



## Mitochondrial DNA D-loop sequence variation among 5 maternal lines of the Zemaitukai horse breed

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### Abstract

Genetic variation in Zemaitukai horses was investigated using mitochondrial DNA (mtDNA) sequencing. The study was performed on 421 bp of the mitochondrial DNA control region, which is known to be more variable than other sections of the mitochondrial genome. Samples from each of the remaining maternal family lines of Zemaitukai horses and three random samples for other Lithuanian (Lithuanian Heavy Draught, Zemaitukai large type) and ten European horse breeds were sequenced. Five distinct haplotypes were obtained for the five Zemaitukai maternal families supporting the pedigree data. The minimal difference between two different sequence haplotypes was 6 and the maximal 11 nucleotides in Zemaitukai horse breed. A total of 20 nucleotide differences compared to the reference sequence were found in Lithuanian horse breeds. Genetic cluster analysis did not show any clear pattern of relationship among breeds of different type.

**Key words:** D-loop, *Equus caballus*, phylogeny, polymorphism.

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### Introduction

Until recently conservation efforts have focused on wild species, but now domesticated animals are recognized as an important part of biodiversity and more efforts to save rare breeds are made. The Zemaitukai horse (*Equus caballus*) is a native breed of Lithuania. As a consequence of unfavourable historical and economic circumstances, the number of Zemaitukai seriously declined and by 2003 the total population size was 147 animals. According to pedigree data, the Zemaitukai horse population now consists of two stallion (male) and five mare (female) family lines (Macijauskiene, 2002). Out of the five mare families the one of Kastanke is represented by significant number of individuals. The least numerous is the Mirta family. The other three are the Zibute, Arabe and Tulpe maternal families. Genetic relationship between Lithuanian and other horse breeds is a point of interest, as the origin of the breed is not exactly known.

Mitochondrial DNA (mtDNA) has strictly maternal inheritance (Hutchinson *et al.*, 1974), which means mtDNA haplotypes should be shared by all individuals within a maternal family line. Mitochondrial DNA is useful for studying the evolution of closely related species and many

studies have focused on the mitochondrial D-loop region, the most variable part of mtDNA (Ishida *et al.*, 1994) due to a higher substitution rate than in the rest of the mtDNA genome (Cann *et al.*, 1984). The entire horse mtDNA sequence has been reported (Xu & Arnason, 1994). Stability of maternal inheritance within documented horse pedigrees has been demonstrated in Lipizzan (Kavar *et al.*, 1999) and Arabian horses (Bowling *et al.*, 2000). Mitochondrial DNA sequence polymorphism has been used to examine genetic relationship within breeds (Hill *et al.*, 2002; Luis *et al.*, 2002), among breeds (Kim *et al.*, 1999; Mirol *et al.*, 2002), between domestic and wild horse populations (Oakenfull & Ryder, 1998) and also to address questions of horse domestication (Lister *et al.*, 1998; Vila *et al.*, 2001).

Here we present a study designed to examine the validity of the breed pedigree, measure mtDNA diversity, and examine interbreed relationships, as this information could be useful in conservation and management of this rare horse breed.

### Materials and Methods

Samples from Zemaitukai (ZO) mare families were: Arabes - Austeja ZRg59, Mirtos - Asveja Zrg173, Kastankes family - Kanarele ZRg40, Zibutes - Zemyna ZRg172 and Tulpes - Tola ZRg217, respectively. Three random samples from other two Lithuanian horse breeds Zemaitukai Heavy Type (ZH) and Lithuanian Heavy Draught (LH)



Zemaitukai Heavy type and three haplotypes for rest of the breeds were observed. A total of 38 nucleotide differences compared to the reference sequence were found representing 9% of the total DNA sequence analyzed. Out of 38 detected mutations, there were 37 transitions and a single deletion in one individual of the Polish primitive horse breed. The deletion was located in stretch of five cytosines at position 15532.

Table 2 shows nucleotide differences between reference sample and horse breeds tested, Kimura's two-parameter genetic distances showed high variation within and between the breeds. Nucleotide diversity ranged from 0.0129 in the Gotland to 0.0244 in Trakehner and Hanovarian horse breeds. Nucleotide diversity of the Zemaitukai was 0.0199. Low nucleotide diversity in Gotland breed is consistent with the low autosomal genetic diversity found in this breed. High nucleotide diversity within Lithuanian horse breeds is consistent with the results obtained by blood typing and DNA typing (Juras *et al.*, 2003). The Zemaitukai horse has high nucleotide and sequence diversity despite having experience recent bottleneck. The intense interbreeding, due to migrations during formation of different horse breeds, or persistence of several mitochondrial archetypes within the breeds might have caused the high intra-breed nucleotide diversity in the horse (Kavar *et al.*, 1999).

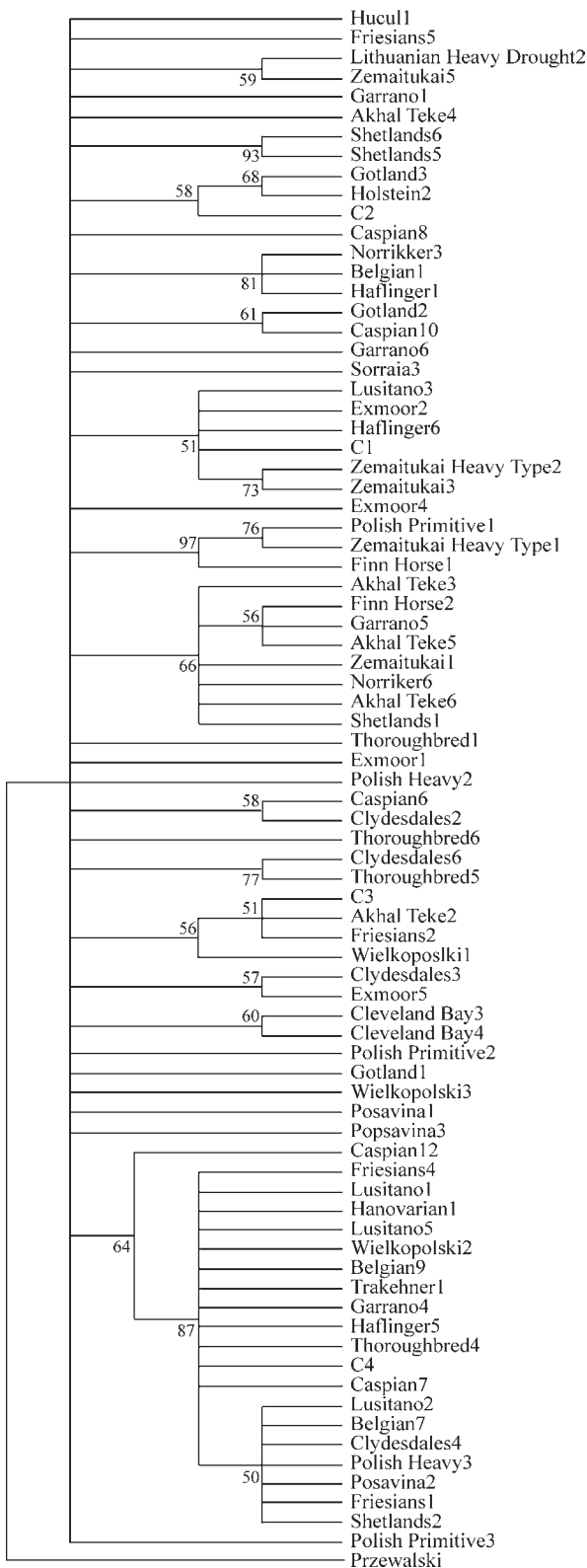
For genetic cluster analysis, sequences of Arabian, Thoroughbred, Friesian, Akhal-Teke, Belgian, Caspian, Cleveland Bay, Clydasdales, Exmoor, Garrano, Haflinger, Lusitano, Noriker, Shetlands and Sorraia horse breeds were obtained from gene bank and included with the breeds sequenced in this study for analysis. The breeds represent a wide geographic area as well as the different horse types. Sequences were truncated to 353 bp for this analysis in order to maximize the number of breeds included. Both maxi-

imum parsimony and neighbour-joining analysis showed similar patterns. After truncating sequences for genetic cluster analysis, breeds that shared the same haplotype were pooled in to groups named C1, C2, C3 and C4. The C1 group contained the Holstein, Trakehner, Lithuanian Heavy Drought, Arabian, Sorraia and Hanovarian breeds. Hanovarian, Trakehner, Zemaitukai and Arabians were put in C2. Haplotypes of Akhal-Teke, Belgian, Haflinger, Zemaitukai, Polish Heavy, Garrano, Cleveland Bay, and Noriker were placed in C3. Hucul, Thoroughbred, Haflinger, Noriker and Lithuanian Heavy drought horse haplotypes formed the C4 group. Genetic cluster analysis did not shown any clear pattern of relationship among the domestic horse breeds whose relationships are well known historically (bootstrap analysis of 1000 replications, Figure 1). Haplotypes from the same breed frequently clustered in separate groups that included breeds of completely different horse breed types. This same pattern has been seen in other studies of horse mtDNA (Vila *et al.*, 2001; Jansen *et al.*, 2002), but are different from ones obtained using blood and protein typing data, were horse breeds clustered corresponding to their known ancestry (Juras *et al.*, 2003). The lack of a pattern may be attributed the high rate of evolution in the control region, which may cause a site to mutate once, and then mutate later changing back to the original haplotype. The result would be the loss of an informative site. Also migration of individuals from one population to another before the establishment of the stud books or at the initial stage of breed formation. The mtDNA analysis provided no new information about the ancestry of the Lithuanian horse breeds.

The analysis of mitochondrial DNA of Zemaitukai horses adds useful information for the effective management and conservation of this rare breed. The mtDNA re-

**Table 2** - Distances calculated by Kimura two-parameter method. Distances within the groups are given in bold. Nucleotide differences between reference and horse groups are given in italic. See text for abbreviations.

|        | X79547 | FH            | GT            | HA            | HO            | HU            | LH            | PH            | PI            | PO            | PV            | TK            | ZH            | ZO            |
|--------|--------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| X79547 |        | <i>14</i>     | <i>10</i>     | <i>17</i>     | <i>10</i>     | <i>11</i>     | <i>16</i>     | <i>16</i>     | <i>13</i>     | <i>15</i>     | <i>16</i>     | <i>17</i>     | <i>14</i>     | <i>20</i>     |
| FH     | 0.0256 | <b>0.0170</b> |               |               |               |               |               |               |               |               |               |               |               |               |
| GT     | 0.0153 | 0.0248        | <b>0.0129</b> |               |               |               |               |               |               |               |               |               |               |               |
| HA     | 0.0194 | 0.0282        | 0.0159        | <b>0.0244</b> |               |               |               |               |               |               |               |               |               |               |
| HO     | 0.0170 | 0.0269        | 0.0137        | 0.0154        | <b>0.0145</b> |               |               |               |               |               |               |               |               |               |
| HU     | 0.0158 | 0.0269        | 0.0149        | 0.0166        | 0.0195        | <b>0.0219</b> |               |               |               |               |               |               |               |               |
| LH     | 0.0186 | 0.0265        | 0.0162        | 0.0165        | 0.0154        | 0.0159        | <b>0.0228</b> |               |               |               |               |               |               |               |
| PH     | 0.0203 | 0.0248        | 0.0167        | 0.0214        | 0.0253        | 0.0158        | 0.0206        | <b>0.0194</b> |               |               |               |               |               |               |
| PI     | 0.0107 | 0.0248        | 0.0145        | 0.0198        | 0.0228        | 0.0141        | 0.0189        | 0.014         | <b>0.0162</b> |               |               |               |               |               |
| PO     | 0.0203 | 0.0191        | 0.0162        | 0.0230        | 0.0236        | 0.0199        | 0.0217        | 0.0178        | 0.0168        | <b>0.0178</b> |               |               |               |               |
| PV     | 0.0219 | 0.0265        | 0.0167        | 0.0220        | 0.0269        | 0.0158        | 0.0211        | 0.0151        | 0.0146        | 0.0179        | <b>0.0178</b> |               |               |               |
| TK     | 0.0203 | 0.029         | 0.0156        | 0.0166        | 0.0162        | 0.0166        | 0.0168        | 0.0211        | 0.0200        | 0.0228        | 0.0212        | <b>0.0244</b> |               |               |
| ZH     | 0.0232 | 0.0170        | 0.0215        | 0.0224        | 0.0183        | 0.0257        | 0.0208        | 0.0257        | 0.0248        | 0.0183        | 0.0273        | 0.0233        | <b>0.0219</b> |               |
| ZO     | 0.0175 | 0.0227        | 0.0153        | 0.0188        | 0.0160        | 0.0192        | 0.0183        | 0.0206        | 0.0190        | 0.0193        | 0.0226        | 0.0193        | 0.0197        | <b>0.0199</b> |



**Figure 1** - Neighbour-joining tree with 1000 bootstrap replication for Lithuanian and other European horse breeds.

sults confirm at least five remaining maternal lineages in Zemaitukai horse breed, which are useful for development of breeding strategies, aimed at evening the genetic contribution of different maternal founding lineages. The mtDNA data also provides additional insights into the genetic diversity of the breed, which, in combination with data from nuclear genes, can be used to maximize the maintenance of genetic diversity within the Zemaitukai horse.

### Accession numbers

GenBank accession numbers AY575103-AY575139 for sequences obtained in this study are as follows: FH1-2, GT1-3, HA1-3, HO1-2, HU1-2, LH1-3, PH1-3, PI1-3, PO1-3, PV1-3, TK1-3, ZH1-2 and Z01-5.

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