

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

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ADDITIONAL KEYWORDS

Ovis aries.

Maternal heritage.

Cytochrome oxidase.

Mitochondrial haplogroup.

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 *Hinf*I 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.

Segregação do MT-COI RFLP em ovinos de Mato Grosso do Sul, Brasil

RESUMO

Pesquisas em regiões do DNA mitocondrial de *Ovis aries* mostraram a existência de haplogrupos de origem asiática e europeia. Assim o objetivo do presente estudo foi aplicar o teste molecular de PCR-RFLP do gene mitocondrial citocromo oxidase I com a enzima de restrição *Hinf*I para caracterizar, em relação aos haplogrupos existentes, algumas raças de ovinos criadas no Estado do Mato Grosso do Sul. Foram analisadas amostras de DNA de 155 animais pertencentes a sete raças de ovinos. Dezesesseis animais foram identificados como pertencendo ao haplogrupo asiático, representados pelas raças Ile de France (n=3), Dorper (n=2), White Dorper (n=9) e Suffolk (n=2). Os outros 139 animais foram identificados como haplogrupo europeu, representantes das raças Pantaneira (n=40), Brazilian Bergamácia (n=21), Ile de France (n=17), Dorper (n=17), White Dorper (n=6), Hampshire Down (n=20) e Suffolk (n=18). Os resultados indicaram que a maioria dos animais pertenciam ao haplogrupo europeu, evidenciando a origem europeia das raças criadas no Estado. A identificação da origem desses animais possibilita melhorar o manejo das populações localmente adaptadas visando sua conservação e o seu aproveitamento no Estado.

PALAVRAS CHAVE ADICIONAIS

Ovis aries.

Herança materna.

Citocromo oxidase.

Haplogrupo mitocondrial.

INFORMACIÓN

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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).

Breed	Animals (n)	Collection site*
Pantaneira (PT)	40	Faz. Experimental UFGD – Dourados/MS; Embrapa Corumbá/MS
Bergamácia Brasileira (BE)	21	Retiro dos Leite – Jardim/MS
Ile de France (IF)	20	Faz. Chancan – Campo Grande/MS
Dorper (DP)	19	Cabanha Morena – Caarapó/MS
White Dorper (WD)	15	Cabanha Morena – Caarapó/MS
Hampshire Down (HS)	20	Faz. Mate Laranjeira – Ponta Porã/MS
Suffolk (SF)	20	Cabanha LCL – Caarapó/MS
Total	155	

*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/ μ L) and quality (260/280 nm ratio) were obtained by spectrophotom-

etry and the integrity was observed by electrophoresis in 2% agarose gel stained with ethidium bromide.

The primers COIF (5'-CAGAGTTTGAAGCT-GCT-3') and COIR (5'-AGCTGACGTGAAGTA-AGC-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 μ L and the amplification mix consisted of: 7,5 μ L of ultra pure water, 1,5 μ L of each primer (10 pmoles), 12,5 μ L of the PCR Master Mix (Fermentas®) and 2,0 μ L of DNA (10-20ng). The digestion reaction was composed of 10 μ L of ultra pure water, 1,5 μ L of buffer 10, 0,2 μ L (10U/ μ L) of the *HinfI* enzyme and 10 μ L 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.

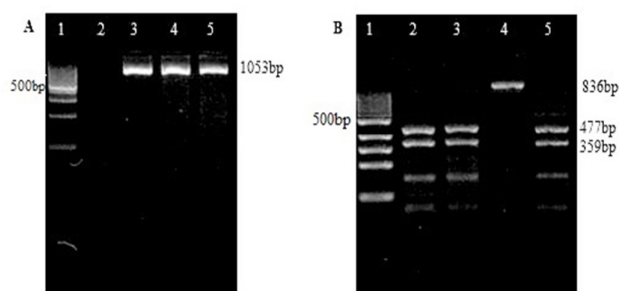


Figure 1. A Electrophoresis on 2% agarose gel of the PCR fragments from the MT-COI 6 gene. Line 1 = ladder 100 bp (Thermo Scientific®). Line 2 = negative control. Lines 3-5 = 1053 bp fragment; **B** Electrophoresis on 2% agarose gel of fragments produced by the restriction enzyme *HinfI* on the MT-COI 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).

(A) Eletroforese em gel de agarose 2% dos fragmentos da PCR do gene MT-COI 6. Linha 1 = marcador molecular de 100 pb (Thermo Scientific®). Linha 2 = controle negativo. Linhas 3-5 = fragmento de 1053 pb; (B) Eletroforese em gel de agarose 2% dos fragmentos da PCR-RFLP produzidos pela enzima de restrição *HinfI* no gene MT-COI 6. Linha 1 = marcador molecular de 100pb (Thermo Scientific®). Linhas 2, 3 e 5 = animais de origem Européia (HB) (fragmentos de 477 e 359 pb). Linha 4 = animais de origem Asiática (HA) (fragmento de 836 pb).

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-Arvizu *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 (*Ovis vignei*) (Paiva *et al.*, 2011). It is known that animals of Dorper and White Dorper breeds arrived in Brazil by imports of embryos and this may have contributed to the observation of greater amount of asian ancestry individuals when compared to other breeds. It is important to note that the Asian haplogroup can also include animals from European origin, therefore this information should be taken under consideration in this kind of analysis in order to correctly classify the animals.

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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BIBLIOGRAPHY

- Bolzan, A.R. 2011. DNA barcode de Drosophilídeos micófagos pertencentes aos gêneros *Hirtodrosophila*, *Mycodrosophila* e *Zigotricha*. 85f (Mestrado). Centro de Ciências Naturais e Exatas. Universidade Federal de Santa Maria. Santa Maria.
- Bruford, M.W.; Bradley, D.G. and Luikart, G. 2003. DNA markers reveal the complexity of livestock domestication. *Nat Rev Genet*, 4: 900-910.
- Crispim, B.A., Silva, D.B.S., Banari, A.C., Seno, L.O. e Grisolia, A.B. 2012. Discriminação alélica em ovinos naturalizados do Pantanal Sul-Matogrossense por meio de marcadores microsatélites. *J Selva Andina Res Soc*, 1: 3-13.
- Hiendleder, S.; Mainz, K.; Plante, Y. and Lewalski, H. 1998a. Analysis of mitochondrial DNA indicates that domestic sheep are derived from two different ancestral maternal sources: no evidence for contributions from urial and argali sheep. *J Hered*, 89: 113-120.
- Hiendleder, S.; Lewalski, H.; Wassmuth, R. and Janke, A. 1998b. The complete mitochondrial DNA sequence of the domestic sheep (*Ovis aries*) and comparison with the other major ovine haplotype. *J Mol Evol*, 47: 441-448.
- Hiendleder, S.; Phua, S.H. and Hecht, W. 1999. A diagnostic assay discriminating between two major *Ovis aries* mitochondrial DNA haplogroups. *Anim Genet*, 30: 211-213.
- Hiendleder, S.; Kaupe, B.; Wassmuth, R. and Janke, A. 2002. Molecular analysis of wild and domestic sheep questions current nomenclature and provides evidence for domestication from two different subspecies. *Proc Biol Sci*, 269: 893-904.
- Mariante, A.S.; Albuquerque, M.S.M.; Egito, A.A. and McManus, C. 1999. Advances in the Brazilian animal genetic resources conservation programme. *AGRI*, 25: 107-121.
- Paiva, S.R.; Silvério, V.C.; Paiva, D.A.F.; McManus, C.; Egito, A.A.; Mariante, A.S.; Castro, S.R.; Albuquerque, M.S.M. and Dergam, J.A. 2005. Origin of the main locally adapted sheep breeds of Brazil: A RFLP-PCR molecular analysis. *Arch Zootec*, 54: 395-399.
- Paiva, S.R.; Facó, O.; Faria, D.A.; Lacerda, T.; Barretto, G.B.; Carneiro, P.L.S.; Lobo, R.N.B. and McManus, C. 2011. Molecular and pedigree analysis applied to conservation of animal genetic resources: the case of Brazilian Somali hair sheep. *Trop Anim Health Prod*, 43: 1449-1457.
- Ulloa-Arvizu, R.; Gayosso-Vázquez, M. y Morales, R.A.A., 2009. Origen genético del ovino criollo mexicano (*Ovis aries*) por el análisis del gen del Citocromo C Oxidasa subunidad I. *Tec Pecu Méx*, 47: 323-328.
- Wood, N.J. and Phua, S.H. 1996. Variation in the control region sequence of the sheep mitochondrial genome. *Anim Genet*, 27: 25-33.