

# 5032 Genetic diversity of the mtDNA in five Italian horse populations



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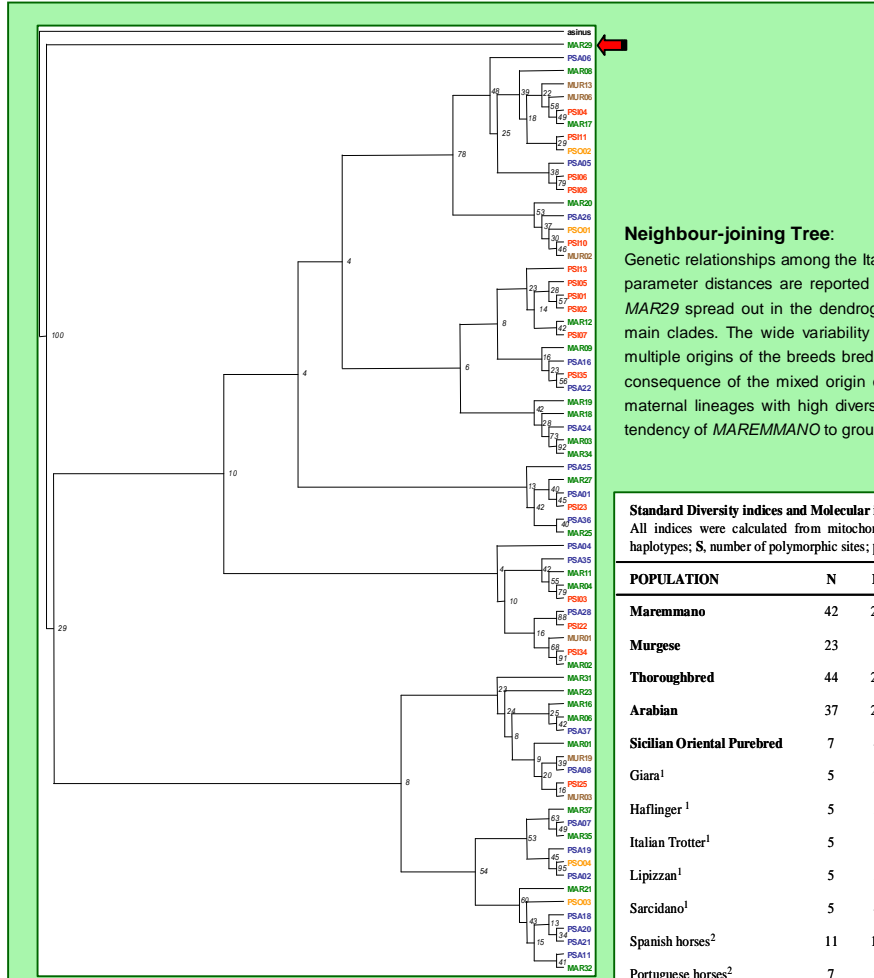
Genetic variability of a 510bp fragment in the D-loop region of the mitochondrial DNA in five horse breeds bred in Italy was analysed. Two breeds are autochthonous: **Maremmano (N=42)** and **Murgese (N=23)**. Other Italian populations were also included: **Arabian (N=37)**, **Thoroughbred (N=44)** and **Sicilian Oriental Purebred (N=7)**. Murgese samples were selected at random, while selection for other breeds has been performed using pedigree information in order to maximise maternal diversity. Genomic DNA was extracted from blood on FTA® papers. PCR primers were designed according to Xu and Arnason (1994). Sequences obtained were aligned and compared with reference sequence from GenBank X79547. For a total of **153 individuals**, 44 polymorphic sites representing 51 haplotypes were observed. Our data were compared with other mtDNA sequences present in literature. The aim of this study is to evaluate the maternal genetic relationships of these breeds in order to increase the information about horse populations bred in Italy and to confirm their multiple origin as reported by other investigators.

### Materials and Methods

The horse's blood was spotted into FTA filter paper (Whatman Bioscience, Cambridge, United Kingdom). For each sample, one FTA disk of 2.4 mm in diameter was punched and it was washed twice with FTA purification reagent (Whatman) for 5 min and then washed twice again with TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) for 5 min and air dried overnight. The washed paper was then used directly as the DNA template in PCR amplification. Hypervariable region of the mtDNA D-loop was amplified from total genomic DNA through primers previously described (Xu et Arnason 1994:forward 5'-CGCACATTACCTGGTCTTG-3', reverse 5'-GAACCAGATGCCAGGTATAG-3') and were synthesized by Invitrogen. PCR reactions were performed in a volume of 50 µl Platinum® Super Mix High Fidelity (Invitrogen). After a hot start of 2 min at 94 °C, 35 cycles were performed, each consisting of a denaturation step (30 s) at 94 °C, an annealing step (30 s) at 55 °C and an extension (60 s) at 72 °C. These cycles were followed by a final primer extension step (10 min at 72°C). The efficiency of the PCR reaction was checked in a 2% agarose gel electrophoresis. DNA sequence analysis was performed from PCR reactions using an automated DNA sequencer (ABI Prism 310 Genetic Analyzer, Applied Biosystems) and the Big Dye terminator V.3.1 staining Kit (Applied Biosystems) according to the manufacturer's instructions. The sequences were aligned using the SeqScape software (Applied Biosystems).



**Figure 1.** Geographical origin of following Italian breeds: Haflinger, Maremmano, Murgese, Cavallino della Giara, Sarcidano, Sicilian Oriental Purebred. For Arabian, Italian Trotter, Lipizzan and Thoroughbred geographical localization is not indicated.



**Neighbour-joining Tree:**  
 Genetic relationships among the Italian horse breeds analysed in the present study obtained using Kimura two-parameter distances are reported in Figure 2. Our mtDNA gene tree revealed that all breeds except sample **MAR29** spread out in the dendrogram clades. Sample **MAR29** appears to be genetically separated from the main clades. The wide variability of the D-loop sequences among our populations might be caused by the multiple origins of the breeds bred in Italy, a previously reported by other authors. These results could be the consequence of the mixed origin of breeds bred in Italy and probably indicate the presence of many ancient maternal lineages with high diversity in mtDNA sequences. Further studies could be performed to verify the tendency of **MAREMMANO** to group in clades with **ARABIAN**.

**Standard Diversity indices and Molecular indices in some Italian Horse populations.**  
 All indices were calculated from mitochondrial D-loop sequences. Abbreviations are as follows: N, size of the sample; K, number of haplotypes; S, number of polymorphic sites; p, Mean number of pairwise differences; H, Gene Diversity.

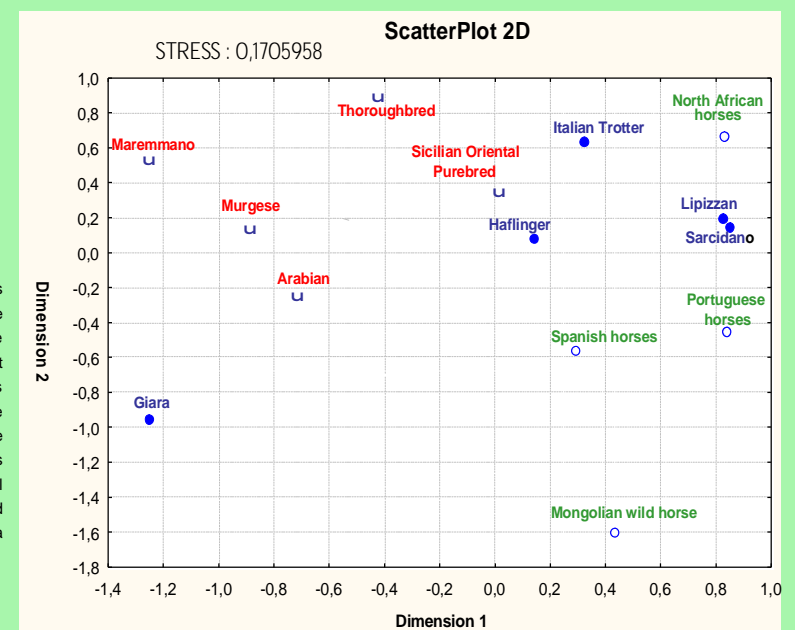
POPULATION	N	K	S	H	p	Nucleotide Diversity
Maremmano	42	29	35	0.9721 +/- 0.0130	6.668990 +/- 3.210582	0.024339 +/- 0.013013
Murgese	23	6	24	0.7154 +/- 0.0752	8.474308 +/- 4.068336	0.018463 +/- 0.009885
Thoroughbred	44	21	29	0.9186 +/- 0.0254	5.947146 +/- 2.892090	0.021705 +/- 0.011720
Arabian	37	21	31	0.9610 +/- 0.0140	8.442943 +/- 3.996559	0.019773 +/- 0.010402
Sicilian Oriental Purebred	7	4	14	0.8095 +/- 0.1298	6.666667 +/- 3.582114	0.016584 +/- 0.010207
Giara <sup>1</sup>	5	2	1	0.6000 +/- 0.1753	0.600000 +/- 0.562226	0.002190 +/- 0.002399
Haflinger <sup>1</sup>	5	5	17	1.0000 +/- 0.1265	6.800000 +/- 3.859230	0.024818 +/- 0.016466
Italian Trotter <sup>1</sup>	5	3	10	0.8000 +/- 0.1640	5.400000 +/- 3.130339	0.019708 +/- 0.013356
Lipizzan <sup>1</sup>	5	5	11	1.0000 +/- 0.1265	4.800000 +/- 2.817368	0.017518 +/- 0.012021
Sarcidano <sup>1</sup>	5	4	10	0.9000 +/- 0.1610	5.600000 +/- 3.234570	0.020438 +/- 0.013801
Spanish horses <sup>2</sup>	11	11	32	1.0000 +/- 0.0388	10.690909 +/- 5.277490	0.028060 +/- 0.015627
Portuguese horses <sup>2</sup>	7	7	22	1.0000 +/- 0.0764	8.190476 +/- 4.328241	0.022751 +/- 0.013772
North African horses <sup>2</sup>	4	3	9	0.8333 +/- 0.2224	5.833333 +/- 3.526594	0.021368 +/- 0.015426
Mongolian wild horse <sup>2</sup>	3	2	1	0.6667 +/- 0.3143	0.666667 +/- 0.666667	0.002130 +/- 0.002656

**Figure 2.** Neighbour-joining tree relating mtDNA haplotypes in horse bred in Italy. The donkey (*Equus asinus*) sequence was used as outgroup. Abbreviations are as follows: **PSI**= Thoroughbred, **MUR**= Murgese, **PSA**= Arabian, **PSO**= Sicilian Oriental Purebred, **MAR**= Maremmano.



**Genetic Diversity :**  
 Standard diversity indices and molecular indices were calculated in the samples analysed in the present study and other samples from literature and GeneBank. Gene Diversity values indicate that **MURGESE** showed the lowest variability. This result could be interpreted by the fact that these individuals were sampled at random from the same farm, while selection for the other breeds used pedigree information to maximise maternal diversity. However, the number of horses analysed in the present study is relevant respect of data present in literature.

**MDS:**  
 In the two-dimensional graph of Slatkin's linearized Fst between horse breeds, Giara and Mongolian wild horses appear to be outgroups respect of other breeds analysed. The Lipizzan sample seem to be closely related to the Sarcidano sample; the distribution of Murgese, Maremmano e Arabian suggests a common tendency of these individuals to group in a unique clade, respect to other breeds located in the upper-right.



In red color are data from Unirelab; in light blue are data from Cozzi et al (2004); in green are following data from Genebank database: Spanish horses AF466006-16; Portuguese horses AY246231-5, AY246243, AY246247; North African horses AY246181, AY246185, AY413660, AY413668; Mongolian wild horses AJ413830-2.

<sup>1</sup> Cozzi et al., *Mitochondrial D-loop sequence variation among Italian horse breeds*. Genet.Sel.Evol.36(2004)663-672  
<sup>2</sup> D-loop sequences selected in GeneBank database.