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# MOLECULAR GENETICS APPROACHES IN FARM ANIMALS IN ROMANIA**

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**Plenary invited paper.

**Abstract:** During the last three decades, major advances have been made in mammalian genetics. New methods have been developed and applied to investigating the genetics of horses and cattle and to improve theirs performance.
For this article we decided to present only the genetic characterisation in horses using microsatellites.
Allele frequencies, the observed heterozygosity (Ho) and expected heterozygosity (He) were calculated using the software program Cervus 2.0. Alleles' size range at individual loci varied between 84 and 253 bp. High level of polymorphism was observed for population of horses studied.
Microsatellite markers are more likely than other methods to detect small differences between populations due to their high levels of allelic variation, being able to discriminate in both overall heterozygosity and mean number of alleles.
**Key words**: farm animal, microsatellite, allele frequencies, genetic characterisation.

## Introduction and Literature Review

During the last three decades, major advances have been made in mammalian genetics. New methods have been developed and applied to investigating the genetics of horses and cattle and to improve theirs performance. The classical method of selection is based on physical observation of inherited traits in adult animals and the careful maintenance of lineage records by breeding organizations.

The recent developments in molecular genetics allowing the detection of genes responsible for economic traits have opened a new area in farm animal selection, including horses and cattle. The PCR technology provides a

sensitive method for parentage verification and individual identification. It can also be used to screen for markers linked to performance traits, genetic disorders, animals with superior traits, such as high milk production and lean carcasses in the case of cattle, or speed and strength in the case of horses, are used as breeding stock for subsequent generations.

Until now, in Romania we did the genetic characterizations of:

i) Five Romanian horse breeds (Thoroughbred, Arabian, Romanian Sport Horse and Hucul) using 17 microsatellite markers;

ii) Romanian Brown and Romanian Black Spotted cattle populations using 11 microsatellites;

iii) Diagnostication of Bovine Leukocyte Adhesion Deficiency, citrullinemia, Deficiency of Uridine Monophosphate Synthase in cattle;

iv) Diagnostication of Severe Combined Imunodeficiency, Junctional Epidermolysis Bullosa and Hyperkalemic periodic paralisys in horses;

v) PCR-RFLP analysis on the β-lactoglobulin and k-casein loci.

For this article we decided to present only the genetic characterisation in horses using microsatellites.

The DNA fingerprinting was the first sensitive DNA-based method for identifying individuals and for studying genetic relationships (*Jeffreys et al.* 1985 a, b). Equine minisatellites were therefore used in early fingerprinting studies to assess relationships within and between members of different horse breeds. Microsatellites are evenly distributed across genome and highly polymorphic. PCR technology provides a sensitive method for parentage verification and individual identification (*Marklund et al.* 1994). It can also be used to screen for markers linked to performance traits or genetic disorders.

# Material and Methods

In our experiment the analyzed blood samples were obtained from: Jegălia haras for Romanian Sport Horse (120 samples); Cislau haras for Thoroughbred (80 samples); Mangalia haras for Arabian (100 samples) and Lucina haras for Hucul (80 samples).

The isolation of genomic DNA from the white blood cells was performed with Wizard Genomic DNA Extraction Kit (Promega).

PCR amplifications were performed in 0.2 mL tubes, in GeneAmp 9700 thermocycler, using 30 cycles with denaturation at 95 ºC (30 s), annealing at 60 °C (30 s) and extension at 72 °C (60 s). The first denaturation step was performed at 95 ºC (10 minutes) and the last extension was to 60 minutes at

72 °C.

Amplification of the STR loci was realized by multiplex PCR using StockMarks Paternity PCR Typing Kit (AppliedBiosystems), according to the procedure recommended by the manufacturer. The reagents in the kit are used to amplify DNA samples using the fluorescent dye-labelled primers specific to the relevant loci. The primers are premixed and the PCR conditions optimized so that the loci can be amplified as multiplex reactions, and then loaded with the GeneScan-500 LIZ Size Standard into one of the ABI PRISM 310 DNA Genetic Analyzer. As the samples migrate past the fluorescence detector, the GeneScan ® Software collects the signal and assigns a base pair size for each sample.

## Results of Investigations and Discussion

In our experiment successful amplification yields allele peaks with the associated PCR stutter bands within a maximum range of eight base pairs from the allele peak. The number of allele peaks depends on whether the individual tested is a heterozygote or homozygote.

Mapping data (*Lindgren et al.*, 1998; *Shiue et al.*, 1999; *Caetano et al.*, 1999) have indicated that the seventeen microsatellite markers used are on different chromosomes. The markers selected have a combined exclusion probability over 99% in a variety of horse breeds including Thoroughbreds and Arabian. In Table 1 are shown expected size ranges for the equine specific loci.

One example of electropherograms of seventeen equine specific loci for three unrelated horses is shown in Figures 1, 2, 3 and 4.

For this StockMarks Kit, all loci are dinucleotid repeats. Occasionally, we observed an allele that falls outside from its expected size range and that overlaps with the expected size range on another locus. Such alleles may be present at low frequencies in the populations.

Allele frequencies, the observed heterozygosity (Ho) and expected heterozygosity (He) were calculated using the software program Cervus 2.0. Alleles' size range at individual loci varied between 84 and 253 bp. High level of polymorphism was observed for population of horses studied. From seventeen microsatellites, fifteen were highly polymorphic and two (HTG4 and HTG7) present low polymorphism. ASB2 and LEX3 were the most variable loci with eleven alleles and high heterozygosity. The average number of alleles and average of observed heterozygosity was 7.65 and 0.683, respectively. A number of loci showed different alleles frequencies

within the horse population. Some alleles present a high frequency (between 0.418 and 0.527) over the population and others, a lower frequency (between 0.004 and 0.009).

For LEX3, ASB2 and HTG10 loci we observed a significant deviation from the Hardy-Weinberg equilibrium. This might be caused by the population substructure (hybridization between subspecies), selection acting on linked loci, a null allele segregating in the population or a sex-linked locus. It is possible that the deviation from Hardy-Weinberg equilibrium across those three loci has shown that the population is genetically subdivided. Perhaps two or more populations have recently been mixed within Romanian Sport Horse breed.

**Table 1: Equine specific loci and expected size range**

| Locus | Dye | Colour | Expected Size Range/ (bp) |
|---|---|---|---|
| VHL20 | 6-FAM | Blue | 83–102 |
| HTG4 | 6-FAM | Blue | 116–137 |
| AHT4 | 6-FAM | Blue | 140–166 |
| HMS7 | 6-FAM | Blue | 167–187 |
| HTG6 | VIC | Green | 74–103 |
| HMS6 | VIC | Green | 154–170 |
| HTG7 | NED | Yellow | 114–128 |
| HMS3 | NED | Yellow | 146–170 |
| AHT5 | VIC | Green | 126–147 |
| ASB2 | VIC | Green | 237–268 |
| HTG10 | NED | Yellow | 83–110 |
| HMS2 | NED | Yellow | 215–236 |
| ASB23 | VIC | Green | 176-212 |
| ASB17 | PET | Red | 104-116 |
| LEX3 | PET | Red | 137-160 |
| HMS1 | PET | Red | 166-178 |
| CA425 | PET | Red | 224-247 |

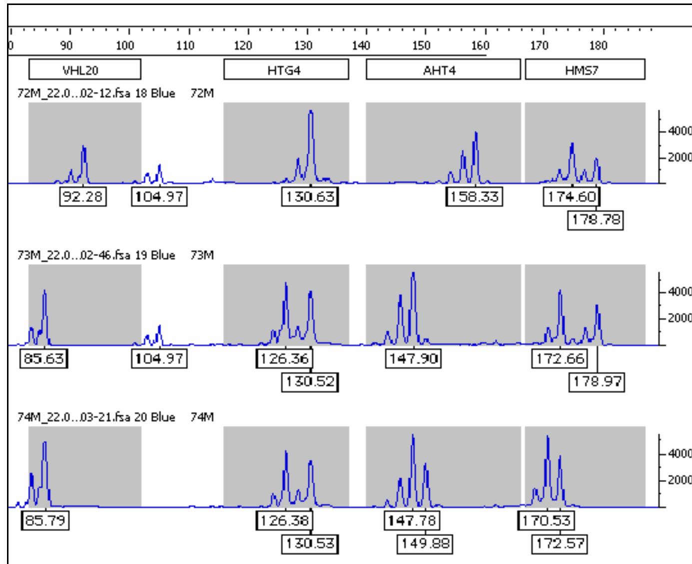


**Figure 1. Genotyper software analysis of PCR amplification products using equine blue markers**

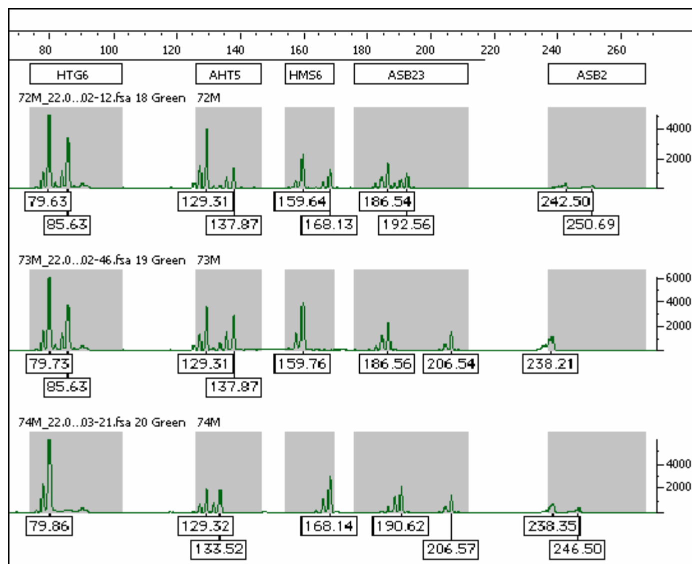


**Figure 2. Genotyper software analysis of PCR amplification products using equine green markers.**

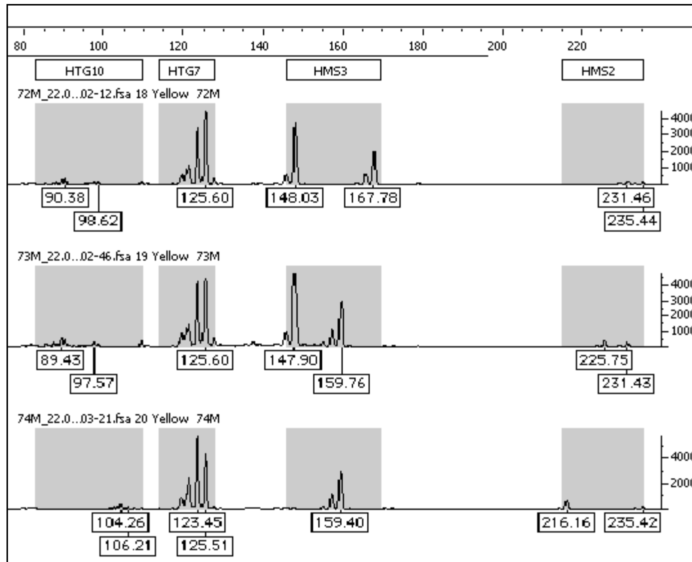


**Figure 3. Genotyper software analysis of PCR amplification products using equine yellow markers.**

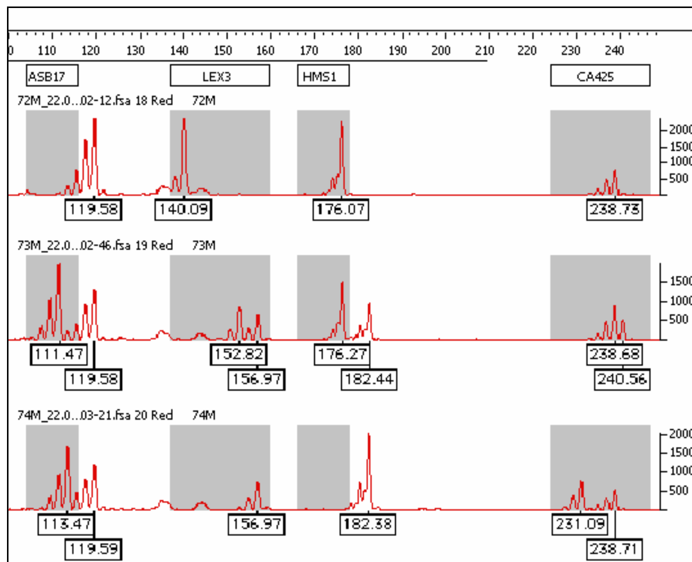


**Figure 4: Genotyper software analysis of PCR amplification products using equine red markers**

## Conclusions

The implications of DNA testing for selective breeding are immense. Horse identification by amplification of the STR loci was realized using modern technology of DNA extraction and automatic genotyping. This technology is for the first time applied in Romania and provides a more efficiently and sensitive method for parentage and individual identification.

Microsatellite markers are more likely than other methods to detect small differences between populations due to their high levels of allelic variation, being able to discriminate in both overall heterozygosity and mean number of alleles.

DNA testing techniques, now available and progressively adopted by most responsible laboratories, can greatly improve the success of parentage tests, providing an excellent alternative to traditional methods.

# MOLEKULARNO-GENETSKI PRISTUPI U OBLASTI STOČARSTVA U RUMUNIJI

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## Rezime

Razvoj molekularne genetike i mogućnost otkirvanja gena odgovornog za ekonomske osobine su otvorili novu oblast u selekciji domaćih životinja, uključujući goveda i konje. PCR tehnologija obezbeđuje metodu verifikacije roditeljstva i pojedinačnu identifikaciju. Može se koristiti za skrining markera koji su vezani za proizvodne osobine i sadržaj mesa u trupovima kod goveda, ili brzinu i snagu kod konja, koji se koriste kao priplodni materijal za naredne generacije.

U ovom radu smo odlučili da predstavimo samo genetsku karakterizaciju kod konja korišćenjem mikrosatelita..

Uzorci krvi analizirani u našem istraživanju su dobijeni od: Jegălia haras za rumunske sportske konje (120 uzoraka); Cislau haras za punokrvne engleske konje (80 uzorakas); Mangalia haras za arapske konje (100 uzoraka) i Lucina haras za Hucul (80 uzoraka).

Identifikacija konja amplifikacijom STR lokusa je urađena korišćenjem

moderne tehnologije DNK ekstrakcije i automatskog genotipiranja. Izolacija genomskog DNK iz belih krvnih zrnaca je urađena korišćenjem Wizard Genomic DNA Extraction Kit (Promega).

Amplifikacija STR lokusa je realizovana višestrukom PCR metodom StockMarks Paternity PCR Typing Kit (AppliedBiosystems). Za ovaj StockMarks Kit, svi lokusi su dinukleotidna ponavljanja.

Ova tehnologija se po prvi put primenjuje u Rumuniji i obezbeđuje efikasniji metod za određivanje roditeljstva i pojedinačnu identifikaciju.

Tehnike testiranja DNK koje su dostupne I progresivno se usvajaju i primenjuju od strane ovlašćenih I odgovornih laboratorija, mogu u velikoj meri da doprinesu uspehu testova roditeljstva, obezbeđujući odličnu laternativu tradicionalnim metodama.

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