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Short Communication

Analysis of polymorphisms in the mitochondrial *ND5* gene in Pantaneira and Creole breeds of sheep

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The aim of this study was to assess genetic variation between a population of Pantaneira sheep in the Brazilian state of Mato Grosso do Sul, and Creole sheep from the south of the country by molecular analysis of the *ND5* gene in mitochondrial DNA. The analysis revealed the presence of 16 haplotypes with all Pantaneira sheep grouped together carrying a single haplotype, and there was no grouping with any of the Creole sheep. The F_{ST} value was 0.44, indicating that there is a genetic difference between the two breeds, which may indicate that both breeds underwent differentiation.

Key words: Ovis aries, ND5 gene, genetic diversity, phylogeny, breed differentiation.

INTRODUCTION

Brazil has many types of domestic animals, including sheep, which originated from breeds brought by colonisers after the discovery. The first steps towards obtaining funding for the improvement, management and conservation of Brazilian naturalised sheep are characterising the diversity of naturalised races, the genetic relationship between them and understanding their origins (Mariante and Cavalcante, 2006). The Creole sheep has been reared for centuries in the Brazilian states of Rio Grande do Sul and Santa Catarina, where there are two known varieties, Fronteira and Serrana (Gonçalves et al., 2010). Considering the geographic distribution of sheep in Brazil and phenotypic similarities between the animals, it is thought that Pantaneira sheep originated from the Creole sheep, and research has been carried out to determine whether the difference between the groups is sufficient for the Pantaneira sheep to be recognised as a separate breed (Paiva et al., 2008). NADH dehydrogenase is one of the main enzymes found in respiratory complexes in mammals. It has 42 polypeptide chains, seven of which are encoded by the mitochondrial genome. The subunit five (*ND5*) was used by Tserenbataa et al. (2004) and by Gonçalves et al. (2010) to study sheep diversity. The former study searched for subspecies of *Ovis ammon* in Mongolia by sequencing this region, and the results suggested the existence of two subspecies (*O. ammon*

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Table 1. DNA sequence comparisons of the different haplotypes found with the ND5 gene.

ammon and O. ammon darwini). In the study by Gonçalves et al. (2010), genetic differentiation was found between animals of the Creole sheep in the south of Brazil belonging to the varieties Serrana and Fronteira. Genetic polymorphisms in mitochondrial DNA (mtDNA) reveal haplotype diversity within species, and are therefore, a useful tool for establishing phylogenetic relationships at the species level (Avise et al., 1987). Therefore, the aim of this study was to assess the variation between a population of Pantaneira sheep in the state of Mato Grosso do Sul and Creole sheep in the south of Brazil through molecular analysis of the mtDNA *ND5* region.

MATERIALS AND METHODS

Blood samples from 19 Pantaneira sheep from the state of Mato Grosso do Sul were collected and stored at 4°C. DNA was extracted in the Animal Biotechnology Laboratory (Faculty of Agrarian Sciences at Federal University of Grande Dourados) using a total blood DNA extraction protocol as described by Crispim et al. (2012). An 657-bp region of the mitochondrial ND5 gene was amplified primers using the ND5 5'-F AATAGTTTATCCAGTTGGTCTTAGG-3' and ND5 R 5'-AAGATTTGTTGGAGATCTCAGGTG-3' (Tserenbataa et al., 2004). Polymerase Chain Reaction (PCR) was performed in a final volume of 25 µL and the amplification mix was made with 7.5 µL of ultra pure water, 1.5 µL of each primer (10 pmoles), 12,5 µL of PCR Master Mix (Fermentas®) and 2.0 µL of DNA (10 to 20 ng). The reaction protocol was 95°C (5 min) one cycle; 95°C (1 min), 52°C (1 min), 72°C (1 min 30 s) 36 cycles and 72°C (10 min) one cycle. The amplified fragments were purified and sequenced by an automatic sequencer ABI 3730 XL (Applied Biosystems). Sequence editing and alignment was performed with the reference sequence AF010406 (Hiendleder et al., 1998) by using the program DNA Alignment (Fluxus Technology Ltd). To compare sequence variability between Pantaneira and Creole breeds, the sequences of 17 Creole sheep were obtained from GenBank with accession numbers EU854593 to EU854607 and EU854609 to EU854610 (Gonçalves et al., 2010). The analysis of molecular variance (AMOVA), the Wright's F-statistics (F_{ST}), haplotype and nucleotide diversities were calculated using the program Arlequin 3.5 (Excoffier and Lischer, 2010). The relationships among the haplotypes obtained were analysed by the construction of networks using the program Network version 4.1.1.2 (Fluxus Technology Ltd) by the Median-Joining method (Bandelt et al., 1999).

RESULTS

A total of 36 sequences were analysed using the mtDNA *ND5* gene. Sixteen haplotypes were found, and all animals of the Pantaneira breed were grouped together with a single haplotype. Table 1 shows DNA sequence comparisons of the different haplotypes. AMOVA revealed that the percentage of variance between the analysed populations was 44.44%, and within populations was 55.56% (p < 0.05). The F_{ST} value found was 0.44, a value that indicates genetic difference according to Hartl and Clark (2010). A significant difference (p < 0.05) was also found between animals of

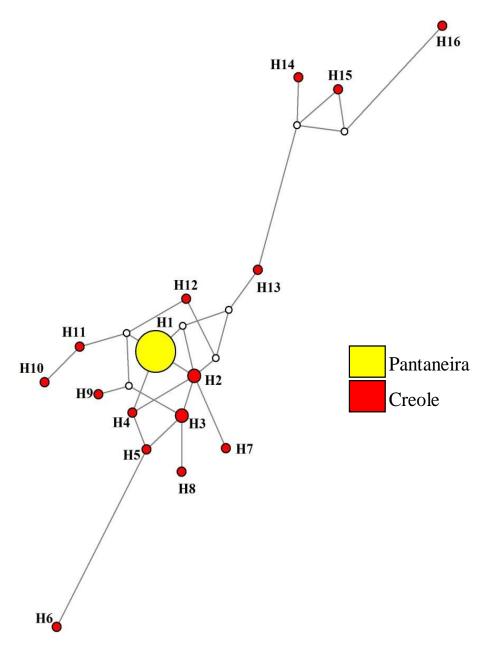


Figure 1. Network constructed by the median-joining method (Bandelt et al., 1999) showing the 16 haplotypes found using the mtDNA *ND5* gene for individuals from the Pantaneira and Creole sheep. The area of the circles for each haplotype is proportional to its frequency. The lengths of the lines are proportional to the mutational steps that separate each haplotype. The white dots are median vectors that represent hypothetical haplotypes introduced by the algorithm used.

the Pantaneira breed and in two varieties of Creole sheep, with the distance between breeds being 0.44, according to the distance method pairwise difference based on F_{ST} values calculated with AMOVA. Figure 1 shows that the haplotype networks constructed for Pantaneira and Creole sheep, based on mutation points present in the sequences, demonstrate the relationship between the different haplotypes. Table 2 shows the haplotype and nucleotide diversities calculated with Arlequin 3.5 for the *ND5* gene.

DISCUSSION

The F_{ST} value found in this study with the *ND5* gene, calculated with AMOVA, was 0.44 and according to Hartl and Clark (2010), F_{ST} values above 0.25 indicate a genetic difference. Therefore, we may say that there was differentiation between Pantaneira and Creole breeds when analyzed with this marker. In addition, Holsinger and Weir (2009) say that if the F_{ST} value is high the allele

Table	2.	Haplotype	(H)	and	nucleotide	(π)	diversities	for	Pantaneira	and
Creole	sh	eep.								

Breed	Н	π
Pantaneira	1.000±0.017	0.000±0.000
Creole	1.000±0.020	0.010±0.006

frequency is different indicating difference between populations, which once again shows that there could be differentiation between the populations studied. The network (Figure 1) revealed that Creole sheep have a different haplotype than Pantaneira sheep since they did not group together, suggesting that differentiation has occurred between these groups, therefore more research would be necessary to know if this would be enough so that the Pantaneira sheep could be acknowledged as a different breed, a suggestion also made by Paiva et al. (2008). Furthermore, several haplotypes in the Creole sheep were close to the one formed by the Pantaneira breed animals which may indicate that, although these animals do not share the same haplotype, they belong to the same haplogroup. The fact that all animals of the Pantaneira breed grouped together in one single haplotype explains the values found for haplotype and nucleotide diversity (Table 2). The geographical region where these animals are found today belonged previously to Paraguay, so it is possible that the Pantaneira breed has been influenced by Paraguayan breeds and the analysis of its mitochondrial genome might confirm this assumption, by showing their genetic distance from the Creole. Thus, the significant differences identified for the mtDNA ND5 gene between Pantaneira and Creole sheep may indicate that differentiation has occurred in both races; however, further research using other markers is required to investigate this further.

Conflict of interest

The authors have no conflict of interests to declare.

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