the offspring, but parentage was not excluded if incompatibility occurred in only one locus.

**DATA ANALYSIS**

The ARLEQUIN package Version 3.1 (Schneider et al., 2005) was used to calculate an exact test for deviation from Hardy-Weinberg equilibrium (HWE), allele and genetics frequencies, heterozygotic deficiency, expected heterozygosity (He) and observed heterozygosity (Ho), and polymorphism information content (PIC).

**RESULTS**

One hundred and fifty seven alleles were detected from the 11 loci surveyed, yielding a mean value of 14.2 alleles per locus. The allele frequencies of 11 microsatellites are listed in table 2. Allele frequencies revealed that not all markers were equally informative. The number of alleles per locus ranged from nine for ETH10 to 17 for BM2113. Tree loci (TGLA227, INRA23 and ETH3) deviated significantly (p < 0.05) from the HWE. A

<table>
<thead>
<tr>
<th>Locus</th>
<th>Size (bp)</th>
<th>Primer sequence2 (5′ - 3′)</th>
<th>Reference</th>
</tr>
</thead>
</table>
| TGLA227 | 64 - 115  | F: CGA AIT CCA AAT CTG TTA AIT TGG C  
R: ACA GAC AGA AAC TCA ATG AAA GCA | Barendse et al., 1992 |
| BM2113  | 116 - 146 | F: CGT GCC TTC TAC CAA ATA CCC  
R: CTT CCT GAC AGA AGC AAC ACC | Bishop et al., 1994 |
| TGLA53  | 147 - 197 | F: GCT TCC AGA AAT AGT TGG CAT CTA  
R: ATC TTC ACA TGA TAT TAC AGC AGA | Barendse et al., 1992 |
| ETH10   | 198 - 234 | F: GTT CAG GAC TGG CCC TGC TAA CA  
R: CCT CCA GCC CAC TTT CTC TCC TC | Toldo et al., 1993 |
| TGLA126 | 104 - 133 | F: CTA ATT TAG AAT GAG AGA GCC TCT T  
R: TGG GTC TCT ATT TCT TGA ATA TTC C | Barendse et al., 1992 |
| TGLA122 | 130 - 193 | F: AAT CAC ATG GCA AAT AAG TAC ATA C  
R: AAT CAC ATG GCA AAT AAG TAC ATA C | Barendse et al., 1992 |
| INRA23  | 193-235   | F: GAG TAG AGC TAC AAG ATA AAC TCC  
R: TAA CTA CAG GGT GTT AGA TGA ACT C | Vaiman et al., 1992 |
| ETH3    | 90 - 135  | F:GAACCTGCCCCTCTCGATTGG  
R: ACT CTG CCT GTG GCC AAG TAG G | Toldo et al., 1993 |
| ETH225  | 133 - 165 | F: GATCACCTGTCGCACTATTTCCT  
R: ACA TGA CAG CCA GCT GCT ACT | Steffen et al., 1993 |
| BM1824  | 170 - 218 | F: GAG CAA GGT GTT TTT CCA ATC  
R: CAT TCT CCA ACT GCT TCC TGG | Bishop et al., 1994 |
| SPS115  | 235 - 265 | F: AAAGTGACACACACAGCTCCTCCAG  
R: AACGAGTGCTCTGCTTTGCGCTTG | Barendse et al., 1992 |

Table 1. Details of the 11 microsatellite loci analyzed. 1 Size in bases pairs. 2 F: Forward; R: Reverse.

<table>
<thead>
<tr>
<th>TGLA122</th>
<th>TGLAS3</th>
<th>ETH22S</th>
<th>INRA23</th>
<th>BM1824</th>
<th>ETH10</th>
<th>ETH3</th>
<th>TGLA126</th>
<th>TGLA227</th>
<th>BM2113</th>
<th>SPS115</th>
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<td>Alle Freq</td>
<td>Alle Freq</td>
<td>Alle Freq</td>
<td>Alle Freq</td>
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<td>Alle Freq</td>
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<tr>
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<td>158</td>
<td>0.3625</td>
<td>114</td>
<td>0.15</td>
<td>112</td>
<td>0.012</td>
<td>118</td>
<td>0.0625</td>
<td>125</td>
</tr>
<tr>
<td>135</td>
<td>0.087</td>
<td>160</td>
<td>0.0187</td>
<td>116</td>
<td>0.012</td>
<td>134</td>
<td>0.056</td>
<td>176</td>
<td>0.0187</td>
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<td>194</td>
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<td>178</td>
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<td>141</td>
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<td>200</td>
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<td>142</td>
<td>0.112</td>
<td>202</td>
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<td>184</td>
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<td>213</td>
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<tr>
<td>149</td>
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<td>182</td>
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<td>144</td>
<td>0.137</td>
<td>204</td>
<td>0.018</td>
<td>186</td>
<td>0.0125</td>
<td>215</td>
</tr>
<tr>
<td>151</td>
<td>0.056</td>
<td>184</td>
<td>0.0125</td>
<td>148</td>
<td>0.012</td>
<td>206</td>
<td>0.162</td>
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<td>0.1375</td>
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<tr>
<td>153</td>
<td>0.081</td>
<td>186</td>
<td>0.0125</td>
<td>150</td>
<td>0.056</td>
<td>208</td>
<td>0.487</td>
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<td>0.1562</td>
<td>219</td>
</tr>
<tr>
<td>157</td>
<td>0.012</td>
<td>190</td>
<td>0.0187</td>
<td>154</td>
<td>0.087</td>
<td>212</td>
<td>0.231</td>
<td>196</td>
<td>0.1062</td>
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<tr>
<td>161</td>
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<td>178</td>
<td>0.018</td>
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<td>216</td>
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<tr>
<td>167</td>
<td>0.012</td>
<td>182</td>
<td>0.087</td>
<td>216</td>
<td>0.075</td>
<td>218</td>
<td>0.0187</td>
<td>135</td>
<td>0.0625</td>
<td>135</td>
</tr>
</tbody>
</table>

Table 2. Allele frequencies of the eleven microsatellite loci analyzed. *Frequencies. *Alleles.
significant deficit of heterozygosity (p < 0.01) was detected in the BM2113, TGLA 53, ETH10, SPS115, TGLA126, TGLA122, ETH225 and BM1824 loci. The mean PIC value was 0.668 and the mean expected heterozygosity value was 0.773. The expected and observed heterozygosity range from 0.411 for TGLA227 to 0.876 for TGLA122 and from 0.334 for BM1824 to 0.785 for ETH3 respectively, the TGLA122 locus showed the highest allele polymorphism, values are shown in Table 3.

### DISCUSSION

Accurate cattle pedigree information is essential for optimal development of breed and selection programs to improve productivity in farm animals. Misidentification of parentage can lead to breeding inaccuracy, causing great financial losses in the beef industry (Geldermann et al., 1986). Microsatellites are the most widely used molecular markers in pedigree control. The use of microsatellites with high polymorphism information content would help to correctly identify individual cattle to improve cattle breeding programs. Cervini et al., 2006, identified the genetic variability of Brazilian Nelore cattle population, detecting that the markers TGLA 227, BM1824 and TGLA53 loci each had one allele with a much higher frequency than the other allele (75 bp, 180 bp, 160 bp respectively). The number of allele per locus ranged from six for TGLA227 to 16 for TGLA122. The TGLA 122 locus showed the highest allele polymorphism, while the INRA023 locus displayed the highest exclusion probability. The mean PIC value was 0.640 and the mean expected heterozygosity value was 0.679.

Little information is available regarding the allele frequencies of the eleven microsatellites studied here as well as for other variability estimates for Brahman cattle. Furthermore, to date in Colombia no estimates exist of the multiplex variability in cattle. Since the evaluation of polymorphism depends strictly on allele numbers and their frequency distribution, estimates of allele frequencies are essential. A comparison of the results obtained for B. indicus Zebu cattle with those of B. taurus breeds (Peelman et al., 1998; Heyen et al., 1997) indicated a difference in variability for some loci, which are highly informative in B. taurus but less informative in B. indicus. Ten microsatellite loci assess the feasibility of applying in

<table>
<thead>
<tr>
<th>Locus</th>
<th>NPA¹</th>
<th>(He)²</th>
<th>(Ho)³</th>
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<tbody>
<tr>
<td>TGLA227</td>
<td>14</td>
<td>0.397</td>
<td>0.411</td>
</tr>
<tr>
<td>BM2113</td>
<td>17*</td>
<td>0.666</td>
<td>0.868</td>
</tr>
<tr>
<td>TGLA53</td>
<td>15</td>
<td>0.712</td>
<td>0.838</td>
</tr>
<tr>
<td>ETH10</td>
<td>9</td>
<td>0.521</td>
<td>0.735</td>
</tr>
<tr>
<td>TGLA126</td>
<td>12</td>
<td>0.614</td>
<td>0.758</td>
</tr>
<tr>
<td>TGLA122</td>
<td>19*</td>
<td>0.666</td>
<td>0.876</td>
</tr>
<tr>
<td>INRA23</td>
<td>16*</td>
<td>0.649</td>
<td>0.870</td>
</tr>
<tr>
<td>ETH3</td>
<td>12</td>
<td>0.785</td>
<td>0.833</td>
</tr>
<tr>
<td>ETH225</td>
<td>17*</td>
<td>0.696</td>
<td>0.746</td>
</tr>
<tr>
<td>BM1824</td>
<td>15</td>
<td>0.337</td>
<td>0.808</td>
</tr>
<tr>
<td>SPS115</td>
<td>11</td>
<td>0.772</td>
<td>0.808</td>
</tr>
</tbody>
</table>

Table 3: Variability measurements of eleven microsatellites molecular markers in Zebu cattle. ¹Number of Average Alleles (*, Alleles more frequencies). ²Expected heterozygosity. ³Observed heterozygosity.
parentage control of beef cattle in Portugal, present in total number of alleles found for the 10 microsatellite markers was 107, the overall of loci mean (na) per locus was 8.20, while it was for TGLAS5: 10.67 and for TGLA227: 10.00 loci, while the lowest mean was observed for BM1824 (5.67). The mean He for the set of 10 microsatellites used was 0.733, all the loci showed high levels of genetic variability, with heterozygosity ranging between 0.587 (ETH10) and 0.837 (BM2113) and in general microsatellites BM2113 and TGLAS5 were the most informative loci (Carolina et al., 2009).

According to Peelman et al., 1998, who studied Belgian cattle, the number of TGLAS5 locus alleles in Holstein Friesian (13 alleles), Belgian Red Pied (12 alleles), East Flemish (12 alleles) and Belgian Blue (10 alleles) cattle were very similar to those found in Nellore cattle (13 alleles). However, we found that the exclusion probability for the TGLAS5 locus in Brazilian Nellore (EP = 0.256) is much lower than in the four Belgian breeds (Holstein Friesian = 0.742, Belgian Red Pied = 0.711, East Flemish = 0.698 and Belgian Blue = 0.682). We obtained similar results for the TGLA227 locus (EP = 0.230), much lower values than those described by Heyen et al., 1997, for Holstein (0.69), Red Angus (0.63) and Gelbvieh (0.68) cattle. Thus, the efficiency of these markers in European B. taurus cattle is not always the same as for Indian B. indicus zebu (Brahman, Nellore).

In the present study we found significant (p<0.01) deviations from HWE for six loci (TGLA122, INRA023, TGLAS3, ETH10, ETH225 and ETH3). Machado et al., 2003, also reported significant deviations from HWE for Brahman and Gyr cattle breeds using microsatellite markers. Almeida et al., 2000, concluded that the TGLA122 locus was in HWE in the Brazilian hybrid bovine breed. We observed deviations from HWE caused by heterozygote deficiency at the TGLA122, INRA023, TGLAS3, ETH10 and ETH225 loci. Beja-Pereira et al., 2003 and Loftus et al., 1999, reported deviations from HWE in other European bovine populations, also caused by heterozygosity deficit, and similar results have been reported by Loftus, 1999, in six populations, including Indian Zebu cattle.

Several factors can lead to heterozygote deficiency including null alleles, assortive mating, the Wahlund effect, selection against heterozygotes, inbreeding, or a combination of these factors. Null alleles are alleles that are not amplified (usually due to a mutation in one of the primer binding sites) and are commonly reported in microsatellite studies as being the source of heterozygosity deficit (Pemberton et al., 1995). The frequency of microsatellite loci containing null alleles has proved to be as high as 30% in humans (Callen et al., 1993).

In paternity tests, an undetected null allele may have profound consequences, since it may cause rejection of an otherwise correctly assigned parent (Holm et al., 2001).

To date, there are no reports of studies on Zebu cattle indicating the presence of null alleles for the markers analyzed, although the presence of null alleles has previously been observed in segregation analyses using other microsatellite loci in Zebu cattle (Tambasco et al., 2000). This possibility cannot be excluded because segregation analysis using the loci evaluated in this study has not yet been undertaken for Zebu cattle. Despite the paucity of information provided by some of the loci analyzed here, the use of this multiplex analysis proved efficient in Zebu characterization and can be used for pedigree verification.

The genetic information like heterozygosity, Hardy Weinberg Equilibrium and allele frequencies allowed relations between this parameter with the inbreeding and other traits productive and evolutionary with high association in the population structure evaluated, the genetic association with the results allows us to find that the level of
variability is directly linked to inbreeding and diversity low, affecting the potential production of different breeds.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


