Maternal and paternal genetic variation in Estonian local horse breeds in the context of geographically adjacent and distant Eurasian breeds

E. Sild, S. Värv, T. Kaart, J. Kantanen, R. Popov and H. Viinalass

Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, Tartu 51014, Estonia. Natural Resources Institute Finland (Luke), Myllytie 1, Alimentum, Jokioinen FI-31600, Finland. Yakutian Research Institute of Agriculture (FGBNU Yakutskij NIISH), ul. Bestyzhevo-Marlinskogo 23/1, Yakutsk 677001, The Sakha Republic (Yakutia), Russia.

Summary

The maternal and paternal genetic variation of horse breeds from the Baltic Sea region, including three local Estonian breeds, was assessed and compared with that of Altai and Yakutian horses. In the mtDNA D-loop region, 72 haplotypes assigned to 20 haplogroups in the nine breeds were detected. In Estonian local breeds, 38 mtDNA haplotypes were found, and five of them were shared by the three breeds. More than 60% of all identified haplotypes were rare. Compared with the Estonian Native and Estonian Heavy Draught breeds, a higher haplotypic diversity was found in the Tori breed (h = 0.969). Moreover, four haplotypes shared among Finnish and Estonian local horse breeds indicated ancient ancestry, and of these, H30 (haplogroup D3) showed global sharing and genetic links between modern Baltic Sea region and Siberian horses, specifically. The studied breed set showed high variability in maternal inheritance and mixed patterns of the international and native breeds of the Siberian and Baltic regions. No variation was found in paternally inherited markers among horse breeds in the Baltic Sea region.

Keywords Baltic Sea region, Estonian local horse breeds, mtDNA, Siberia, Y chromosome

Genetic characterization of farm animal breeds is an important task in the conservation of animal genetic resources at the national and international levels. Variations in mtDNA and Y chromosomal markers in farm animals have been used to investigate domestication, phylogenetic relationships among populations, and temporal and spatial changes in maternal and paternal lineages (Kantanen et al. 2009; Rannamae et al. 2016). Genetic variation in mitogenomes is typically interpreted as reflecting the survival of ancestral variability in modern breeds rather than being a result of animal breeding and artificial selection (Cieslak et al. 2010). Although high mtDNA variation in horse breeds has been observed in terms of haplotype and haplogroup diversity, the Y chromosomal variation has been low, and it appears that very few stallions contributed to the paternal genetic diversity of horses (Lindgren et al. 2004; Wallner et al. 2004, 2017; Brandariz-Fontes et al. 2013; Kreutzmann et al. 2014).

Comparative analysis of male-specific diversity based on sequencing data in American, Asian and European breeds detected that the Asian breeds in particular showed more male lineages compared with the European breeds (Felkel et al. 2018).

Based on the local native horse population in the Estonian territory in the nineteenth century, three different breeds were developed, and of these, the Estonian Native breed has maintained some indigenous characteristics (e.g. dorsal stripe and smaller body size compared with the Tori and Heavy Draught breeds). As described by Petersen et al. (2013) and Sild et al. (2019), the genetic relationship between North European and Central Siberian horses exists on the basis of autosomal markers. Here, our aim was to investigate genetic diversity and relationships using maternal and paternal genetic markers in Estonian, Finnish, Latvian and Siberian (Altai and Yakutian) breeds.

Hair or blood samples were collected from 259 horses of nine breeds (the pedigree information for the Altai horse was not known, the samples from Yakutian horses were collected from three different villages and the horses from the rest of the breeds were unrelated considering at least two generations; Table S1) and analysed for the mtDNA D-loop sequence and five loci of Y chromosome-specific
microsatellites (Table S2). For mtDNA D-loop sequences, the alignment and nucleotide position determination were based on reference sample X79547 (Xu & Arnason 1994). 

**Table 1** mtDNA diversity estimates in the analysed breeds

| Breed             | n  | nH  | n_unique   | h      | p     | Tajima's D |
|-------------------|----|-----|------------|--------|-------|------------|
| Altai             | 11 | 7   | 3          | 0.909  | 0.017 | 0.03       |
| Arabian           | 29 | 9   | 1          | 0.882  | 0.016 | 0.64       |
| Estonian Heavy    | 30 | 13  | 3          | 0.871  | 0.011 | -0.66      |
| Draught           |    |     |            |        |       |            |
| Estonian Native   | 40 | 19  | 6          | 0.947  | 0.016 | 0.43       |
| Horse             |    |     |            |        |       |            |
| Finnhorse         | 37 | 18  | 6          | 0.926  | 0.016 | -0.04      |
| Latvian           | 22 | 17  | 8          | 0.978  | 0.018 | 0.15       |
| Tori              | 40 | 25  | 8          | 0.969  | 0.015 | -0.08      |
| Trakehner         | 23 | 16  | 5          | 0.964  | 0.016 | 0.24       |
| Yakutian          | 27 | 11  | 6          | 0.789  | 0.012 | -0.13      |
| All               | 259| 72  | -          | 0.964  | 0.016 | -0.09      |

n, sample size; nH, number of haplotypes; n_unique, number of unique haplotypes; h, haplotype diversity; p, nucleotide diversity.

with the neutral mutation model, the lowest (−0.70, Estonian Heavy Draught) and highest (0.43, Estonian Native) values indicated differences in population genetic processes.

Focusing only on Estonian breeds, it follows that, of the 110 horses representing Estonian Native, Estonian Heavy Draught and Tori breeds, 42 (38%) carried one of the five haplotypes shared (Fig. 1a, b). There were 38 different haplotypes in the three Estonian local breeds. In total, 63% of the haplotypes were rare and detected in only one or two horses; of these haplotypes, the highest number was counted in the Tori breed (14; Fig. 1b, Table S5). The variation in modern Estonian local breeds is probably, to some extent, due to the preference for various mare types at the breed’s formation stage.

For both the Estonian Native and Estonian Heavy Draught, the breeds’ gene pools have been influenced by major declines in numbers. In the case of the Heavy Draught, there has also been some introgression from other cold-blooded breeds, and for the Estonian Native, one of the influencing factors has been temporal population expansion.

In total, 20 haplogroups were determined (Table S6), and the number of haplogroups ranged from seven (Altai and Yakutian) to 14 (Tori and Trakehner). One haplogroup (D3) was common in all nine breeds and was the most frequent in the Yakutian Horse (44%). Haplogroup D3 with F, B1 and X3c1 included five common haplotypes shared among the Estonian local breeds.

The occurrence of haplogroups A, B1 and X3c1 could be interpreted as a genetic contribution of the oldest/ancient native matriline in the Baltic Sea region. Dating back to the first archaeological equine findings in Estonia, the sequences in these horses determined by Vilà et al. (2001) corresponded most likely to haplogroup X3c1. Haplogroup X3c1 (appearing in 1500 BC according to Cieslak et al. (2010) showed a similar proportion (12–17%) in Estonian...
local breeds and the Latvian Horse. The constructed haplogroup network (Fig. S1) showed the grouping of horses from both native and international breeds into the same haplogroups.

None of the studied breeds showed a high frequency of unique alleles and genetic distinctiveness in the mtDNA data, but the distribution of haplotypes revealed relationships among geographically distant breeds. Only one haplotype (H18 of haplogroup F) was shared across all local breeds in the Baltic Sea region. Four different haplotypes were shared among the Finnhorse and Estonian local breeds (Fig. S2), with the predominating maternal line (haplogroup D3) pointing to global sharing and to genetic links between the Baltic Sea region and Siberian horses, specifically.

In conclusion, the studied breed set showed high variability in maternal inheritance, and mixed patterns of the international and native breeds of the Siberian and Baltic regions were revealed in the haplotype network. Further analysis is needed to determine the male-specific phylogeny of Baltic horse breeds accurately.

All breeds in the Baltic Sea region (except the Estonian Heavy Draught breed) showed more maternal line variation compared with Siberian Altai and Yakutian horses, indicating multiple origins in the Baltic Sea region. Although the maternal variation of the ancestral population of Estonian breeds that served as a primary genetic resource during breed formation has been lost from the Estonian Native breed, it has been maintained by rare haplotype distribution within modern Tori horses. Matrilineal information could be used for better management of endangered breeds and for the amendment of conservation programmes.

Acknowledgements

We are grateful to Agnes Viluma who provided us with the Latvian Horse samples. This work was supported by institutional research funding IUT-2 of the Estonian Ministry of Education and Research, and the Academy of Finland (The Arctic Ark—project in the ARKTIKO research program, decision no. 286040).

References

Bandelt H.-J., Forster P., Sykes B.C. & Richards M.B. (1995) Mitochondrial portraits of human populations. *Genetics* **141**, 743–53.

Brandariz-Fontes C., Leonard J.A., Vega-Pla J.L., Backström N., Lindgren G., Lippold S. & Rico C. (2013) Y-chromosome analysis in Retuertas horses. *PLoS ONE* **3**1, e64985.

Cieslak M., Pruvost M., Benecke N., Hofreiter M., Morales A., Reissmann M. & Ludwig A. (2010) Origin and history of mitochondrial DNA lineages in domestic horses. *PLoS ONE* **5**, e15311.

Felkel S., Vogel C., Rigler D. et al. (2018) Asian horses deepen the MSY phylogeny. *Animal Genetics* **49**, 90–3.

Fu Y.X. & Li W.H. (1993) Statistical tests of neutrality of mutations. *Genetics* **133**, 693–709.

Jukes T.H. & Cantor C.R. (1969) Evolution of protein molecules. In: *Mammalian Protein Metabolism* (Ed. by H.N. Munro), pp. 21–132. Academic Press, New York, NY.

Kantanen J., Edwards C.J., Bradley D.G. et al. (2009) Maternal and paternal genealogy of Eurasian taurine cattle (*Bos taurus*). *Heredity* **103**, 404–15.

Kimura M. (1983) *The Neutral Theory of Molecular Evolution*. Cambridge University Press, Cambridge, UK.

Kreutzmann N., Brem G. & Wallner B. (2014) The domestic horse harbours Y-chromosomal microsatellite polymorphism only on two widely distributed male lineages. *Animal Genetics* **45**, 460.

Librado P. & Rozas J. (2009) *DnaSP v5*: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–2.

Lindgren G., Backström N., Swinburne J., Hellborg L., Einarsson A., Sandberg K., Cothran G., Vila C., Binns M. & Ellegren H. (2004) Limited number of patrilines in horse domestication. *Natural Genet*ics **36**, 335–6.

Lynch M. & Crease T.J. (1990) The analysis of population survey data on DNA sequence variation. *Molecular Biology and Evolution* **7**, 377–94.

Peakall R. & Smouse P. E. (2012) *GENALEX 6.5*: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* **28**, 2537–2539.
Petersen J.L., Mickelson J.R., Cothran E.G. et al. (2013) Genetic diversity in the modern horse illustrated from genome-wide SNP data. PLoS ONE 8, e54997.

Rannamäe E., Lõugas L., Niemi M., Kantanen J., Mäldre L., Kadõrova N. & Saarma U. (2016) Maternal and paternal genetic diversity of ancient sheep in Estonia from the Late Bronze Age to the post-medieval period and comparison with other regions in Eurasia. Animal Genetics 47, 208–18.

Sild E., Roomi K., Värv S., Roed K., Popov R., Kantanen J. & Viinlass H. (2019) Genetic diversity of Estonian horse breeds and their genetic affinity to northern European and some Asian breeds. Livestock Science 220, 57–66.

Vilà C., Leonard J. A., Götheström A., Marklund S., Sandberg K., Liden K., Wayne R. K. & Ellegren H. (2001) Widespread origins of domestic horse lineages. Science 291, 474–477.

Wallner B., Piumi F., Brem G., Müller M. & Achmann R. (2004) Isolation of Y chromosome-specific microsatellites in the horse and cross-species amplification in the genus Equus. Journal of Heredity 95, 158–64.

Wallner B., Palmieri N., Vogl C. et al. (2017) Y chromosome uncovers the recent oriental origin of modern stallions. Current Biology 13, 2029–35.

Xu X.F. & Arnason U. (1994) The complete mitochondrial-DNA sequence of the horse, Equus caballus—extensive heteroplasmy of the control region. Genetics 148, 357–62.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Median joining network of haplogroups (nomenclature according to Cieslak et al. (2010), based on 43 haplotypes (calculated from 41 polymorphic sites, with four hotspots removed).

Figure S2 Euler diagrams of Estonian and Finnish local breeds presenting (a) the number of horses according to their haplotype shared by or unique to different breeds and (b) the number of haplotypes common or unique to different breeds (EstN, Estonian Native Horse; EstH, Estonian Heavy Draught; Tori, Tori Horse; Finn, Finnhorse).

Table S1 Sample information of studied horse breeds.

Table S2 Primers used for Y-chromosomal markers and mtDNA D-loop sequence [between nucleotides 15 343 and 15 852 (509 bp; HVR1 region)].

Table S3 MtDNA D-loop HVR1 region variation and derived haplotypes (N = 72) based on 45 polymorphic sites.

Table S4 Y-chromosome microsatellite genotyping data.

Table S5 Haplotypic distributions of mtDNA.

Table S6 Occurrence of haplogroups (nomenclature used by Cieslak et al. 2010).