Variation Of Coat Color Genotypes Pinpoints Down The Roots Of Horse Domestication

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The transformation of wild animals making animals accessible for human nutrition was an essential prerequisite for our modern days' human societies. Among the early domesticated animals, two species differ from all others by having been bred not mainly as food resource; the dog and the horse. While the dog may arguable have been the first domestic animal, and may be considered human's closest companion, horses attained their special role as animals of transportation and especially warfare, changing societies on a continent-wide scale be it with Alexander the Greta's or Genghis Khan's armies invading most of Asia and Eastern Europe or Francisco Pizarro destroying the Inca Empire with about 30 mounted warriors. The horse was a costly and prestigious animal in all times, featured in gifts from one sovereign to another as nobleman's mark (Levine 1999, Hyland 2003). For these reasons, deciphering the spatial and temporal origin of domestic horses is of key importance for understanding the origin of modern human societies and has consequently attracted considerable scientific as well as public interest since centuries. Despite the pivotal role, horses have played in the history of human societies; the process of their domestication is not well understood. The huge variability of mitochondrial (mt) haplotypes (Vila et al. 2001, Jansen et al. 2002, Keyser-Tracqui et al. 2005, McGahern et al. 2006), contrasting with many other domesticated species have been prevented any conclusions so far. Unfortunately, the apparent lack of phylogeographic structure of mt haplotypes the in recent breeds (Vila et al. 2001) as well as in early domesticated horses from the beginning of the 3rd century BC (Keyser-Tracqui et al. 2005) blurred any conclusions on their domestication. In this study, we extended the ancient mtDNA sample set of Eurasian steppe horses by sequencing a highly variable part of the control region from more than 100 ancient horses, including both wild and early domestic horses covering a geographic range from China to Spain and spanning a time frame from the Pleistocene to the Iron Age. Unfortunately, mtDNA results do allow only the exclusion of Iberian Peninsula as centre of horse domestication (as recently also found by Lira et al. 2010), because the observed complex pattern of mtDNA haplotypes suggests that present day diversity existed already within separated wild populations before their domestication. Moreover, we find no significant geographical structure of mtDNA, preventing any conclusions about the centers of domestication, as well as about the question whether domestication of the horse took place once at a single place or independently in several locations as argued by some authors (Vila 2001). In fact, the combination of high genetic diversity within individual populations in combination with the lack of a phylogeographic pattern in pre-domestication horse mitochondrial DNA makes it unlikely that light can be shed on the mystery of horse domestication using mtDNA. Also studies of the paternal variability failed to detect place and time of horse domestication because there is zero variation of the heterosomal part of horse y-chromosome (Lindgreen et al. 2004).

Therefore, we chose to follow a new approach. Artificial domestication experiments on rats and foxes (Belyaev and Trut 1975) have shown that even if not intended, coat colour variation will increase rapidly in domesticated species. Moreover, modern domesticated animals are characterized by a huge variability of coat colorations. As coat color is an easily detectable and selectable phenotypic trait, we believe that this trait was an important goal of selective breeding since ancient times and consequently this trait has great capability to elucidate horse domestication. While such an ancient DNA approach would have been deemed illusory a few years ago, recent progress in ancient DNA extraction and amplification, have allowed the analysis of targeted nuclear DNA sequences even from non-permafrost specimens. As the amplification success of ancient DNA decreases rapidly with increasing amplification length, especially the analysis of single-nucleotide-polymorphisms (SNPs), which requires extremely short amplifications, is a promising approach for ancient DNA studies targeting nuclear genes. We adapted these SNP analyses on the prerequisites of ancient DNA investigations. Not surprisingly, success rate of aDNA amplification depended on the climate (91% in Siberia, 52% in East Europe and 35% in Spain). As samples for the wild populations, we analyzed bones from Late Pleistocene and Early Holocene horses originating from Siberia, East and Central Europe and the Iberian Peninsula. All Siberian and European Pleistocene horses had the *non-black* allele (*A*) for the *ASIP* gene. Therefore, bay seems to be the primary *wildtype* for these regions during the Late Pleistocene. Iberian Early Holocene wild horses shared both the non-black allele *A* as well as the black allele *a* resulting in four black and four bay horses. No chestnut allele *e* in the responsible *MC1R* locus was observed within Late Pleistocene or Early Holocene horses. Analysis of Neolithic and Copper Age horses, representing the millennia before the beginning of domestication, as far as this is known from archeological findings produced similar allelic patterns. Unfortunately, this period is characterized by a lack of horse bones in the archaeological record of some regions, which prevents the inclusion of Siberian horses from the 6th up to 3rd mill. BC. Lowland horses from East Europe (Romania, Ukraine; 5th-4th mill. BC) revealed a mixed population of bay and black phenotypes. All of these horses are regarded as wild animals as they clearly predate the earliest unambiguous evidence for horse husbandry in this region (Benecke 1999, 2002, 2006; Benecke & Driesch 2003). As black horses are missing during the Late Pleistocene, their occurrence in the Early Holocene might be likely the result of postglacial immigration and supplementation. The opposite explanation, human selection, is less likely because we would expect also other colorations as only black and bay horses during the 5th and 4th mill. BC. A large proportion of wild black Mesolithic/Neolithic horses were also found for the Iberian Peninsula and no shift was observed for Bronze Age horses. In contrast, a rapid, significant shift and an increase in color variants (including dilutions and spottings) are demonstrated for Siberia and East Europe starting in the 3rd mill. BC. First evidence for the chestnut phenotype was observed in Siberian horses and later in East European horses. Chestnut is recessive against black. The chestnut (*fox*) allele (*e*) turned up in a Romanian sample from the late 5th mill. BC for the first time; the first chestnut phenotype observed in a Siberian horse dates to the 3rd mill. BC. The chestnut phenotype increased very rapidly at the Bronze Age. In the same way, the number of bay horses drops down during Bronze Age and Iron Age. Mutations responsible for dilution or spotting phenotypes appeared later in domestic horses. Both cream dilution (buckskin) and silver dilution (black silver) (800–400 BC) were observed in Siberian horses for the first time. However, they never reached the same frequency as chestnut. In the complex of coat color pattern we observed the Tobiano genotype in a Chinese horse (1200-800 BC) demonstrating its ancient history. Tobiano is widespread today. Chestnut Sabino genotype was discovered in a Middle Bronze Age (1410-1250 BC) Armenian horse. The Sabino phenotype is European coloration known from Scandinavian horses of 18th centuries for example but *SB1* mutation (*Kit*) causing Sabino pattern is only found in North American horses today. Overo which is not common in Europe but locally present in American breeds today is considered as a “new” mutation because we did not found it in our ancient samples. In contrast to Siberia and East Europe, we observed only black or bay phenotypes in Spanish samples until medieval times. No change in genotype distribution was observed during the Neolithic or the Bronze Age. As sample size for this periods is quite small depending on storing conditions of ancient bones this might be purely coincidental but taking their mt haplotypes under consideration, we found no evidence for independent horse domestication at the Iberian Peninsula.

So far research has been focused on detection of domestic animals at a side but selective breeding causes the difference between taming and domestication resulting in a massive transformation of both genotypes and phenotypes. By addressing such traits of human selection, research can move behind the simple designation of remains as domestic or wild addressing the more complex goals of animal husbandry. We conclude that coat coloration appears to be such a powerful marker of horse domestication. Based on our results, horse domestication started in Eurasian steppe region and Balkan Peninsula about 5000 years ago. Filling existing gaps, we recommend to study more samples from the transition to Copper/Bronze Age allowing a more precisely estimation of the starting date in the various regions of Eurasia. Nevertheless our data give a very clear cut between wild and domestic horses for the first time.

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