MICROSATELLITE DIVERSITY OF HORSE BREEDS IN CZECH REPUBLIC

DIVERZITA MIKROSATELITNÝCH MARKEROV U PLEMIEN KONÍ V ČESKEJ REPUBLIKE

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ABSTRACT

In the present study was estimate the genetic diversity and relationships among nine horses breeds in Czech and Slovak Republic.

In conclusion, the main objective of study was to show the level of genetic distance among the horse breeds with different history of breeding of each country. Furthermore, it should be clarified whether these populations and subpopulations are distinct enough from each other to justify defining separate breeds. This research concerns the variability of microsatellite markers in genotypes of horse.

We compared the genetic diversity and distance among nine horse breeds Czech and Slovak Warmblood both of Czech origin, Slovak Warmblood of Slovak origin, Hucul, Hafling, Furioso, Noriker, Silesian Noriker and Bohemian-Moravian Belgian Horse.

In total, 932 animals were genotyped for 17 microsatellites markers (AHT4, AHT5, ASB2, HMS3, HMS6, HMS7, HTG4, HTG10, VHL20, HTG6, HMS2, HTG7, ASB17, ASB23, CA425, HMS1, LEX3) recommended by the International Society of Animal Genetics.

In the different population size, the allele frequencies, observed and expected heterozygosity, test for deviations from Hardy-Weinberg equilibrium and Polymorphism information content have been calculated for each breed. We analyzed genetic distance and diversity among them on the base of the dataset of highly polymorphic set of microsatellites representing all autozomes using set of PowerMarker v3.25 analysis tools and Structure 2.2. programme for results comparison.

Key words: microsatellite, horse, diversity

INTRODUCTION

Introduct on genetic characterization is important to guard breed integrity and conserve breed identity. Furthermore, it is a prerequisite for managing genetic resources (Bjørnstad&Røed 2002). Molecular techniques have been widely used to analyse phylogenetic relationships among various animal groups and different breeds. Microsatellite loci comprise an attractive potential resource to determine population histories and evolutionary processes, as these loci permit simple and accurate typing in combination with high levels of polymorphism and widespread distribution in the genome. The usefulness of microsatellite markers has been documented in many previous equine population genetic studies (e.g. Cañon *et* al. 2000; Aranguren-Méndez *et* al. 2001; Bjørnstad&Røed 2001; Cunningham *et* al. 2001). The present study is focused on using microsatellite markers to characterise genetic structure of horse populations in Czech Republic and Slovak Republic.

MATERIAL AND METHODS

Hair, blood and sperm samples were collected from 932 unrelated individuals of Czech Warmblood, Slovak Warmblood, Slovak Warmblood origin from Slovakia, Hucul, Hafling, Furioso, Noriker, Silesian Noriker and Bohemian-Moravian Belgian Horse. Genomic DNA was isolated using the JETQUICK Tissue DNA Spin Kit and JETQUICK Blood&Cell Culture DNA Spin Kit (Genomed GmbH, Germany), by following the Protocol Handbook. Determination of microsatellites was performed using the PCR reaction with 17-plex horse genotyping kit designed by Applied Biosystems StockMarks® (Applied Biosystems, Foster City, CA, USA). The genotyping of microsatellite markers was performed on ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) by fluorescent fragment analysis and detected by software GeneScan® 3.7 NT. Alleles were asigned by GENOTYPER. Alleles were assingned to alphabetical symbols (B, C, F, G, H, I , J, K, L, M, N, O, P, Q, R, S). Genetic variability of each horse breeds was calculated as number of alleles (N_A) per microsatellite, observed heterozygosity (H_o), expected heterozygosity (H_E), Polymorphism information content (PIC) under Hardy-Weinberg equilibrium (Table 1). PowerMarker v 3.25. was used for noted analysis. Nei's standard distance was measured for graphs construction using PhyloDraw V0.82 programme and Structure 2.2 programme for figure construction.

The breeds were divided into two subgroups warmbloods (Czech Warmblood, Slovak Warmblood, Slovak Warmblood origin from Slovakia, Hucul, Furioso) and coldbloods (Noriker, Silesian Noriker Bohemian-Moravian Belgian horse, Hafling).

RESULTS AND DISCUSSION

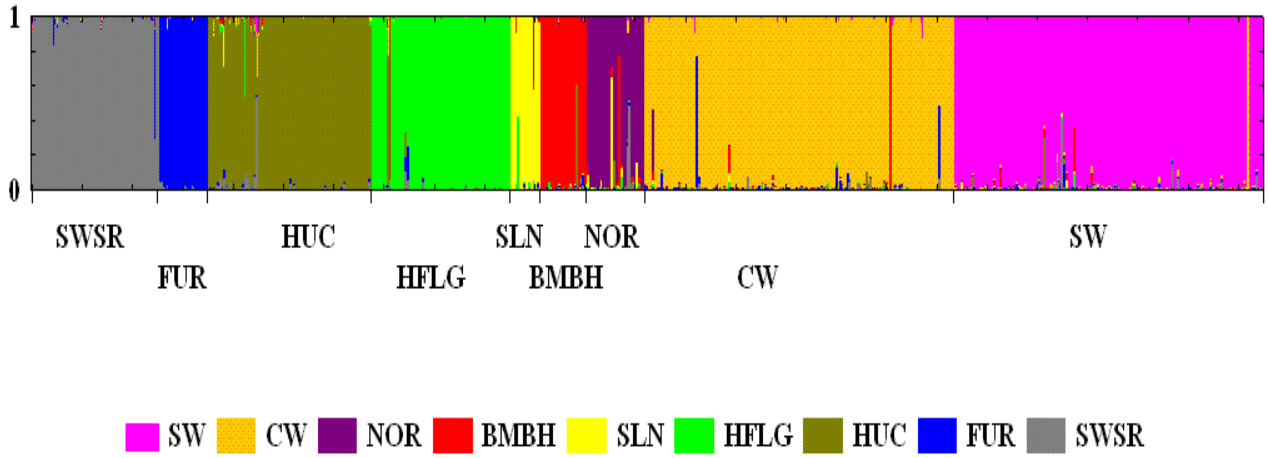
The total number of alleles was 168 across the 17 microsatellites. The number of alleles per locus ranged from 6 (HMS6) to 15 (ASB17). In the data set of all individuals, the average number of alleles was 7.150. The average observed heterozygosity of warmblood horses ranged from 0.716 (Czech Warmblood) to 0.947 (Furioso), in the coldblood

populations varied from 0.711 (Bohemian-Moravian Belgian Horse) to 0.781 (Table 1). Gene diversity (H_e) varied in warmblood horses from 0.748 (Hucul) to 0.777 (Slovak Warmblood origin from Slovakia) and coldblood breeds from 0.686 (Hafling) to 0.722 (Noriker). The Polymorphism information content PIC ranged from 0.641 (Hafling) to 0.743 (Slovak Warmblood origin from Slovakia).

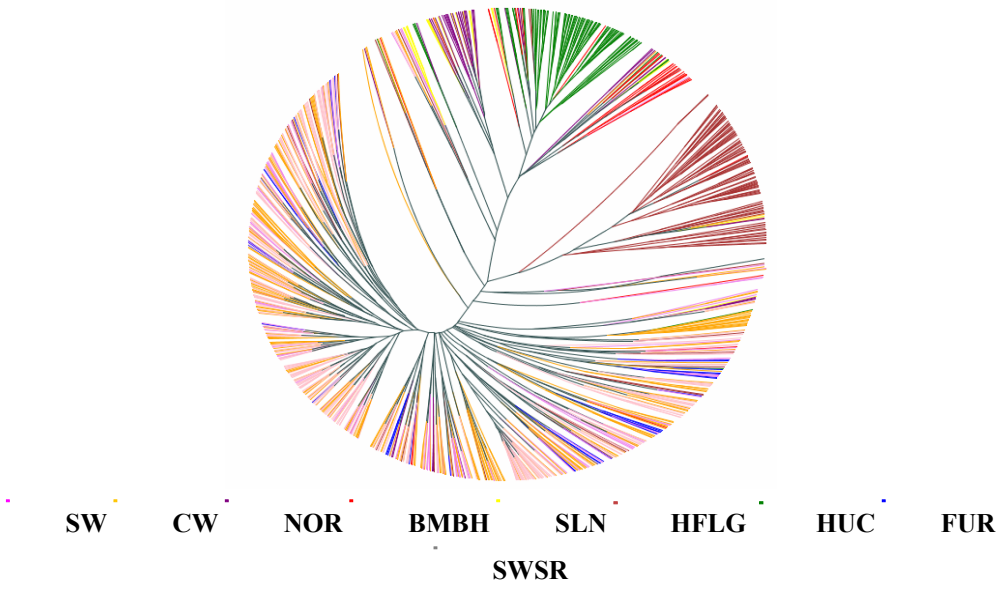
Table 1 Average number of alleles per locus, Heterozygosity H_o-observed. H_e-expected heterozygosity, Average polymorphism information content PIC

Breed	**Average no. of alleles per locus**	**H_o**	**H_e**	**PIC**
Czech Warmblood (CW)	8.294	0.716	0.759	0.724
Slovak Warmblood (SW)	7.882	0.730	0.750	0.710
Slovak Warmblood origin from Slovakia (SWSR)	8.058	0.921	0.777	0.743
Hucul (HUC)	8.235	0.757	0.748	0.716
Furioso (FUR)	6.059	0.947	0.755	0.721
Hafling (HFLG)	6.647	0.781	0.686	0.641
Noriker (NOR)	6.706	0.741	0.722	0.682
Silesian Noriker (SLN)	5.823	0.744	0.707	0.664
Bohemian-Moravian Belgian Horse (BMBH)	6.647	0.711	0.716	0.681

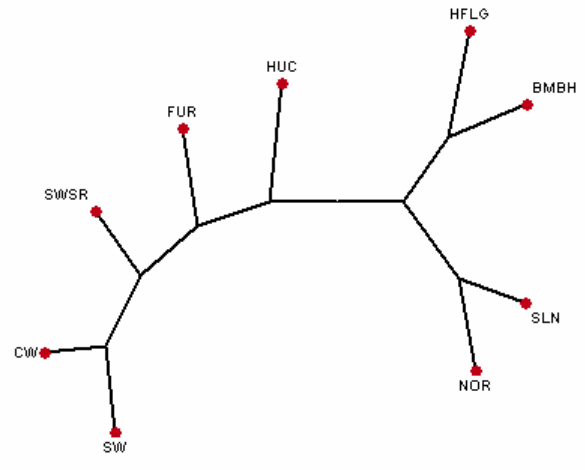
Figure 1 The diversity and population structure of nine horse breeds using Structure 2.2. programme.Each individual is represented by one thin vertical line and each breed is characterized by one color.



Graph 1 The UPGMA dendograms was construced from Nei's standard genetic distance (1972), summarizing genetic distance among 932 horses based on 17 microsatellites.



Graph 2 The neighbour-joining trees was construced from Nei's standard genetic distance (1972) mine horse populations.



SUMMARY

The Bayesian approach detects not only population structures by the identification of clusters, but also allows a probabilistic estimate of the proportion with which an individual belongs to one of the inffered clusters (Glowatzki-Mullis et al. 2005). However, a many andmixtures of different memberships were found in breeds. Hafling horses had partial membership with Bohemian-Moravian Belgian Horse what was unexcepted result.We identified a potential outbreeding in these two breeds as a result of deficient pedigree verification or non correct purebreeding. The Czech Warmblood, Slovak Warmblood, Slovak Warmblood origin from Slovakia and Furioso clustered together. Warmblood horses in Czech and Slovak Republic are formed from local breeds with high introgression of European Warmblood, in Czech Republic is unbalanced mating and in Slovakia is mating based on the Furiosso breed (Figure 1). Graph 1,2 shows high similarity among Czech Warmblood, Slovak Warmblood, Slovak Warmblood origin from Slovakia and Furioso populations. because of opened populations for a number of generations and similar history of breeding programs. Another possible reason of high diversity is a small effort for breeding programe in these populations. Graph 1 shows the Hucul breed is the most homogenous from all of the breeds, as we expected. The Hucul stud book is considered as closed, pureblooded breeding is using strictly pure lines of Hucul breed.

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