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Analysis of Arabian stallion lines with Y chromosomal markers

Diplomarbeit

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vorgelegt von
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1 Introduction and literature survey

The Arabian horse is a very old breed, with its origin on the Arabian Peninsula (Hendricks 1995). From the very beginning, breeders aimed to obtain a pure and noble horse without foreign influence. When Arabian horses became popular around the world, Arabian stallions were used to enhance and refine many other breeds. So far the origin of the Arabian horse has been studied with autosomal microsatellite markers (Khanshour et al. 2013) and Arabian matrilines have been investigated by mitochondrial sequencing (Glazewska et al. 2007).
Nowadays, it is possible to trace patrilinies on the genetic level through single nucleotide polymorphisms (SNPs) on the Y chromosome. With this method, this thesis aims to unravel the ancestry and paternal relationship among imported Arabian patrilines.

1.1 Definitions of different Arabian breeds

When talking about Arabians, one must differentiate between Original Arabians, Arabian Horses in general, Anglo-Arabians, Shagya-Arabians and Partbred Arabians.

The Original Arabian is one of the oldest horse breeds in the world, with its origin on the Arabian Peninsula. The main characteristics of this breed are endurance, durability and speed, which makes Original Arabians ideal for racing, over short as well as long distances (Metz 2007). Their character is defined by good nature, despite being temperamental and courageous. Their exterior is marked by a short, but strong back and hard, well-formed hooves. Tail and mane are silky and the head is considered noble-featured with a small mouth and large, dark eyes (Edwards and Langrish 2003). The studbook of Original Arabians is closed, meaning that a horse will only be considered “Original” if all ancestors were Original Arabians (OX). On the Arabian Peninsula these horses are called “asil”, which translates to “noble”, reflecting the traditional fanaticism for pure blood of the Arabian tribes. (Flade 1990).

Arabian Horses are mainly used as riding horses. They have some typical Arabian characteristics but are usually a bit taller than Original Arabians. The main difference, however, is, while Original Arabians have to exhibit a pure pedigree, Arabians are bred on the basis of Original Arabians or Shagya-Arabians, but show ancestors in their pedigree that are not approved as Original Arabians (Schuster 1992).

Shagya-Arabians are riding horses with an ‘Arabian appearance’, who are in general taller than Original Arabians. This breed originated in the stud of Bábolna (Hungary) and Shagya-Arabians were also bred in Radautz (Austria, today Romania). Shagya-Arabians trace back to

Original Arabians through the paternal as well as the maternal line. (Schuster 1992) In the 18th century Arabian stallions were crossbred with heavy local horses, because the army needed more horses to replace those that had fallen in battle. The name Shagya-Arabians was only adopted in 1978 by the international breeding association, named after the stallion Shagya (born in 1830), a very appreciated horse with many high quality offspring (Metz 2007).

Anglo-Arabians originate from crossing Original Arabians and English Thoroughbreds. The breeding goal is an elegant riding horse, combining all positive characteristics of the Arabian as well as the English Thoroughbred. The breed originates in the French stud Pompadour, where the breeding of Anglo-Arabians was started in 1836 (Edwards and Langrish 2003).

Another term will be mentioned in this paper: Partbred Arabian. These horses have to have at least one Original Arabian, Shagya-Arabian, Arabian or Anglo-Arabian in the second generation of its pedigree (Schuster 1992).

1.2 Brief history of importing Arabian horses to Europe

The Europeans first sporadically encountered oriental horses when the Saracens tried to spread their religion over the whole world from the eighth century onwards and started to conquer parts of Europe (Schiele 1967). During the time of the crusades, crusaders first observed Arabian horses in large groups. Arabian horses soon became a popular loot, however, in the beginnings the Arabians imported to central Europe were only used for royal parades, as the knights with their heavy armour needed big, heavy horses to carry them. After the development of firing arms and the decrease of heavily armed knights, small, agile horses became popular. First, European armies solved the problem with Spanish and Neapolitan horses, but they could not stand a chance against the horses of the Turkish army. During the following wars, especially the Napoleonic war, the Arabian horse proved its superiority over the European ones.

To rebuild horse breeding in Europe after the heavy losses during the wars of the 18th and 19th century (especially the Napoleonic war which ended in 1815), governments and sovereigns sent missions to the Arabian Peninsula in order to obtain oriental stallions to breed many noble horses in a short period of time. But the expeditions to the Arabian Peninsula were expensive and not without risks, so European studs tried to build their own stock of purebred Arabians to become independent from expeditions. The main task of Arabian horses, especially stallions, was to enhance the characteristics of European horses, so few studs bred them for their own sake (Schiele 1967).

Many different countries all over Europe imported Arabian horses and built their own studs from the 19th century onwards, so it would be beyond the scope of this project to mention all of them. The focus will be on Poland, Germany and Austria, since the horse samples analysed in this study were obtained from these three countries. However, the Hungarian stud Bábolna shall be mentioned as well, since it is the origin of the Shagya-Arabian breed.

1.2.1 Poland

Pedigrees of Arabian horses in Poland date back to 1803, when Count Waclaw Rzewuski went on an expedition to Arabia, organised by Prince Jerome Sanguszko. He lived with the Bedouin for a few years and brought back many noble stallions as well as mares, destined for studs in Poland, Russia and Germany (Archer 1998). The Sanguszko family owned many horses, including Arabian horses, so the Slawuta Stud south-east of Kiev was founded. In 1845 it was split into two studs, with the second being established at Antoniny.

By continually adding desert-bred Arabians to these studs, their horses became famous throughout Europe and attracted many visitors and buyers. Such as an American-born sculptor, who purchased a son of the imported stallion Ibrahim (born in 1899), the famous Skowronek (born in 1909), and brought him to England.

Unfortunately, both studs were ruined due to political changes in 1917. After the First World War the breeding of purebred Arabians was continued by State Studs, such as Janow-Podlaski, Nowy Dwor, Albigowa and Michalow. In 1926 the Polish Arab Association was founded, who assorted information for the Polish Stud Book and introduced races for Arabians to test constitution, soundness and temperament.

Polish breeding of Arabians flourished between the wars but was heavily damaged during the Second World War. Yet, after the war, huge efforts were made to rebuild the studs, back to its former excellence. In the 1970s Polish Arabians had become very popular throughout Europe as well as in the USA. Two famous stallions from that time were Doktryner (born 1950; Ilderim line) and Trypolis (born 1937; Krzyżyk line). The demand grew rapidly, so Janow-Podlaski organised Annual Prestige Sales, where several stallions were sold, which resulted in a loss of valuable sires. Some famous stallions derive from this period, such as Witraz (born 1938; Kuhailan Kharas line), Comet (born 1953; Kuhailan-Abu-Urkub line), Bandos (born 1964; Ibrahim line) or Palas (born 1968; Saklawi I line) (Archer 1998).

1.2.2 Germany

Breeding of purebred Arabians started in 1816 when King William I of Württemberg founded the Royal Stud at Weil (Archer 1998). At Marbach a national stud already existed, where Arabian stallions had already been used for breeding purposes. But King William I wanted purebred Arabians as well as Partbreds. The most famous stallion imported to Germany was Bairactar (born 1813), who sired a large number of sons and daughters. One of his descendants, Amurath (born 1881), was very influential on European Arabians. After breeding at Weil for some years, he was sold to Radautz.

After William's death the number of Arabians dwindled, as the demand for strong working horses grew and breeding goals shifted towards Thoroughbreds. During the great depression of the 1930s most horses were moved from Weil to Marbach, where the State Stud was rebuilt and the number of purebred Arabians increased again (Archer 1998). The environment and the climate there was optimal for breeding Arabian horses. After the Second World War Marbach desperately tried to find a successor for Jasir, the only stallion that had moved from Weil and was used for breeding in Marbach. The stud re-established ties to breeders in Egypt and two sons of Nazeer (Saklawi I line) were purchased in Egypt and added to the stock (Schiele 1967).

After the Second World War several small private breeders established independently all over Germany and a German stud book for Arabian horses was set up (Schiele 1967).

1.2.3 Austria

Today, there is no State Stud in Austria, that breeds Arabians or Shagya-Arabians and breeding of Arabians and Shagya-Arabians is solely carried out by private breeders throughout the country, who are members of the Austrian Arabian Breeding Association (https://www.araber-zuchtverband.com/z%C3%BCchter/; Access 10.07.2018). However, in the Austrian Empire, there were several State Studs importing and breeding Original Arabians, so this passage will focus on them, until the end of the First World War and the fall of the monarchy.

Apart from the Bábolna stud (see below), the military stud at Radautz (in today's Romania) was very important in the Austrian monarchy. Radautz was founded in 1792 when the army encountered a severe lack of horses after the Seven Years' War (1756-1763) (Schiele 1967). The main goal was to breed horses of the Arabian type with local mares to get agile, yet taller and stronger Arabians. In the first half of the 19th century three successful lines were established: Gidran (line sired by Siglavy Gidran, born in 1810), Dahoman (son of Kidwan al Harzan;

born 1846) and Shagya (born 1830). Especially the descendants of Dahoman and Shagya were very popular and successful amongst riders and breeders.
Another stallion who had a great impact on the Partbred Arabians was Amurath (Bairactar line), purchased from the stud Weil in Germany. His descendants have also influenced the Holstein and Hanoverian breed in Germany at that time.
Stallions as well as mares in Radautz and Bábolna had to pass a performance test before they were used for breeding. The summit of breeding in Radautz was reached within the last ten years before the First World War, between 1904 and 1914, with more than 1000 horses. Those horses had to be evacuated to the centre of Austria when the war began in 1914. Many horses were sold or dispersed into the succession states after the war. Radautz was officially closed as an Austrian state stud in 1919, but still exists today (Schuster 1992).

In the Hungarian part of the Austrian monarchy, two private studs were also successful in the 19th century: Ürmeny and Puszta Lengyeltóty. However, both were closed after one generation, so for systematically planned breeding, the State Studs were of greater significance (Schiele 1967).

In today's Serbia the stud Borike was founded in 1895 close to Sarajevo, after the region was annexed by the Austrian monarchy in 1878. The breeding stock came from Syria and Bábolna, some stallions from Poland and Radautz as well. After 1918, the stud was continued by the Yugoslavian government, together with a few others, but after 1945 Borike was the only stud in Yugoslavia breeding Arabian Horses (Schiele 1967).

1.2.4 Bábolna stud (Hungary)

Its turbulent history goes back to 1789, when the stud was founded as a branch of the stud Mezöhegyes. From 1807 onwards Bábolna became an independent stud (Schiele 1967, Schuster 1992), but at that time there was no defined breeding aim.
The stud was destroyed by Napoleon's army in 1809 and rebuilt in 1816. Then it was decided to produce horses for the army, who stand out with endurance, speed and good temper, in Bábolna. This should be achieved by mating the local mares with Arabian stallions.
After a heavy setback caused by dourine, an expedition was sent to Syria in 1836 to buy Arabian horses. Amongst the imports was the stallion Shagya (born 1830). He would become the founder of the most successful line of the stud and after him the Shagya-Arabian breed would be named.
The following years were marked by more expeditions on one hand and setbacks, such as

illness and war, on the other.

In 1869, two years after the Austro-Hungarian Compromise, the stud was given to the Hungarian government, who sent a committee to examine the stock at the stud. Many of the Original Arabians were sorted out, but the “half-bloods”, as they were called back then, were allowed to stay. A performance test was introduced, especially for young mares, a principle that is still in use today.

In 1885 an expedition brought several horses from the Orient, amongst them the stallions O Bajan (born 1881) and Koheilan Adjuze (born 1876), who both founded very successful lines. O Bajan was mainly used for breeding Shagya-Arabians and Koheilan Adjuze for Original Arabians. After an expedition in 1897, the stallion Hamdani Semri (born 1890) was imported, who also founded a Shagya-Arabian stallion line.

During the following years, the stud continued to grow and was very successful, even throughout the First World War. In 1919 the stud had to be evacuated, except for pregnant mares and those who had new-born foals. These were confiscated by the Romanian army.

From 1927 onwards, stallions had to pass performance exams before they were used for breeding. The performance test for mares was continued as before. Until today, the years between the two World Wars are considered the summit of breeding at Bábolna.

In 1944 the eastern front came so close to the stud, so that the horses had to be evacuated once again. Half of the stock was brought to Germany, the other half remained in Hungary, yet neither of them was really safe. The ones in Germany were sold by the Americans in 1945 and the ones remaining in Hungary were brought to Austria, where they got dispersed all over the country.

From the 1960s onwards, the stud was reconstructed. For some time mainly Original Arabians were bred, but in 1986 the stud returned to breeding Shagya-Arabians, which had become popular in other European countries. By joining the International Shagya-Arabian Association the stud laid the headstone for successful international collaboration (Schuster 1992).

1.3 Famous imported stallions

The lines of Original Arabians have traditionally been named after the founder sire. Unlike in other breeds (e.g. Haflinger, Noriker) there are not a few defined lines in Arabians, but numerous families that emerged, were cross-bred with each other, diminished or got sometimes renewed. This leads to some Arabian lines lasting longer than others (Flade 1990).

In this paragraph, some imported stallions, whose descendants are represented in the samples of this project will be introduced briefly (see

Table 8: Number of samples representing each stallion line).

1.3.1 Bairactar

Bairactar was born in 1813 and imported to Germany by Baron von Fechtig in 1816. He was the most important foundation sire at Weil (Archer 1998) and one of his descendants (Amurath "Weil", 1881) was sold to the Austrian state stud Radautz, where he sired 315 offspring (Schiele 1967).

Figure 1: Bairactar, the most important foundation stallion at Weil (http://in-the-focus.com/wp-content/uploads/2015/03/Bairactar-Litho-unbekannt-600px.jpg; Access 10.07.2018)

1.3.2 Ilderim

Ilderim was born in 1896 in Turkey and imported to Slawuta in Poland in 1900, where he became the founder sire of a line of Original Arabians. He was later sold to Borike (Austria, today Yugoslavia). Two of his descendants (Aquinor and Doktryner; born in 1951 and 1950) became two well-known stallions at Janow and Michalow (Schiele 1967).

1.3.3 Koheilan Adjuze

Koheilan Adjuze was born in 1876 as a descendant of the Anazeh tribe and purchased during an expedition of Mihai Fadlallah el Hedad in 1885 and brought to Bábolna. His sons spread

over Eastern Europe to Poland, Russia, Romania and a few other countries. His son Koheilan I (born 1887) won at the world exhibition in Paris in 1900 in the category ‘half-breds under 160cm’ and a gold medal in the Grand Championat (Schiele 1967).

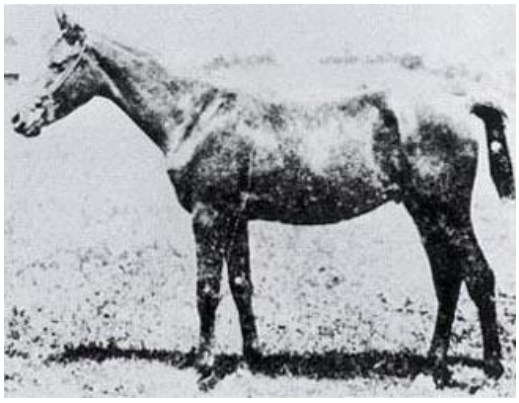

Figure 2: Koheilan Adjuze, imported to Europe in 1885 (http://www.athenaarabians.com/Artiklar_hingstar/K_Adjuzestallions/koheilan_adjuze_oa.jpg; Access 10.07.2018)

1.3.4 Mersuch

Mersuch was born in 1898 and imported to Bábolna in 1902 together with Siglavy Bagdady (born 1895), who both sired popular Original Arabian lines. Descendants of Mersuch were also bred in the Romanian State Stud Mangalia (Schiele 1967).

1.3.5 O Bajan

O Bajan was born in 1880 (Schiele 1967) or 1881 (https://www.allbreedpedigree.com/o+bajan; Access 10.07.2018) and was one of the most valuable stallions ever imported to Hungary (Schiele 1967). In 1885 he was brought to Bábolna by Fadlallah el Hedad (Archer 1998), where he lived until his death in 1910 and sired 312 offspring. One of his sons won at the world exhibition in Paris in 1900 in the category ‘Original Arabians’ against opponents from all over the world (Schiele 1967).

1.3.6 Shagya

Shagya was born in 1830 and imported to Bábolna in 1837. He was greatly admired by Prince Pückler-Muskau when he visited the stud in 1839, not knowing that one day there would be a breed named after him. Shagya’s sons soon became famous for their good looks, their endurance and their toughness. Shagya’s descendants were carefully selected and interbred, soon becoming very popular throughout Europe (Archer 1998).

1.4 Particularities of the inheritance of the Y chromosome

The Y chromosome of the horse is in total 45-50 Megabases (Mb) long, consisting of an approximately 12 Mb long euchromatic region, a small pseudoautosomal region (PAR) and a large heterochromatic region. (Janecka et al. 2018)

The PAR is located terminally next to the euchromatic region (Raudsepp et al. 2004) and is the only part of the Y chromosome that recombines with the X chromosome. But most of the Y chromosome is made up by the male-specific part, that does not recombine. This region is called either ‘nonrecombining region of the Y chromosome’ (NRY) or ‘male-specific region of the Y chromosome’ (MSY) (Jobling and Tyler-Smith 1995). The NRY is passed from father to son unaltered, except for spontaneous mutations which occur rarely (Jobling and Tyler-Smith 2003). The same principle applies to the mitochondrial DNA (mtDNA), which is inherited maternally without meiotic recombination (Jobling and Tyler-Smith 1995). Both systems (NRY and mtDNA) are passed clonally from generation to generation. Therefore, mtDNA and Y-chromosomal haplotype (HT) distributions mirror the paternal or maternal ancestry of populations. With MSY HT analysis wrong paternities, that happened even multiple generations back in time, can be uncovered.

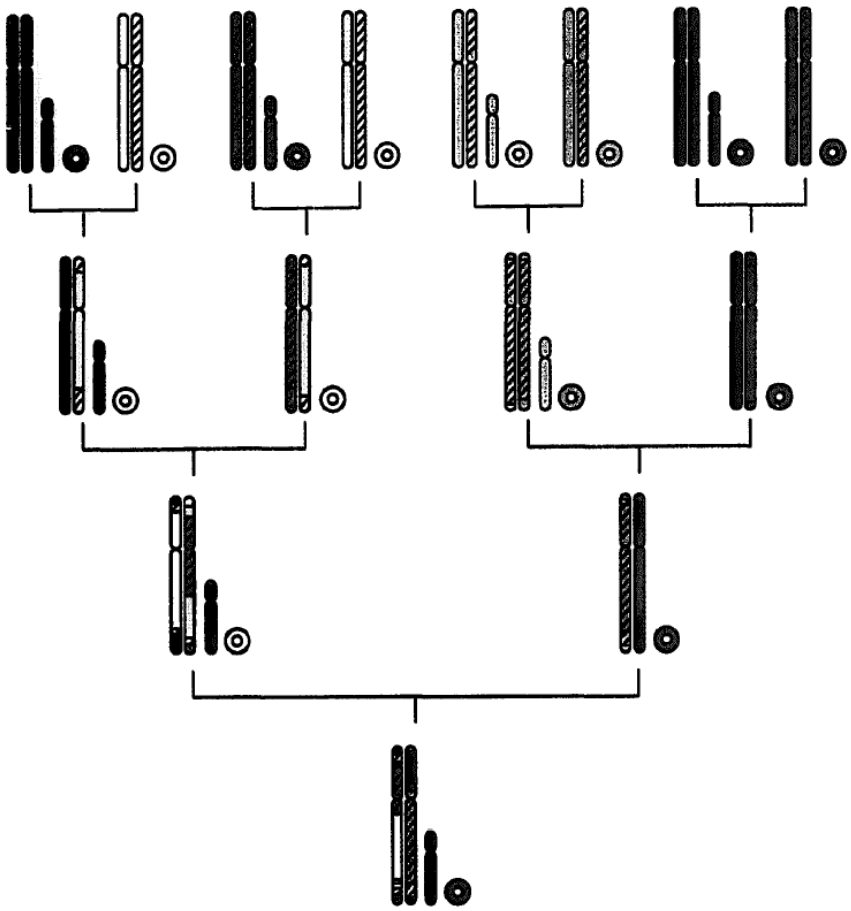

Figure 3: Inheritance of autosomal (large pair of chromosomes), Y chromosomal (small chromosome) and mtDNA (circle) (image from Jobling and Tyler-Smith 1995).

Figure 3 depicts the inheritance of autosomal, Y chromosomal and mtDNA. While autosomal chromosomes recombine during meiosis, the NRY and the mtDNA do not. The individual at the bottom can trace its autosomal DNA back to several origins (represented by the different shades of grey), but the Y chromosome and the mtDNA only have a single ancestor (Jobling and Tyler-Smith 1995).

1.5 Studying patrilinies with Y chromosomal markers

The NRY became an important tool in human population genetics to combine molecular and genealogical information (Jobling and Tyler-Smith 2003). Whereas horses have been found to show much diversity in maternally inherited mtDNA (Jansen et al. 2002), genetic diversity on the NRY is scarce (Lindgren et al. 2004).

The limited diversity on the horse Y chromosome is a result of the domestication and breeding process. One study (Lippold et al. 2011) found that wild horses in the past had a much greater

Y chromosomal diversity than domestic horses. The extreme reduction of Y chromosomal diversity in modern domestic horses can be explained by the limited number of stallions today's breeds derive from (extreme founder effects) (Wallner et al. 2013). As pedigrees show, few selected stallions were mated with as much mares as possible, the mares often coming from different backgrounds (for example Arabian stallions with local Hungarian mares; see 1.1 Definitions of different Arabian breeds1.1 and 1.2.4 Bábolna stud (Hungary)) (Lippold et al. 2011).

In 2013, Wallner et al. identified six Y chromosomal haplotypes (HT; linked group of alleles inherited from a single parent) for the domestic breeds and two HTs for Przewalski's horses based on biallelic markers. The number of modern domestic horse HTs was extended in 2017 (Wallner et al. 2017) by screening whole genome next-generation sequencing (NGS) data of 52 males. Based on a 1.46 Mb spanning reference, they detected 49 single nucleotide variants (SNV) and three indels (insertion or deletion of bases in the genome) which formed 24 HTs found in 57 different breeds. According to this study, all central European and North American breeds cluster into a recently established 'crown-haplogroup' (HG; marked as ALST, Oriental in Figure 4). Within the crown-haplogroup, one branch originates from the English Thoroughbred founders (red dotted line in Figure 4) whereas one indicated HG found mainly in Original Arabians (greed dotted line in Figure 4). However, some Arabian samples clustered at the basal node of the T haplogroup (green circle in Figure 4).

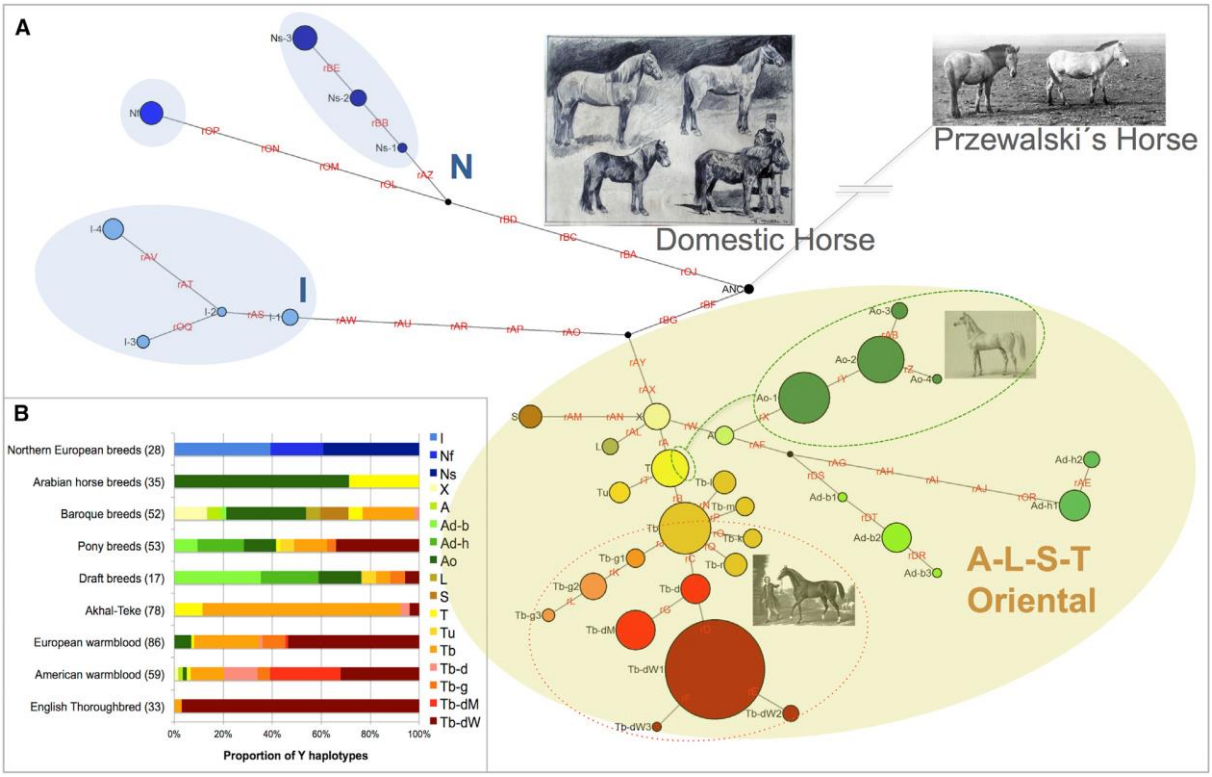

Figure 4: MSY HTs detected in 363 purebred horses of 57 breeds; (figure from Wallner et al. 2017)

Figure 4 shows the phylogenetic tree of the 24 HTs detected by Wallner et al. 2017 (A). The green dotted circle indicates the HG with Original Arabian ancestry, the red dotted circle marks the HG influenced by English Thoroughbreds. (B) shows the relative number of MSY HTs in each breed or group of breeds (Wallner et al. 2017).

Felkel et al. (2018) extended the phylogeny from Wallner et al. 2017 by additionally analysing 52 horses from European, American and Asian breeds. 101 new variants were detected (61 only in Asian breeds), resulting in 42 HTs in domestic horses (depicted in Figure 5; newly defined HTs are in bold print). The high diversity of Asian breeds could be explained by less intense artificial selection compared to highly selected European breeds (Felkel et al. 2018).

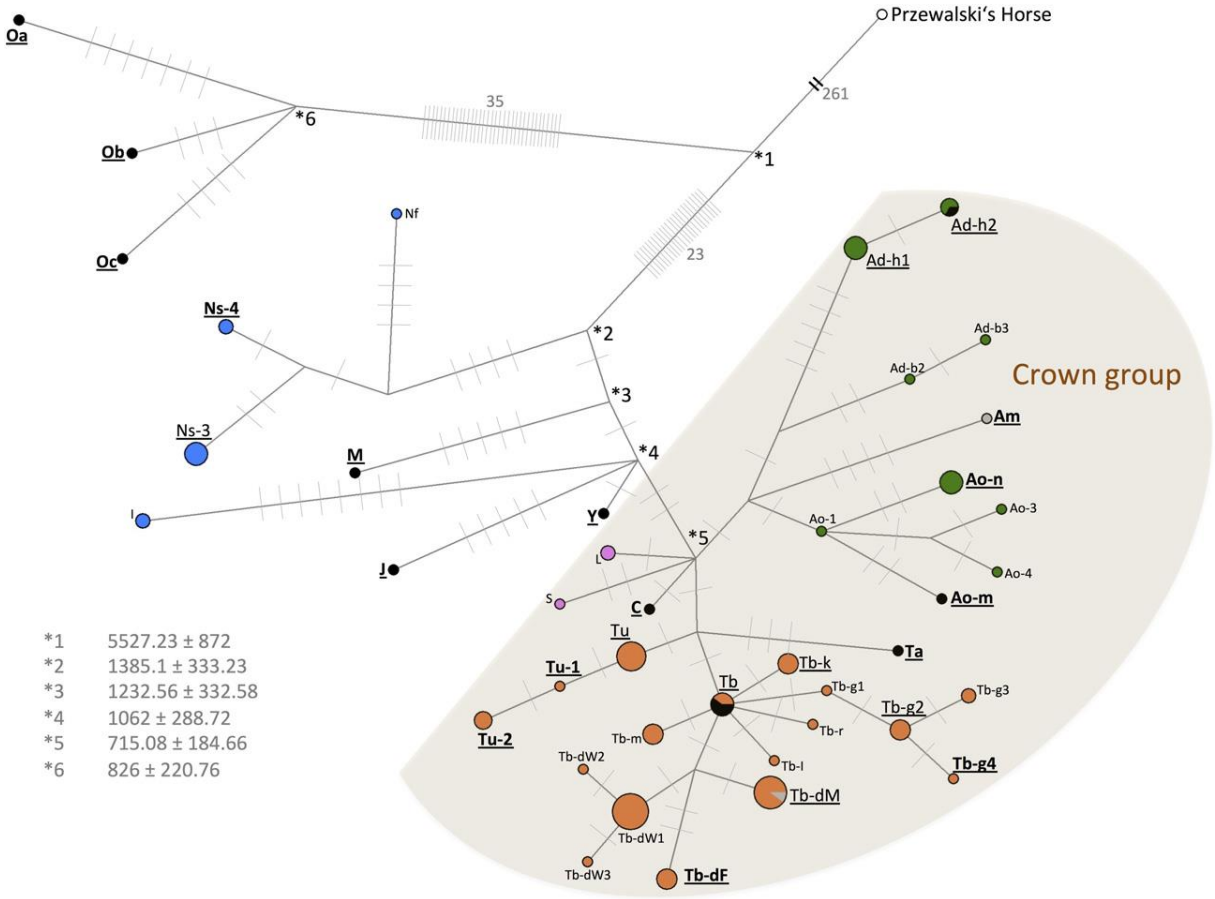

Figure 5: Phylogenetic tree as described by (Felkel et al. 2018)

Figure 5 shows the HTs described by Felkel et al. 2018. The shaded area marks the ALST-crown group. HTs newly described in this study are in bold print, for example HT Ta, which was found in Arabian samples (Felkel et al. 2018).

1.6 Thesis and aim of this project

So far, Wallner et al. (2017) and Felkel et al. (2018) detected two HTs being specific for Arabian horses (Ta and Ao). However, the number of samples analysed in these studies was rather limited and did not cover the full spectrum of sire lines tracing back to imported Arabian stallions.

The aim of this project is to determine a HT spectrum of the imported Arabian stallions in a more representative sample set. The question is – do they carry only HTs Ao and Ta or are other HTs present also. The sampleset consisted of a representative sample of the Polish

Arabian breeding stock and a selection or Arabian and Shagya-Arabian stallion actively breeding in Austria and Germany. We followed the hypothesis that Ao and Ta will be the predominant HTs.

The Polish samples were provided by Dr. Monika Stefaniuk from the Department of Horse Breeding at University of Agriculture in Krakow and Dr. Monika Bugno-Poniewierska, National Research Institute of Animal Production. The Austrian and German samples were provided by the ‘Österreichischer Araberzuchtverband’ and the ‘Haupt- und Landesgestüt Marbach’. Full contact details to collaborators are given in the Appendix. This study was financially supported by the Austrian Federal Ministry for Sustainability and Tourism (DAFNE 101184/2; Title: Characterisation of stallion lines in Austrian horse breeds with Y-chromosomal markers).

This study has been approved by the Ethics Committee of the University of Veterinary Medicine in Vienna regarding the Guidelines of Good Scientific Practise and the national law. The identity of the probed animals will remain anonymous.

2 Material and Methods

2.1 Material

2.1.1 Chemicals and reagents

Table 1: Chemicals and Reagents

Chemical	**Producer**
Sodium Chloride (NaCl)	Roth®, Austria
Sodiumcitrate	Sigma-Aldrich®, Austria
Buffer G	nexttec, Germany
Proteinase K	nexttec, Germany
Prep Solution	nexttec, Germany
Tris	Sigma-Aldrich®, Austria
EDTA	Roth®, Austria
DTT	nexttec, Germany
Proteinase K	Qiagen, Germany
PBS	Sigma-Aldrich®,Austria
Buffer AL	Qiagen, Germany
Ethanol	Roth®,Austria
Buffer AW1	Qiagen, Germany
Buffer AW2	Qiagen, Germany
Buffer AE	Qiagen, Germany
2x KASP® Master mix	LGC genomics®, Great Britain
KASP® Assay mix	LGC genomics®, Great Britain
DNA isolated from cells of the root of a hair	Different sources
Agarose peqGOLD ELECTRAN®	VWR Life Science, Germany
Atlas ClearSight DNA Stain	Bioatlas, Estonia
Bromophenol Blue	Sigma-Aldrich®, Austria
Glycerine	Roth®, Austria
Gene Ruler DNA Ladder Mix	Thermo Scientific™, USA

2.1.2 Devices

Table 2: Devices

Device	Producer
Vortexer (MS 2 Minishaker®)	IKA®, Germany
Thermomixer compact	Eppendorf, Austria
Plate spinner (Labnet MPS 1000 Mini plate spinner®)	Sigma-Aldrich®, Austria
Centrifuge	Eppendorf, Austria
Pipettes	Gilson®, USA
Electronic pipette	Eppendorf, Austria
CFX Real-Time System C1000 Touch®	Bio-Rad, USA
Molecular Imager® Gel Doc™ XR System	Bio-Rad, USA

2.1.3 Material

Table 3: Material

Material	Producer
96 well plate white	Bio-Rad, USA
Eppendorf Tubes	Eppendorf, Austria
Microseal® B Adhesive Optical Sealer	Biozym, Germany
nexttec-columns	nexttec, Germany
DNeasy Mini spin column	Qiagen, Germany
2 ml collection tube	Qiagen, Germany

2.1.4 Software and Programs

Table 4: Software and Programs

Software	Producer
Excel®	Microsoft Office®, USA
Bio-Rad CFX Manager 3.1®	Bio-Rad, USA
Image Lab™ 5.1	Bio-Rad, USA

2.1.5 Used samples

In total, samples from 116 male horses from 25 different sire lines were selected considering pedigree information. Closely related samples were avoided by choosing one of paternal brothers randomly. The sample set harbours 57 Original Arabians, 48 Shagya-Arabians, nine Partbred Arabians and two Anglo-Arabians. They were chosen to give a representative overview over Arabian stallions in Austria (55 samples), Germany (14 samples) and Poland (46 samples). Table 5 to Table 7 show how many samples of each country, breed and type were analysed. Table 8 shows how many samples of each stallion line were analysed.

Table 5: Origin of samples used

Origin of samples	**No. of samples**
Austria	55
Germany	14
Poland	46
USA	1
Total	116

Table 6: Breed of samples used

Breed	**No. of samples**
Original Arabian	51
Shagya-Arabian	48
Arabian	6
Partbred	9
Anglo-Arabian	2
Total	116

Table 7: Type of samples used

Type of sample	**No. of samples**
DNA Agrobiogen	60
Hair	10
Blood (only from Poland)	31
Semen (only from Poland)	15
Total	116

Table 8: Number of samples representing each stallion line

Founder (year of birth)	No. of samples
A Saglawi DB (1885)	4
Amer (1984)	2
Bairactar (1813)	6
Dahman-Amir (1897)	1
Felhaan Alshawaf*	1
Hadban (Ibn Galal) (2002)	1
Hadban (1891)	3
Hamdani el Samra*	1
Hamdani Semri (1830)	8
Ibrahim (1899)	5
Ilderim (1896)	5
Kidwan al Harzan (1836)	2
Koheilan Adjuze (1876)	5
Krzyżyk (1869)	2
Kuhailan-Abu-Urkub*	2
Kuhailan Kharas (1900)	8
Mahmoud Mirza (1851)	1
Mersuch (1889)	1
Mirage (1919)	2
O Bajan (1881)	7
Saklawi I (1886)	34
Shagya (1830)	10
Shuwayman Sabbah (1895)	3
Souakim (1894)	1
Telmese (1903)	1
Total	116

*Birth year unknown

2.1.5.1 Type of sample material

The majority of the samples were extracted DNA samples from paternity testing, provided by Agrobiogen (Germany) upon permission of the Austrian Arabian Breeding Association. Blood samples were leftovers from initially collected samples for paternity testing and were provided by the Polish collaborator (Dr. Monika Stefaniuk, Department of Horse Breeding at University of Agriculture in Krakow and Dr. Monika Bugno-Poniewierska, National Research Institute of Animal Production). Semen was also provided for some samples, when blood was not available from the Polish collaborator. Hair root samples were collected from the horses' manes with the approval of the owner.

Of the 46 samples provided by the collaborator in Poland, 15 were semen and 31 were blood samples. They were collected at the studs of Janow and Michalow, the centre of Arabian

horse breeding in Poland. The samples represent the following sire lines: Amer (1984), Bairactar (1813), Felhaan Alshawaf, Ibrahim (1899), Ilderim (1896), Krzyżyk (1869), Kuhailan-Abu-Urkub, Kuhailan Kharas (1900), Mirage (1919) and Saklawi I (1886).

2.2 Methods

2.2.1 Extraction of genomic DNA

DNA was extracted from hairroots, whole blood or semen, using two different commercially available Kits and protocols.

2.2.1.1 Nexttec 1-step DNA Isolation Kit

The principle of the protocol is to first digest the cells using Proteinase K to release the DNA from the nucleus and in the second step separate the DNA from the remnants of the lysate.

Blood

Prior to digestion, blood samples had to be cleaned from the haemoglobin first (because haemoglobin blocks the PCR reaction). This was achieved by mixing 50 µl defrosted blood with 1 ml SSC (0.15 M NaCl, 0.015 M $Na_3C_6H_5O_7$) in a 1.5 ml Eppendorf tube. After vortexing, the sample was centrifuged for five minutes at 8600 rcf (relative centrifugal force). Afterwards the excess fluid (contains haemoglobin) was discarded, ensuring that the cell pellet remained in the tube. The washing steps were repeated at least twice until a white cell pellet remained.

Hair

At least ten hairroots were cut into a 1.5 ml Eppendorf tube.

Semen was thawed and 50 µl were placed in 1.5 ml Eppendorf tubes.

The following steps were the same for all sample types.

First, the Lysis Buffer was prepared by mixing 140 µl Buffer G and 10 µl Proteinase K per sample in a larger tube. 150 µl of the Lysis Buffer was added to each sample (to the sperm samples 1.5 µl DTT were additionally added) and the tubes were incubated on a thermomixer at 56°C and 800 rpm (rounds per minute) for two hours.
The nexttec columns were prepared by placing 350 µl Prep Solution on the column, closing the lid, letting it rest at room temperature for five minutes and then spinning it for one minute at 400 rcf. The columns were placed in a new tube and 100 µl of the digested cell lysate was placed on the prepared columns. After letting it rest for three minutes at room temperature, the

columns were spun at 700 rcf for one minute. Finally, the column got discarded and the sample diluted with 200 µl 1 x TE (10 mM Tris-HCl, 0,1 mM EDTA).

2.2.1.2 DNeasy® Blood & Tissue Kit from Quiagen

The DNeasy® Blood & Tissue Kit (Qiagen) was used for one sample (see 3.2.3 Other Haplotypes).

Using the Quick-Start Protocol available at https://www.qiagen.com/at/resources/resource-detail?id=63e22fd7-6eed-4bcb-8097-7ec77bcd4de6&lang=en (Access 19.07.2018). 50 µl blood were used as input and the instructions of the protocol were followed.

2.2.2 Agarose Gel Electrophoresis

A standard Gel Electrophoresis was performed on a one percent Agarose Gel and the gel image of the results was taken with Image Lab™ 5.1.

2.2.3 Quantitative Real-Time PCR and KASP®-Technology

Quantitative Real-Time PCR (qPCR) is a very sensitive technique to analyse and quantify gene expressions, which can be visualized directly ("real-time") on a computer. (Thornton and Basu 2015) Its basics have been described in Gadkar and Filion 2014 and how they have revolutionized analysis in Molecular Biology.

2.2.3.1 Mechanism of KASP®

The competitive allele-specific PCR (KASP®) is a type of qPCR that uses a fluorescence based reporting system to screen single nucleotide polymorphisms (SNPs) or short insertions or deletions (InDels). (Bernardo et al. 2015, He et al. 2014)

First a SNP-specific KASP®-Assay and the KASP® Master Mix (containing the dyes and the DNA template) are mixed with the DNA sample. During a thermal cycle reaction, the template is denatured. The two allele specific forward primers from the KASP®-Assay bind to the corresponding allele. Each allele specific forward primer carries a unique tail sequence, one homologous to a FAM™- and one to a HEX™-dye labelled oligo, which are included in the Master mix. Upon binding of the primer to template DNA, its tail is added to the synthetized strands during the following rounds of PCR cycles, when the complement of the sequence is generated. After that, single stranded dye labelled oligos can bind to the tail, are no longer quenched and therefore emit fluorescence. Through the competitive binding of the allele-specific primers, the assay distinguishes between the two alleles. If the locus is homozygous, only one fluorescent dye is detected, in case of a heterozygous locus a mixed signal will be generated.

(https://www.lgcgroup.com/kasp/#.Wzt-3yDLhPY, accessed 03.07.2018) Figure 6 shows the principle of KASP® genotyping.

The assays got designed according to the SNP info from Wallner et al. 2017 and Felkel et al. 2018. Then they were purchased from LGC genomics®.

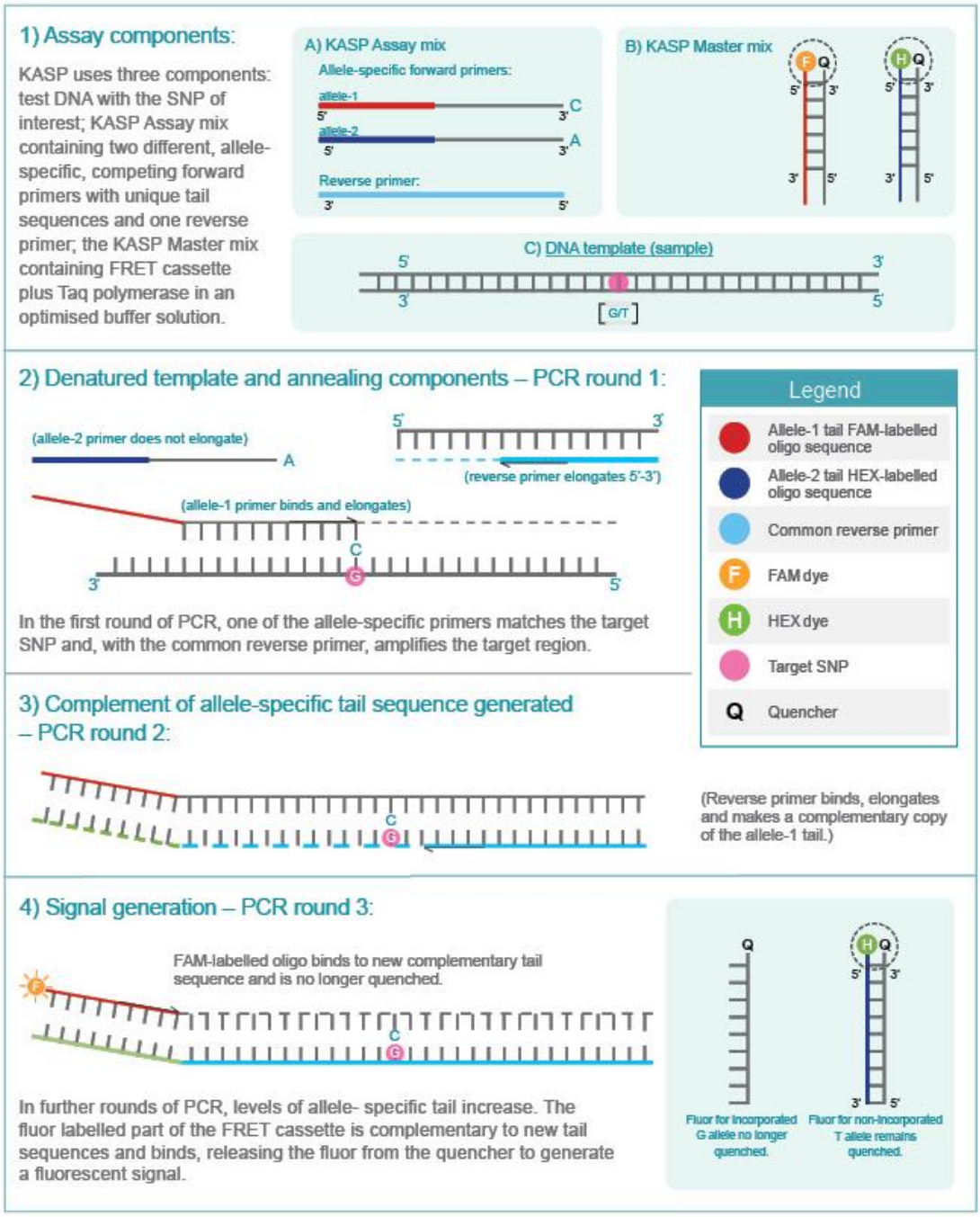

Figure 6: Schematic representation of the mechanism of the KASP®-Assay. https://www.lgcgroup.com/LGCGroup/media/website-content/Products/Genotyping/KASP/how-does-kasp-work.jpg (Access 03.07.2018)

2.2.3.2 Executed procedure

The SNP analysis was formed for 21 purchased KASP® Assays for variants relevant for this project (the exact sequences of the used Assays can be found in the Appendix). qPCR was performed on a white 96 well plate. A Mastermix was prepared and the components were calculated in an Excel® sheet (Figure 7). For each assay the appropriate amount of 2 x KASP® Master mix and KASP®-Assay mix were placed in a 1.5 ml Eppendorf tube and mixed by vortexing and centrifugation. Then 3 µl Mastermix was placed into the wells and 3 µl of the DNA sample were added, adding up to a total of 6 µl fluid per well. For each analysis run, two positive controls for the two different alleles (FAM™- and HEX™-allele), two non template controls (NTCs) and a female sample as a negative control were analysed together with the test samples having unknown allelic state.

Table 9: Components of the KASP® Mastermix

Component	**Amount for one well (in µl)**
DNA	3
2xKASP® Master mix	3
KASP®-Assay mix	0.084

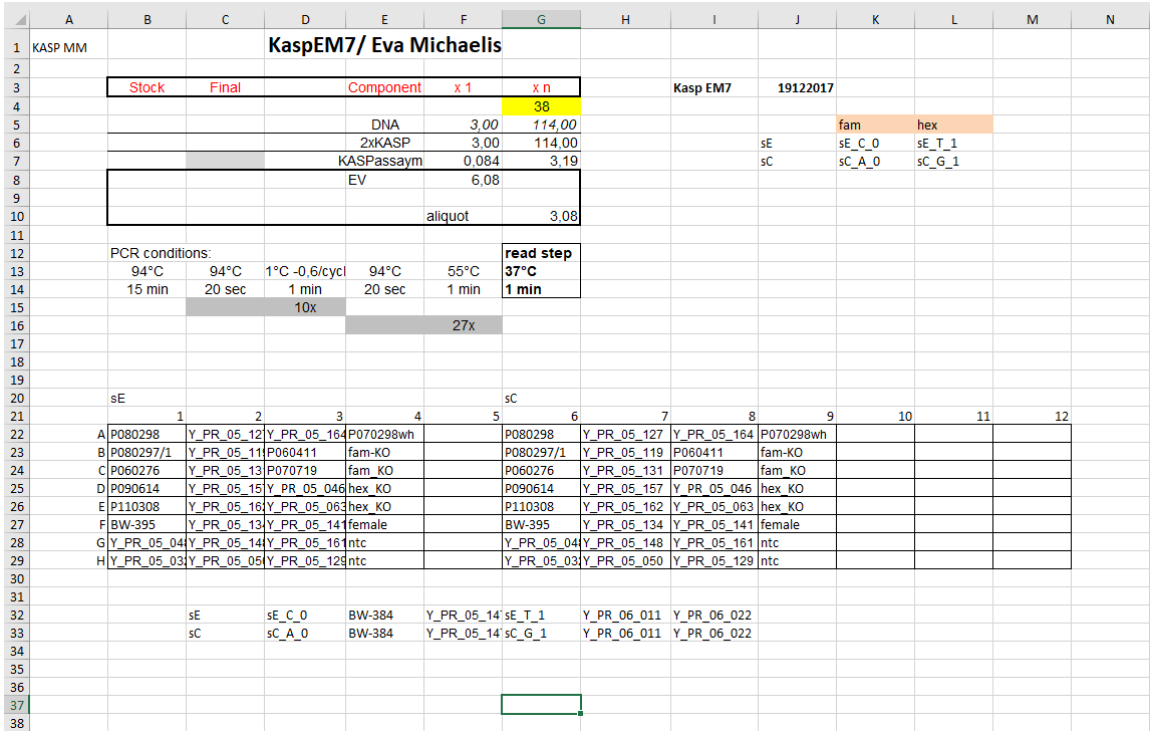

Figure 7: Example of a Mastermix sheet

Genomic DNA of male horses with known HT were used as positive controls. Table 14 provides an overview of the controls for the respective markers. As the work advanced, samples whose HT had been determined in the beginning were taken over as controls as well. The DNA of a Lipizzan mare (166) served as negative control.

In the next step the plate was sealed with an adhesive optical sealer, spun for a few seconds in a special centrifuge and then the qPCR process was started in the CFX Real-Time System C1000 Touch® from Bio-Rad. The used PCR protocol of the Institute of Animal Breeding and Genetics of the Vetmeduni Vienna was the same as the standard protocol recommended by LGC Genomics® (Table 10). To get more precise results two recycling steps were added (Table 11). The recycle protocol used at the Institute was identical to the one recommended by the producer.

Table 10: KASP® standard protocol recommended by the producer (LGC Group. https://www.lgcgroup.com/LGCGroup/media/PDFs/Products/Genotyping/KASP-quick-start-guide.pdf?ext=.pdf&ext=.pdf, Accessed 03.07.2018)

Step	Description	Temperature	Time	Number of cycles per step
1	Activation	94°C	15 minutes	1 cycle
2	Denaturation	94°C	20 seconds	10 cycles
	Annealing/Elongation	61-55°C	60 seconds (drop 0.6°C per cycle)	
3	Denaturation	94°C	20 seconds	26 cycles
	Annealing/Elongation	55°C	60 seconds	

Table 11:KASP® recycle protocol as recommended by producer (LGC Group. https://www.lgcgroup.com/LGCGroup/media/PDFs/Products/Genotyping/KASP-quick-start-guide.pdf?ext=.pdf&ext=.pdf, Accessed 03.07.2018)

Step	Temperature	Time	Number of cycles
Denaturation	94°C	20 seconds	3
Annealing/Elongation	57°C	60 seconds	

The results were visualized in Cluster plots with Bio-Rad CFX Manager 3.1® and the data exported to and processed in Excel® (see Results). (https://www.lgcgroup.com/LGCGroup/media/PDFs/Products/Genotyping/KASP-quick-start-guide.pdf?ext=.pdf&ext=.pdf, Accessed 03.07.2018)

2.2.3.3 Executed qPCRs

The assays were selected based on Felkel et al. 2018. Figure 8 shows a simplified phylogenetic tree based on the work of Felkel et al., which the following approach was based on.

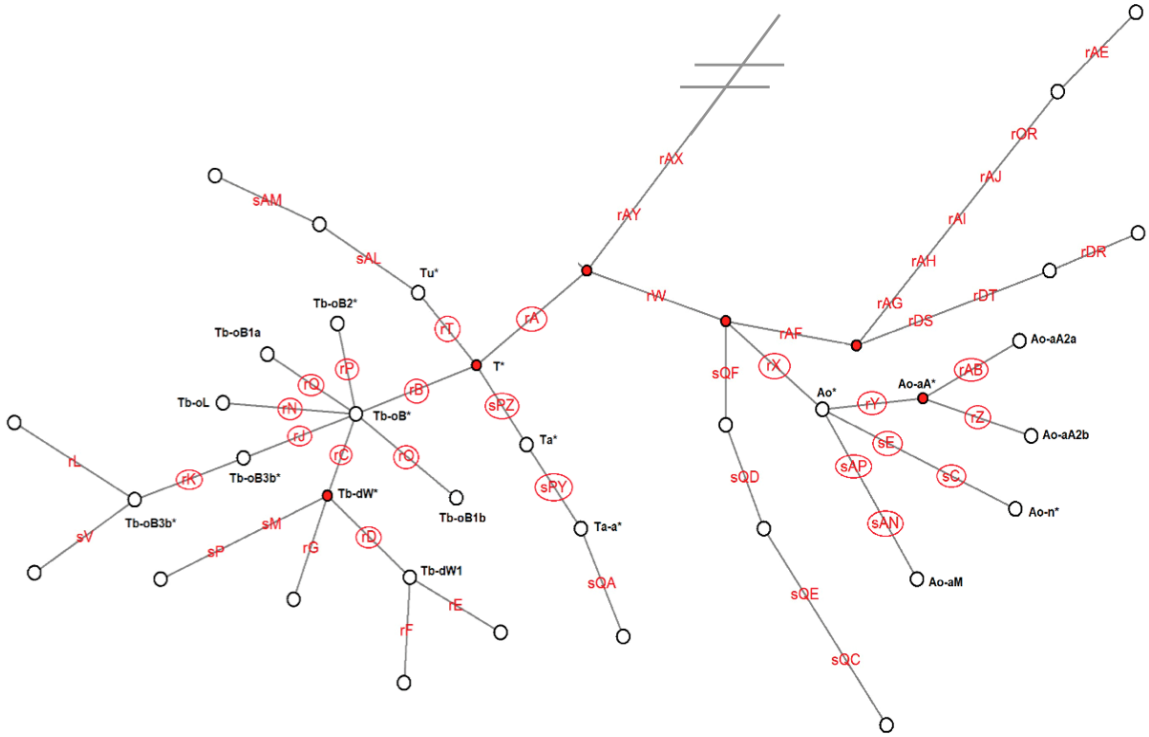

Figure 8: Phylogenetic tree after Felkel et al. 2018 inlcuding markers. Tested variants in context of this article are marked with a red circle.

Allele and haplotype nomenclature

The result was noted as Assay_0 (for example rX_0), if the allele was present in its ancestral form, according to the haplotype phylogeny. The derived allele is the variant in which a mutation has taken place, compared to the ancestral allele and was noted as Assay_1. In a first step, all samples were tested for rA and rX to roughly assign them to haplogroup A or T and to decide which further markers should be genotyped. If the result showed the ancestral allele on a variant basal to a haplogroup, the variants further down the branch were not tested for.
In case of rX_1 the next marker was rY followed by rZ and rAB if the derived allele (_1) was present at rY. If rY would be _0 sE, sC, sAN and sAP were genotyped (see Figure 8). Based on these results the HT of the respective samples was identified. In case of a sample not carrying any derived allele, the HT after the last derived allele was marked by an asterisk (for example Ao*,Table 13)
If the samples showed rA_1, they were tested for sPZ and sPY which define HT Ta. In some cases, the rA-assay revealed the derived allele but sPZ and sPY were ancestral.

Those samples were examined with the rT and rB assay. If they showed rT_1 no further examinations were carried out, but in case of rB_1 the following assays were also tested: rC, rD, rP, rO, rJ, rK, rQ and rN (see Figure 8).

Some samples had been previously analysed in Wallner et al. 2013, Wallner et al. 2017 and Felkel et al. 2018. The data were thankfully provided by Doris Rigler, who was responsible for the genotyping in these projects.

Table 12 shows the allele variations of each assay and Table 13 the compound HTs. The full DNA sequences of the different variants are listed in the appendix.

Table 12: Used assays

Assay	**Contig:pos_Yref2016**	**Allele1 FAM**	**Allele2 HEX**
rA	contigE_00017_1_6200_for_Y50869:1790	rA_A_1	rA_T_0
rX	contig4755:817	rX_G _0	rX_T_1
rY	contig12513:407	rY_ACC_0	rY_AC_1
rZ	contig17307_96	rZ_G _0	rZ_A_1
rAB	contig4734_1723	rAB_A_0	rAB_T_1
sPY	contig12570:15	sPY_T_0	sPY_C_1
sPZ	contig1670:11510	sPZ_A_0	sPZ_T_1
rC	contig12148:320	rC_T_0	rC_C_1
rD	contigE_00003_11200_14040 for_45288_HT5_HT6:337	rD_TT_0	rD_DEL_1
sAP	contig13667:93	sAP_A_0	sAP_T_1
sAN	contig1031:381	sAN_G_0	sAN_T_1
rB	contig697:412	rB_C_0	rB_G_1
rT	contig10304:68	rT_C_0	rT_T_1
sC	contig3599:1483	sC_A_0	sC_G_1
sE	contig5219:170	sE_C_0	sE_T_1
rO	contig1548:343	rO_C_0	rO_T_1
rJ	contig4106:526	rJ_G_0	rJ_C_1
rQ	contig1782:1077	rQ_T_0	rQ_C_1
rP	contig15338:132	rP_T_0	rP_G_1
rN	contig3106:1055	rN_G_0	rN_A_1
rK	contig7946:1401	rK_A_0	rK_C_1

Table 12 lists the used assays and the alleles linked to FAM™ and HEX™. _0 stands for the ancestral variant, _1 for the derived variant.

Table 13: Relevant haplotypes and corresponding alleles

Haplotype	**Alleles**			
Ao*	rX_T_1			
Ao-aA*	rX_T_1	rY_AC_1		
Ao-aA2a	rX_T_1	rY_AC_1	rAB_T_1	
Ao-aA2b	rX_T_1	rY_AC_1	rZ_A_1	
Ao-n*	rX_T_1	sE_T_1	sC_G_1	
Ao-aM	rX_T_1	sAN_T_1	sAP_T_1	
Ta*	rA_A_1	sPZ_T_1		
Ta-a*	rA_A_1	sPZ_T_1	sPY_C_1	
Tu*	rA_A_1	rT_T_1		
Tb-oB*	rA_A_1	rB_G_1		
Tb-dW*	rA_A_1	rB_G_1	rC_C_1	
Tb-dW1	rA_A_1	rB_G_1	rC_C_1	rD_DEL_1
Tb-oL	rA_A_1	rB_G_1	rN_A_1	
Tb-oB1a	rA_A_1	rB_G_1	rQ_C_1	
Tb-oB1b	rA_A_1	rB_G_1	rO_T_1	
Tb-oB2*	rA_A_1	rB_G_1	rP_G_1	
Tb-oB3*	rA_A_1	rB_G_1	rJ_C_1	rK_C_1

Table 14: Used controls for the respective markers

Assay	**Haplotype/ Haplogroup**	**Sample Number**
rX	Ao*	P070784, BW-383, Y_PR_05_124, Y_PR_05_125
rY	Ao-aA*	P070784, BW-383
rAB	Ao-aA2a	BW-387
rZ	Ao-aA2b	Y_PR_03_044
sE	Ao-n*	Y_PR_06_011, Y_PR_06_022
sC	Ao-n*	Y_PR_06_011, Y_PR_06_022
sAP	Ao-aM	No controls available
sAN	Ao-aM	No controls available
rA	T	Y_PR_02_009, P070172, Y_PR_05_145, Y_PR_05_146
sPZ	Ta*	Y_PR_02_009, P070172, Y_PR_05_147, Y_PR_05_153
sPY	Ta-a*	Y_PR_02_009, BW-381
rT	Tu*	Y_PR_02_006, FM2001
rB	Tb-oB*	Y_PR_04_002, Y_PR_04_010

rP	Tb-oB2*	No controls available
rC	Tb-dW*	Y_PR_11_024, P102777, Y_PR_04_033
rD	Tb-dW1	Y_PR_11_024, P102777, Y_PR_04_033
rN	Tb-oL	169
rQ	Tb-oB1a	P050035/4, P070301
rJ	Tb-oB3b*	169
rO	Tb-oB1b	FM1178, FM1785
rK	Tb-oB3b*	Y_PR_11_001, Y_PR_11_004
	Female	166, BC3

This table provides an overview about the assays and controls with known HT that were used.

2.3.4 Analysis of the data

The acquired dataset was collected in an Excel®-sheet and the HT-distribution was analysed for the different stallion lines and the different breeds. For the stallion lines it was determined whether all the sampled horses from one stallion line carried the same HT. Then the number of stallion lines showing each HT was determined. Combined with the number of samples analysed from each stallion line, a pie chart was created for each HT. The pie chart was then inserted into the phylogenetic tree after Felkel et al. 2018.

The breeds represented by the sampleset were analysed for the number of HTs found in each breed and the number of samples with each HT. These informations were combined into a bar chart.

3 Results

116 samples were analysed with qPCR for 21 Y-chromosomal markers in total and according to their allelic states, each horse was assigned to a HT.

3.1 Display and handling of the data

The results of each qPCR analysis run were separated according to the markers analysed for better outline and illustration in Cluster plots (Figure 9). The data were also exported to Excel® as shown in Figure 10 and further processed. If a result was negative or dubious, it was marked as ‘NO_CALL’. If a sample had more than two ‘NO_CALL’ results it got excluded from further analysis. Considering the pedigree information, a closely related sample, if available, was chosen as a replacement (see Table 15). A detailed list of all samples and their results are provided in the appendix.

The NTC and the female sample always showed NO_CALL.

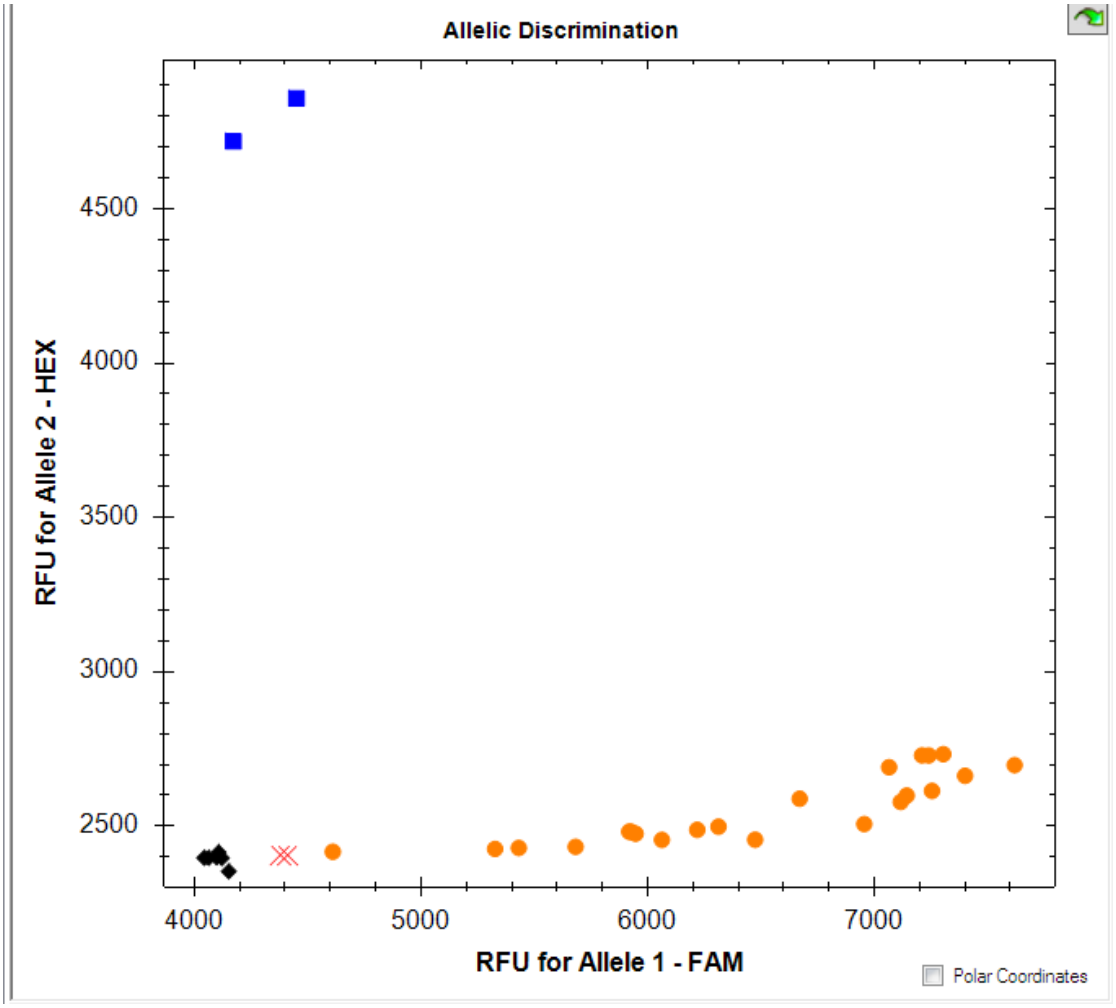

Figure 9: Cluster Plot of KaspEM7 for Assay sC

Figure 9 gives an example of the qPCR results in a Cluster Plot. The orange dots stand for samples emitting a FAM™ signal (carry allele 1), the blue squares stand for samples emitting a HEX™ signal (allele 2; in this case only two positive controls), the black squares stand for samples not emitting any signal and the red crosses stand for samples that emitted a signal not strong enough to be thoroughly detected. Those samples were counted as NO_CALL, as well as the samples not emitting any signals (black squares). The NTCs and female controls always clustered as black squares at the bottom left. As the Y chromosome is hemizygous in every male individual, no heterozygous outcomes were detected.

	B	C	D	E	F	G	H
1	Well	Sample	Call		RFU1	RFU2	
2	F06	BW-395	Allele 1	sC_A_0	5927,25	2481,63	
3	A06	empty	No Call		4121,02	2396,57	
4	B09	fam KO	Allele 1	sC_A_0	6670,43	2588,98	
5	C09	fam KO	Allele 1	sC_A_0	6312,38	2498,44	
6	F09	female	No Call		4107,05	2415,31	
7	D09	hex KO	Allele 2	sC_G_1	4450,26	4857,94	
8	E09	hex KO	Allele 2	sC_G_1	4172,01	4718,89	
9	G09	ntc	No Call		4043,57	2397,27	
10	H09	ntc	No Call		4063,24	2397,21	
11	C06	P060276	Allele 1	sC_A_0	7618,53	2697,92	
12	B08	P060411	Allele 1	sC_A_0	5431,15	2429,50	
13	A09	P070298	No Call	sC_NO_CAL	4151,82	2353,72	
14	C08	P070719	Undetermined	sC_NO_CAL	4411,41	2406,47	
15	B06	P080297	Allele 1	sC_A_0	4610,78	2417,20	
16	D06	P090614	Allele 1	sC_A_0	6955,15	2506,90	
17	E06	P110308	Undetermined	sC_NO_CAL	4379,71	2407,01	
18	H06	Y_PR_05_032	Allele 1	sC_A_0	7400,13	2663,26	
19	D08	Y_PR_05_046	Allele 1	sC_A_0	6474,01	2456,21	
20	G06	Y_PR_05_048	No Call	sC_NO_CAL	4097,83	2399,01	
21	H07	Y_PR_05_050	Allele 1	sC_A_0	5326,51	2426,35	
22	E08	Y_PR_05_063	Allele 1	sC_A_0	7116,31	2578,47	
23	B07	Y_PR_05_119	Allele 1	sC_A_0	5681,99	2433,18	
24	A07	Y_PR_05_127	Allele 1	sC_A_0	6062,74	2455,70	
25	H08	Y_PR_05_129	Allele 1	sC_A_0	5918,40	2482,26	
26	C07	Y_PR_05_131	Allele 1	sC_A_0	7254,20	2614,40	
27	F07	Y_PR_05_134	Allele 1	sC_A_0	6218,43	2488,23	
28	F08	Y_PR_05_141	Allele 1	sC_A_0	5946,55	2475,67	
29	G07	Y_PR_05_148	Allele 1	sC_A_0	7064,84	2691,02	
30	D07	Y_PR_05_157	Allele 1	sC_A_0	7210,14	2729,44	
31	G08	Y_PR_05_161	Allele 1	sC_A_0	7239,58	2729,25	
32	E07	Y_PR_05_162	Allele 1	sC_A_0	7303,81	2733,35	
33	A08	Y_PR_05_164	Allele 1	sC_A_0	7142,72	2599,33	
34							
35			KaspEM7				
36			sC				
37			allel1=fam=	sC_A_0			
38			allel2=hex=	sC_G_1			
39							

Figure 10: Example of a results sheet in Excel® (KaspEM7, assay sC)

Figure 10 shows displays the qPCR results in Excel®. In this example Allele 1 is linked to the ancestral allele (*_0). The samples that could not be determined in the reading process were counted as NO_CALL.

In the beginning 110 samples were analysed. Of those 14 needed to be excluded because qPCR failed in two or more assays. For six of the failed samples, closely related replacement

samples derived from a paternally closely related individual, were collected. Of the six replacement samples, five yielded results, one had NO_CALLS. This sample was excluded from analysis as well. For the remaining 101 samples the HT was allocated according to the allelic state of the genotyped variants (Figure 8). 63 individuals were assigned to HG Ao (HT Ao* or Ao-aA*), 30 to HG Ta (HT Ta* or Ta-a*), six to HG Tb (HT Tb-oB* or Tb-dW), one to HT Tu* and one could only be clustered roughly because it remained at the root of HG T with showing only the derived allele of variant rA (noted in results as T*). Figure 11 shows an overview of the HT distribution in the probed samples.

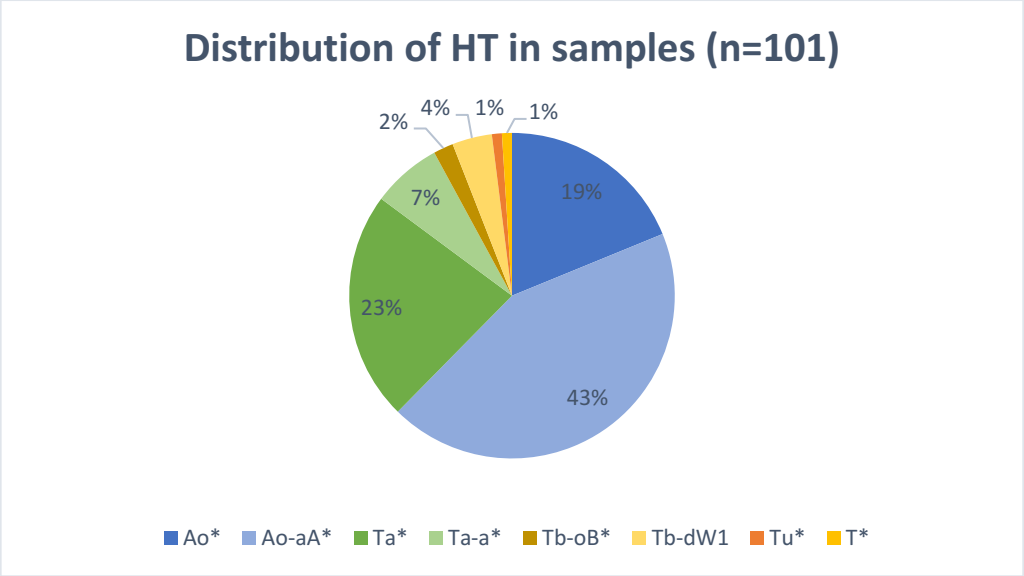

Figure 11: Distribution of haplotypes in 101 samples

Table 15: Excluded samples and their replacement samples (if available)

Sample Number	Replacement sample
P080269	No replacement
Y_PR_05_120	No replacement
P080268	No replacement
P070740	P080268
P080194	No replacement
Y_PR_05_117	No replacement
P070298	Y_PR_05_041
P070719	No replacement
Y_PR_05_040	Y_PR_05_036
Y_PR_05_034	P070784
Y_PR_05_042	Y_PR_05_049
Y_PR_05_123	Y_PR_05_126
P080265	No replacement
P110308	No replacement
Y_PR_05_048	No replacement

However, five samples were not excluded from the final analysis despite having one or two NO_CALLS. These samples were namely BW-382, P060881 (Saklawi I line), P080297 (Kidwan al Harzan line), P070864 (Souakim line) and Y_PR_05_116 (Kuhailan-Abu-Urkub line).

Samples BW-382 and P060881 had a NO_CALL for marker rZ and because all the other samples examined for rZ had the ancestral allele, it was assumed that these samples would be no exception. P060881 also had a NO_CALL for marker rAB, but the derived allele at this variant was not detected in any of the samples, so it was not examined further in this case. Sample P080297 had a NO_CALL for sAP and sAN. The derived allele at these variants were not detected either in any other of the examined Arabian samples, so the sample was not excluded. P070864 had a NO_CALL for sPY. After repeating the qPCR three times it was left this way. A result at this marker would have been important for P070864, to determine if the sample has HT Ta* or Ta-a*. In the analysis the sample was counted as Ta*. Keeping in mind that the sample was the only one of this stallion line (Souakim line) and the results could not be confirmed with paternal relatives. Sample Y_PR_05_116 had only a NO_CALL for marker rP in the end, but other variants needed numerous runs to get a result, indicating that DNA quality of this sample was very bad. Isolating the sample two more times, even with another isolation kit (see 2.2.1.2) did not improve the results. During most runs, the sample only yielded a result after the second recycle. After a meeting with my tutor Barbara Wallner, we agreed to include the sample despite the difficulties, as it was one of only two samples of the Kuhailan-Abu-Urkub line and the other sample had yielded a different HT. Barbara Wallner suggested that the original blood sample probably contained heparin to block blood coagulation, but it would also inhibit the PCR.

3.2 Linking Y-chromosomal HTs with stallion lines

3.2.1 Haplogroup Ao

HG Ao could be assigned to 63 samples of 15 different stallion lines. It could be further divided into HT Ao* (rX_1 but rY_0) and Ao-aA*(rX_1 and rY_1) (see Figure 8). A few more HTs would have been possible, like Ao-aA2a, Ao-aA2b, Ao-n* and Ao-aM (see Table 13: Relevant haplotypes and corresponding alleles) but these HTs were not detected in the Arabian horses tested.

Of the 63 samples, 19 samples were assigned to Ao* and 44 to Ao-aA*. Horses from seven stallion lines show HT Ao*, namely those founded by Hadban, Kidwan al Harzan, Krzyżyk, Kuhailan Kharas, Mirage, Shuwayman Sabbah and Telmese. Lines founded by A Saglawi DB, Felhaan Alshawaf, Hadban (Ibn Galal), Ibrahim, Ilderim, Koheilan Adjuze, Mahmoud Mirza and

Saglawi I mainly carried Ao-aA*. However, in the Saglawi I line there were also three samples with HT Ao* and one with Tu*. In the A Saglawi DB line one sample was assigned to HT Ta* and one from the Ilderim line to Ta-a*. Table 16 provides an overview of the number of samples that were examined for each stallion line and their respective HTs.

P060881 (Saklawi I line) had a NO_CALL for the rAB and rZ assay but was not excluded since all the other assays had worked. BW-382 (Saklawi I line) had a NO_CALL for rZ and P080297 (Kidwan al Harzan line) had a NO_CALL for sAP and sAN, but they were not excluded either for the same reasons.

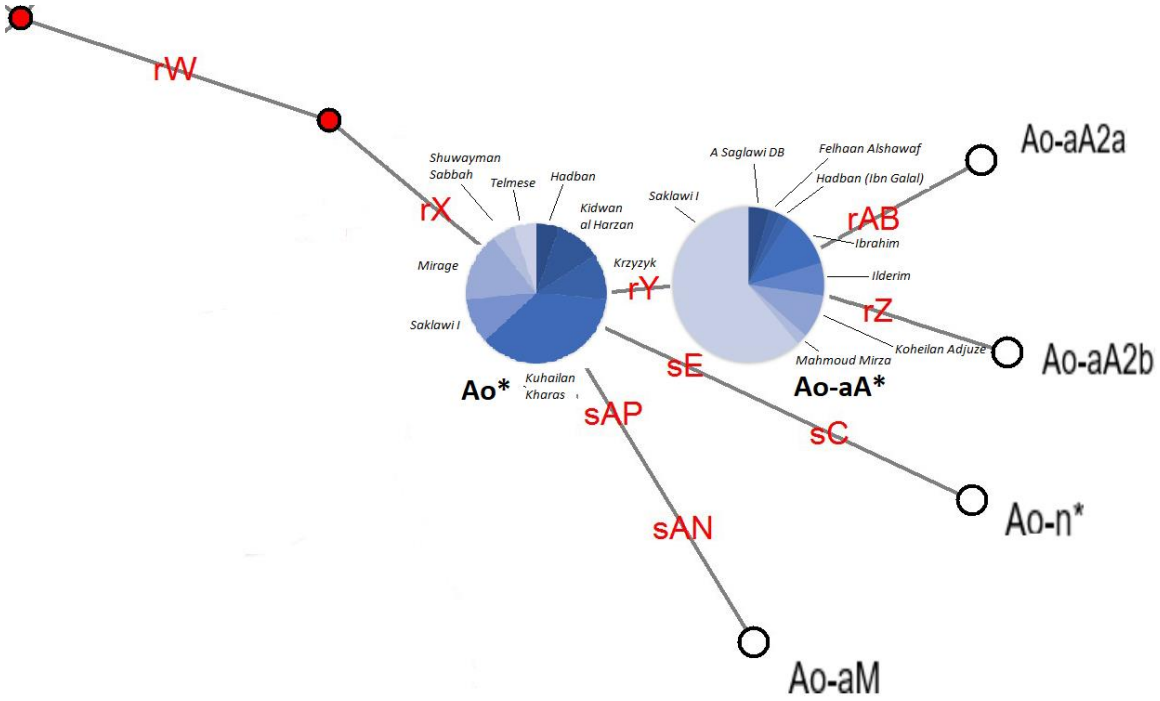

Figure 12: Distribution of the samples assigned to Ao HTs. The figure is modified after Felkel et al. (2018); the size of the circles correlates with the number of samples that showed the HT (Ao: 19 samples, Ao-aA*: 44 samples). The subcolours of the pie charts show which stallion lines, the samples with the respective HT belonged to. HTs not detected in the analysis are shown as white circles.*

3.2.2 Haplogroup Ta

HG Ta was found in 30 samples, representing nine stallion lines. If both, the sPZ and the sPY assay, showed the derived allele (sPZ_1 and sPY_1), which was observed in seven samples, they were summarized as Ta-a*. 23 samples had the derived allele at sPZ but the ancestral allele at sPY, they therefore cluster at Ta*.

The stallion lines which showed the Ta* HT were founded by Dahman-Amir, Hamdani Semri (except for one sample with HT Ta-a*), O Bajan, Shagya, Souakim and one sample from A Saglawi DB line, a line mainly showing Ao-aA*.
The seven samples showing Ta-a* represented the lines Bairactar, Hamdani Semri, Mersuch and one sample assigned to Ilderim (Sample Y_PR_02_009), a line mainly showing Ao-aA* (also see Table 16).

Sample BW-380 from the Bairactar line could only be assigned to T*, since the assay rA showed the derived allele, but all other assays tested (sPZ, rT, rB, rC and rD) showed the ancestral allele (_0) although repeated once.

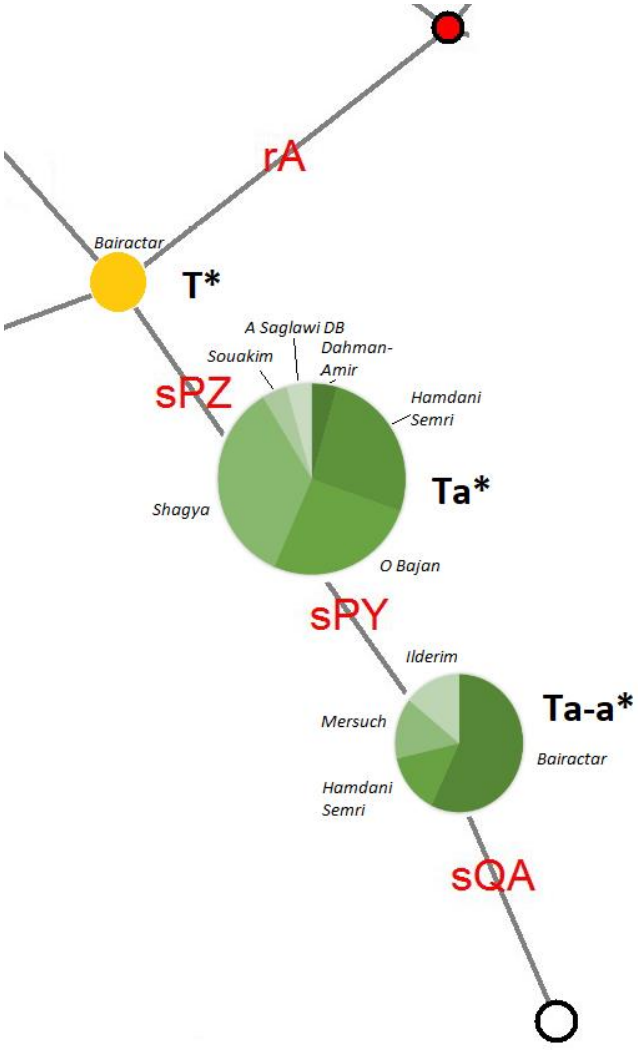

Figure 13: Distribution of the samples with HT Ta , modified after Felkel et al. (2018); the size of the circles correlates with the number of samples that showed the HT (Ta: 7 samples, Ta-a*: 23 samples). The subcolours of the pie charts show which stallion lines the samples belong to.*

3.2.3 Other Haplotypes

A few samples showed HTs previously not assigned to Arabians. One sample clustered as Tu*, two as Tb-oB* and four had Tb-dW1.

P090273 from the Saklawi I line was assigned the Tu* HT (rA_1, sPZ_0 and rT_1).

The two samples clustering at Tb-oB* were one from the Hamdani el Samra line (P112348) and one from the Kuhailan-Abu-Urkub line (Y_PR_05_116). Both samples showed the derived alleles at the rA and rB locus, yet all other possible assays of this part of the phylogenetic tree yielded the ancestral alleles.

Four samples clustered at HT Tb-dW1. They carried the derived alleles at rA, rB, rC and rD. Two were descendants of the stallion Amer (Y_PR_05_137 and Y_PR_05_138) and the only two samples of this line. Sample Y_PR_05_039 descended from Kuhailan-Abu-Urkub (the other sample of this line showed the Tb-oB* HT, as mentioned above) and Y_PR_05_061 was an outlier of the Shagya line, which mainly showed Ta*.

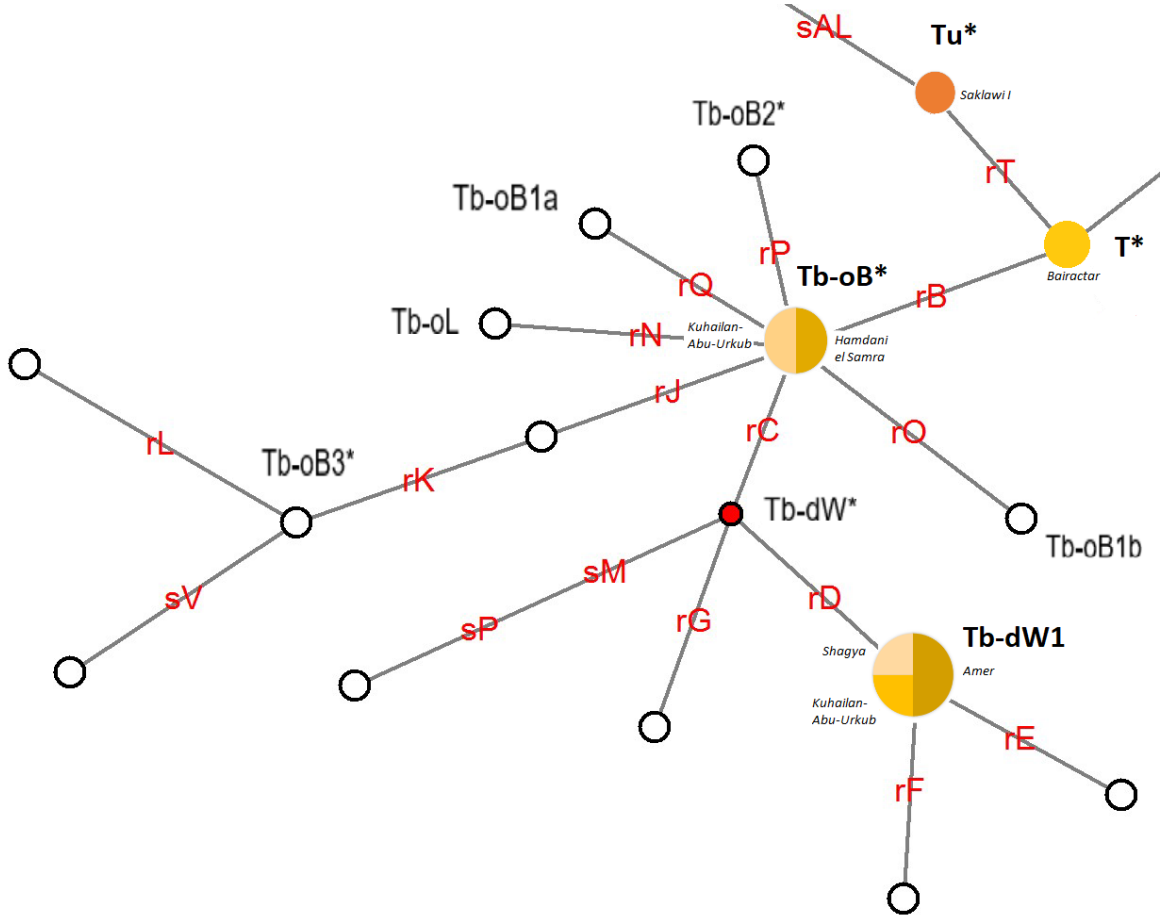

Figure 14: Distribution of the non-Arabian HT, modified after Felkel et al. (2018); the size of the circles correlates with the number of the samples with the HT (T: 1 sample, Tu*: 1 sample, Tb-oB*: 2 samples, Tb-dW: 4 samples). The subcolours of the pie charts show which stallion lines, the samples with HT Tb and Tu belonged to.*

Table 16 summarizes the number of samples representing each stallion line and the observed HTs.

Table 16: Number of samples representing each stallion lines, the observed HTs and the breeds the samples derive from

Founder	**Number of samples**	**Observed HTs**	**Breed**
A Saglawi DB	3	Ao-aA* (2), Ta* (1)	Shagya-Arabian (2), Partbred (1)
Felhaan Alshawaf	1	Ao-aA*	Original Arabian
Hadban (Ibn Galal)	1	Ao-aA*	Shagya-Arabian
Ibrahim	5	Ao-aA*	Arabian (2), Original Arabian (2), Partbred (1)
Ilderim	4	Ao-aA* (3), Ta-a* (1)	Arabian (1), Original Arabian (3)
Koheilan Adjuze	4	Ao-aA*	Original Arabian (1), Shagya-Arabian (3)
Mahmoud Mirza	1	Ao-aA*	Shagya-Arabian
Saklawi I	31	Ao* (3), Ao-aA* (27), Tu* (1)	Arabian (2), Anglo-Arabian (1), Original Arabian (25), Shagya-Arabian (2), Partbred (1)
Hadban	1	Ao*	Shagya-Arabian
Kidwan al Harzan	2	Ao*	Shagya-Arabian
Krzyżyk	2	Ao*	Original Arabian
Kuhailan Kharas	7	Ao*	Arabian (1), Original Arabian (4), Shagya-Arabian (1), Partbred (1)
Mirage	2	Ao*	Original Arabian
Shuwayman Sabbah	1	Ao*	Shagya-Arabian
Telmese	1	Ao*	Anglo-Arabian
Bairactar	5	Ta-a* (4), T* (1)	Original Arabian
Mersuch	1	Ta-a*	Shagya-Arabian
Dahman-Amir	1	Ta*	Partbred
Hamdani Semri	7	Ta* (6), Ta-a* (1)	Shagya-Arabian
O Bajan	6	Ta*	Partbred (1), Shagya-Arabian (5)
Shagya	9	Ta* (8), Tb-dW1 (1)	Shagya-Arabian
Souakim	1	Ta*	Shagya-Arabian
Amer	2	Tb-dW1	Original Arabian
Kuhailan-Abu-Urkub	2	Tb-dW1 (1), Tb-oB* (1)	Original Arabian (1), Partbred (1)
Hamdani el Samra	1	Tb-oB*	Partbred
Total	101		

3.3 Distribution of HTs in the different breeds

The sample collection represents males from five different breeds: Original Arabians (OX), Arabians, Anglo-Arabians, Partbred Arabians and Shagya-Arabians (see also Table 6). Original Arabians showed a clear trend to HG Ao, with 40 out of 48 samples (~ 83 %) having either Ao* or Ao-aA*. A large portion of Shagya-Arabians showed one of the HTs associated with HT Ta. In 23 out of 37 samples (~ 62 %) either Ta* or Ta-a* was found. The Arabians mainly showed HG Ao (four out of six samples) as well as the Partbred Arabians (four out of eight samples), while the Anglo-Arabians only showed HG Ao. HTs Tu*, Tb-oB* and Tb-dW1 were not found to be enriched in one breed but appear in nearly the breeds (see Figure 15).

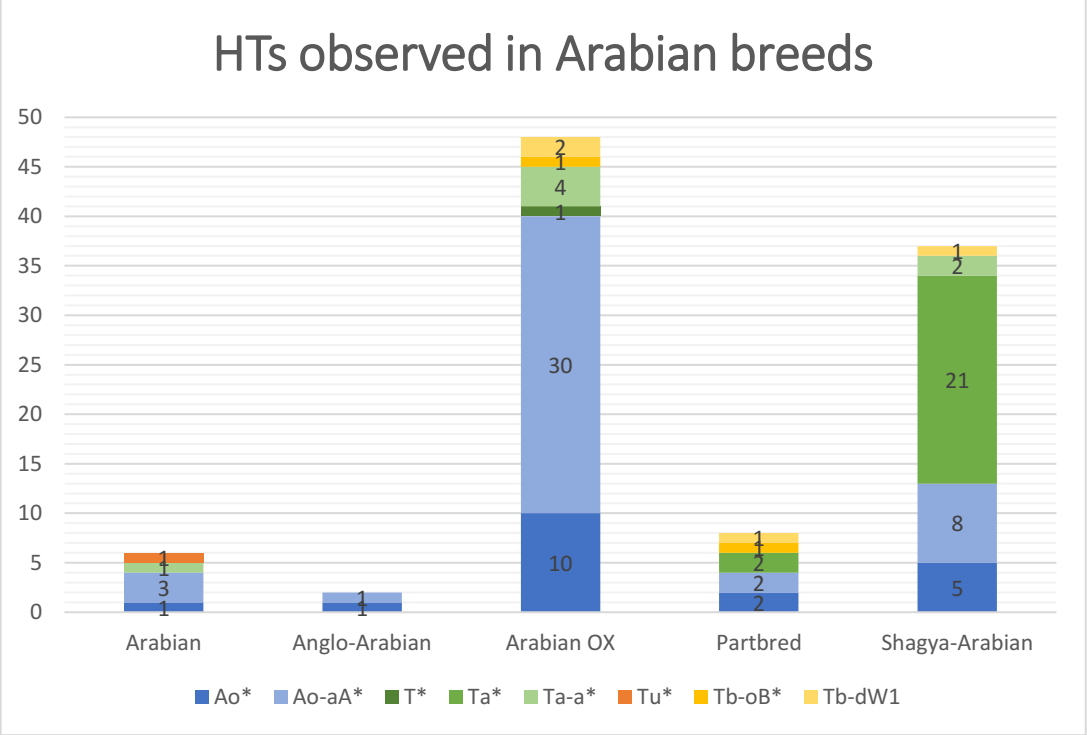

Figure 15: Distribution of HTs in the different breeds represented by sampleset. The bars represent the number of samples with the respective HT.

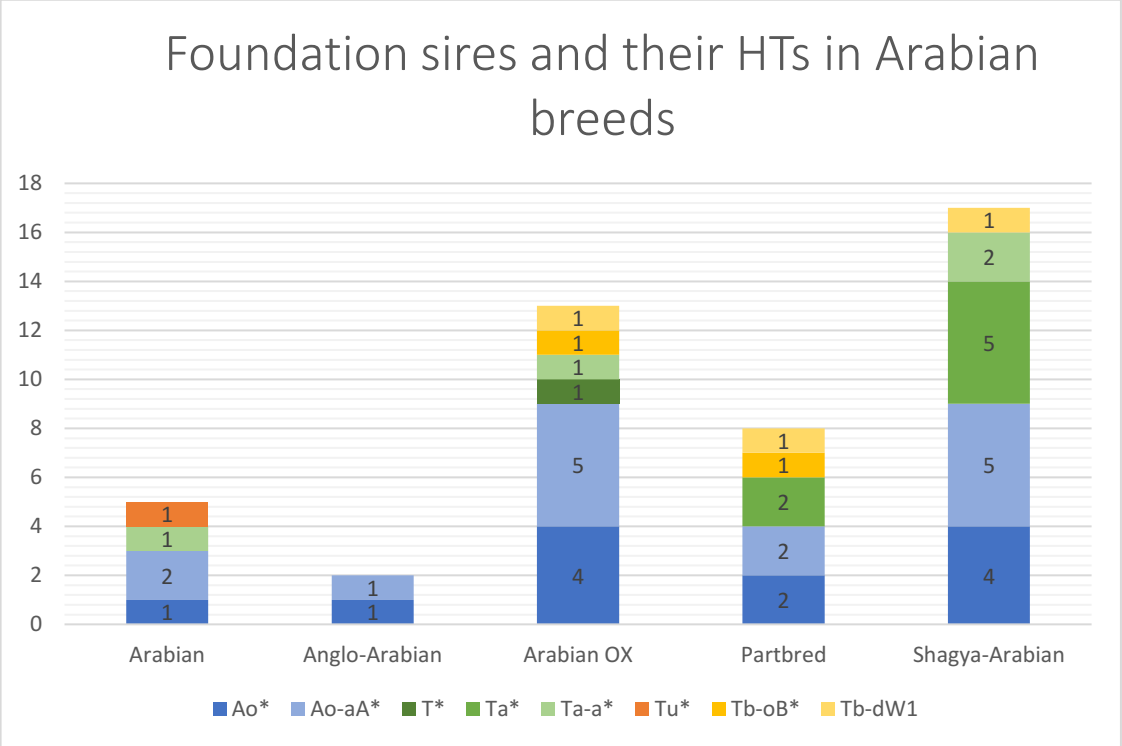

Figure 16: Number of foundation sires and their respective HTs in the different breeds represented by sampleset. The bars represent the number of stallion lines and colours their respective HT.

4 Discussion

Despite the exceptionally long breeding history of Original Arabians and their dispersion over the whole word, Wallner et al. 2017 and Felkel et al. 2018 found only two Y-chromosomal HTs in a small sample group of descendants of imported stallions. In this project, a collection of present Arabian males being representative for the male progeny of many imported sires, was analyzed using KASP® technology.

4.1 MSY and imported Arabian stallion lines

When Wallner et al. first genotyped Original Arabians, they named HG Ao after them, as this HG seemed to be specific for and the only one appearing in Arabians. Yet, they found some Arabian horses clustering at the basal node of the T HG (Wallner et al. 2017), which were assigned to the new HT Ta after further investigations by Felkel et al. (2018).

So far, the findings of this study accord with the former findings from Wallner et al. (2017) and Felkel et al. (2018). The most frequent HTs were Ao-aA*, Ta*, Ao* and Ta-a*, and only a few samples had HTs apart from these two HGs.

Additionally, HG Ta was split into two subgroups: Ta* and Ta-a*. Felkel et al. (2018) found HG Ta in a single Arabian sample and identified three markers that determine this HT. Two of these markers (sPY and sPZ) were tested for and all samples that were found clustering in HG Ta carried the derived allele of sPZ (sPZ_1), but only some of them had the derived allele at sPY (sPY_1). It was concluded that the mutation at sPZ occurred earlier in history than the mutation at sPY, and sPZ is therefore closer to the root of the phylogenetic tree. Accordingly, two subgroups were defined: Samples clustering at Ta* show sPZ_1 and sPY_0 and samples clustering at Ta-a* show sPZ_1 and sPY_1.

As described above in the results, some HTs had a higher frequency in certain breeds than in others. The Original Arabians in this sample set show a remarkable tendency to HT Ao-aA* (see Figure 15). However, of the 30 Original Arabian samples with this HT, 27 belong to the Saklawi I-line and therefore this line is overrepresented in the sample set. Deep pedigree re-construction suggests that the enormous impact of Nazeer (born in 1934; a descendant of Saklawi I) is responsible for the high frequency of HT Ao-aA* amongst Original Arabians bred in Europe.

Shagya-Arabians were the only breed with a majority of HG Ta. This is mainly because some imported sires with this HT were only present in this breed (see Table 16).

The reason for observing two predominant, distantly related MSY HGs in imported Arabians needs to be addressed in future studies. Perhaps there was substructure in the source population and expeditioners, who bought Arabian stallions to breed them with European mares (for example the Shagya-Arabian breed in Bábolna; see Introduction), focused on different characteristics in the horses, than expeditioners, who bought Arabian stallions to breed Original Arabians (for example in Weil). On the other hand, it could also be that in the source population of the imported horses these two HGs were present at equal frequencies. To address this further, MSY HG distribution in the Middle East at the time of the import needs to be determined. This can be performed by the inclusion of ancient and historic samples, like Gaunitz et al. 2018 did with Botai horses or Librado et al. 2017 with Bronze and Iron Age horses.

Partbred Arabians show an even mix of several HTs, mostly “Arabian” HTs (Ao*, Ao-aA*, Ta*) but also others like Tb-oB* and Tb-dW. Per definition, Partbred Arabians need an Original Arabian, Shagya-Arabian or Anglo-Arabians ancestor in the second generation of their pedigree, no matter which breed they were mated with. However, the pedigree of the tested horses claimed that their paternal ancestors were all Arabians. If the pedigrees are correct, even those Partbred Arabians should not have HT Tb-dW1 or Tb-oB*, as these HTs indicate a Thoroughbred or Akhal-Teke ancestry (Wallner et al. 2017).

Tested Arabians had a HT spectrum similar to Original Arabians. This suggests, that they were as purely bred as the Original Arabians, but their ancestry is not “asil” enough to be considered an Original Arabian.

Both Anglo-Arabians investigated had an “Arabian” HT. In this breed it would be least startling to find a “Thoroughbred” HT. But according to pedigree in the two tested horses, the English Thoroughbred influence is on the maternal side, which is typical for the first generation of Anglo-Arabians and does not influence the MSY HT at all. As long as no Thoroughbred stallion is introduced to the line, the male offspring will have an ‘Arabian’ HT. From the pedigree one of the Anglo-Arabians is a descendant of Saklawi I line and the other of Telmese and the Y HT data match with the pedigree.

4.2 Other haplotypes in Arabian horses

Seven horses had HTs other than HGs Ao and Ta. Two had Tb-oB*, four had Tb-dW1 and one had Tu*. Those results were unexpected, as those three HTs had been associated with different breeds previously.

In the paper from 2017, Wallner et al. described the HT Tb-dW1 as specific for a widely distributed English Thoroughbred line tracing back to the stallion Whalebone (1897), while Tb-oB* (in their paper referred to as basal HT Tb) was also found in horses tracing back paternally to the English Thoroughbred founder Byerly Turk (1680) as well as other breeds, especially the Akhal-Teke. The Akhal-Teke is the remnant of the influential and now extinct Turkoman horse. There is an ongoing debate on the history of Turkoman and Arabian horses (Hendricks 1995). Under the aspect of the similarity in the history of these two horse types (both are horses from the desert), it could be that horses of Turkoman origin were assigned as 'Arabians' and vice versa. Głażewska (2010) also found the mtDNA of Arabian and Turkoman horses to be quite similar, so maybe those two breeds have a common ancestry. It cannot be yet distinguished if the presence of Tb-oB* in Kuhailan-Abu-Urkub and Hamdani el Samra is due to a recent cuckoldry or because the imported horse already carried this HT.

The presence of Tb-dW1 in certain lines is easier to interpret, as this is a very young HT that occurred in 1800 in the English Thoroughbred. Both samples of the Amer line showed Tb-dW1, which is typical for the descendants of the Thoroughbred stallion Whalebone (born 1807). Most likely those two individuals have a recent paternal ancestor, who was an English Thoroughbred. However, the ancestry of Amer himself is heavily doubted amongst breeders. Scientific proof cannot be found, but there are lots of rumours and theories circulating. A blog entry from Edouard al-Dahdah from 2008 states that "Amer is the biggest scandal in modern Arabian horse breeding" (http://daughterofthewind.org/note-on-amer-saudi-race-stallion/; Access: 27.12.2018). The finding of HT Tb-dW1 in both available samples supports the theory that the horses of this line are influenced by English Thoroughbreds to a certain extent. Tb-dW1 in two other horses (one from the Shagya and one from Kuhailan-Abu-Urkub) can also be interpreted as wrong paternities or mix-up of samples (see below 4.3).

4.3 Technical challenges

Since mainly DNA extracts from backup samples for paternity testing were used, a certain proportion of samples were expected to have low DNA quality.

Of the 116 samples analysed, 15 needed to be omitted from the final analysis, as the PCRs yielded no results in two or more loci. This could be due to DNA damage from long storage or a mistake in the process of DNA extraction. To not lose too many samples, a few specimen were exempted from the rigorous sorting out (as described in 3.1)

Further it was observed in some lines (see Table 16) that single horses do not cluster in concordance with the majority of samples of the particular line. One question that remains, is whether the outliers are sampling errors or cuckoldries. Since the samples used, were collected by many different people, the possibility that mistakes were made during the collection and handling of these samples or during DNA extraction cannot be excluded. Fresh tissue samples of these horses would be needed to confirm or reject the observed inconsistencies.

4.4 The Arabian horse from the MSY perspective – open questions and potentials

By extending the sample size and sampling closely related individuals of one line a detailed HT- and pedigree overlay (as shown for Darley Arabian in Wallner et al. 2017) a more detailed picture could be drawn for particular lines in the future. Then it would also be possible to find the source of outliers in certain Arabian lines. In case of a confirmed cuckoldry, an inclusion of information from studbooks should make it possible to predict potential fathers. Especially in the case when several stallions with different HTs were used for breeding in a stud at the same time.

However, all the findings in this thesis do only apply to the Arabian population of Austria, Germany and Poland. For this project, no samples from other countries were investigated, which would be highly appreciated for future studies.

A more complete understanding of the ancestry of the Arabian horse can only be achieved by including Arabian males from the Arabian Peninsula. Those samples should be collected from different breeding regions to get a full view on the Y-chromosomal lineages preserved in the Arabian horse.

Maybe the picture would turn out to be different to the patterns observed in Arabians imported to Europe. An inclusion of samples from skeletons of known origin, could further refine the view on the development of the breed in its original breeding area as well as the global influence of the Arabian horse.

5 Abstract

The male specific part of the Y-chromosome is inherited without recombination from the father to the son. Therefore, this part of the genome perfectly matches the paternal line and polymorphic genetic markers on the Y-chromosome can be used to genetically trace male genealogies of populations. The Arabian horse is one of the oldest breeds in the world. Present European Arabians trace back paternally to a few sires, that were imported from the original breeding area on the Arabian Peninsula during the last 200 years. The aim of the imports was to breed Arabian horses in Central Europe and to refine local horse populations with these stallions.

In this study Y-chromosomal haplotyping was performed, using qPCR and the KASP® technology, to investigate the paternal origin of Arabian Sire lines. It was aimed to verify whether the two Y-chromosomal haplogroups recently described in a few Arabian males would also be the only ones in a larger sample group. 116 samples from five Arabian associated breeds from three countries were tested with 21 markers and eight haplotypes from three haplogroups were distinguished.

The predominance of the previously described haplogroups (Ao and Ta) in European Arabians was confirmed. A few unexpected findings were made, namely haplotypes not expected in Arabian horses. Such observations are most likely outliers due to pedigree errors or during the procedure of handling the samples. Whether those unexpected haplotypes have already been carried by the imported sires, could be topic of future studies.

Zusammenfassung

Der nicht rekombinierende Abschnitt am Y-Chromosom wird ohne Rekombination vom Vater an den Sohn weitervererbt. Dadurch spiegelt dieser Teil des Genoms perfekt die väterliche Herkunft wider und mit polymorphen genetischen Markern am Y-Chromosom können somit väterliche Genealogien einer Population genetisch nachvollzogen werden. Der Araber ist eine der ältesten Pferderassen der Welt. Die heutigen Araber in Europa gehen zurück auf wenige Gründerhengste, die aus dem ursprünglichen Zuchtgebiet auf der Arabischen Halbinsel in den letzten 200 Jahren nach Europa importiert wurden. Das Ziel dieser Importe war es, sowohl Araber in Europa zu züchten, als auch die örtliche Pferdepopulation mit diesen Hengsten zu veredeln.

In dieser Studie wurden Y-chromosomale Haplotypen untersucht, unter Verwendung von qPCR und KASP® Technologie, um die väterliche Herkunft von arabischen Hengstlinien zu untersuchen. Das Ziel war es zu verifizieren, ob die zwei kürzlich in arabischen Hengsten beschrieben Haplogruppen, auch in einer größeren Probenmenge die Einzigen sein würden. Es wurden 116 Proben fünf Araber-assoziierter Rassen aus drei Ländern mit 21 Markern untersucht und zwischen acht Haplotypen aus drei Haplogruppen unterschieden.

Das Überwiegen der kürzlich beschriebenen Haplogruppen (Ta und Ao) in europäischen Araberhengsten wurde bestätigt. Es wurden aber auch unerwartete Entdeckungen gemacht, nämlich Haplotypen, die in arabischen Pferden nicht zu erwarten waren. Diese Beobachtungen sind vermutlich Ausreißer durch Fehler in den Stammbäumen oder im Prozess der Bearbeitung der Proben. Ob diese unerwarteten Haplotypen schon bei den importierten Hengsten vorhanden waren, könnte das Thema einer zukünftigen Studie sein.

6 List of abbreviations

HG	Haplogroup
HT	Haplotype
Imp	Imputed
InDels	Insertions and deletions
KASP®	Competitive allele-specific PCR
Mb	Megabases
MSY	Male-specific region of the Y chromosome
mtDNA	Mitochondrial DNA
NC	NO_CALL
ND	Not done
NGS	Next-generation sequencing
NRY	Non-recombining region of the Y chromosome
NTC	No template control
OX	Original
PAR	Pseudoautosomal region
qPCR	Quantitative real-time PCR
rcf	Relative centrifugal force
rpm	Rounds per minute
SNP	Single nucleotid polymorphism
SNV	Single nucleotid variant

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8 Illustration directories

9 List of Tables

10 Note of thanks

Finally, I want to thank everyone who was directly or indirectly involved in the working process.

Most importantly, I want to thank my mentor Dr. med. vet. Barbara Wallner, who supported me all the way through. If I needed explanations, samples or motivation, she was always there to help and cheer me up. Especially during exam times, she was very understanding, accepting me to lay down the work for a few weeks to focus on studying. Thank you for all your kind support during the last few months!

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Special thanks go out to my colleague and friend Christina Hartsleben. Thank you for getting me interested in this topic and helping me think through some issues to find a solution.

Last but not least I want to thank my family and my boyfriend for their mental support during my time in Vienna. Thank you for enduring my moments of frustration, even if the reason was not always clear, and for being with me through all the ups and downs.

11 Appendix

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11.2 Sequences of the used assays

rX contig4755:817

ACATGCAATTTTCAACCTGCACTACCTGATCAACGAAATCAAAATAAATTCCGCACAT-
ACCCATATTATTTCCTATTTTTTTTTTTTCTTTCTTGTATGACACAAGCTTCTTGTCACATT
CGAGTCAGGGTCCATTAGAGTTTTATCTTT[G/T]CGTGTGGAATAAGATGAC-
CAATGTGGTGATTTCTGTTCTGCACTTGTCAGAGTCCCCA-
TAGTTATAAATAAAATCATCTGATGAAGGTTTAAGAGCACTGGTAGTATTTTGCATCAGC
CAAGGGAGTATGAGTCTTAGAAATTGTTTTCC

rY contig12513:407

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CCATAACCCCTCCTCCTAGAACCACCTCTTGATACT[ACC/AC-]ACCACAGTGGCAAGAA-
GAGGGGCTCTGAAGAGTGTGTGCCACTATTTTCTCATA-
GAGCTTTAATGTGGTTGACTTTGTGCTTGCCTGGGGTGGGAACATCATAGTTGATTTCTA
GATTTTCTCACGAAAGAAACTGGCACATGTTTTG

rZ contig17307_96

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CATATTTA-
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rAB contig4734_1723

ATACATTTACCTTTACTTTTATGTAATTCATTTTATTGAAATTGGTTATTATAGCAATT-
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CAGAGACATTCATGTAATACTTCCTTACC[A/T]GACGATAATCCTCTCAGATTTTAAACTA-
GAAACAATCTGTACTAAGTACGCAAATGCCTG-
GACACTGCCTCTATTTCAGTATAGATCCAGGCAGGTAAGTATTAAATCCTTTCTGGTTCA
TTTCATAATTTTTTTTAGATTTTTCTCTTG

sE contig5219:170

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GA-
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GGTATGTCAATATTCAGCTACCTGCTACTTTTT-
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sC contig3599:1483

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sAN contig1031:381

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sAP contig13667:93

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AAATATCTA

rA contigE_00017_1_6200_for_Y50869:1790

ATACTTGAGAGCACATGTTGCAAAGCCAAGATGCAGGGCCTAAGTTGTTCGCAGAGAG-
CAA-
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AGGCTTCCATGAATGACTCTCGAGTTCTACA[T/T]TCACAGTTTGAGATTACTGTTTA-
GAAAGCACACTTCTGTTGGTTGCAGGCGTGAATAACAG-
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CGGGTGGACACTGGACACCACCAGTCAG

SPY contig12570:15

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SPZ contig1670:11510

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CTCCAAACAATATATTAGTGGGCCTACACTAAGCAC[A/T]CGAGGACTCTGACAAAC-
CTCAGAGGACCCACTCACCTCAGGGGATCCAG-
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rT contig10304:68

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AACC

rB contig697:412

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rD contigE_00003_11200_14040for_45288_HT5_HT6:337

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rQ contig1782:1077

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GGACATTTGGCAATAGCTGGAGACATATTTAGTTG

rO contig1548:343

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TAAACTCCACAAATAGGCACAGTGTCTCCTA-
GAAATGTTTACCTTCTGGAGATAACACATACTATTAATCATTCTCCTCCAGATAAGACATA
TTATGTCACTAGAAAGTCAAATGCTTTCCCGCGGGGA

rJ contig4106:526

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TAAAAATGCCACAGCAATGACAAACTGCTTCAAGTTTCTGAAGACTCTCATTTTCTTTATC
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CAGTGTGCTCAAATATCTTTTACCACAC

rK contig7946:1401

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CTAGCTCTCGTCAGGGAGGAAGAGGGAAAAGTGCTTGGGTAAGTATGGGAAAGAGACA
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GACTCCTGACTTATAAAGGTGTTTATGAGAT-
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rP contig15338:132

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TAATCAAATTTAGCTCATAGGTTTTTCAGATCAGTCATCTAAAACTATTGTGTTTCTATTG
GTTTTAACATCGCCGCCTTTTTT[T/G]TTTGTTTGTTTTGGGGGATGTTTTTCCTGTG-
CAAAAAGAAAGTGGTGTCTTACATAGCTT-
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TGCTCTAATTGAACCCTACATTGTTCAAGT

rN contig3106:1055

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TCATAAAATCACCTCCTG-
TATCATTTCATTTAAATGAAATGTCCAGAATATGCAAATCTGTCTAGAGAAAAAATAGTGA
TTGCCAAGGGCTGGGAAAGGGGAAAATGGGGATTGACTGCAAATGAGT

11.3 Tables with results

In the following tables, each sample will be listed with the results of the analysed assays. The tables will be separated after the HG the sample was sorted into. To list all 21 assays for each sample would make the table very big and confusing, so I will limit the tables to the assays relevant for each HG. In some cases, other were tested as well, but since they were relevant for a different HG, the corresponding allele was ancestral (_0).

For some assays the statistical tool of imputation (imp) was used to limit the number of PCRs carried out. This tool lets you assume the results of assays not done yet, based on results of assays already analysed. If a sample shows the derived allele (_1) for an assay on one branch of the phylogenetic tree (e.g. sPZ), the assays on another branch (e.g. rX, rY, etc.) would be ancestral. Thus, these PCRs do not need to be carried out and the results can be imputed as ancestral (_0).

Some assays are marked as 'not done' (ND) because the previous findings suggested that they would show the ancestral allele.

The assay defining the HT is marked with a green background. Samples that were excluded from the final analysis are marked with a yellow background. Assays with NO_CALL (NC) are marked with orange background.

Table 17: Results of samples in HG Ao

Sample Nr.	Founder sire	rW	rX	rY	sE	sC	rZ	rAB	sAP	sAN	HT	Notes
BW-367	Ibrahim	A_1	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
BW-382	Saklawi I	A_1	T_1	AC_1	ND	ND	NC	A_0	ND	ND	Ao-aA*	
BW-383	Saklawi I	A_1	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
BW-395	Telmese	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
BW-XX	Saklawi I	A_1	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
P060276	Kuhailan Kharas	A_1	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
P060411	Kuhailan Kharas	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
P060881	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	NC	NC	ND	ND	Ao-aA*	
P070210	Koheilan Adjuze	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
P070298	Koheilan Adjuze	A_1_imp	T_1	NC	NC	NC	ND	ND	ND	ND		rA_NC
P070568	A Saglawi DB	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	

P070719	Kuhailan Kharas	A_1_imp	T_1	ACC_0	C_0	NC	ND	ND	NC	NC		
P070740	Hadban	ND	NC	ND	ND	ND	ND	ND	ND	ND		rA_NC
P070784	Saklawi I	A_1	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
P080268	Hadban	ND	ND	NC	ND	ND	ND	ND	ND	ND		sPZ_NC
P080269	A Saglawi DB	ND	NC	NC	ND	ND	ND	ND	ND	ND		
P080297	Kidwan al Harzan	A_1	T_1	ACC_0	C_0	A_0	ND	ND	NC	NC	Ao*	
P090271	Ibrahim	A_1	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
P090614	Shuway-man Sab-bah	A_1	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
P110308	Shuway-man Sab-bah	A_1	T_1	ACC_0	C_0	NC	ND	ND	NC	NC		
Y_PR5_32	Hadban	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
Y_PR5_33	Mahmoud Mirza	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_34	Saklawi I	ND	NC	NC	ND	ND	ND	ND	ND	ND		
Y_PR5_41	Koheilan Adjuze	A_1_imp	T_1_imp	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_42	Saklawi I	ND	NC	NC	ND	ND	ND	ND	ND	ND		
Y_PR5_43	Koheilan Adjuze	A_1_imp	T_1_imp	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_46	Kuhailan Kharas	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
Y_PR5_48	Shuway-man Sab-bah	A_1_imp	T_1	ACC_0	C_0	NC	ND	ND	NC	NC		
Y_PR5_49	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_50	Kidwan al Harzan	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
Y_PR5_57	Koheilan Adjuze	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_63	Saklawi I	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
Y_PR5_65	Hadban (Ibn Galal)	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_67	Ibrahim	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_69	A Saglawi DB	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_108	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_109	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_110	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_112	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_113	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_114	Felhaan Alshawaf	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_117	Ilderim	A_1_imp	T_1_imp	AC_1	ND	ND	NC	NC	ND	ND		

Y_PR5_118	Ilderim	A_1_imp	T_1_imp	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_119	Kuhailan Kharas	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
Y_PR5_122	Ibrahim	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_123	Saklawi I	ND	NC	NC	ND	ND	ND	ND	ND	ND		
Y_PR5_124	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_125	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_126	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_127	Krzyzyk	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
Y_PR5_129	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_131	Kuhailan Kharas	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
Y_PR5_133	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_134	Mirage	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
Y_PR5_135	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_136	Ilderim	A_1_imp	T_1_imp	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_139	Ibrahim	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_140	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_141	Saklawi I	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
Y_PR5_142	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_143	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_144	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_145	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_146	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_148	Mirage	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
Y_PR5_149	Ilderim	A_1_imp	T_1_imp	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_150	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_154	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_157	Kuhailan Kharas	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
Y_PR5_158	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_159	Saklawi I	A_1_imp	T_1	AC_1	ND	ND	G_0	A_0	ND	ND	Ao-aA*	
Y_PR5_161	Saklawi I	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
Y_PR5_162	Kuhailan Kharas	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	
Y_PR5_164	Krzyzyk	A_1_imp	T_1	ACC_0	C_0	A_0	ND	ND	A_0	G_0	Ao*	

Table 18: Results of samples in HG Ta

Sample Nr.	Founder sire	rA	sPZ	sPY	HT
BW-380	Bairactar	A_1	A_0	T_0	T*
BW-381	Bairactar	A_1	T_1	C_1	Ta-a*
BW-384	Bairactar	A_1	T_1	C_1	Ta-a*
P060304	A Saglawi DB	A_1	T_1	T_0	Ta*
P060305	O Bajan	A_1	T_1	T_0	Ta*
P060313	O Bajan	A_1	T_1	T_0	Ta*
P060334	Shagya	A_1	T_1	T_0	Ta*
P060358	Hamdani Semri	A_1	T_1	T_0	Ta*
P060414	Dahman-Amir	A_1	T_1	T_0	Ta*
P060614	Hamdani Semri	A_1	T_1	T_0	Ta*
P070172	O Bajan	A_1	T_1	T_0	Ta*
P070173	Shagya	A_1	T_1	T_0	Ta*
P070263	O Bajan	A_1	T_1	T_0	Ta*
P070326	Shagya	A_1	T_1	T_0	Ta*
P070864	Souakim	A_1	T_1	NC	Ta*
P080194	Hamdani Semri	A_1	T_1	NC	
P080265	Shagya	NC	NC		
P080566	Shagya	A_1	T_1	T_0	Ta*
P080710	Hamdani Semri	A_1	T_1	T_0	Ta*
P090413	Hamdani Semri	A_1	T_1	T_0	Ta*
P090611	Shagya	A_1	T_1	T_0	Ta*
P100191	Shagya	A_1	T_1	T_0	Ta*
P102340	Hamdani Semri	A_1	T_1	T_0	Ta*
Y_PR2_9	Ilderim	A_1	T_1	C_1	Ta-a*
Y_PR5_31	Shagya	A_1	T_1	T_0	Ta*
Y_PR5_36	O Bajan	A_1_imp	T_1	T_0	Ta*
Y_PR5_38	Hamdani Semri	A_1	T_1	T_0	Ta*
Y_PR_40	O Bajan	A_1	NC	T_0	
Y_PR_045	Hamdani Semri	A_1	T_1	C_1	Ta-a*
Y_PR5_51	Shagya	A_1	T_1	T_0	Ta*
Y_PR5_58	O Bajan	A_1	T_1	T_0	Ta*
Y_PR5_59	Mersuch	A_1	T_1	C_1	Ta-a*
Y_PR5_120	Bairactar	NC	NC	NC	
Y_PR5_147	Bairactar	A_1	T_1	C_1	Ta-a*
Y_PR5_153	Bairactar	A_1	T_1	C_1	Ta-a*

Table 19: Results of samples in HG Tb

Sample Nr.	Founder sire	rA	rT	rB	rC	rD	rP	rN	rQ	rJ	rK	rO	HT
P090273	Saklawi I	A_1	T_1	C_0	ND	ND	ND	ND	ND	ND	ND	ND	Tu*
P112348	Hamdani el Samra	A_1	C_0	G_1	T_0	TT_0	T_0	G_0	T_0	G_0	A_0	C_0	Tb-oB*
Y_PR5_39	Kuhailan-Abu-Urkub	A_1	C_0	G_1	C_1	DEL_1	ND	ND	ND	ND	ND	ND	Tb-dW1
Y_PR5_61	Shagya	A_1	C_0	G_1	C_1	DEL_1	ND	ND	ND	ND	ND	ND	Tb-dW1
Y_PR5_116	Kuhailan-Abu-Urkub	A_1	C_0	G_1	T_0	TT_0	NC	G_0	T_0	G_0	A_0	C_0	Tb-oB*
Y_PR5_137	Amer	A_1	C_0	G_1	C_1	DEL_1	ND	ND	ND	ND	ND	ND	Tb-dW1
Y_PR5_138	Amer	A_1	C_0	G_1	C_1	DEL_1	ND	ND	ND	ND	ND	ND	Tb-dW1