

Genetic relationship between Mongolian and Norwegian horses?

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Summary

Human populations of Central Asian origin have contributed genetic material to northern European populations. It is likely that migrating humans carried livestock to ensure food and ease transportation. Thus, eastern genes could also have dispersed to northern European livestock populations. Using microsatellite data, we here report that the essentially different genetic distances D_A and $(\delta\mu)^2$ and their corresponding phylogenetic trees show close associations between the Mongolian native horse and northern European horse breeds. The genetic distances between the northern European breeds and Standardbred/Thoroughbred, representing a southern-derived source of horses, were notably larger. We suggest that contribution of genetic material from eastern horses to northern European populations is likely to have occurred.

Keywords dispersal, horse breed history, microsatellites, migration, Mongolian horse, Northern European horse breeds.

Migration is an important force shaping the social structure, evolution and genetics of populations. Migrating people conceivably carried livestock to ensure food and transportation, thus generating associations between human and livestock movements (Medjugorac *et al.* 1994). Deep traditions for horse breeding exist in the central Asian steppes, exemplified through the great expansions of the Mongolian Empire under Attila (5th century) and Genghis Khan (13th century), conducted on horseback. Compatible with what partly could be the origin of the northern European human populations (Zerjal *et al.* 1997), central Asian populations could also have played a notable role in the genetic constitution of northern European horses. The origins of at least two native Norwegian horse breeds, the Nordland/Lyngen horse and the Fjord horse, have been expected to include an eastern component (e.g. Roaldsøy 1969; Edwards & Ceddes 1987). The Vikings introduced the horse, probably of Norwegian origin, to Iceland a 1000 years ago (Lie 1973; Adalsteinsson 1981). The Icelandic horse has since been isolated, and thus might be a key for determining the origin of the Norwegian horse breeds. In this study, we

examined the genetic distances between these northern European breeds and the Mongolian native horse by microsatellite genotyping. Thoroughbred, having an oriental origin, and Standardbred trotter, a breed developed by substantial crossings, were included to survey a southern impact vs. an eastern impact of northern European horses.

The data material covered 334 animals representing the four native Norwegian breeds Fjord horse (40), Nordland/Lyngen horse (30), Døle horse (40) and Coldblooded trotter (44), together with Icelandic horse (37), Standardbred (41), Thoroughbred (40) and Mongolian native horse (62). The Norwegian breeds and the Icelandic horse are referred to as northern European breeds. Blood samples were the DNA source for all breeds except the Mongolian horse, where hair samples were collected from the three areas Bulgan, Karakorum and Terelj in Mongolia.

The following 26 microsatellites were analysed: *AHT4*, *AHT5*, *ASB2*, *ASB17*, *HMS2*, *HMS6*, *HMS7*, *HTG4*, *HTG6*, *HTG7*, *HTG14*, *LEX20*, *NVHEQ5*, *NVHEQ11*, *NVHEQ18*, *NVHEQ21*, *NVHEQ29*, *NVHEQ40*, *NVHEQ43*, *NVHEQ54*, *NVHEQ70*, *NVHEQ79*, *NVHEQ82*, *NVHEQ100*, *UCDEQ425* and *VHL20* (references and laboratory protocols given in Bjørnstad *et al.* 2000). The genetic distance parameters D_A (Nei *et al.* 1983) and $(\delta\mu)^2$ (Goldstein *et al.* 1995) were chosen to estimate the interbreed distances because their mathematical and biological bases differ essentially. Random genetic drift is assumed to account for breed differences under D_A evolving under the infinite allele model, while

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Accepted for publication 2 August 2002

$(\delta\mu)^2$ was developed to account for the particular mutation behaviour of microsatellites evolving in a stepwise process, utilizing information of the number of repeated units. The parameters, their corresponding neighbour-joining phylogenetic trees (Saitou & Nei 1987) and bootstrap replications ($n = 1000$) performed to estimate the robustness of the trees, were calculated in 'njbaf' (<http://cib.nig.ac.jp/dda/ntakezak.html>). The trees were visualized using TreeView (Page 1996).

The two distance algorithms were highly correlated ($r_{sp} = 0.76$, $P < 0.0001$). Thus, only their general pattern will be discussed. All the northern European breeds had considerably smaller genetic distances to the Mongolian native horse than to Thoroughbred/Standardbred (Table 1). In the $(\delta\mu)^2$ tree, the Mongolian native horse was included within the panel of northern European breeds (Fig. 1a), while it was located outside this cluster in the D_A tree (Fig. 1b). The corresponding UPGMA trees (Sokal & Michener 1958) clustered the Mongolian native horse together with the northern European breeds (trees not shown). The standard errors of Nei's standard genetic distance (1972) further revealed non-overlap of the distance estimates measured to Mongolian native horse as opposed to Standardbred and Thoroughbred for the Icelandic and Fjord horse. Also for the Nordland/Lyngen horse, Døle horse and Coldblooded trotter the distance to Mongolian native horse was considerably smaller than that to Standardbred and Thoroughbred, but the standard errors of the distances overlapped to some degree for these breeds (data not shown).

The distances between the Mongolian native horse and the Norwegian breeds were at a similar level as the distances between the Norwegian breeds and the Icelandic horse, presumably separated by about 1000 years. The Icelandic horse had short distances to the Mongolian native horse. Because the Icelandic horse represents ancient Norwegian horses the close associations between Icelandic horse and Mongolian native horse support eastern influence on northern European horses. This interpretation is in

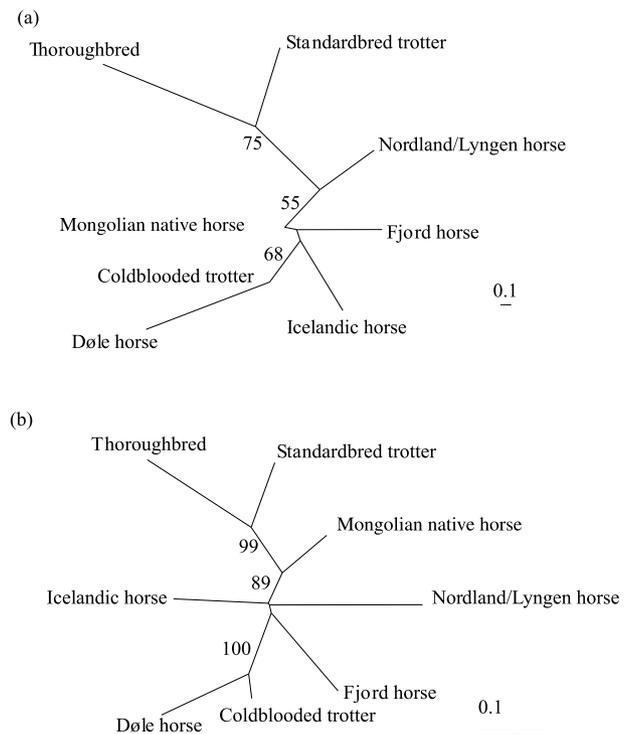


Figure 1 Neighbour-joining trees summarizing the genetic distances $(\delta\mu)^2$ (a) and D_A (b) between eight horse breeds estimated from microsatellite data. Bootstrap values above 50% are indicated on the branch nodes.

accordance with the presumed history of two of the Norwegian breeds, the Fjord horse and the Nordland/Lyngen horse. The presence of primitive phenotypes in the Fjord horse, such as a dark eel stripe along the back and occasionally transverse stripes on the legs, suggests that the breed is old and could be traced directly back to the Asiatic wild horse (Edwards & Ceddes 1987). Further, one of the theories of the origin of the Nordland/Lyngen horse, the northernmost horse breed of Scandinavia, implies eastern

Table 1 Matrix of D_A (above diagonal) and $(\delta\mu)^2$ (below diagonal) genetic distances between eight horse breeds based on 26 microsatellites¹.

	ICE	FJO	NOR	CTR	DØL	MON	STB	THB
Icelandic horse		0.201	0.225	0.171	0.232	0.150	0.233	0.316
Fjord horse	1.775		0.238	0.185	0.232	0.177	0.249	0.279
Nordland/Lyngen	1.598	2.326		0.239	0.290	0.237	0.270	0.328
Coldblooded trotter	0.880	1.513	1.985		0.110	0.179	0.228	0.279
Døle horse	2.857	2.289	2.855	1.237		0.266	0.323	0.338
Mongolian native	0.914	0.867	1.381	0.759	1.758		0.162	0.221
Standardbred	3.169	2.923	1.956	2.874	4.532	1.885		0.176
Thoroughbred	4.431	3.126	3.275	3.775	4.956	2.296	2.305	

¹One locus, *NVHEQ18*, was omitted from the $(\delta\mu)^2$ calculation as large alleles, primarily present in the Døle horse, seriously affecting this distance parameter.

immigration in early times (Roaldsøy 1969). Two dramatic population declines during the last century (Tyssø 1948) could have enlarged the genetic distance between the Nordland/Lyngen horse and the Mongolian native horse. Contribution of eastern genes to northern European horse populations is in accordance with an eastern immigration route for northern Scandinavian cattle breeds (Kantanen *et al.* 2000), and the direction of genetic contribution is compatible with human genetic studies (Zerjal *et al.* 1997).

The large genetic distances from Thoroughbred to the northern European breeds could partly be explained by restricted genetic variation, because of its origin from a limited stock of founders (e.g. Cunningham 1991). However, genetic drift and founder effects alone could not be responsible for these large genetic distances. The fact that all distance estimates were smaller for the comparisons between the northern European breeds and the Mongolian native horse, as opposed to the distances to both Standardbred and Thoroughbred, suggests that more fundamental causes are needed to explain this unidirectional pattern.

The Mongolian native horse has a substantial level of genetic variability (Nozawa *et al.* 1998; Bjørnstad 2001). Areas where domestication has taken place expose higher levels of genetic variability and have a greater probability of acting as gene reservoirs than other populations (Zohary 1996). Thus, the history of the Mongolian horse could have significant bonds to horse domestication, although domestication of horses involved a large number of founders recruited over an extensive period of time from throughout the Eurasian range of the horse (Vilà *et al.* 2001). The smallest genetic distance estimates in this study were detected for the comparisons involving the Mongolian native horse. As well as having short distances to the northern European breeds, it had relatively short distances also to Standardbred and Thoroughbred. Vilà *et al.* (2001) reported that high levels of mtDNA variation, both within and between horse breeds, may reflect a bias towards females in dispersal/trading and breeding. This, together with the high level of polymorphism within the Mongolian native horse and its short distances observed in the present study to both the northern European breeds and also to Standardbred, may suggest that the Mongolian horse has had a major impact on a wide range of breeds, and that central Asia could have been a centre of dispersal of horses through trading and human migration.

In conclusion, two fundamentally different distance measures suggest genetic associations between northern European horse breeds and the Mongolian native horse. Further analyses incorporating old breeds like the Gotlandsruss and the Finn horse, together with breeds representing intermediate locations between Mongolia and northern Europe, and more breeds representing a southern

origin, would establish valuable information on horse breed history and development.

Acknowledgements

We thank Stefan Adalsteinsson, Kaj Sandberg and two anonymous referees for valuable comments on earlier drafts of the manuscript, and Ellen Gunby for laboratory assistance. The Norwegian Research Council and The Norwegian Trotter Association funded the study.

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