

Review paper

NEW EVIDENCES ON DOMESTICATION OF THE HORSE (*Equus ferus caballus*) AND ORIGINE OF DOMESTIC MOUNTAIN PONY*Trailović R.¹, Đermanović V.*², Mitrović S.², Savić M.¹, Dimitrijević V.¹*¹Faculty of Veterinary Medicine University of Belgrade, Bulevar Oslobođenja 18, 11000 Belgrade, Serbia²University of Belgrade, Faculty of Agriculture, Institute for Zootechnique, Nemanjina 6, 11080, Zemun, Serbia*Corresponding author: djermanovic@agrif.bg.ac.rs**Abstract**

The results of draft genome research of permafrost horse bone dated approximately 560-780 (kyr BP) have been published recently. According to the data representing the oldest full genome sequence compared to the modern genomes of domesticated horse, Przewalskii and donkey it was suggested that the *Equus* lineage gave rise to contemporary horses, zebras and donkeys some 4.0 to 4.5 million years ago, twice increasing the assumed time to the common ancestor. Upon the phylogenetic and divergence analyses it was estimated that the Przewalskii and domestic horse populations diverged some 38-72 kyr BP and that there are no evidences that admixtures between Przewalskii and domestic horse populations occurred post-divergently thus Przewalskii's involvement in the process of horse domestication has been excluded.

The new data on ancient equide genome question the conventional theory on the origin of Domestic mountain pony. Upon the protein variability of the Domestic mountain pony the influence of diluvial genome on the population was confirmed and this finding differed from the conventional theory stating *E. f. Przewalskii* and *E. ferus caballus* as ancestors of the autochthonous population. The role of different ancestral horses in evolution of our autochthonous population is therefore unclear.

The questions emerging from the most recent genetic and proteomic research in concern with the autochthonous populations are presented in the paper.

Key words: *fossil DNA, horse, wild ancestors***Introduction**

Although the fossil findings of equine ancestors made these mammals the model for evolutionary processes, the domestication process remained unsatisfactory cleared until recently. The evidences on horse domestication, found in grassland at the space between nowadays Russia and Ukraine, were calibrated to be 5-6 million years old, while the artifacts speaking of the utilization of these animals belong to the period 6,000-3,000 years B.C. Other centers of horse domestication were determined on different geographic regions so it was considered that domesticated horse arose through independent domestication events that have involved different ancestral subspecies of wild horse. The multicentric and polyphyletic origin can be the major factor contributing extensive diversity in domesticated

horse. It was also recognized that mutations occurred rarely during domestication so separate equine gen pools were geographically differentiated (Ellegren, 2002).

Anyway the importance of the horse in transportation and human migrations through history led to crossing between the separate domesticated populations and thus influencing the gene pool of the original domesticated breeds and types (Kaminski and de Andres Cara, 1986).

The origin of horse populations in Europe, North Africa and in West Asia is traceable back to the prehistoric forms. Therefore the contemporary horse breeds are classified as occidental, oriental and horses of East and South-East Asia. The pre-domestication horses belonged to the four (4) original types, i.e.: horse of North-West Europe, which assembled to contemporary Exmore pony (type 1); a larger pony of North Europe and Asia similar to Highland pony (type 2); horses inhabiting Central Asia (type 3) probably ascending Akhalteka and similar breeds; and horses of West Asia (type 4) as probable progenitor of Kaspian pony and Arab. Oriental horses descend from two ancestral forms: Tarpan (*E. ferus ferus*) and Przewalskii (*E. Przewalski*), while occidental horses have developed from diluvial forest pony. The dualistic evolutive approach has been undoubtedly incorporated into the existing theory on autochthonous horses of the Balkan (Savić et al., 2007).

Studies on polymorphism in horses lead to the development of evolutive-genetics as new discipline allowing testing and clarification of the evolutive process including effects of domestication and selective breeding. The data obtained by testing of genetic variability could be applied for characterization of the breeds and interpretation of evolutive-genetic relationship between the different population and breeds, even in absence of pedigree data (Langlois, 2005). The fact that wild ancestors of domesticated horses have been extinct their traits could be traced only by comparisons of the different domesticated breeds as carriers of the original wild genetic variance (Sponenberg, 2000). Only recently, the variability of the DNA molecule allowed investigation of the variability in the fossils, so allowing the comparison of the ancient and contemporary animals on the level other than comparison of the skeletons (Ludovic et al., 2013; Trailović et al., 2013a and 2013b).

Genetic variability applied in the studies of the equine evolution

Genetically controlled variability is profoundly studied in domestic animals and applied in characterization of different breeds and types, aiming to evaluate origin and course of domestication, and to establish population data necessary for preservation and/or conservation of rare and unique traits that can influence adaptation and unpredictable selective challenges in the future. The data concerning genetic characterization are obtained by evaluation of biochemical, physiological and DNA polymorphism (Trailović et al., 1994 and 1998; Trailović, 2009). Genetic polymorphism has been applied in the research of genetic variability in wild equines, while DNA variability and sequencing allowed insight in genetic constitution of the equines before domestication (Ellegren, 2002; Ludovic et al., 2013). Polymorphism of the different proteins, blood groups, MHC markers, DNA markers together with genome mapping and research of the genome sequence allowed studies of the interbreed divergence. The genome sequencing and equine gene mapping gave rise to the profound investigation of the genetic traits of the ancient equines by DNA analyses of the fossil remnants (Ludovic et al., 2013).

The evaluations of genetic variability on different levels show that some breed specific alleles reflect the heritage and therefore indicate possible ancestry of the modern breeds of horses (Trailović, 2009, Trailović et al., 2013a and 2013b). Although numerous allelic

forms in modern equine species are common, some alleles are species specific and therefore reflect divergence of the species (Put and Whitehouse, 1983; Nozawa et al., 1998; Cho and Cho, 2004). Research of genetic polymorphism and collection of the data gave rise to the debate on the role of Przewalskii horse during domestication.

Evaluations of the biochemical polymorphism revealed high degree of homology between Przewalskii and domestic horse breeds, although frequent alleles in Przewalski are rare in any of the domestic horse population studied and some Przewalski characteristic alleles have been revealed both by blood typing and by genotyping of the remaining wild horse (Putt and Whitehouse, 1983; Bowling and Ryder, 1987). It was suggested that Przewalski and domesticated horses had separated before domestication events and that the separate evolution gave rise to speciation of the two horse species.

During the last decade equine gene mapping allowed presentation of the medium dense gene map and revealed genome sequence in horses. The methodology of DNA studies has developed enough to allow amplification and sequencing of ancient DNA.

By comparison of genomic data obtained on Late Pleistocene horse remnants and modern genomes of five domestic horse breeds (*E. ferus caballus*), Przewalskii (*E. Przewalski*) and donkey (*E. asinus*) the evolution of different equines was recalibrated and the role of the different ancient horses in domestication was clarified (Ludovic et al., 2013). The discovery and sequencing of the genomic DNA in Late Pleistocene horse fossil confirmed that Przewalskii is the last surviving wild horse population. The comparative analyses of the genome sequence of the Late Pleistocene horse and modern genomes of five domestic breeds, Przewalskii horse and donkey suggested that *Equus* lineage that gave rise to all contemporary horses, zebras and donkeys originated 4.0-4.5 million years before present (myr BP), twice the time that were accepted as the distance to the most recent common ancestor of the genus *Equus*. It was estimated that Przewalskii's and domesticated horse populations diverged 38-72 kyr BP and no admixture between the two diverging populations was evidenced. The results obtained by Ludovic et al. (2013) exclude Przewalskii horse from the domestication process.

The novel finding on *Equus* evolution posed several questions on ancestry of our autochthonous Yugoslav mountain pony, as well as the other horses on the Balkan.

The ancestry of autochthonous Yugoslav Mountain pony

Yugoslav Mountain pony is autochthonous horse breed that inhabits mountains South of Sava and Danube on West Balkan. It is believed that all mountain ponies of former Yugoslavia descended from Tarpan (*Equus ferus*) and Przewalskii (*Equus Przewalskii*).

Although the dispersed natural habitat of this breed is biogeographically divided by natural barriers, all ponies on the mountains in Serbia and Montenegro were considered to belong to autochthonous Yugoslav Mountain pony breed and the data concerning biogeographic differentiation of local ecotypes are missing. There is evident lack of interest reflected in few scientific data about this very adaptive breed which indicates the degree of endangerment of these unique animals (Mitrovic et al., 2011). On the other hand mountain ponies in B&H have been described in details by the beginning of the 20th century and besides numerous ecotypes, Bosnian pony was developed as highly selected breed.

Recently, some physiological, morphological, and reproductive characteristics of Autochthonous Yugoslav mountain pony have been described (Trailović et al., 1998;

Trailović, 2009; Đermanović et al., 2010; Djermanovic et al., 2012; Trailović et al., 2013a and 2013b).

The functional polymorphism within the breed was evaluated in aim to confirm the dualistic origin of autochthonous breed upon biochemical variability, to investigate genetic divergence between original population of Yugoslav Mountain pony and descending selected breed Bosnian pony, and also in aim to establish the data for further evaluation of physiology of the breed and to obtain information on functional variability in aim to promote conservation (Trailović, 2009).

During the investigation polymorphism in eight protein systems: albumin, transferring, protease inhibitors, Xk, vitamin D binding protein, esterase, phospho-glucose dehydrogenase and glucose-phosphate isomerase was established. A total of 33 functional alleles was segregating in Domestic mountain pony compared to 26 in Bosnian pony. The observed loss of functional polymorphism in descending population reveals restriction of the gene pool that could be the consequence of the limited number of founding animals and selective breeding. However none of the Przewalskii horse specific biochemical markers was determined in autochthonous population, thus the dualistic origin could not be confirmed upon the investigated biochemical polymorphism. Unexpectedly the rare Al¹ isoprotein, which is considered to be the heritage of diluvial ancestors (Sandberg, 1972) was determined in Domestic mountain pony. The ancestral influence of the diluvial genome on gene pool of autochthonous horse in Former Yugoslavia was not described until our investigation. The introduction of diluvial horses to the Balkans could occur during prehistoric nomad migrations and certainly in Roman Empire.

Conclusion

Very recent evidences obtained by genome sequence analyses of early Middle Pleistocene horse allowed recalibrating of evolution of the genus *Equus* and profound comparison of the prehistoric and contemporary horses thus clarifying the lineage of all contemporary horses. These results also indicated that no recent admixture between the domestic horse breeds and Przewalskii occurred after the divergence of the species some 38-72 kyr BP (Ludovic et al., 2013), so that it was cleared that Przewalskii horse could not be involved into the horse domestication.

The novel finding therefore poses questions on the existing theory concerning the evolution of autochthonous Yugoslav mountain pony especially when Przewalskii is involved in ancestry. However the ancestral role of Tarpan is not questionable, but the original finding of the distribution of the diluvial genome markers in the autochthonous population of Yugoslav mountain pony however demands further research on routes and period of introduction of diluvial horse genome on the Balkans.

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