COMMUNICATION

Genetic analysis of the Sicilian Oriental Purebred horse

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ABSTRACT - Sicilian Oriental Purebred is a small breed considered at critical risk status. In order to evaluate the genetic diversity of the extant selection nucleus, two different molecular markers were used. Twelve microsatellite loci were characterized to assess autosomal variability of a dataset including two other Sicilian autochthonous horse breeds for comparison. Sicilian Oriental Purebred showed lower levels of diversity when compared with Sanfratellano and Sicilian Indigenous and a definite distinctiveness as revealed by genetic distances. Mitochondrial D-loop region (397 bp) was analysed in 20 Sicilian Oriental Purebred and other autochthonous horses to investigate matrilineal genetic diversity. Only one haplotype was found in Sicilian Oriental Purebred whereas high diversity was noticed in the other breeds. The unique haplotype overlapped with many database sequences (GenBank) including five Sanfratellano horses.

Key words: Sicilian Oriental Purebred horse, Genetic diversity, Microsatellites, MtDNA.

Introduction – Sicilian Oriental Purebred horse belongs to the Italian Stud Book since the 1875. The origin of this breed is supported by clear historical information which proves the importation of Oriental horses from Syria and Mesopotamia to Sicily occurred since 1864 (Balbo, 1995). The introduction of this genetic type was initially managed by some Sicilian breeders with the purpose of breeding purebreds to improve local horse populations. Nowadays, the breed is under special legal protection, since it counts an enrolled section nucleus of 40 mares and 15 stallions and the main breeding goal is the equestrian competition based on long distance riding (endurance). Together with Sicilian Oriental Purebred two other autochthonous horses, historically bred in Sicily, were included in this analysis for comparison: Sanfratellano and Sicilian Indigenous. Sanfratellano is mainly reared in the Natural Park of Nebrodi and represents a rare example of semi-feral autochthonous horse. Sicilian Indigenous instead is an heterogeneous population originating from ancient local mares and sires crossed during ages with uncertain breeds. Two different classes of genetic markers were used to examine the autosomal genetic variability (microsatellites) and matrilineal genetic diversity (mtDNA) of Sicilian Oriental Purebred in comparison...
with the other Sicilian autochthonous horses. Both markers were already used combined with historical information, to evaluate genetic relationships among European breeds (Pérez-Gutiérrez et al., 2008; Aberle et al., 2007).

**Material and methods** – Blood samples were collected in Sicily from 50 Sicilian Oriental Purebred (SOP) horses, nearly the entire Stud Book-registered population, and from 61 Sanfratellano (SAN) and 30 Sicilian Indigenous (SIC). DNA was extracted and amplified by standard methods. A set of 12 microsatellites markers (HTG6, HTG10, VHL20, HTG7, HTG4, AHT5, AHT4, HMS3, HMS6, HMS7, HMS2 and ASB2) was genotyped using an ABI PRISM® 377 genetic analyser equipped with GeneScan® software (Applera Biosystems). Individual multi-locus genotypes were analysed by GENALEX ver. 6.1 software to calculate the main parameters of genetic variability. The FSTAT software (Goudet, 2001) was used to calculate the allelic richness (AR) and to estimate Wright's F-statistics. Reynolds' (D_R) population distance was obtained by PHYLIP software (Felsenstein, 2005). Proportion of shared alleles distance (Dps') calculated by MICROSAT 1.5b (Minch et al., 1998), was chosen as distance among individuals. Mitochondrial control region (D-loop) was amplified by PCR using primers designed according to the published horse sequence (X79547). The amplicon of 397 bp (15382-15788) was purified and sequenced using the BigDye Terminator Kit (Applied Biosystems) by an ABI PRISM® 310 Genetic Analyser. The aligned sequences were edited in MEGA 4 (Tamura et al., 2007) in order to identify the polymorphic sites and to achieve the BLAST search.

**Results and conclusions** – A total of 113 alleles were characterized: 80 in SOP, 85 in SAN, 96 in SIC. SOP showed lower levels of diversity when compared with the other Sicilian horses (table 1), probably because of the very small size of the breed selection nucleus.

However the diversity indexes were higher than those observed in other endangered European horse breeds (Luis et al., 2007). In SOP 10 private alleles were identified all with a low frequency, except for one (9%) at HMS3 locus. D_R distances were: 0.101 between SAN and SOP, 0.042 between SAN and SIC, 0.081 between SOP and SIC; individual tree, built on Dps', showed 41/50 SOP clearly grouped in a unique cluster (data not shown). Both population and individual genetic distances discriminate SOP from the other Sicilian autochthonous horses. Only one mtDNA haplotype was found in the entire sample of SOP (Table 2), whereas more were found in the other Sicilian horses: 11 in SAN and 13 in SIC. The

| Breeds                      | Sample size | Ho     | He     | MNA    | AR    | FIS     |
|-----------------------------|-------------|--------|--------|--------|-------|---------|
| Sicilian Oriental Purebred  | 50          | 0.704±0.02 | 0.702±0.04 | 6.7±0.51 | 6.01  | -0.002  |
| Sanfratellano               | 61          | 0.739±0.03 | 0.751±0.02 | 7.1±0.07 | 6.06  | 0.016   |
| Sicilian Indigenous         | 30          | 0.727±0.02 | 0.803±0.02 | 8.0±0.56 | 8.00  | 0.096   |
lack of polymorphism in SOP does not allow to accomplish the molecular diversity analysis for this breed. The unique haplotype identified in the breed overlapped (BLAST search) with GenBank sequences belonging to many widespread breeds (Arab, Barb, Andalusian, Thoroughbred). In our sample this haplotype matched only with five SAN but not with SIC horses. The absence of the SOP haplotype in SIC population could be explained assuming that SOP selection started from a small nucleus of Oriental mares imported from Syria and Mesopotamia during the 19th century, as supported by historical documentation. Then a bottleneck event could be supposed to justify the presence of a unique female ancestor. The presence of the SOP haplotype within SAN horses suggests that a relevant part of the population shares a common female founder with the Oriental ones.

Research supported by Istituto di Incremento Ippico per la Sicilia, project "Recovery, enhancement and safeguarding program of Sicilian Oriental Purebred".

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