



PHYLOGENETIC STUDY ON THE MAIN BRAZILIAN NATURALIZED SHEEP BREEDS

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INTRODUCTION

The domestic sheep *Ovis aries* was introduced in Brazil by the Portuguese settlers and, given an extensive raising system, they have been apparently subject to natural selection pressure for almost 500 years. Some of their specific adaptations granted them the "naturalized Brazilian" attribute (Mariante *et al.*, 1999). These breeds were raised for subsistence needs, and they were not highly valued (Morais, 2000). A reconstruction of the phylogeny is essential for the conservation of the genetic structure of herds and for sound management. Mitochondrial DNA data traced back the origin of domestic sheep to Europe, Asia and China (Wood e Phua, 1996; Hiendleder *et al.*, 1998; Guo *et al.*, 2005). Patterns of variation of the second half of the mitochondrial D-loop were characterized, as well as the distribution of the variability among breeds. Based on these results, it was proposed a hypothesis on the geographic origin of the ancestors of the naturalized Brazilian sheep.

MATERIAL AND METHODS

Samples and genetics analyses of mtDNA. Forty-nine individuals from the following naturalized breeds: Santa Inês, Morada Nova, Bergamácia, Crioula Lanada,, Rabo Largo, Brazilian Somalis were sampled. Data were compared to the following commercial breeds: Ile de France, Corriedale, Hampshire, as well as to the African breeds Damara e Dorper. DNA was extracted from leukocytes with a non-organic protocol modified by (Miller, et al., 1998) and fragments were amplified with PCR standard protocols. The forward primer was INT (5'-ATCCATTCCCTAGTCAACATGCGTATC-3') and the reverse as described by Wood e Phua (1996) and Hiendleder *et al.* (1998). Amplified products were purified with Promega (Wizard® SV Gel and PCR Clean-Up System) and sequenced in a *ABI Prism 3700*. Sequences were aligned using MEGA v.3.0 (Kumar *et al.*, 2004), with the Genbank haplogroup B sequence AF010406 (sensu Hiendleder *et al.*, 1998) as a blueprint, and sequences from Europe, Oceania (Wood e Phua, 1996; Hiendleder *et al.* 1998; Meadows, *et al.* 2005; Zardoya, et al., 1995) and Mexico (AY582800 - AY582820).

Statistical analyses. Reduced median networks (Bandelt *et al.*, 1999) were drawn using the program Network 4.1.1.2 (www.fluxus-engineering.com) with default parameters.

RESULTS AND DISCUSSION

A 600bp fragment was obtained from a total sample of 49 individuals. Alignment with Genbank sequences was possible for only 524 bp. Nucleotide diversity in Brazilian sheep was significant among breeds (0.005), an intermediate level considering the ones reported by Meadows *et al.* (2005) (0.00318) and Pedrosa *et al.* (2005) (0.00881). These intermediate values were considered as high values, due to their recent origin. Thirty-eight haplotypes were identified out of 106 sequences (Table 1).

According to the estimated network (Figure 1), haplotypes H17 and H27 were more generalized haplotypes and central relative to the other haplotypes. The relative position of haplotype H17 is congruent with the hypothesis formulated by Hiendleder *et al.* (1998) placing wild sheep and the Mouflon as sharing ancestors of *Ovis aries*. In Brazil, breeds irradiated from two ancestors, with H27 and H28 representing European and Brazilian ancestors respectively. Sharing H15, H17 e H27 haplotypes suggest a founder effect of the European breeds no the Brazilian sheep. H15 was highly frequent among the Mexican Creoles (15), woolen Brazilian Creoles (4), Bergamacia (1), Corriedale (3) and the Aragonese (1) suggesting a common history between the Creoles and the Iberian breeds. In Latin America, shared haplotypes are explained by a recent demographic expansion, whereas derived haplotypes such as H18, H19, H30, H31, H38 indicate later genetic structure. The recently introduced African breeds (H17, H36 e H37) also shared the European H17 haplotype. Homoplasies in the network arrangement (Figure 1) may indicate recent expansion, recent and incipient differentiation of the Brazilian breeds. Our results underline multiple stock introductions along the colonization process and a detection of older and more recent lineage-splitting events.

CONCLUSION

The second half of the D-loop mitochondrial DNA was more informative and showed less homoplasies than the first half of the region. It was concluded that there was an evidence for a common origin of the South and Meso American woolen creole sheep.

Table 1. Frequency and distribution of haplotypes in the D-loop mitochondrial DNA region, in 23 sheep breeds from Brasil, Mexico, Africa, Europe and Oceania.

Haplotypes	AF010406	Île de France	Hampshire	Corriedale	R. Aragonesa	Crioula Mexicana	Crioula Lanada	Bergumacia	Morada Nova	Rabo Largo	Somalis	Santa Inês	Damara	Dorper	Lataxa	Suffolk	Vepsia	Oxfordown	Tyrolean	Mouflon	Me, Ro, Coo ^A	Total ^B	
1	1																					1	
2																						1	1
3																						1	1
4																						1	1
5																						1	1
6																						1	1
7																						1	1
8																						1	1
9																						1	1
10																						2	2
11																						1	1
12																						3	3
13																						1	1
14																						2	2
15				3	1	15	4	1														24	24
16						1																1	1
17			1			3							1	1							2	8	
18						1																1	1
19						1																1	1
20														1								1	1
21															1							2	2
22																1						1	1
23																	1					1	1
24																		1			1	2	2
25																		1				1	1
26																				1		1	1
27		1		1			1	2	2	3	2									1		12	12
28							3	1	3	2	4											13	13
29							1		1													2	2
30							1															1	1
31							1															1	1
32		1										1										2	2
33		1																				1	1
34			1																			1	1
35				1																		1	1
36													1									1	1
37													3									3	3
38							1															1	1
T ^C	1	3	3	4	1	21	6	7	3	6	5	7	3	2	2	2	2	2	2	1	3	22	106

^AMe= Merino, Ro= Romney and Coo= Coopworth, sequences of Wood & Phua, (1996); ^BTotal frequency of the more frequent haplotypes; ^CTotal frequencies of all haplotypes within breeds.

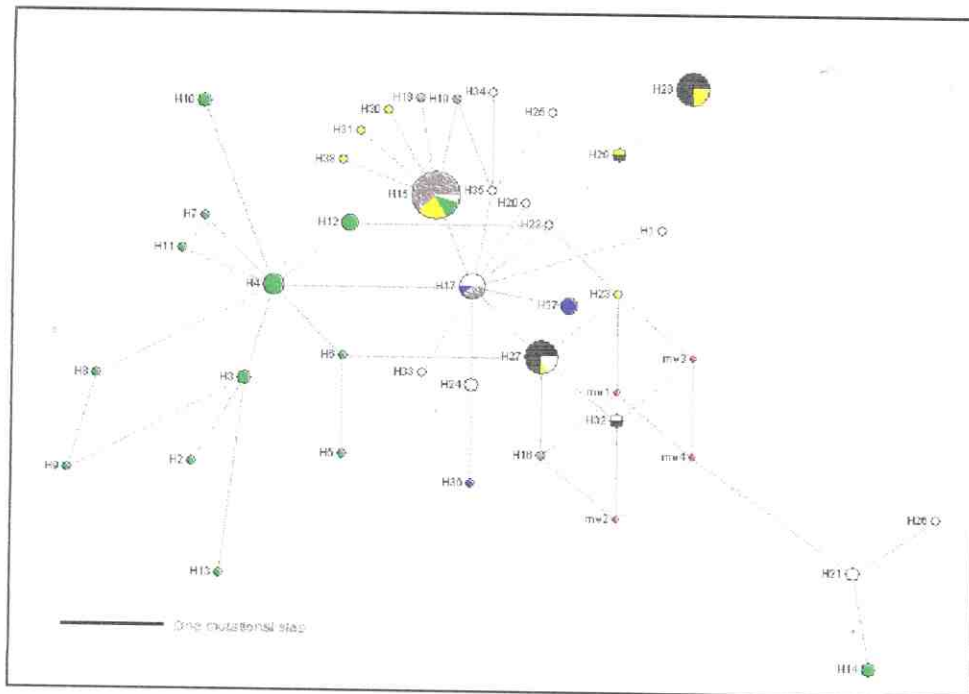


Figure 1. Reduced Median network of Brazilian sheep mtDNA. Circles represents haplotypes; sizes proportional to frequency. *mv are median vector that represents hypothetical haplotypes. ● Brazilian hair breeds; ● Brazilian wool breeds; ○ European breeds; ● Oceania breeds breeds; ○ Mexico Creole breed; ● African breeds.

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