

GENETIC DIVERSITY ANALYSIS OF THE 3 PORTUGUESE NATIVE HORSE BREEDS INFERRED FROM MICROSATELLITE DATA

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Introduction

The importance of domestic animal genetic resources is being recognized in many countries. There is an increasing interest to survey and document native livestock breeds, to protect them even without commercial interest, to maintain their cultural value and their possible importance in modern genetic improvement programs (1,2).

There are 3 autochthonous breeds in Portugal:

- the *Lusitano*, the classical type of Iberian riding horse, is considered one of the First saddle horse of the World and detains a significant genetic contribution to many of today's most important breeds, especially those of the New World. It's the most managed and widely represented Portuguese breed, with the oldest Studbook.
- the *Garrano*, a Celtic brown pony living free in the northern mountainous region, is recently being registered at a Studbook.
- the *Sorraia*, probably the primitive type of southern Iberian horse, mouse or yellow dun colored, is now a rare and very inbred breed, being a closed population since 1937, tracing back to only 12 founders.

All the breeds are considered to be of important economic, genetic and cultural value and have an EC program to incentive the breeders to preserve them.

Reproductive isolation, a consequence of specific management and Studbooks rules, reduces effective population size and avoids migration, contributing to genetic subdivision and increasing inbreeding and genetic drift effects. In conservation genetics the main objective is to preserve variability within populations under the hypothesis of correlation between genetic variation and populations viability.

With this work, as part of a conservation management plan, we intend to characterize and to compare the genetic structure of the three Portuguese native horse breeds based on DNA markers - *microsatellites* - and to analyse the genetic divergence between them.

Materials and Methods

183 Lusitano horses, 31 Sorraia horses and 167 Garranos were genotyped for 9 dinucleotide horse microsatellites *loci*: ASB2 (with the sequence modified by Perkin-Elmer for use with Stockmar's kit (Brandt, com. pess.), HMS2, HMS3, HMS7, HTG4, HTG6, HTG10, LEX023, VHL20 (3,4,5,6,7). DNA was obtained from whole blood samples using a high salt extraction procedure⁸. PCR was conducted in 10 µl reactions using a MJ-Research PTC-100 thermocycler. Fluorescence labelled and unlabelled primer's concentration ranged from 0.5 to 1.3 µmol. Microsatellites HMS7 and HTG4 were amplified in a multiplex reaction.

PCR products were separated using an automated fluorescence 4200S Li-COR sequencer. Alleles were scored with RFLPScan 3.1⁹, by reference to the Li-COR STR marker.

Observed (Ho) and expected (He) heterozygosity, polymorphism information content (PIC) exclusion probability (Pe), deviation from Hardy-Weinberg equilibrium (HWE), with pooling) and F-Statistics were calculated for all polymorphic *loci*. Genetic resemblance between breeds was estimated by Nei's 1972 coefficient, Fst distance and by principal co-ordinate analysis of a binary data matrix (presence / absence of each possible allele).

Computations were performed with Cervus 1.0¹⁰, BIOSYS-1 release 1.7¹¹, Genetic Data Analysis 1.0¹² and NTSYS1.8¹³.

Results

Genetic Variability and HWE

The statistics of genetic variation in each breed are presented in Tab. 1; Tab. 2 contains the F-statistics results; allele frequencies are graphically represented in Fig.1 and the dendrograms using Fst and Nei's 1972 distance values are showed in Fig. 2.

✓ Lusitano and Garrano breeds are **highly polymorphic** for the 9 microsatellite *loci* analyzed, with number of alleles ranging from 5 to 13 and from 7 to 12, respectively.

✓ Sorraia breed, as expected due to the high level of inbreeding of this population (Oom *et al.* 1991)⁴, presents a **number of alleles extremely reduced**, ranging from 2 to 5, and **lower values of PIC and heterozygosity**.

✓ This panel of 9 *loci* proves to be very useful for paternity assignment for Lusitano and Garrano breeds (**PE = 0.999**). For the Sorraia breed, due to the reduced number of alleles and the extremely heterogeneous distribution of allele frequencies, this set of microsatellites is not so efficient (**PE = 0.963**).

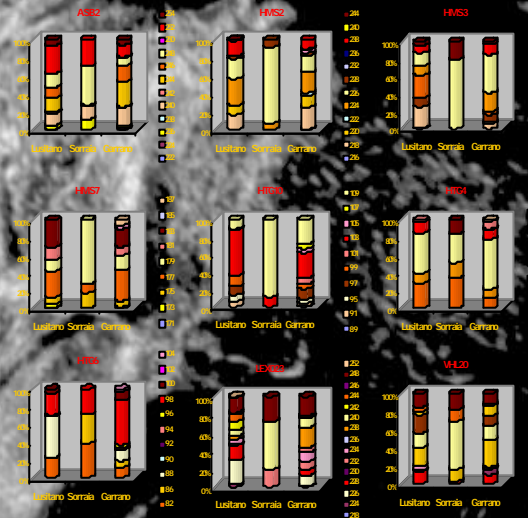


Fig.1- Allele frequencies of the nine microsatellite *loci* in the 3 breeds analysed

Tab. 1: Number of alleles (NA), polymorphic information content (PIC), observed heterozygosity (Ho), expected heterozygosity (He) and probability of exclusion (PE) for the analysed *loci*.

	LUSITANO					SORRAIA					GARRANO				
	NA	PIC	Ho	He	PE	NA	PIC	Ho	He	PE	NA	PIC	Ho	He	PE
ASB2	10	0.759	0.732	0.786	0.600	5	0.641	0.710	0.703	0.443	11	0.792	0.784	0.819	0.638
HMS2	8	0.763	0.770	0.796	0.594	3	0.243	0.258	0.263	0.133	10	0.800	0.814	0.826	0.650
HMS3	8	0.792	0.770	0.818	0.638	2	0.277	0.355	0.337	0.138	8	0.697	0.635	0.732	0.521
HMS7	7	0.738	0.672	0.774	0.562	3	0.389	0.452	0.439	0.224	8	0.765	0.784	0.794	0.604
HTG10	7	0.645	0.612	0.674	0.471	2	0.180	0.097	0.204	0.090	10	0.784	0.868	0.810	0.633
HTG4	5	0.634	0.667	0.687	0.438	4	0.673	0.677	0.736	0.472	7	0.600	0.653	0.630	0.421
HTG6	6	0.616	0.661	0.673	0.413	3	0.588	0.645	0.662	0.367	10	0.670	0.689	0.698	0.499
LEX023	13	0.831	0.792	0.850	0.701	3	0.535	0.581	0.614	0.326	12	0.838	0.844	0.857	0.711
VHL20	10	0.823	0.732	0.845	0.684	5	0.611	0.742	0.662	0.422	10	0.818	0.856	0.839	0.680
Mean	8.22	0.7320	0.712	0.767	0.499	3.33	0.460	0.502	0.514	0.262	9.67	0.751	0.776	0.778	0.599

Lusitano not in HWE for ASB2 (P<0.05), HMS3 (P<0.05) and VHL20 (P<0.05). Sorraia not in HWE for HTG10 (P<0.001). Garrano not in HWE for HMS3 (P<0.01) and VHL20 (P<0.05).

Statistics of Genetic Differentiation

✓ Nei's genetic distance, appropriate for long-term evolution analysis, clusters Lusitano and Sorraia breeds, which agrees with the hypothesis that considers the Sorraia an close related and an ancestral of the Lusitano.

✓ The Fst Distance, more appropriate for short-term evolution analysis, namely reflecting genetic divergence only due to drift¹⁵, reveals a separation of Sorraia horse from the other two breeds, now closer, which is in agreement with the extremely reduced effective size of the breed.

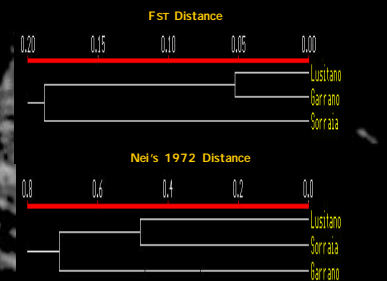


Fig.2- Dendrograms obtained by the UPGMA method using Fst distance and Nei's 1972 genetic distance values

✓ The principal co-ordinate analysis shows the Sorraia breed as an isolate group, while the Garrano overlaps the Lusitano distribution, confirming the particularity of the Sorraia's genetic structure.

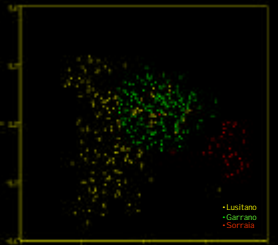


Fig.3- Diagram derived from the principal co-ordinate analysis. OTU's were plotted against co-ordinates 1 and 2 (Simqualgorithm-Jaccard coefficient)

Tab. 2: F-statistic estimates by locus

Locus	Fis	Fst	FIT
ASB2	0.051	0.0686	0.1162
HMS2	0.0236	0.09	0.1112
HMS3	0.0872	0.1413	0.2161
HMS7	0.0698	0.0841	0.148
HTG10	0.0191	0.1459	0.1622
HTG4	0.0067	0.044	0.0504
HTG6	0.0179	0.136	0.1515
LEX023	0.0435	0.0817	0.1216
VHL20	0.0493	0.039	0.0864
All	0.0418	0.0926	0.1306

✓ A general deficit of heterozygotes of 4.2%, on average, exists overall populations. The deficit of heterozygotes, considering the 3 breeds as a whole, was equal to 9.3%. The average genetic differentiation between breeds was 13% which indicates low differentiation.

Final remarks

- ✓ The microsatellite DNA approach may be an effective way to get data for genetic populations studies.
- ✓ The low number of alleles present in Sorraia breed was an expected result due to the high level of inbreeding coefficient, founder effect and genetic drift.
- ✓ The cluster analyses corroborates the idea that the Sorraia is genetically closed to the Lusitano regarding long-term evolution, also becoming evident the strong effect of genetic drift.
- ✓ The significant between-populations Fst estimates (P<0.01) indicates a relatively low gene flow between breeds.
- ✓ Only 13% of the total genetic variation is due to breed differentiation, the remaining 87% correspond to differences among individuals, which reveals enough variability for future management plans.

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