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Mitochondrial sequence variation in ancient horses from the Carpathian Basin and possible modern relatives

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Whatever the place, ethnic changes always leave their footprint in the local culture and genetic makeup and the same applies to the different types of horses moving with their owners. This thesis is concerned with the mitochondrial control region genotypes of ancient and modern horses from the Carpathian Basin, where in the late 9th cenury the incoming pagan Hungarian tribes permanently changed the population.

Studies of mitochondrial DNA have shown that modern horses are descended from at least 77 different wild mares, with a last common ancestor over 300,000 years ago, and so probably inhabiting very different regions. Despite this matrilineal genetic diversity, correlations between modern breeds of horses and mitochondrial genotype are often uncommon. This is probably because horse-trading and horse-stealing, sometimes over long distances, have been popular and profitable for a long time.

To determine genetic diversity and origin of horse populations in the Carpathian Basin at the time of the Avars and of the Hungarian Conquest, mitochondrial DNA analysis was undertaken on 31 archaeological horse remains, excavated from authentic, well-dated Avar and pagan Hungarian burial sites. Based on a supposed relationship, modern Hucul and Akhal Teke horses were included in the analysis. To reveal relationships to other ancient and recent breeds, mtDNA sequences from 79 breeds representing 913 individual specimens were combined with our sequence data. Sequences were aligned and truncated to a length of 247 bp to accomodate published short sequences (nucleotide positions 15495-15740 of reference sequence X79547).

Estimation of standard diversity measures, such as haplotype diversity (h) and nucleotide diversity (π) were performed in DnaSP 4.50.2.

To compare our samples with other modern and ancient horse sequences, 921 previously published equine mtDNA CR sequences with fully overlapping standard 247 bp lengths were obtained from the database (http://www.ncbi.nlm.nih.gov/Genbank).

Median-joining network was constructed using the NETWORK 4.5.1 software (Fluxus Technology Ltd. at www.fluxus-engineering.com) to reveal approximate genealogical relationships among the haplotypes found in our ancient and recent breeds and the haplogroup-indicating sequences.

Both genetic distances and haploltype-based methods indicate a clear separation between horses of the Avar and Hungarian leading nobles. Avar sequences were genetically heterogeneous, closely related to Eastern breeds; (with Mongolian and north Russian Vyatskaya groups). This Asian relationship can also be seen in the genetic distance matrix and the haplotype network.

By contrast, beside the great heterogenity and unique haplotypes, the ancient Hungarian horses showed a relatively close relationship with the Turkoman Akhal Teke and Norwegian Ffjord. It confirms the assumption of Hecker (1955) and is an admixture to the clew up of the Hungarian origin. After a short communication of Bjørnstad (2003), different distance measures suggest genetic associations between northern European horse breeds and the Mongolian native horse. By all means, the high variability of Hungarian horse haplotypes may be connected with the well-attested, continent-wide raiding habits of the ancient Hungarians. The Hucul data show no such relationship. Our results show that the ethnic changes induced by the Hungarian Conquest in the late 9th century were accompanied by a similar change in the stables of the Carpathian Basin.

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Isolation and characterization of bacterium and phage isolates which have biocontrol ability against *Pseudomonas* strains pathogenic to *Pleurotus ostreatus*

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Pleurotus ostreatus is one of the most extensively cultivated mushrooms in the world; however significant loss of crop and quality arises from bacterial diseases causing by different bacterial pathogens. The yellowing of *Pleurotus ostreatus* and the brown blotch disease of *Agaricus bisporus*, caused by *Pseudomonas tolaasii* is well known (Lee and Cha 1998). The bacterium produces the toxins tolaasins that disrupt the cellular membrane by forming pores (Rainey et al. 1992).

Ps. tolaasii can be identify easily, with tolaasin toxin gene specific primers. This method is much more reliable than the white line test. The *Ps. tolaasii* is able to make a phenotypic switch; this variant form is nonpathogenic and differs from the wild type in a range of biochemical and physiological characteristics (Cutri et al. 1984).

Moreover other fluorescent pseudomonads such as *Ps. agarici*, *Ps. constantinii* and *Ps. gingeri* can cause various symptoms as well. The same degree of discoloration may be caused by dissimilar species of pseudomonads, suggesting that the factors are not exclusive to a particular pseudomonad species (Godfrey et al. 2001).

Many investigations have been carried out to find an appropriate method for preventing or controlling this disease. There are trials to use of chemical wash formulations, including such chemicals as calcium chloride, sodium hypochlorite, hydrogen peroxide, bronopol and antibiotics in watering mushrooms, but none of them has been found to be fully effective and non-toxic to humans (Wong and Preece 1985).

Biological control by competition has also been investigated. Potential bacterial control agents have been isolated and shown to be active antagonists (Nair and Fahy 1972).

In this study, the *Pseudomonas* strains of an infected oyster mushroom farm in Hungary were investigated. Sixty strains were isolated on Gould's S1 medium, which consistently gives high selectivity and good recoveries of fluorescent pseudomonads with samples obtained from a variety of habitats. S1 medium has several advantages over other media used for the isolation of fluorescent pseudomonads. The identification of the strains was carried out by sequencing a part of the *rpoB* gene or in some other cases a part of the 16S rDNA gene. The *rpoB* gene codes for the RNA polymerase β-subunit, it is a highly conserved essential gene, so it could be used for bacterial identification. The pathogenicities of the strains were tested on yeast extract media in Petri-dishes, in direct confrontation tests.

To find and effective antagonistic agent, we isolated and evaluated fluorescent pseudomonads, bacilli and lytic bacteriophages, against *Pseudomonas tolaasii*, and other pathogenic *Pseudomonas* strains.

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Interacting protein partners on Drosophila telomeres

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The *proliferation disrupter* (*prod*) gene in *Drosophila* encodes a 346- amino-acid protein that localizes strongly to the centric heterochromatin of the second and third chromosomes as well as to >400 euchromatic sites, and all telomeres. In *Drosophila melanogaster*, three telomeric domains can be distinguished by DNA sequence and by proteins associated with them: the end of the chromosomal DNA molecule (capping complex), the retrotransposon array consisting of three non-long terminal repeat retrotransposons, *HeT-A*, *TART*, and *TAHRE* (HTT), and the subtelomeric repetitive telomere associated sequences, repetitive region (TAS). Chromosome length in *Drosophila* is maintained by targeted transposition of the three telomere-specific non-long terminal repeat retrotransposons, HTT, to the chromosome end.

Immunofluorescence stainings of different mutant telomeres clearly showed, that Prod binds to the *HeT-A* element of the HTT array. We could also show that Prod binding represses *HeT-A* transcription, nevertheless does not influence HTT length. Reduction of Prod levels in heterozygous *prod*^{k08810} null mutant flies results in elevated levels of *HeT-A* transcripts in ovaries as well as in third instar larvae, while it has no effect on genomic *HeT-A* copy number, which we used as a measurement for HTT length.

To identify proteins that may interact with Prod, we performed a yeast two-hybrid screen, using the Clontech Matchmaker cDNA library and the entire *prod* cDNA as bait. About 120 000 cDNA clones were tested and about 100 potential Prod interactors were identified. Prod was found 4 times, suggesting that Prod interacts with itself. We also identified Z4 as one of the interacting proteins, which is one of the few proteins known to be associated with the HTT telomeric domain. The Chromator protein, which has previously been shown to co-immunoprecipitate with Z4 and co-localize with Z4 in interbands of polytene chromosomes and at some telomeres, was also found to interact with Prod. We could verify the latter interaction with co-immunoprecipitation experiment.

Proteins Uba2 and Lesswright were the strongest interactors of Prod in the yeast two hybrid screen.Uba2 is the E1 SUMO activating enzyme regulating the initial steps of sumoylation, - a posttranslational protein modification system modifying protein activity - while the second step is performed by the E2 ubiquitin-conjugating enzyme encoded by the lesswright gene in *Drosophila*. Computer sequence analysis showed that Prod (and Chromator) have potential sumoylation sites. This raised the possibility that either Prod itself is sumoylated or sumoylation enzymes are recruited by Prod to sumoylate Prod-associated proteins. Our immunostainings confirm this notion, demonstrating that most if not all sumoylated proteins on telomeres are located on the HTT array.