

*EVALUATION OF PHYLOGENETIC CONNECTIONS IN LIVESTOCK BREEDING*

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Livestock breeds have been formed by centuries of human and natural selection. Different breeds have been selected to fit several environmental conditions and human needs. The genetic diversity found in domestic breeds allows farmers to develop new use of molecular markers for evaluation of genetic diversity.

Monitoring of genetic polymorphisms within populations is an important component of breed maintenance and reproductive programs in many agricultural species, including horses. Characterization of the population genetic structure can become the first step toward breed preservation and restoration, and contributes to advancing breeding programs (Iwanczyk, et al., 2006). This is particularly important in countries such as the Ukraine, where a method of validating horse identification through genetic testing has not been resolved. The between-breed differences in phenotypic and morphophysiological traits are readily discernible; however, the gene pool differences among the breeds still remain poorly investigated (Stolpovskii, 2010).

Genetic markers provide information about allelic variation at a given locus. The increasing availability of molecular markers in farm animals allows the detailed analyses and evaluation of genetic diversity and furthermore the detection of genes influencing economically important traits.

The majority of molecular markers used nowadays with high-throughput systems are microsatellite markers (simple tandem repeat, STR). Variable number of tandem repeats (VNTRs), random amplified polymorphic DNA (RAPD) markers, single strand conformation polymorphisms (SSCPs), restriction fragment length polymorphisms (RFLPs) and amplified fragment length polymorphisms (AFLPs) markers are not commonly applied in farm animals.

As microsatellites are also frequently polymorphic and evolutionarily conserved in the eukaryotic genome, they provide useful markers for comparative studies of genetic variation, parentage testing, and studies of gene flow (Bjornstad and Roed 2001; Cunningham et al. 2001; Tozaki et al. 2001a) and have recently been the markers of choice for analyses of population structure in wild and domesticated species (Bowcock et al. 1994; Kim et al. 2001; Takahashi et al. 1998). The genetic relationships of many horse populations in Europe have been investigated recently using microsatellites (Canon et al. 2000; Vila et al. 2001). Canon J, Checa ML, Carleos C,

Vega-Pla JL, Vallejo M, and Dunner S, 2000. The genetic structure of Spanish Celtic horse breeds inferred from microsatellite data Vila C, Leonard JA, Gotherstrom A, Marklund S, Sandberg K, Liden K, Wayne RK, and Ellegren H.

Molecular markers are used in genetic diversity studies which give us information about the difference between breeds and the domestication process. Genetic characterization is the first step in breed conservation and may have implications for future breeding strategies. Ease and accuracy of typing, together with high levels of polymorphism and spread distribution in the genome, make microsatellite loci an attractive potential source of information about population histories and evolutionary processes. Microsatellites have been successfully applied to parentage and relatedness testing in horses (Bowling et al. 1997; Marklund et al. 1994) and their usefulness for estimating genetic distances among closely related populations has been documented (Takezaki and Nei 1996).

Horse breeds around the world have been analyzed by microsatellites, including the Przewalski horse (Breen et al. 1994), Spanish Celtic breeds (Canon et al. 2000), Norwegian breeds (Bjornstad et al. 2000; Bjornstad and Roed 2001), and various European and Asian breeds (Tozaki et al. 2003; Vila' et al. 2001). In the present study, we estimated the genetic diversity of the four native breeds, investigated the effect of genetic bottlenecks, and estimated the amount of genetic differentiation between these breeds. We applied three different approaches to evaluate the distribution of genetic diversity and corresponding conservation priorities. This information can be helpful for the correct management of the four native horse populations located in the Cantabrian-Pyrenean area Ishida et al. (1995) Ishida N, Oyunsuren T, Mashima S, Mukoyama H, Saitou N, 1995.

A considerable number of genetic distance studies for several livestock species were carried out during the last years by research teams from all over the world. They were mainly based on microsatellite loci, although a number of other polymorphic systems such as protein polymorphisms, blood groups, or other molecular marker systems were alternatively used (Baumung et al., 2004).

The use of microsatellites has become a standard method to estimate neutral genetic diversity in livestock. Peter et al. (2007) analysed the population structure and the genetic diversity of 57 European sheep breeds derived from 15 European countries. The analyses were based on microsatellite typing followed by principal component analysis and Bayesian model based clustering respectively. As a result of this study, distinct groups of sheep could be identified: Middle Eastern fat tailed sheep, south eastern European sheep and north western/western European sheep.

However, a recent study using the protein polymorphisms of 22 loci from 2,415 horses belonging to 34 horse populations in Japan and Asia (Nozawa et al. 1998) was inconsistent with this two-wave migration. The phylogenetic relationships among Japanese horses could not correspond

to the geographical distribution in Japan (Nozawa et al. 1998). Nozawa K, Shotake T, Ito S, and Kawamoto Y, 1998. Phylogenetic relationships among Japanese native and alien horses estimated by protein polymorphisms. The difference between the phylogenetic relationship and the geographical distribution might be a consequence of a small population size, a bottleneck effect, and low polymorphism for protein loci. Recently many microsatellites were isolated from horse genome DNA (Tozaki et al. 2000a,b,c, 2001b).

Additionally within diversity and phylogeny studies specific mtDNA and Y chromosome markers are used for the identification of maternal and paternal lineages. Additionally within diversity and phylogeny studies specific mtDNA and Y chromosome markers are used for the identification of maternal and paternal lineages.

Mitochondrial DNA sequences of various species of the genus *Equus* with special reference to the phylogenetic relationship between Przewalskii's wild horse and domestic horse. J Mol Evol conducted a phylogenetic study of thoroughbreds, Japanese (Hokkaido) horses, Mongolian horses, and Przewalskii's wild horses using the mitochondrial DNA (mtDNA) D-loop region. The study suggested that the Asian horses were similar to each other and distinct from thoroughbreds.

Formerly, testing blood groups and plasma protein was used to verify parentage and breed composition. The development of DNA-technologies has allowed for the development of more advanced methods of identification. Genotyping of animals by DNA microsatellite loci is widespread. However, these test systems can be not always applied for the analysis of aboriginal breeds due to poor investigation of their genomes with respect to the microsatellite alleles and their frequencies. The high cost of the equipment and kits also makes their use problematic for mass population studies. Nevertheless, other DNA-genetic methods of identification such as Inter-Simple Sequence Repeat (ISSR) analysis are available and are more adaptable to the Ukrainian equine industry. This technology is more easily adapted to the study of unique breeds where nucleotide sequences are unknown and less expensive than Simple Sequence Repeat (SSR)-PCR techniques used for microsatellite genotyping. The ISSR-PCR method of amplifying intermicrosatellites fragments of DNA allows estimation of genomic variability between two inverted SSR-loci (Zietkiewicz, et. al., 1994; Borner, et. al., 2002; Bardukov, 2010; Metlitzka, 2012). ISSR-PCR is characterized by reproducibility and can be effectively used to examine genetic variation within and across breeds, and enable authentication of species and populations when compared with other methods of multiloci profiling (Stolpovskii, 2010, Feofilov, 2011).

Analysis of the spectra of amplification products of the regions between the inverted repeats of microsatellite loci was used for identification of interspecific and interbreed differences in cattle (subfamily Bovinae) (Azari, et. al., 2006; Stolpovskii, et. al., 2011), yaks (Azari, et. al., 2006), sheep (Stolpovskii, 2010), deer (Kol, and Lazebnyi, 2006) and other species.

ISSR DNA markers have been applied to the determination of polymorphism and genetic variety in plant-growing (Raina, et al., 2001; Bento, et al., 2008), but they have not been as broadly applied in livestock experiments. According to our results, this type of marker is useful for determination of polymorphism and genetic distance in horse breeding.

The last 10 years in world molecular genetics is sanctified as studying of single nucleotides polymorphism (SNP). Their enormous amount in the genomes of every type of agricultural animals and newest technologies for genotyping by this markers force other types of markers out of genetic variety of kinds and breeds researches sphere.

As well as in the sheep breeding, cattle breeding and pig breeding of study of genetic variety of breeds of horse comes true as a rule in national and regional scales without bringing in research of genetic resources from other regions. It is necessary bringing in of different standards of genetic material and application of standard set of markers for the deeper and generalized understanding of different types of domestic animals variety. Then possible combining of results got after such analysis in different regions and within the limits of analyses.

Such analysis of genetic variety and genetic distance between breeds for the use of different types of markers enables placing of priority at the decision of breed maintenance problem

Thus, determination of genetic polymorphism within the limits of populations is the important constituent of support of breed and genesial programs of many agricultural kinds.

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