

DNA on the hoof

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Left: The head of a horse drawn on the wall of Rouffignac Cave, SW France, some time between 20,000 and 12,000 years ago. In external features, these animals resemble in some ways the living Przewalski horse (right). However, morphological studies (by L. Kaagan) suggest that the Przewalski horse is not a completely unaltered relic of the Late Pleistocene horse represented in cave art.

Horses have an enduring fascination. The variety of domestic breeds ranges from semi-wild ponies to heavy draught horses. Przewalski's horse of Mongolia is the only remaining 'wild' horse, though some have suggested that it too had domestic origins. What are the true relationships among these forms? Can genetics, archaeology and palaeontology go hand-in-hand to help us understand the origins of domestic horses from their wild ancestors?

We have sequenced a horse gene believed to evolve rapidly and at a fairly constant rate. Comparing the sequence among 31 modern horses has allowed us to draw up a tree of relationships. These data have produced some fascinating results:

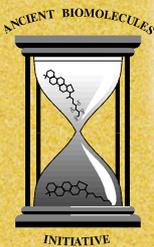
- There is a remarkable amount of genetic diversity among domestic breeds, which has its roots up to 100,000 years ago, long before true domestication began about 5,000 years ago.
- In general, horses which are thought to be similar types, e.g. Shire and Suffolk Punch (so-called 'heavy horses'), do not appear genetically related, and may simply have converged by selective breeding.
- 'Native' ponies, such as the Exmoor, Shetland and Iceland, do not appear

'primitive' in a genetic sense: they may simply have become secondarily adapted to a rigorous environment.

- Przewalski's horse is genetically distinct, but no more divergent from domestic breeds than the latter are among themselves.
- Taken together, these facts are consistent with palaeontological evidence for a vast population of wild horse across Eurasia and N. America in the 'Last Cold Stage' about 100,000 -12,000 years ago). This population accumulated considerable genetic diversity, and from it were derived both the wild Przewalski horse, and populations from which domestic animals were ultimately bred.

We also tested fossil and archaeological material for the presence of ancient

DNA. Several ancient specimens produced data, including a 100-year-old piece of skin of the tarpan from European Russia, a supposedly wild horse which became extinct at the turn of this century; and a foot bone of a wild horse excavated at Kent's Cavern in Devon, radiocarbon-dated to 12,250 years ago. Short DNA sequences from these bones have been verified as horse by comparison with modern animals, but we have not so far obtained usable ancient DNA from other material, including 5,500-year-old archaeological material from Kazakhstan, a supposed centre of horse domestication. Our results point the way to further work, and to combining results from the ancient and modern samples in the analysis.



Molecular Signatures from the Past

The Ancient Biomolecules Initiative is a five-year programme to understand the fate of biological molecules in archaeological and fossil materials, and to explore the applications of this new knowledge. The Initiative is funded by the Natural Environment Research Council.

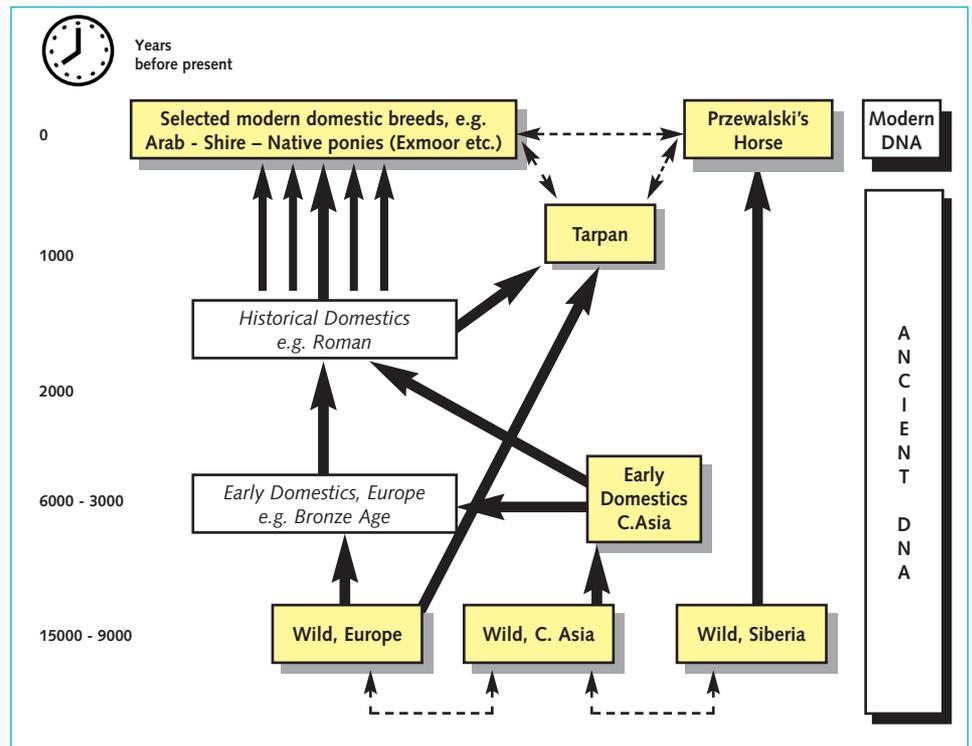


DNA on the hoof: the science in detail

A 608 base pair (bp) segment of mitochondrial D-loop DNA was sequenced for 31 modern horses. They had a remarkable degree of genetic diversity, although haplotype diversity does not in general correlate with phenotypic breeds. Forty ancient samples, of varying provenance and burial history, were analysed for DNA, of which two yielded a usable 90bp segment of 16S ribosomal gene: historical material of the Russian tarpan, and a 12,250 year old bone from a British cave deposit.

Modern horses

The 608bp D-loop sequence on modern horses was used to draw up trees by parsimony and maximum likelihood methods, using onager (*E. asinus*) as outgroup. Both methods, including a range of most-parsimonious trees, gave essentially similar topologies, with Przewalski's horse forming a clade distinct from most but not all domestics. Together



A Russian photograph, dating from 1895, of the very last tarpan, whose skeleton is preserved in Moscow and from which DNA was obtained in this study.

with its behavioural and karyotypic distinctiveness, and similar morphology to Late Pleistocene horses, the data are consistent with a wild origin of Przewalski's with some late gene flow from domestics, but a feral origin cannot be excluded.

There were 50 variable sites within the D-loop sequence of domestic and Przewalski's horses, the 29 individuals encompassing a remarkable 19 haplotypes and up to 3.3% sequence divergence.

This is far more than can have arisen since any plausible date for domestication, and must derive ultimately from genetic diversity within the vast Eurasian Pleistocene horse population, a diversity maintained by the selection and expansion of domestic horse breeds. Domestication

from diverse wild stocks is likely.

Our results also show poor mapping of haplotypes onto phenotypic breeds, and are in several ways interestingly similar to recent work on domestic dogs and their wolf ancestors (Vila et al., Science (1997) 276:1687).

Ancient horses

We first amplified a short (90bp) fragment of 16S gene from the ancient samples, as a test for the presence of DNA. We successfully obtained sequence for a 100-year-old specimen of the tarpan, and a bone of a Late Pleistocene wild horse from Kent's Cavern, directly radiocarbon dated to 12,250±110 before present. The preservation of DNA in the latter specimen is interesting and perhaps surprising, as the bone would have been deposited in wet sludge under the cool conditions of the last ice age, then preserved over the millennia under probably alternating damp and dry conditions though at relatively unvarying temperature. There are no sequence differences between the Tarpan, Kent's bone, Przewalski's horse, and published Late Pleistocene frozen Siberian and domestic horses. Subsequently we designed four pairs of horse-specific primers for informative regions (90-140bp in length) of our D-loop sequence, and tested them on

Schematic diagram representing possible routes to domestic horses. Broad arrows indicate various alternative ancestral pathways. Dashed lines represent possible gene flow among broadly contemporaneous populations. Populations from which samples were analysed in the present study are highlighted in yellow.

all our ancient samples, but so far these have not yielded good product, though we are continuing in this effort. We are confident of ultimately incorporating ancient and modern sequences in a single tree.

Methods

The 40 ancient bone and tooth samples were crushed to a fine powder in a freezer mill, and DNA extracted using the silica/guanidinium thiocyanate method. Best results were achieved by running a Taq polymerase PCR product on an agarose gel, cutting out the band and eluting. This was then amplified using Pfu polymerase. Resulting PCR products were cloned and sequenced.

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