**H3.6**

# 55th Annual Meeting
# of the European Association for Animal Production

## Bled, Slovenia
## September 5th – 9th, 2004

## Commision on Horse Production
## Session 3
## *Endagered Horse Breeds and Genetic Distance*
## *(H*+ Rare Breeds International)*

## H3.6
# Genetic characterisation of Pentro young horses by microsatellite markers

*D. Iamartino[1*], M. Fidotti[1], N. Miraglia[1], F. Pilla[1]*
*[1]Molise University, Departement of Animals, Vegetables and Environmental Sciences, Via De Sanctis, 86100 Campobasso, Italy*

## *Abstract*

The Pentro horse population is an autochthonous breed from breeding area characterized by climatic and geographic peculiarity and today it counts about 400 heads. Because of its peculiar adaptive ability, the “Pentro Horse” must be considered a genetic resource which needs to be preserved. Moreover, the horse pasture is very important for the ecological equilibrium of the area. The genetic structure of Pentro population was studied by means of microsatellite markers (Bruzzone *et al*, 2003) with the aim of the analysis of its biodiversity and relationship versus other horse breeds. The data introduced in this work regard the genetic and morphological assessment of Pentro young horses assigned to the reproduction demonstrating again the uniqueness and the identity of the Pentro group. A total of twelve microsatellite loci were used to score 23 Pentro young horses and then the obtained data were used to compare the genetic structure of the Pentro horse to other six Italian horse breeds (Maremmano, Murgese, TPR, Halflinger, Trottatore and Bardigiano). The genetic distance among individuals was calculated as the proportion of shared alleles, (Dps=1-Ps) according to Bowcock *et al*.(1994) using the Microsat software (Minch et al, 1995). The Neighbour-Joining (N-J) tree computed on individual genetic distance showed that all Pentro young horses clustered together.

## *Introduction*

The remains of an autochthonous horse population called “Cavallo Pentro” is bred in the wild in the mountainous area of the Molise region in Italy. The population is well adapted to the difficult environment of the “Pantano della Zittola”, its breeding area. This is a wide plain of about 2200 hectares, located along the border of the Abruzzo National Park in the mountainous territory between the regions of Abruzzo and Molise. Living conditions in this area are very difficult because of the harsh climate; the winter is cold with floods and abudant snow while the summer is very dry. Parasites are among the principal causes of death during this season . Moreover, the presence of predator, mainly wolves and bears from the nearby National Park, represent a constant danger for the livestock.

Historical informations about this population, together with morphological data and the genetic characterisation by microsatellite markers carried out on all the horses, adults and young horses, for the constitution of an “Registro Anagrafico”of the “Pentro Horse” in the context of Italian equine autochthonous populations at extinction risk. In this paper are reported data on genetic molecular analysis of the selected young horses according to the morphology.

## *Material and Methods*

Morphological and genealogical data were collected and blood samples were taken from a total of 23 Pentro young horses. In addition, six Italian horse breeds (Maremmano – 20 individuals, TPR – 20 individuals, Murgese – 20 individuals, Haflinger – 20 individuals, Trottatore – 20 individuals and Bardigiano – 20 individuals) were used as a comparison.

DNA samples were obtained from lymphocytes with the classical phenol-chloroform extraction protocol (Sambrook et al., 1989).

Microsatellites genotypes were determined at each of twelve loci (HTG10, VHL20, HTG7, HTG4, AHT5, AHT4, HMS3, HMS6, HMS7, LEX003, HMS2, ASB2) for a total of 241 horses with a protocol of multiplex-PCR (Blasi et al., 1999). For each locus the chromosomal location, the primer reference and size range in the studied populations are reported in Table1. Primer sequences and PCR amplification conditions are available from the authors. Genotypes were scored with an ABI PRISM 377 automatic sequencer and the GENESCAN and GENOTYPER computer packages.

Genetic distance by Nei (1987) was used for the breed comparison, while distances among individuals were calculated as the proportion of shared alleles, (Dps=1-Ps) according to Bowcock et al. (1994) using the MICROSAT program (Minch et al., 1995). Neighbour-Joining diagram (Saitou et al., 1987) were constructed on genetic distances using the PHYLIP package ver. 3.5c (Felsenstein, 1993). The program TreeView (Page, 1996) was used to visualize the diagram.

## *Results and Discussion*

In order to test the uniqueness of the selected offsprings, genetic distance among indviduals were calculated as the proportion of shared alleles and used to build a N-J diagram. This method, proposed by Bowcock et al. (1994) for the study of human populations was recently used on horses as a statistical test for breed assignment (Bjornstad et al., 2001). Biornstad's study (2001) shows how 10 microsatellite markers are enought to give a result of a 75% in terms of correct assignment while raising the number of markers up to 20 increases the successful assignment to 95%.

The tree shows a high degree of structuring of the breeds. All the breeds display a good level of correct clustering (Figure 1). The Pentro horse group together in 100% of the cases. These results confirm the hipothesis of the Pentro horse being a genetic unit well differetiated from all the breeds analyzed in this work. This result is even more interesting if the introduction of genetic material from some of those breeds in the Pentro population is taken into account. The Pentro horse, despite all the foreign genetic material which has been introduced, has conserved its uniqueness. This introduction did not cause a total fading of the morphological characteristics of the original population.

## *Conclusions*

Interbreeding is a common practice between the breeders and it is used to modify the characteristics of breeds according to the needs and also to expand the size of population when it is becoming too small. This practice is useful in a sense to preserve from excessive inbreeding, but it is also deleterious where small endagered populations are concerned. In this paper the case of the Pentro horse is presented, but other breeds seem to suffer from problems of this nature.

Molecular tool showed to be useful to assist the offsprings selection.

The uniqueness of the Pentro horse resides in its adaptive ability, which preserved it from complete "extinction" throught mixing with other breeds. The preservation of not only a natural, but also a social and cultural environment, is essential for the conservation of livestock genetic resources.

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**Table 1.** References, chromosomal location, and size range for the 12 microsatellite loci.

| Locus | Reference | Chrom. | Size range (bp) |
|---|---|---|---|
| HTG10 | Marklund et. al 1994 | 21 | 88-116 |
| VHL20 | Van Hearingen et. al 1992 | 30 | 87-107 |
| HTG7 | Marklund et. al 1994 | 4 | 114-132 |
| HTG4 | Ellegen et. al 1992 | 9 | 125-139 |
| AHT5 | Binns et. al 1995 | 6 | 127-145 |
| AHT4 | Goddard et. al 1998 | 24 | 146-164 |
| HMS3 | Guerin et. al 1994 | 9 | 152-170 |
| HMS6 | Guerin et. al 1994 | 4 | 157-171 |
| HMS7 | Guerin et. al 1994 | 1 | 173-185 |
| LEX03 | Coogle et. al 1996 | X | 195-217 |
| HMS2 | Guerin et. al 1994 | 10 | 215-239 |
| ASB2 | Breen et. al 1997 | 15 | 219-255 |

**Figure 1.** N-J diagram of distances among single individuals constructec using the proportion of shared alleles
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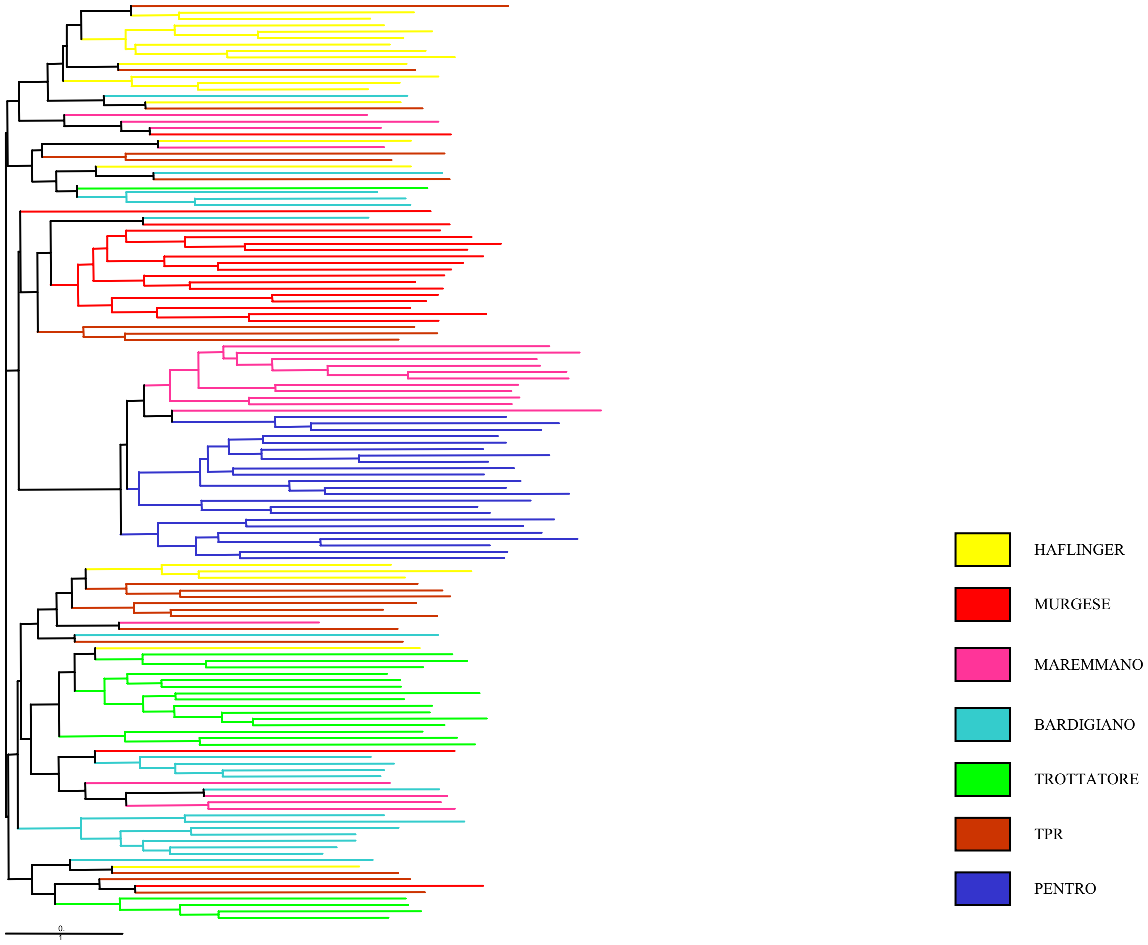

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*Corresponding author*: Daniela Iamartino.Facoltà di Agraria, Università degli Studi del Molise.Via De Sanctis, 86100 Campobasso, Italy. Tel: +39 0874 404671; Fax: +39 0874 404855; Email: iamartino@unimol.it