

## **Gavalda Kinga**

Assignment for the Journal Club,  
Kabai's class, 2006

### **DNA techniques**

#### **1. Mitochondrial D-loop sequence variation among Italian horse breeds**

Maria Cristina Cozzi, Maria Giuseppina Strillacci, Paolo Valiati, Barbara Bighiognolia, Mario Cancedda, Marta Zanotti  
(Genet. Sel. Evol. 36 (2004) 663–672)

The aim of this study was to investigate genetic diversity of the mtDNA D-loop hypervariable region in seven Italian horse populations, in order to evaluate their matrilineal relationships. Mitochondrial DNA (mtDNA) analysis has often been used in evolutionary studies.

The genetic variability of the mitochondrial D-loop DNA sequence in seven horse breeds bred in Italy was analysed. Five unrelated horses were chosen in each breed and twenty-two haplotypes were identified.

Total DNA was extracted from peripheral blood samples of five horses for each of the following breeds: Giara (GRH), Haflinger (HFL), Italian trotter (ITR), Lipizzan (LPZ), Maremmano (MAH), Sarcidano (SRH) and Thoroughbred (TBH).

The D-loop region was amplified by the polymerase chain reaction (PCR) using two primers specifically designed from a published horse sequence (GeneBank X79547).

Mitochondrial DNA sequences were compared with a reference sequence from a “Swedish horse” (GeneBank X79547).

The mitochondrial DNA D-loop region is very polymorphic. They identified 22 haplotypes among the 35 horses. Haflinger, Lipizzan, Maremmano and Thoroughbred showed the highest variability (5), while Giara showed the lowest variability. The clustering of haplotypes shows seven clades A to G.

The distribution of our haplotypes in the different clades suggests that the modern horse mtDNA sequences do not define monophyletic groups. In particular compared to wild progenitors, modern horse populations are not derived from a single stock of wild horses. The horse domestication probably involved several distinct populations.

## **2. Mitochondrial DNA analysis of horses recovered from a frozen tomb (Berel site, Kazakhstan, 3rd Century BC)**

C. Keyser-Tracqui, P. Blandin-Frappin, H.-P. Francfort, F.-X. Ricaut, S. Lepetz, E. Crubézy, Z. Samashev, B. Ludes  
(Animal Genetics, 36 (2005) 203–209)

In this study, the D-loop hypervariable region of the mtDNA was used to investigate the genetic diversity of ancient equine remains recovered from a Scythian grave. The aim was to test the possibility of a matrilineal relationship between the horses, which had been adorned with similar harnessing and ornament before burial.

In 1998, a frozen Scythian burial place, dating from the beginning of the 3rd century BC, was discovered under a stone tumulus (kurgan) near the village of Berel. Owing to the good preservation of these bodies remains, numerous biological analyses were possible, including DNA typing. Sequence polymorphism of the mitochondrial DNA D-loop was used to determine the genetic diversity of horses recovered from a Scythian princely tomb.

The northern half of the tomb was found to contain 13 horses, sacrificed with all their equipment and adornment, and arranged on two levels most of them were protected by the frost, which even preserved their skin and the colour of their coat.

The first three of them, wore large (70 cm) gilded wooden ibex horns whereas the others were decorated with pendants, bits and other wooden ornaments.

In an attempt to determine if the eclectic style of the decorative elements found on the horses could really be related to the diverse origin of these horses, mitochondrial DNA (mtDNA) analyses were undertaken on the 13 equine skeletons found in Berel and the mtDNA sequences obtained were compared with those of other breeds from disparate provenances.

Considering the manner with which the horses had been ornamented, their results surprisingly did not reveal any relation between the mtDNA haplotypes (or even haplogroups) obtained and the decorative elements described

Two horse specimens from Yakutia were also included in the molecular analysis. Both gave positive amplification results. But the exact origins of the Yakut horse remain unclear. There seems to be two main theories. The first is that the animal is related to the Mongolian horse. The second theory is that the Yakut horse is related to the Pleistocene horse of northeast Asia. The mtDNA sequences of the two specimens studied matched neither the Mongolian sequences, nor the Pleistocene ones (from permafrost deposits in Alaska) available in GenBank.

Phylogenetic trees were constructed using the maximum parsimony and neighbour-joining method, the statistical confidence of each node being estimated by 1000 random bootstrap runs. All modern sequences were from GenBank.

They finally conserved 133 haplotypes representing 300 sequences. Maximum parsimony and neighbour-joining trees were constructed to address the relationships between the ancient specimens studied and the breeds used for comparison. The neighbour-joining tree shows that

the 15 ancient Asian horse's sequences obtained did not form a separate cluster. Nine of them were distributed into five of the six clades described by Vila' et al. (2001).

It was interesting to determine if the ancient sequences obtained from the Scythian equine samples could be linked to one or two particular breeds. With this objective, we compared the ancient horse samples to a data set composed of horses mtDNA sequences retrieved from GenBank. Because the Akhal-Teke is an ancient horse breed originating from Asia and known to have been used by nomadic tribes as a war-horse, we expected, for example, to find homologies between the Scythian sequences and this horse breed.

Whereas no matches were found with the Akhal-Teke specimens, some complete matches were observed with Chinese Guanzhong and Tuva horses as well as with Anatolian horses. Nonetheless, interpretation of these results is difficult considering that the Scythian sequences were either identical or similar to a wide range of other sequences from domestic horses of various and more distant origins. Consequently, no relationship between the ancient samples studied and some particular breed could be clearly established.

## **Phylogeny**

### **Horse genomics and reproduction**

James D. Murray

(Theriogenology 58 (2002) 281-293)

Construction of genetic maps of an animal like horse now not only possible but also rapidly advancing. The principal mapping strategies being used are linkage, somatic cell hybrid (SHC), radiation hybrid (RH panels), in situ hybridization (physical mapping).

Prior to 1990, only 25 genes were assigned into the horse to five autosomal linkage groups and the X chromosome. The linked loci were the genes for red blood cell antigen epitopes, serum proteins, and coat color.

In the mid-1990s, an international group of scientists interested in mapping the horse genome initiated the International Equine Gene Mapping Workshop (IEGMW), a loose consortium with the specific goal of developing a linkage map for the horse genome.

This paper describes the principal techniques used in mapping the horse genome:

#### 1. Linkage maps

The linkage map of the horse is still in the preliminary development phase.

The horse has 64 chromosomes, 31 autosomal pairs plus the X and Y, for a total of 33 linkage groups. There are two requirements for linkage mapping: genetic variation and families. In the case of the horse, complex mapping families and complex mapping functions have been used based on maximum likelihood estimations of the fraction of gametes.

DNA-based markers are the markers of choice for linkage mapping (for example: RFLPs, Microsatellites, SSCPs, SNPs, AFLPs, RAPDs).

The most easily obtained mapping populations for the horse are stallion-derived half-sib families.

## 2. Physical maps

Physical maps are genetic maps that are derived from information other than recombination (linkage mapping) and often contribute considerable information concerning comparative mapping prior to the development of gene-dense linkage maps.

There are several methods that can be used to develop physical maps of the horse. These methods are:

- somatic cell hybrids (SCH): SCH panels are produced by fusing primary cells from a species of interest with cells from a mouse or hamster transformed cell line.
- radiation hybrids (RH): an RH is a special type of SCH panel, in that cells from the animal of interest are first irradiated prior to fusion to the rodent cells.
- in situ hybridization: it can be used to map either a single piece of DNA that contains a marker, gene, or other regions of interest or it can be used to map chromosomal homologies between species.
- Zoo-FISH (Zoo for cross species and FISH for fluorescent in situ hybridization): in this case probes specific to the unique sequence fraction of a single chromosome are labeled with a fluorescent tag and hybridized to horse metaphase chromosome spreads.

## 3. Comparative maps

Comparative gene maps are based on typing genes, as opposed to markers occurring in non-coding DNA, as they tend to be highly conserved at the DNA sequence level to preserve gene function.

Comparative mapping in the horse began when coding genes first began to be mapped and linkage groups identified.

The number of coding genes placed on the horse linkage map is too small to allow a meaningful comparative gene map to be compiled.

## 4. Genome scanning panel

Prior to the availability of a linkage and comparative maps for the horse, the only avenue of access to the genome data from other species for the identification of a gene controlling a trait of interest was through the candidate gene approach. This means where the action of known genes in other species are compared to the phenotype of the species under study.

## 5. Expressed sequence tags (ESTs)

ESTs provide sequence and probe material specific to the majority of genes expressed in a given tissue and provide the basis for microarray analysis of global gene expression.

The development of genetic maps of the horse genome is now well underway. The data now available are the product of a strong international collaborative effort being focused on the horse genome and bode well for the continued, rapid, and strong development of horse genomic resources. The recent development of RH-SCH panels and equine EST libraries will greatly aid the rapid development of an ordered comparative map for the horse.