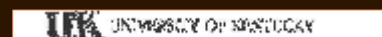


# Variation of Major Histocompatibility Complex (MHC) microsatellites in Portuguese and South American horse breeds.

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## Introduction

The major histocompatibility complex (MHC), a genetic region composed of clustered genes, is one of the most important genetic systems for infectious disease resistance in vertebrates [1], therefore playing a central role in the immune response.

The high variability in many MHC genes, the highest known in any vertebrate genes, is thought to be an essential aspect of the ability of an organism to recognize different parasites [2]. The favored hypothesis is that heterozygotes can recognize a larger suite of pathogens than can homozygotes [3] and consequently have higher fitness.

Decreased genetic variability at MHC *loci* may be particularly harmful for small populations and endangered species by increasing their susceptibility to infectious disease [4].

The importance of domestic animal genetic resources is nowadays widely recognized. There is a major interest to survey and document livestock breeds, in an attempt to preserve their genetic patrimony and cultural value. In conservation genetics, the main goal is to preserve variability within populations, under the hypothesis that genetic variation is directly implicated in populations' viability.

It is therefore relevant to analyze the levels of variability underlying the genetic legacy of domestic animal breeds, namely the horse, in order to establish a conservation strategy that allows the retention of maximum genetic diversity. The amount and type of MHC variation, including its allelic diversity, is of particular interest in such conservation studies for its recognized role in the survival of populations.

There are presently three autochthonous horse breeds in Portugal, the Lusitano, the Garrano and the Sorraia, being the Lusitano the most common one and the Sorraia the most endangered one. The relationship of the Iberian horse breeds (Portuguese and Spanish) with the South American ones is historically documented by the description of horses' transportation through the navigator Christopher Columbus in 1493 and during the subsequent Spanish and Portuguese colonization period [5].

## Aims

Analyze the genetic variation of the MHC region in the 3 Portuguese autochthonous and in 6 South American horse breeds by screening 4 microsatellite *loci* physically proximal to the MHC genetic region. By documenting the extent and type of MHC variation in this way, insight can be provided on the frequency of alleles that are described as being associated to potential resistance or susceptibility to new pathogenic agents, other than evaluating the capacity for future adaptive change within breeds. Because of the diversity and broad geographic distribution of equine breeds, studies of MHC diversity within this species should provide considerable insight into natural levels of variability and effects of varied environments and management policies on MHC allelic diversity.

## Preliminary Results

✓ Mean number of alleles scored in each population varies from 3.25 in the Sorraia breed to 7.25 in the Pantaneiro breed (Figure 3).

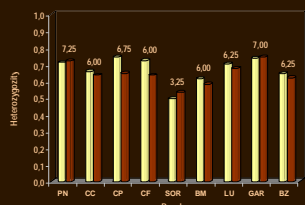


Figure 3. Expected and observed heterozygosities ( $H_e$  and  $H_o$ , respectively) for each of the analyzed breeds. Mean number of alleles is indicated on top of bars.

✓ Observed heterozygosity ranges between 0.535 and 0.747, being highest in Garrano and lowest in Sorraia while unbiased expected heterozygosity ( $H_e$ ) ranges from 0.496 in Sorraia to 0.746 in Colombian Paso Fino (Figure 3).

✓ No significant departures from Hardy-Weinberg assumptions were found in any of the nine breeds analysed ( $p > 0.006$  for all).

✓  $F_{IS}$  values obtained reveals that, for the overall set of MHC-linked microsatellites, no breed displays statistically significant results for heterozygote deficit or excess. The Sorraia breed shows highest  $F_{ST}$  values of all pairwise comparisons ( $F_{ST} > 0.110$ ) (table 1).

✓ Significant between-breed  $F_{ST}$  estimates were found for every breed pair except for Campolina and Pantaneiro.

✓ Genotypic disequilibrium analysis was found to be significant for one of the analysed pairs of *loci*: HTG5 and UM11 ( $p < 0.05$ ) (table 2).

Table 1. Pairwise  $F_{ST}$  estimates calculated over the four MHC-linked microsatellite *loci* for the nine horse breeds studied (above the diagonal). Below the diagonal is each value's statistical significance after standard Bonferroni corrections.

	BZ	CP	CC	CF	BM	PN	GAR	LU	SOR
BZ	0.000	0.052	0.028	0.072	0.067	0.052	0.063	0.029	0.205
CP	*	0.000	0.046	0.056	0.024	0.059	0.061	0.126	
CC	*	*	0.000	0.091	0.063	0.053	0.083	0.070	0.163
CF	*	*	*	0.000	0.077	0.028	0.057	0.031	0.166
BM	*	*	*	*	0.000	0.078	0.061	0.075	0.196
PN	*	NS	*	*	*	0.000	0.067	0.053	0.112
GAR	*	*	*	*	*	*	0.000	0.033	0.191
LU	*	*	*	*	*	*	*	0.000	0.198
SOR	*	*	*	*	*	*	*	*	0.000

\* Significance at the 5% nominal level ( $p < 0.006$ )  
 NS - non-significant ( $p > 0.006$ )

Table 2. Linkage disequilibrium probabilities between the four MHC-linked microsatellite *loci*.

	HMS42	HTG5	LEX52	UM11
HMS42	-			
HTG5	0.287	-		
LEX52	0.759	0.188	-	
UM11	0.605	0.038*	0.065	-

\* Significance at the 5% nominal level ( $p < 0.05$ ).

## Material & Methods

### • Sampling (figure 1)

Portugal: Garrano (30), Lusitano (30) and Sorraia (38).

South America: Pantaneiro (31), Chilean Criollo (25), Campolina (25), Colombian PF (25), Mangalarga (30), Brazilian Criollo (20).

### • Type of Samples & DNA Extraction Methods

- ✓ Blood → High salt extraction procedure [6]  
 QIAmp DNA Blood Mini Kit (Qiagen)  
 Puregene Genomic DNA Isolation Kit (Gentra Systems)
- ✓ Hair → Chelex-100 [7]

### • Molecular Markers

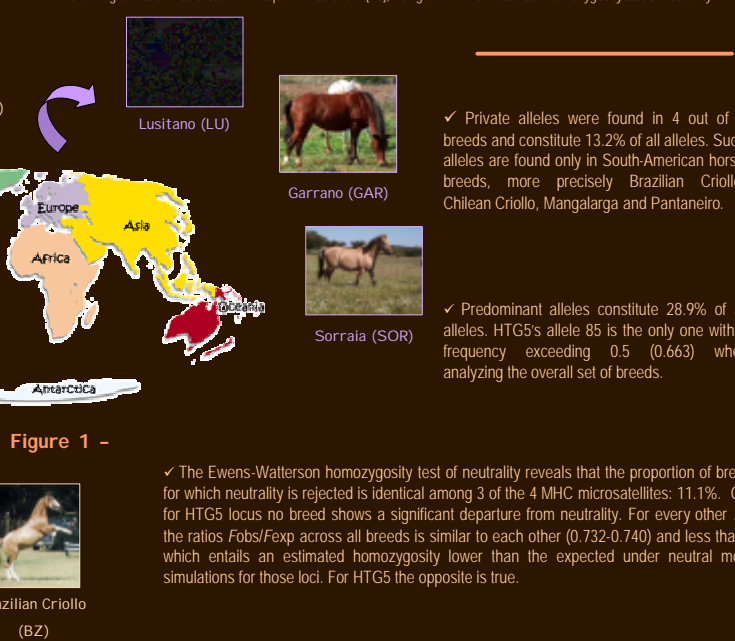
- ✓ Dinucleotide microsatellites HTG5 [8], HMS42 [9], LEX502 [10] and UM011 [11], mapping to equine chromosome 20 [12] (Figure 2).

### • Methodology

- ✓ Samples were amplified by PCR using fluorescent labelled primers. The PCR products were separated in a 0.25 mm thick 6% Long Ranger Polyacrilamide gel using a 4200S LI-COR sequencer. Gel image results were analyzed with RFLPscan 3.1 (Scanalytics, CPS Inc., USA).

### • Estimated Genetic Parameters

- ✓ Observed and unbiased expected heterozygosity ( $H_o$  and  $H_e$ , respectively) and mean number of alleles *per* population were estimated with Microsatellite Toolkit 3.1 [13].
- ✓ Departures from Hardy-Weinberg equilibrium (HWE) were tested using GENEPOP 3.4 software [14]. The alternative hypothesis of heterozygote excess or deficit was tested through Wright's  $F_{IS}$ . Standard Bonferroni procedure was applied to adjust the  $p$ -value.
- ✓ Estimations of allele frequencies *per locus* in each breed and of inter-breed differentiation through  $F_{ST}$  were performed with FSTAT 2.9.3 [15].
- ✓ Linkage disequilibrium tests between the four microsatellites were computed in GENEPOP 3.4 software [14].
- ✓ Balancing selection was tested in Arlequin 2.0 software [16], using the Ewens-Watterson homozygosity test of neutrality.



## Discussion

- ✓ Two more allelic variants than it was first reported by [17], were found in the present work for *loci* HMS42, HTG5 and UM11.
- ✓ A high number of alleles was detected for *loci* LEX52 (13) and UM11 (12), what is in accordance to the expected high levels of variability for the MHC region.
- ✓ Results show that the Portuguese breeds Garrano and Lusitano, along with the South American Pantaneiro, have the highest genetic variability, what is in agreement with the large population size of these breeds.
- ✓ The Sorraia population seems to be the less variable for the four analyzed *loci*, supporting previously obtained evidence of a debilitated genetic patrimony within this breed [18, 19]. This decreased levels of genetic variability may suggest a reduced ability to respond to different pathogens and parasites at the MHC level and a subsequent higher fragility to infectious diseases.
- ✓ The lack of significant genetic differentiation between Pantaneiro and Campolina suggests significant outcrossing between both breeds, as previously suggested by [20].
- ✓ The highest levels of genetic differentiation found between the Sorraia and the remaining horse breeds, suggests a considerable degree of isolation of the Sorraia to all the remaining horse breeds. This emphasizes the consequences of a very small founder population and a very small sized current population. It may also be the result of the selective breeding regime in which this breed has been managed for the past 70 years
- ✓ The occurrence of private alleles exclusively in South-American horse breeds is probably an indicator of other breeds' influence over their genetic patrimony, such as the Spanish breeds which are historically related with the previous.
- ✓ Associations of the MHC-linked microsatellites to MHC genes and to pathogenic responses should be investigated. Indeed, the high frequency of allele HTG5-85 found in the Portuguese and South American horse breeds should be analysed in future studies since it has been suggested to be associated with levels of IgE antibody [17].
- ✓ The absence of deviations from Hardy-Weinberg equilibrium and the fact that only 11.1% of the analysed breeds rejected the neutrality predictions suggests that there are probably other evolutionary forces influencing these horse breeds genetic variability, namely processes of random genetic drift and artificial selection resulting from breeding management policies.

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