# The potential of Y-chromosomal markers for individual lineage tracking in horses

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### **Background and Motivation**

Stallion lines play an important role in horse breeding and in general their genealogies are accurately documented in pedigrees. But when the origin of a stallion line is ambiguous implementing genetic information could be revealing. The male specific region of the Y chromosome (MSY) is passed directly without recombination from fathers to sons and therefore perfectly mirrors paternal genealogies. Polymorphic markers on the MSY have been used to trace paternal origins in many species, but in horses this has been hampered by the low Y-chromosomal sequence diversity. Recently, we generated a 1.46 MB spanning non-repetitive MSY reference and based on this reference we generated the first horse Y-chromosomal phylogeny [1]. We showed that almost all Y-chromosomal haplotypes (HTs) in modern horse breeds group together in a very young, recently introduced haplogroup. Here we apply Y-chromosomal haplotyping to characterise stallion lines using two different approaches: first by genotyping MSY variants and second by de-novo ascertainment of haplotypes using whole genome NGS data.



## 1: Tracing stallion lines by MSY genotyping - American Saddlebred

#### **Historic Background**

The American Saddlebred paternally traces its registry through two foundation sires, both English Thoroughbred crosses. Today the most prevalent is the 'Chief' line which is recorded through *Bourbon Chief, 1883* and this stallion traces back through *Messenger, 1780* to *Darley Arabian,1700*. The second line 'Denmark' is far less prevalent today and traces back through *Herod,1758* to *Byerley Turk,1680*.



#### Methods

We collected hair samples from 21 present American Saddlebred stallions. Seven representatives from the 'Denmark' and 14 from the 'Chief' line were obtained by a pedigree based sampling scheme. Paternal genealogies of the sampled stallions back to the founder stallions were deduced from pedigrees. 13 MSY-SNVs, specific for English Thoroughbred derived HTs, were genotyped using LGS-KASP technology and the results were implemented in the horse MSY network.

#### Results

The seven stallions representing the 'Denmark' line had HT Tb. This HT has also been carried by *Byerley Turk*. The members of the 'Chief' line had Tb-dM. This subtype derived from Tb-d has been alreay assigned to the line of *Messenger* - due to the fixation of Tb-dM in the Standardbred. The MSY haplotyping confirmed the ancestry of the two American Saddlebred stallion lines documented in the pedigree.





Pedigree reconstruction and Y-HTs implemented into the published MSY network. Orange (American Saddlebred) and blue (Franches Montagnes) indicate the Y-HTs observed in this study. Broken frames show newly defined with denovo mutations starred.

# 2: Ascertaining Y-HTs by NGS resequencing - Franches Montagnes

#### Historic background

The Franches Montagnes (FM) breed was developed at the end of the 19<sup>th</sup> century by crossing native Swiss Jura mares with English Thoroughbred and Anglo Norman stallions. Two stallion lines are retained from this period, one tracing back to *Vaillant*, *1891* and the second to *Imprevu*, *1886*. Since 1950 breeding efforts were made to make the Franches-Montagnes horse a lighter, modern-type leisure horse by careful crossbreeding with warmblood stallions, namely *Aladin*, *1964*, *Noé*, *1984* and *Qui-Sait*, *1985*.

#### Methods

We mapped whole genome NGS data from 21 present FM males representing

#### Results

All four FM stallion lines cluster within the T-clade of the modern horse crown group and are clearly separated based on their Y HTs. In total we detected eight Y-HTs with only three described previously (Tu, Tb-r and Tb-g2). The five newly identified HTs are based on de-novo mutations that occurred within FM genealogies and are therefore unique for FM sublines.

For the two recently established FM lines, Y-haplotpying is in agreement with pedigree records. Tb-d has been shown to be the HT of *Darley Arabian* and Tb-r the HT of *King Fergus*. For the founders with ambiguous ancestry, we show that *Imprevu's* descendants carry a HT most probably derived from *Godolphin Barb* (Tb-g2). *Vaillant's* descendants carry the rare HT Tu. No heavy horse HTs (Ad) were detected in the FMs. Accordingly, the MSY matches the documented origin of FM stallion lines - paternally tracing back to English Thoroughbreds.

four stallion lines to the 1.46 MB nonrepMSY reference. After variant calling, HTs were inferred and implemented into the modern horse MSY network. Paternal genealogy of the samples was reconstructed from the pedigree.

# **Conclusion and Perspectives**

So far only the Y-chromosomal HTs of the English Thoroughbred stallion lines are characterised in a fine-grained way. For most other breeds Ychromosomal variants indicative for focal lines are still limited. Here, we show that the 1.46 Mb horse MSY reference together with NGS data enables the ascertainment of biallelic markers specific also for recently established lines. In current projects we ascertain diagnostic Y-chromosomal SNVs for numerous stallion lines to make genetic stallion-line tracing feasible over a broad range of breeds.

#### References

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#### Acknowledgement

We thank the American Saddlebred association and Steven Gaw for providing samples, pedigree information and images. We thank the Swiss Franches-Montagnes association for providing pedigree information.