Segregation of MT-COI RFLP in sheep from Mato Grosso do Sul, Brasil

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SUMMARY

Research conducted in different regions of the mitochondrial DNA of Ovis aries showed the existence of Asian and European haplogroups. The study aimed at applying the PCR-RFLP molecular test of mitochondrial gene cytochrome oxidase I with the restriction enzyme HinfI to molecularly characterize, over the existing haplogroups, some sheep breeds used in the State of Mato Grosso do Sul. DNA from 155 animals belonging to seven sheep breeds was analysed. Sixteen animals were identified as belonging to the Asian haplogroup, represented by Ile de France (n=3), Dorper (n=2), White Dorper (n=9) and Suffolk (n=2) breeds. The other 139 animals were identified as belonging to the European haplogroup, representative from the breeds Pantaneira (n=40), Brazilian Bergamácia (n=21), Ile de France (n=17), Dorper (n=17), White Dorper (n=6), Hampshire Down (n=20) and Suffolk (n=18). The results indicated that most animals were identified as belonging to the European haplogroup, highlighting the European origin of the State’s breeds. Origin identification of these animals allows a better management of the locally adapted populations seeking their conservation and better usage in the State.

PALAVRAS CHAVE ADICIONAIS

Ovis aries.
Herança materna.
Citocromo oxidase.
Haplogrupo mitocondrial.

INTRODUCTION

Brazil has several breeds of sheep, including the animals that developed from breeds brought by settlers soon after the discovery. Over the years, these animals were under the action of natural selection of environmental and climatic conditions, resulting in breeds that are now considered naturalized, locally adapted or native (Mariante et al., 1999), however few studies have been conducted in order to discover the origin of these animals.

Wood and Phua (1996) and Hiendleder et al. (1998a), demonstrated the existence of at least two major haplogroups in Ovis aries from the control region (D-loop) of mitochondrial DNA (mtDNA) sequencing; one of european origin and another, probably of asian origin. These results can also be interpreted as two independent domestication events that have occurred for domestic...
species (Bruford et al., 2003). Furthermore, Hiendleder et al. (1999) developed a test based on Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) of mitochondrial cytochrome C oxidase I gene (MT-COI 6) with the restriction enzyme HinfI (extracted from bacteria Haemophilus influenzae Rf) in order to more easily identify these two haplogroups HA (Asian origin) and HB (European origin).

The study of mtDNA region, which can be called DNA barcoding, uses partial DNA sequences of the MT-COI 6 gene to identify and designate both new species as previously described, helping to unravel the diversity (Bolzan, 2011).

Given the above, this study aimed to use PCR-RFLP from MT-COI 6 gene using HinfI restriction enzyme to molecularly characterize, over the existing haplogroups, some sheep breeds used in the State of Mato Grosso do Sul.

MATERIALS AND METHODS

The locality of origin of each breed and the number of researched animals were described in Table I. Except for the animals of the Pantaneira breed, the animal collections of other breeds were all made in single herds which may have influenced the results.

Table I. Breeds used in the experiment, number of animals and collection site (Raças utilizadas no experimento, quantidade de animais e local de coleta).

<table>
<thead>
<tr>
<th>Breed</th>
<th>Animals (n)</th>
<th>Collection site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pantaneira (PT)</td>
<td>40</td>
<td>Faz. Experimental UFGD – Dou-rados/MS; Embrapa Cuiabá/MS</td>
</tr>
<tr>
<td>Bergamácia Brasileira (BE)</td>
<td>21</td>
<td>Retiro dos Leite – Jardim/MS</td>
</tr>
<tr>
<td>Ile de France (IF)</td>
<td>20</td>
<td>Faz. Chancan – Campo Grande/MS</td>
</tr>
<tr>
<td>Dorper (BP)</td>
<td>19</td>
<td>Cabanha Morena – Caarapó/MS</td>
</tr>
<tr>
<td>White Dorper (WD)</td>
<td>15</td>
<td>Cabanha Morena – Caarapó/MS</td>
</tr>
<tr>
<td>Hampshire Down (HS)</td>
<td>20</td>
<td>Faz. Mate Laranjeira – Ponta Porã/MS</td>
</tr>
<tr>
<td>Suffolk (SF)</td>
<td>20</td>
<td>Cabanha LCL – Caarapó/MS</td>
</tr>
<tr>
<td>Total</td>
<td>155</td>
<td></td>
</tr>
</tbody>
</table>

*The blood of Pantaneira breed animals was collected from two separate farm herds, but it was found that there were no genetic differences between them and, therefore, they were analyzed as one group. Both herds in which the collection was made consisted of animals purchased from various locations.

DNA extraction was performed using a whole blood DNA extraction protocol described by Crispim et al. (2012). The quantity of DNA (ng/µL) and quality (260/280 nm ratio) were obtained by spectrophotometry and the integrity was observed by electrophoresis in 2% agarose gel stained with ethidium bromide.

The primers COIF (5’-CAGAGTTTGAAGCTGCT-3’) and COIR (5’- AGCTGACGTGAAGTACG-3’), described by Hiendleder et al. (1999), were used to amplify a 1053 base pair (bp) fragment of the MT-COI 6 gene. This fragment contains the polymorphic site, previously identified through sequencing by the same author, of the HinfI enzyme in positions 5562-5566.

The Polymerase Chain Reaction (PCR) was performed in a final volume of 25 µL and the amplification mix consisted of: 7,5 µL of ultra pure water, 1,5 µL of each primer (10 pmoles), 12,5 µL of the PCR Master Mix (Fermentas®) and 2,0 µL of DNA (10-20ng). The digestion reaction was composed of 10 µL of ultra pure water, 1,5 µL of buffer 10, 0,2 µL (10U/µL) of the HinfI enzyme and 10 µL of the amplified product.

The PCR was performed using initial denaturation at 94°C for 5 min, amplification at 94°C for 30s, 57°C for 1 min, 72°C for 1 min (37 cycles) e final extension at 72°C for 5 min. The digestion reaction with enzyme was performed on a thermocycler at 37°C for 2 hours.

Figure 1. A Electrophoresis on 2% agarose gel of the PCR fragments from the MT-COI 6 gene. Line 1 = ladder 100 bp (Thermo Scientific®). Line 2 = negative control. Lines 3-5 = 1053 bp fragment; B Electrophoresis on 2% agarose gel of fragments produced by the restriction enzyme HinfI on the MT-COI 6 gene. Line 1 = molecular marker 100 bp (Thermo Scientific®). Lines 2, 3 and 5 = animals of European origin (HB) (477 and 359 bp fragments). Line 4 = animals of Asian origin (HA) (836 bp fragment).

RESULTS AND DISCUSSION

A 1053 bp fragment was obtained in the MT-COI 6 gene PCR. The fragments resulting from the digestion reaction were analyzed according to the results found by Hiendleder et al. (1999) wherein the presence of the 836 bp fragment represented the animals from asian.
origin (HA) and two fragments of 477 bp and 359 bp, the animals of european origin (HB) (figure 1).

Small fragments of 144 bp and 73 bp were also observed in the gel that were additional sites of the enzyme digestion, but these fragments were considered non-diagnostic polymorphisms and they were not included in analyzes (Hiendleder et al., 1999).

HA and HB are the most frequently identified haplogroups and group the animals with asian (Ovis orientalis) and european (Ovis musimon) origin, respectively. Both were first identified by Wood and Phua (1996) and classified by Hiendleder et al. (1998b), but it has been located in all geographic regions where Ovis aries was sampled.

From the total of 155 animals, 16 were identified as belonging to HA, represented by the breeds Ile de France (n=3), Dorper (n=2), White Dorper (n=9) and Suffolk (n=2). The remained 139 animals were identified as belonging to HB, representatives of breeds Pantaneira (n=40), Brazilian Bergamácia (n=21), Ile de France (n=17), Dorper (n=17), White Dorper (n=6), Hampshire Down (n=20) and Suffolk (n=18). All animals from the Pantaneira, Bergamácia and Hampshire Down breeds belonged to the european haplogroup, while 60% of the White Dorper breed was asian.

Considering that the colonization of Brazil was performed by European, most animals (n=139) were identified as haplogroup HB. These sheeps provided wool and meat and it was common to be taken on long journeys with the settlers and adaptive processes and natural selection resulted in the formation of various sheep breeds locally adapted in Brazil (Paiva et al., 2005). Our results about mitochondrial haplogroups could be compared with local Mexican sheep (Creole, Chiapas and Pelibuey breeds) that revealed the genotype B of the COX1 gene (Ulloa-Arvizua et al., 2009).

The animals of exotic breeds, imported by Brazil in the early XX century, Dorper, White Dorper, Ile de France and Suffolk belonged to both haplogroups: HA (n=16) and HB (n=58). The White Dorper breed was the group that had more animals of the Asian haplogroup than of the european haplogroup, nine out of the total of 15 (60%). This breed, like the Dorper, is from South Africa and the cross between the exotic breed Dorset Horn (coming from the southwest of England) and the adapted Blackhead Persian (from South Africa, known in Brazil as Somalis). The Blackhead Persian breed is African, but it is believed that the breed that gave rise to it has been the asian Urial head Persian breed is African, but it is believed that the breed that gave rise to it has been the asian Urial

CONCLUSION

The origin of sheep from some of the breeds in the State of Mato Grosso do Sul is important because these are part of the genetic heritage of the State and by knowing their phylogeny it is possible to improve the management of these breeds, aiming its conservation and the use of the productivity of these animals in our environment. The study with the MT-COI RFLP gene indicated the applicability of this molecular tool to classify most of the animals as belonging to the European haplogroup, highlighting the European origin of the State breeds.

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